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Abstract Booklet

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Abstracts

(In order of author's last name)

Clinical Translation of Autologous Gamma/Delta-Enriched Chimeric Antigen Receptor (CAR)-T Cells to Target Prostate Stem Cell Antigen (PSCA) in Patients with Bone Metastatic Prostate Cancer

Presenter Name: Daniel Abate-Daga

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Bone metastatic castration-resistant prostate cancer (M1b CRPC) remains a highly fatal disease with significant associated morbidity. PSCA is expressed in >90% of mCRPC, with limited basal expression in normal tissues. A recent clinical trial of alpha/beta CAR-T cells targeting PSCA showed feasibility and safety, with evidence of antitumor activity. Based on our preclinical results we successfully completed an investigational new drug (IND) application to conduct a Phase 1 study (NCT06193486) in M1b CRPC to assess the safety of autologous gamma/delta-enriched T cells genetically modified to express a CAR targeting PSCA, in patients who receive zoledronic acid as standard of care. We postulate that due to the bone tropism of zoledronate, CAR-T cells will be activated by bone-resident tumor cells through two mechanisms: 1) activation of the CAR by PSCA, and 2) activation of the endogenous gamma/delta TCR by zoledronate-induced accumulation of phosphoantigens. We anticipate that this tissue-restricted dual activation will reduce the risk of systemic toxicities. Primary objective is to assess the safety and tolerability. Secondary objectives include antitumor efficacy based on prostate specific antigen (PSA) response, radiographic progression free survival and conversion of circulating tumor cells from above 5 to below 5/ml of peripheral blood.

Enrolled patients undergo apheresis at day -14. Lymphodepletion chemotherapy with cyclophosphamide 500 mg/m2 and fludarabine (30 mg/m2) is given at days -5, -4, and -3. Fresh gamma/delta-enriched CAR T cells targeting PSCA will be infused at day 0. Zoledronic acid will be given before apheresis and resumption of zoledronic acid after CAR-T infusion would be at the discretion of the treating physician. Patients will be infused with escalating doses of CAR T cells to establish the maximum tolerated dose (MTD): 1 × 105, 3 × 105, 1 × 106, 3 × 106, and 5 x 106 CAR T cells/kg, with a dose-limiting toxicity period from day 0 to day 28 post-infusion. Following determination of MTD, an expansion phase will be initiated. As of February of 2025, four patients have been treated, corresponding to dose levels 1 and 2, with no reported dose-limiting toxicities. Preliminary correlative analysis of post-infusion samples suggests persistence of infused gamma/delta T-cells in circulation for at least two weeks post infusion. The results of IND-enabling studies as well as preliminary correlative observations will be discussed.

Reciprocal Impacts of γδ IEL on Epithelial Cells in Human Intestinal Organoid co-Cultures

Presenter Name: George Adams

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

The gut epithelium harbours intraepithelial lymphocytes (IEL) which have putative roles in supporting normal epithelial development and barrier integrity. Many IELs express T cell receptor (TCR) γδ and the human Vγ4 subset recognises the epithelium through direct interactions between TCRV□4 and BTNL3 expressed on intestinal epithelial cells (IEC). Moreover, our group's previous work has implicated dysfunction of this interaction in Crohn's disease progression. These and other data have fuelled the hypothesis that TCRV□4 IEL promote gut integrity and repair. To test this, a 3D in vitro intestinal co-culture system between γδ IEL and intestinal organoids was established.

After much trial-and-error, conditions were found that permitted human IEL co-culture with organoids derived from either ileum biopsies or iPSC. Wide-field fluorescence microscopy demonstrated clear integration of the IEL within the epithelial barrier of both types of organoid. Moreover, IEL recovered from the organoids appeared healthy and showed a bona fide IEL phenotype by comparison with primary IEL maintained in culture with cytokines. In addition to this impact of IEC on IEL, there was an apparent reciprocal impact of co-cultured γδ T cells on epithelial growth, with an increase in the proportion of cells expressing EpCAM and other markers.

These results confirm the utility of a human organoid system for dissecting γδ interactions within the epithelium. Data already obtained support a role for IEL in supporting epithelial growth that may promote gut integrity and wound healing germane to the implication on BTNL variants in disease. This can be further analysed by gene-editing BTNL genes within the organoids.

Expansion and Characterization of KIR-enriched Polyclonal γδ T cells with Enhanced Anti-cancer Immunity

Presenter Name: Yeganeh Almasi

Yeganeh Almasi, University of Ottawa, Children's Hospital of Eastern Ontario Research Institute
Mahya Razmi, University of Ottawa, Children's Hospital of Eastern Ontario Research Institute
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Primary Theme: Cancer Therapies

Abstract

γδ T cells show great promise in cancer immunotherapy due to their HLA class I-independent tumor recognition, rapid expansion, cytotoxicity, cytokine production and ability to infiltrate peripheral tissues. Clinical trials have primarily focused on the Vγ9Vδ2 subset, which can be efficiently expanded in vitro using phosphoantigens (pAgs). However, this approach yields γδ T cells with limited diversity and may suppress NK cell activity, potentially leading to suboptimal clinical outcomes. Polyspecificity emerges as a key feature in the antigen recognition of γδ T cells, and a polyclonal γδ T cell pool encompassing all subsets could provide a compelling alternative to pAg-stimulated γδ T cells. To this end, we have developed a novel protocol that expands γδ T cells by approximately 1500-fold. This method generates a diverse population of γδ T cells, including Vδ1+, Vδ2+, and Vδ1/2^{-} subsets, and selectively enriches for killer cell immunoglobulin-like receptor (KIR)+ cells, which we have demonstrated to possess enhanced effector potential. These expanded cells persist in vitro for up to 30 days, exhibit potent effector functions, and express minimal levels of checkpoint inhibitors. Additionally, they retain a broad repertoire of Natural Killer (NK) cell receptors, enabling them to effectively recognize and eliminate a wide range of cancer cells, mediate strong antibody-dependent cell cytotoxicity (ADCC), and respond robustly to IL-15 stimulation. These characteristics make them ideal candidates for combination with trispecific engagers designed to enhance tumor targeting. Such engagers bind CD16 on γδ T cells to promote ADCC, target tumor-associated antigens for precise recognition, and incorporate an IL-15 crosslinker to drive expansion and sustain activation, thereby maximizing antitumor efficacy. This advanced cell product holds significant therapeutic potential, offering a powerful approach to improving anti-cancer immunity and enhancing clinical outcomes across diverse patient populations, irrespective of donor-patient compatibility.

Tiny Warriors: Unraveling the Frontline Role of Neonatal γδ T Cells in Early-Life Battles

Presenter Name: Vicente Almeida

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Primary Theme: Infection Responses

Secondary Theme: Development & Differentiation

Abstract

Neonates, especially those born prematurely, are more susceptible for infectious diseases than adults. Human γδ T cells are among the first T cells generated in the fetal thymus, where they can get functionally programmed to respond immediately to any stimuli in the periphery. As such, they may represent an important line of “first responders” in neonatal perturbations. However, the mechanisms that determine the responsiveness of neonatal γδ T cells to inflammation and infection, and how this relates to their prematurity status, remain mostly unknown. Here, we aim to characterize γδ T cells in the first days of life from healthy and diseased neonates, born either at term or preterm, including the most prevalent neonatal disorders: infections and perinatal asphyxia. Peripheral blood mononuclear cells were isolated from the neonates during routine blood sampling and analysed by flow cytometry or single cell RNA sequencing. Our data suggests that γδ T cells, but not αβ T cells and NK cells, can respond to neonatal disturbances such as prematurity, infection, and metabolic acidosis/asphyxia. We show that the prematurity state at birth is a key determinant of the postnatal adaptation of γδ T cells, as reflected by increased expression of CD127 and CD25 in γδ T cells from preterm neonates. This expression directly correlated with the capacity of these cells to be activated, proliferate, and release cytotoxic mediators in vitro. During neonatal disturbances, we observed a preferential response of Vδ2 γδ T cells to pathogen-confirmed infection, while Vδ1 γδ T cells appear to respond to all remaining sterile inflammation stimuli. Deeper characterization at a single cell level indicated a response of γδ T cells against all neonatal perturbations, with NK cells responding mainly to infection and αβ T cells remaining naïve during the time period analysed. We could additionally characterize an undescribed AREG+ γδ T cell population in the bloodstream with the transcriptional profile of tissue-resident cells, which requires further validation. Altogether, we show the immune system adapts in response to early life disturbances mainly by the modulation of the γδ T cell pool, with NK and αβ T cells not responding in such a pleiotropic manner. Overall, we expect that our findings will shed light on the functional diversity of neonatal γδ T cells at different levels of inflammation, and how this might differ among term and preterm neonates.

Technical Advancements in Nano-Neoantigen Activated γδ T Cells for Tumor Immunotherapy

Presenter Name: Mohammed Alnaggar

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Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

The development of immunotherapy has marked a revolutionary step in cancer treatment, allowing for more targeted and personalized approaches. γδ T cells, a unique subset of T cells, have emerged as a promising therapeutic tool due to their ability to recognize and kill tumor cells without relying on traditional antigen presentation pathways. Neoantigens, specific to tumor cells, offer a promising target for cancer immunotherapy. Combining these with γδT cells, which possess natural cytotoxic properties, represents a novel therapeutic approach. Nano-neoantigen technology enhances the effectiveness of this treatment by increasing antigen load and activation in γδT cells, leading to enhanced tumor-targeting abilities.The neoantigen peptides (P001) were efficiently loaded onto γδT cells, achieving a loading rate of over 60% after just 30 minutes, which increased to 100% after 4 hours of incubation. Following activation with nano-neoantigens, γδT cells exhibited a significant upregulation of key markers, including CD80 (increasing from 5% to 70%), CD86 (from 0.38% to 50%), and HLA-DR (from 0.4% to 86.3%). Moreover, co-incubation with CD3+ T cells resulted in enhanced proliferation of both CD4+ T cells (from 26% to 52%) and CD8+ T cells (from 16% to 33%), contributing to a boost in adaptive immunity as shown in Fig 1. Finally, at an effector-to-target ratio of 50:1, γδT cells activated by nano-neoantigens demonstrated a tumor cell killing efficiency of 90%, which significantly outperformed control groups, as illustrated in Fig 2. The integration of nanotechnology with neoantigen-loaded γδ T cells marks a significant advancement in cancer immunotherapy. This approach enhances antigen loading, improves cell maturation, and activates the immune system, increasing the specificity and efficacy of treatments. Its tumor-killing potential supports its viability as a therapeutic option. Future research will focus on optimizing these techniques in clinical settings and validating their long-term safety and effectiveness through larger clinical trials. Ultimately, the combination of γδ T cells and nanotechnology paves the way for personalized, targeted cancer therapies with improved patient outcomes.

Keywords: γδ T cells, tumor immunotherapy, nano-neoantigen, CD8+ T cells, adaptive immunity, tumor killing

Fig 1: Nano-neoantigen-activated γδT can effectively induce the proliferation of CD4+ T cells and CD8+ T cells, thereby enhancing the adaptive immune response.

Fig 2: γδT activated by Nano-neoantigen improves the specific killing of tumor cells by inducing the activation and proliferation of CD8+ T cells

Microbiota-induced TCR and IL-15 signaling enhances CD39 expression on γδ intraepithelial lymphocytes

Presenter Name: Sara Alonso

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

γδ intraepithelial lymphocytes (IEL) serve as the first line of defense against pathogen invasion. We recently identified a hyperproliferative γδ IEL (γδHYP) phenotype that is transmissible via the gut microbiota and protects against enteric infection. Despite increased IEL number, γδHYP mice display no overt intestinal pathology; therefore, we hypothesized that additional regulatory mechanisms may prevent aberrant IEL activation. Bulk transcriptomics of sorted WT and γδHYP IELs revealed that γδHYP IELs upregulate Entpd1 (CD39) (padj<0.0001), which was supported by increased surface expression of CD39 on γδHYP IELs compared to WT (p<0.0001). CD39 is an ectoenzyme that hydrolyzes extracellular ATP into ADP/AMP, which in turn is broken down by CD73 into immunoregulatory adenosine. To investigate the molecular mechanisms driving enhanced CD39 expression, we performed CITE-seq and found that CD39neg γδ IELs exhibit a more naïve-like phenotype whereas CD39hi γδ IELs upregulated genes downstream of T cell receptor (TCR) activation and IL-15 signaling (padj<0.05), suggesting a mature IEL phenotype. ELISA of jejunal lysates revealed a substantial increase in IL-15 receptor complex (IL-15RC) expression in γδHYP mice relative to WT (p<0.001), with the frequency of CD122+ (IL-2Rβ) cells highest among CD39hi γδ IELs (p<0.0001). Culturing CD39neg γδ IELs with 100 ng/ml IL-15RC induced CD39 (p<0.001), whereas antibody-mediated blockade of CD122 in vivo led to an 80% reduction of CD39hi γδ IELs in γδHYP mice (p<0.0001). Further, TCRγδ MFI was lowest among CD39neg (p<0.01) and CD39int (p<0.05) γδHYP IELs, demonstrating that these cells are responsive to antigen in vivo. Internalization of the TCRγδ decreased the frequency of CD122+ (p<0.05) and CD39hi γδHYP IELs (p<0.0001). Oral administration of broad-spectrum antibiotics to WT and γδHYP mice had a similar effect (p<0.0001), indicating that γδHYP-associated commensals likely activate the TCR. Lastly, we found that CD39hi γδ IELs from both phenotypes produce less IFNγ and TNF upon stimulation relative to CD39neg and CD39int γδ IELs (p<0.0001). Overall, our study identifies a novel mechanism by which an altered microbiota leads to the expansion of γδ IELs with regulatory potential. Further understanding of the mechanisms regulating γδ IEL effector function may ultimately allow fine tuning of mucosal immunity to ensure protection against intestinal injury or infection while limiting aberrant cytotoxicity.

Multimodal Single-Cell Profiling Reveals Distinct Gamma Delta T Cell Subsets with Pathogenic and Protective Functions in Ulcerative Colitis

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Primary Theme: Tissues & Inflammation

Secondary Theme: Ligands & Repertoires

Abstract

This study provides new insights into the role of γδ T cells in ulcerative colitis (UC) using single-cell multimodal profiling. Existing research in both mice and humans generally portrays γδ T cells as protective in inflammatory bowel diseases (IBD) — primarily due to a focus on intra-epithelial lymphocytes (IEL). To date, no single-cell study has been conducted to comprehensively assess the roles of γδ T cells in IBD.

To adress this issue, we conducted an unbiased single-cell study of γδ T cells isolated from colonic tissue and peripheral blood mononuclear cells (PBMC) of healthy donors and UC patients by performing single-cell RNA sequencing (scRNA-seq), single-cell T cell receptor (TCR) sequencing and cytometry by time of flight (CyTOF).

We identified two distinct γδ T cell subsets in the inflamed colon of UC patients: a memory-like (TCF-1+ PD-1+) population and a cytotoxic (GZMB+ PRF1+ TBX21+) subset. These subsets showed clonal overlap based on TCR sequencing data, indicating a shared developmental origin. Both populations expanded in active UC and contracted with treatment, linking them to disease progression and rendering them potential therapeutic targets.

Notably, the TCF-1+ PD-1+ memory-like subset closely resembles stem-like CD8+ T cells previously identified in chronic infections, cancer, and UC. Our findings suggest that this subset represents a γδ T cell counterpart, challenging the prevailing view that γδ T cells are primarily protective. Instead, our data highlight the additional existence of pathogenic γδ T cell populations that contribute to the disease.

Furthermore, we found a marked depletion of protective CD103+ γδ IELs, correlating with reduced expression of butyrophilin-like molecules (BTNL3, BTNL8) in inflamed intestinal enterocytes of UC patients. In contrast, the up-regulation of BTN3A1 and BTN3A3 in enterocytes was associated with the cytotoxic γδ T cell phenotype. Recovery of the normal γδ T

cell composition in patients responding to therapy suggests that these subsets could serve as both biomarkers and therapeutic targets.

Our study provides a comprehensive single-cell landscape of γδ T cells in UC, revealing their protective and pathogenic roles. These findings open new avenues for therapeutic interventions targeting distinct γδ T cell subsets.

Clonal Selection Defines a Hyporesponsive Human Vγ9+Vδ2+ γδ T Cell Compartment

Presenter Name: Daniel Arsovski

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

The human Vγ9$^+$Vδ2$^+$ T cell compartment is pivotal in antimicrobial and antitumor immunity, yet its functional heterogeneity remains poorly understood. Here, we reveal that clonal expansion within this compartment is tightly associated with a phosphoantigen (PAg)-hyporesponsive state. Using flow cytometry, single-cell TCR sequencing, and bulk transcriptomics, we characterise a distinct subset of Vγ9$^+$Vδ2$^+$ T cells identified by a CD27negCD28neg phenotype that further diverges to express CD16 and is critically linked to its TCR reactivity. These CD16$^+$ Vγ9$^+$Vδ2$^+$ T cells display highly clonal γδ TCR repertoires and exhibit markedly reduced activation, proliferation, and TNF-α production in response to HMB-PP and Plasmodium falciparum-infected red blood cells, as compared to their CD27$^+$CD28$^+$ counterparts. γδ TCR transduced cell lines demonstrated that while TCRs from non-CD16$^+$ cells retain robust PAg reactivity, those derived from CD16$^+$ cells are intrinsically hyporesponsive. Transcriptomic analysis further distinguishes these cells by a cytotoxic, NK-like gene signature marked by high perforin and granzyme B, alongside markers of exhaustion, and lower expression of granzyme K. Importantly, we show environmental exposures shape this phenotype, where Malian children in malaria-endemic regions exhibit increased frequencies of CD16$^+$ Vγ9$^+$Vδ2$^+$ T cells compared to age matched Australian peers. Moreover, HCMV reactivation in HSCT recipients was linked to the expansion of this hyporesponsive subset, and tissue analyses revealed an enrichment of CD16$^+$ Vγ9$^+$Vδ2$^+$ T cells in the gut, and clonally expanded cells in other peripheral tissues. Collectively, our findings highlight an axis of differentiation within the Vγ9$^+$Vδ2$^+$ T cell compartment that is inextricably linked to TCR reactivity.

Upgrading TEGs with an anti-BTN3A-Chimeric Costimulatory Receptor Offers Two Key Advantages: Superior Tumor Control and Extended TEG Longevity

Presenter Name: Dennis Beringer

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Primary Theme: Cancer Therapies

Secondary Theme: Ligands & Repertoires

Abstract

Targeting of solid tumors with T cell based therapies remains challenging, highlighting the need for improvements in developing more effective therapies that overcome the limitations encountered to target this type of tumors as limited presence of tumor-associated antigens or immunosuppressive tumor microenvironment. In our previous study, we showed that addition of 103-4-1BB, an anti-BTN3A chimeric coreceptor (CCR), to αβT cells engineered to express a tumor reactive Vγ9δ2-TCR (TEG-103-4-1BB) significantly improved tumor control in solid and hematological in vivo models compared to our original TEG1. We characterized the underlying mechanism of the superior activity of TEG-103-4-1BB, by dissecting the importance of different CCR building blocks; the anti-BTN3A antibody fragment (scFv) & the intracellular signaling domain. We established that the BTN3A epitope of the anti-BTN3A-CCR impacted its functionality in TEGs, only the use of anti-BTN3A scFv-103.2 resulted in significant enhanced tumor control and proliferation, while all other tested anti-BTN3A-scFv were not enhancing TEG activity due to overlapping epitopes the Vγ9δ2-TCR. In addition we showed that the 103-chimera increases the interaction between TEGs and tumor cells, resulting in enhanced early-stage T cell effector functions that were independent of the costimulatory intracellular signaling domain. Sustained long-term T cell responses were fully dependent on the intracellular signaling domain and the cis-interaction of the 103-chimera with BTN3A on the TEG surface induces prolonged survival of TEGs without TCR activation. In conclusion, the unique properties of the 103-4-1BB CCR provided TEGs not only with better tumor control though trans interaction with BTN3A on the tumor cells, but also with longevity due to BTN3A binding in cis.

Alternative Expansion Methods Influence Vγ9Vδ2 T Cell Biology

Presenter Name: Adi Berman

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Primary Theme: Ligands & Repertoires

Secondary Theme: Cancer Responses

Abstract

Vγ9Vδ2 T cells, the predominant subset of γδ T cells in peripheral blood, have been of scientific and clinical interest given their ability to recognize and kill tumor cells. Expanding Vγ9Vδ2 T cells ex vivo is a critical step in generating cells for basic discovery and for developing effective immunotherapies. Efforts have focused on optimizing effective expansion methods to yield high quantities of cytolytic Vγ9Vδ2 T cells. The two most widely used expansion methods utilize either the small molecule zoledronate to activate BTN2A1/BTN3A1 surface expression and Vγ9Vδ2 TCR engagement, or TCR targeting antibodies to directly activate Vγ9Vδ2 T cells. However, it is unknown how alternative expansion protocols can influence the phenotype, functionality, and cytotoxic capabilities of Vγ9Vδ2 T cells. Therefore, we characterized Vγ9Vδ2 T cells expanded through either of these two methods to compare their cell yield, activation states, and effector functions. Cells expanded by either method showed quantitative and temporal changes in the surface expression of activation markers. Additionally, Vγ9Vδ2 T cells expanded by TCR antibody-based activation exhibited higher cytotoxicity against an ovarian cancer cell line, relative to zoledronate expanded cells. Together, our data indicate that systematic evaluation and characterization of these expansion methods are essential to understanding the biology of Vγ9Vδ2 T cells and for further development of Vγ9Vδ2 T cell-based immunotherapies.

ADI-270, an Armored Allogeneic Anti-CD70 γδ CAR T Cell Therapy, Demonstrates Robust CAR-dependent and CAR-independent Anti-Tumor Activity Against Hematologic and Solid Tumor Models

Presenter Name: Arun Bhat

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Background: Unlike conventional αβ T cells, γδ T cells possess both innate and adaptive immunity that enable targeting and killing of tumors in an MHC-independent manner. Together with their natural tissue tropism and antitumor activity, γδ T cells have the potential to effectively traffic to tumor sites and reduce antigen escape. We developed ADI-270, a novel allogeneic γδ T cell product engineered with a third-generation CAR targeting CD70 using its natural receptor (CD27). In addition, ADI-270 is armored with a dominant negative TGFβ receptor (dnTGFβRII) to resist the immunosuppressive effects present in the tumor microenvironments.

Methods: Vδ1 T cells engineered to express an anti-CD70 CAR and dnTGFβRII were generated from healthy donor human PBMCs. We compared the functionality of ADI-270 to clinically relevant benchmarks representing scFv-based anti-CD70 αβ CAR T cells using in vitro cytotoxicity assays against tumor cells. Human tumor xenografts in NSG mice were used to assess in vivo trafficking and efficacy of ADI-270. CITE-seq was used to profile gene and surface expression levels of ADI-270.

Results: ADI-270 exhibited potent in vitro cytotoxicity against tumor cells expressing varying levels of CD70 and maintained effector function in the presence of TGFβ1. Compared to αβ CAR T cell benchmarks, ADI-270 exhibited increased potency against CD70-low tumors and in CD70+/- cell mixtures, as well as activity against CD70- tumor cells, indicating both CAR-directed and CAR-independent killing mechanisms. Despite this increased potency, ADI-270 demonstrated a lower inflammatory cytokine profile when cocultured with CD70+ tumor cells

compared to αβ CAR T benchmarks. Additionally, ADI-270 showed rapid tumor homing and inhibited tumor growth in CD70+ human tumor xenografts, including those with low and heterogenous CD70 expression. Analysis of transcriptomes obtained by scRNAseq using UMAP dimensionality reduction and cluster analysis confirmed ADI-270 cell population homogeneity.

Conclusion: These results support the enhanced potency of ADI-270 against multiple CD70-low to CD70-negative tumor cell models compared to scFv-based αβ CAR T cell benchmarks. The anti-tumor activity of ADI-270, which includes both CAR-directed and CAR-independent mechanisms, highlights the potential to overcome the challenges with antigen heterogeneity. The promising preclinical efficacy and safety profile support the clinical development of ADI-270 to target multiple CD70+ cancers.

Dual modulation of cytotoxic and checkpoint receptors tunes the efficacy of Delta One T cells against colorectal cancer

Presenter Name: Rafael Blanco-Domínguez

Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Colorectal cancer (CRC) remains a major challenge for immunotherapy, as only a minority of CRC, displaying microsatellite instability (MSI), responds to current checkpoint inhibition. Vδ1+ γδ T cells, endowed with robust HLA-I-independent and neoantigen-independent cytotoxic functions, coupled with natural tropism for the gut and tumors, emerge as promising mediators of CRC immunotherapy. We developed a Vδ1+ γδ T cell-based adoptive cell therapy, named Delta One T (DOT) cells, that has been translated to the clinic for hematological malignancies, but has never been applied in solid tumors. Here we demonstrate the capacity of DOT cells to target CRC cell lines and patient-derived specimens and organoids in vitro (both MSI and MSS); and to control tumor growth in an orthotopic xenograft model of human CRC. Notwithstanding, we found tumor-infiltrating DOT cells to exhibit a dysregulated balance of cytotoxic and inhibitory receptors that paralleled that of endogenous Vδ1+ TILs and limited their cytotoxic functions. Critically, we unveil two strategies to maximize DOT-cell efficacy: upregulating CRC expression of ligands for the key activating receptor, NKG2D, upon butyrate administration; and blocking the immune checkpoints TIGIT and PD-1, which synergistically drive DOT-cell dysfunction. These findings open new avenues for DOT-cell-based combinatorial approaches for CRC treatment.

Building a Multimodal Map of Early Human T Cell Development to Unravel the Molecular Mechanisms of the αβ vs. γδ Lineage Decision

Presenter Name: Lena Böhme

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Primary Theme: Development & Differentiation

Secondary Theme:

Abstract

αβ and γδ T cells, though distinct in their T cell receptors and functional properties, develop from common multipotent bone marrow-derived precursors. Their differentiation and lineage bifurcation take place in the specialised microenvironment of the thymus, where the immature thymocytes undergo TRD, TRG, and TRB locus rearrangements to form a functional γδ- or pre-TCR, facilitating their commitment to either the γδ or αβ lineage. Previous work from our lab identified unique changes in the chromatin landscape upon commitment of human thymocytes to the γδ lineage, which were not observed in β-selected cells, hinting at lineage-specific molecular programmes. However, the precise mechanisms – including the transcriptional regulators – governing this fate decision in human thymocytes remain poorly understood, largely due to challenges in the detection of rare or transient differentiation stages.

To enhance our understanding of the αβ vs. γδ lineage bifurcation process and uncover the molecular drivers underlying this pivotal milestone in the development of T cells, we leveraged cutting-edge single-cell technologies to profile immature human postnatal thymocytes at unprecedented resolution.

By employing a combination of 166-plex CITE-seq and scTCR-seq, we were able to map the T cell differentiation trajectory and pinpoint the αβ/γδ bifurcation point, which included an immature γδTCRlow/- population with high predicted γδ T fate potential. We further identified transcriptional shifts associated with β-selection or γδ lineage differentiation and uncovered gene regulatory networks with selective activity in lineage-committed cells or bipotent progenitors. We are now further dissecting these networks using paired scRNA-seq and scATAC-seq data, which can help reveal stage- or lineage-specific regulatory regions and verify relationships between transcription factors and predicted target genes through motif enrichment analysis. Promising transcription factor candidates can then be validated via gene editing in in vitro co-culture systems to assess their potential role in the αβ/γδ lineage decision.

This comprehensive multimodal profiling approach has yielded new insights into the molecular processes taking place in immature human thymocytes as they differentiate along the αβ or γδ T cell lineage and will help unravel the drivers behind the lineage decision.

γδ T cells for Cancer Therapy: Lessons Learned From a Case

Presenter Name: Anna Bold

Anna Bold, Lothar Marischen, Marco Bardenbacher, Jovana Ilic, Laura Wahl, Martin Wilhelm, Stefan Knop,
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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

γδ T cells exhibit cytotoxic effects on cancer cells and are thus suitable candidates for cellular immunotherapy against several types of malignancies. We have shown in the past that the allogeneic transfer of γδ T cells is feasible without relevant side effects and that monoclonal antibodies, such as the anti-CD20 obinutuzumab, can enhance their antibody-dependent cellular cytotoxicity (ADCC). Therefore, we treated a patient with refractory B cell lymphoma, who was not eligible for any other treatment, with haploidentical γδ T cells. These were stimulated with zoledronate and interleukin-2 in vivo and obinutuzumab was administered to the patient. The patient had a complete remission within a few weeks, but unfortunately relapsed with CD20 negative B cell lymphoma after four months.

Here, we summarize what we learned from this case and which strategies we chose to optimize the cellular treatment with γδ T cells. This includes the need for repeated administration of γδ T cells. As this is not possible by an in vivo stimulation, we established an ex vivo cultivation protocol that leads to a cell product consisting of more than 90% γδ T cells and an improved anti-tumor activity compared to γδ T cells generated by our previous protocol. As seen in this case, antigen loss is a common obstacle of cancer therapy with therapeutic antibodies. Since the combination of two antibodies with different targets may prevent this resistance mechanism, we investigated the effect of a combination of two antibodies on the anti-tumor activity of γδ T cells and could show that the ADCC of γδ T cells is not reduced compared to single antibody use. Finally, we investigated the effect of different bispecific antibodies on the anti-tumor activity of γδ T cells. We could show that bispecific antibodies significantly increase the anti-tumor activity of γδ T cell compared to the respective controls and even to an associated monoclonal antibody to an extent.

The next step is now to check whether our in vitro findings lead to an optimization of cellular therapy with γδ T cells in vivo.

IL-17-Microbiota Crosstalk Regulates Peripheral Nerve Regeneration

Presenter Name: André Bombeiro

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

Peripheral nervous system (PNS) injuries represent a significant clinical challenge, often leading to chronic pain, impaired motor function, and substantial economic burdens. Following PNS injury, immune cells play a crucial role by clearing debris, modulating inflammation, and promoting nerve regeneration through the release of growth factors. While the contributions of macrophages and neutrophils are well-reported, the involvement of T cells, particularly IL-17-producing T cells, remains poorly understood. IL-17A (referred to as IL-17), traditionally associated with inflammatory diseases, has also been implicated in tissue repair. Of note, these functions are often regulated by the gut microbiota, which can directly promote IL-17 production. Conversely, IL-17 shapes the microbiome composition, as shown in the context of neuroinflammation of the central nervous system. Here, we hypothesized that IL-17 impacts peripheral nerve regeneration following injury in a microbiota-dependent manner. Using flow cytometry, we identified γδ T cells as the primary source of IL-17 in the crushed sciatic nerve of mice. IL-17-producing γδ T cells (γδ17 T cells) accumulated in the injured nerve early in the regenerative process, peaking at 3 days post-injury (dpi). This accumulation was seemingly triggered by microbiota derived cues, as it was abolished in mice treated with broad-spectrum antibiotics. To further unveil the functional role of IL-17 in nerve regeneration, we used IL-17-deficient (Il17-/-) and IL-17-competent (Il17+/+) mice. When housed separately from their Il17+/+ littermates, injured Il17-/- mice displayed incomplete motor recovery, along with elevated electrophysiological activity in the sciatic nerve. Importantly, these differences were abrogated when Il17-/- and Il17+/+ mice were housed together, thus sharing the microbiome composition. Further mechanistic analysis showed that separated Il17-/- mice exhibited a dysregulated expression of the regeneration marker growth associated protein 43 (GAP43) and poorer axonal myelination, both of which were restored by co-housing. In summary, our findings reveal a bidirectional crosstalk between IL-17 and the gut microbiota that impacts sciatic nerve regeneration, shedding light on novel potential therapeutic targets for diseases linked to peripheral neurodegeneration.

CCR7+LAG3+ γδ T Cells May Be Tumor Reactive in Neuroblastoma

Presenter Name: Anne Borst

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Background: High-risk neuroblastoma (HR NBL) patients have a survival rate of ~50%, highlighting an urgent need for novel treatments. Recent implementation of immunotherapy in the treatment regimen improved survival with ~15%. HR NBL contains few immune infiltrates and expresses low major histocompatibility complex I (MHC-I). Recently, it was shown for other MHC-I negative tumors that γδT cells play a key role in anti-tumor activity. We therefore aimed to gain insights in γδT cells in NBL to ultimately generate γδT cell-based therapy.

Methods: Resected tumors after chemotherapy from 19 patients were digested and stained with 35 antibodies, and analyzed by spectral flow cytometry. Healthy donor blood from 12 adults was used as control. Manual gating was done using FlowJo software. Unsupervised clustering was performed using the R package cyCONDOR. Single-cell T cell receptor sequencing (scTCRseq) was performed on blood and tumor samples at diagnosis and resection from 10 HR NBL patients using the 10x genomics platform.

Results: ~10% of T cells in the tumor microenvironment (TME) of NBL were γδT cells. In scTCRseq data, Vδ1+ cells were enriched in tumor versus blood and significantly higher in tumors post-chemotherapy, while Vδ2+ showed the opposite pattern. Vδ3+ cells were rare but also enriched in tumors. Based on flow cytometry data, γδT cells separated into three clusters, expressing either high KLRB1, CCR7/LAG3, or CD45RA. CCR7+LAG3+ γδT cells expressed higher levels of multiple immune checkpoints, including PD-1, TIGIT, and TIM-3, and of activation markers CD137 and CD25 compared to KLRB1+ and CD45RA+ γδT and to αβT cells, possibly suggesting tumor reactivity. Although in general γδT cells had low expression of GZMB and perforin compared to healthy adult blood γδT cells, KLRB1+ and CCR7+LAG3+ γδT cells had a slightly higher expression compared to CD45RA+ γδT cells. We performed an interaction analysis based on the expression of immune checkpoint receptors on γδT cells and their ligands on other cells in the TME. The highest interaction probability was detected between tumor and γδT cells via the CD200/CD200R axis. In addition, a high probability was observed with M2-like macrophages via the TIGIT/PVR axis, possibly indicating that these interactions inhibit γδT cell functions.

Conclusion: CCR7+LAG3+ γδT cells in NBL are possibly tumor reactive yet inhibited by immune checkpoints. These insights may pave the way for γδT cell-based immunotherapies for NBL.

The αβ T and B Cell Lineages have Multiple Impacts on γδ T Cell Subsets

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Whereas studying γδ T cells in isolation is appropriate, it is equally important to understand the cells' integration with the two other classes of adaptive lymphocytes: αβ T cells and B cells. In this regard, our group published some years ago that γδ T cells in TCRβ-/- mice differ from those in w.t. mice because they lack trans-conditioning, specifically, the impact of TCRαβ + DP thymocytes on developing γδ thymocytes (PMID:14502287, PMID 15591166). That work highlighted the integration of lymphocyte lineages and had practical implications because it was commonplace for various experimental studies to use γδ T cells from TCRβ-/- mice because of the ease of γδ cell isolation. This study greatly expands this approach, using s.c.RNASeq coupled with validation experiments to compare intestinal, splenic and immature thymic γδ cells from w.t.., TCRβ-/- mice, and B cell-deficient mice, at steady-state and following challenge. The steady-state data will be presented here, showing overt differences between γδ cells derived from the different strains.

Emphasising the impact of the αβ lineage on the cells' cytolytic and functional potentials, gut γδ IEL from TCRβ-/- mice showed down-regulation of genes encoding granzymes, Ccl4, Ccl3 and CD8α while upregulating Prss2, Xcl1, Ltb, Cd226, Nfkb1, Kit and Cd160 amongst others. Strikingly, differentially expressed genes (DEGs) of gut γδ IEL from B cell-deficient mice were completely different including up regulation of Jak3, Lat, Tgtp2, and Itgb2.

γδ splenocytes from TCRβ-/- mice also showed downregulation of cytotoxicity-related genes, Klrb1c, Tyrobp, and Klrk1 and genes encoding TCR-associated functions, whereas those from B cell-deficient mice showed upregulation of S100a9, S100a8, Ifitm2 and Ifitm3, all implicated in the interferon response.

The downregulation of cytotoxicity-associated genes was already apparent in HSA+ γδ thymocytes from TCRβ-/- mice, while upregulated DEGs were related to selection and signalling. γδ thymocytes from B cell-deficient mice were phenotypically distinct from those in w.t. and TCRβ-/- mice. In sum, these data are developing a high-resolution map of the impact of the αβ T and B cell lineages upon γδ T cells; promote the validity of the concept of integrated immunology; and are relevant to better understanding different from of immunodeficiency.

Understanding CAR Signaling in γδ T Cells to Enhance Metabolism and Function

Presenter Name: Xiomar Bustos Perez

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Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

We have previously shown that Vd2 γδ CAR-T cells targeting prostate stem cell antigen (PSCA) can induce tumor regressions, in a mouse model of bone-metastatic castrate-resistant prostate cancer. While we test the safety of this approach in a phase I clinical trial (NCT06193486), we sought to systematically map the signaling events triggered by the CAR, with the ultimate goal of identifying actionable targets to enhance Vd2 γδ CAR-T cells.

To that end, we performed a mass spectrometry-based analysis of the CAR signalosome, using αβ CAR-T cells as a benchmark. As a result, we identified 323 phosphorylation events that were differentially abundant between γδ and αβ T cells (fc ≥ 1.5, Welch's t-test p value < 0.05). Glycolysis and gluconeogenesis pathways were significantly overrepresented within differentially phosphorylated proteins, with a trend suggesting inhibition of the pathway. Further functional analyses, including SeaHorse and flow cytometry, confirmed that γδ present lower glycolytic capacity and glucose uptake than αβ CAR-T cells. γδ CAR-T cells also displayed lower mitochondrial mass, consumed less oxygen, and had lower spare respiratory capacity than αβ CAR-T cells. We identified Thioredoxin-Interacting Protein (TXNIP) as a potential contributing factor to these metabolic properties. TXNIP is known to regulate the intracellular oxidative stress response and glucose metabolism. Interestingly, γδ CAR-T cells showed hypophosphorylated TXNIP in tyrosine 349. Phosphorylation of this residue induces TXNIP degradation and, consistently, we found that γδ CAR-T cells expressed higher levels of TXNIP than αβ T cells. We next hypothesized that modulation of TXNIP expression in γδ T cells would enhance their metabolic fitness and therapeutic potential. γδ T cell expansion in the presence of a small molecule inhibitor of TXNIP (SRI-37330 hydrochloride) resulted in T cells with greater respiratory capacity and increased glycolytic rate. We are conducting further assays to test the impact of TXNIP inhibition over γδ CAR-T cell antitumor activity.

Ongoing and future efforts are focused on validating TXNIP as a target to improve the function of γδ CAR-T cells, and on designing cGMP-compatible processes to modulate TXNIP expression.

Elucidation of the Molecular Underpinnings of γδ Lineage Commitment Using Genome Wide Approaches

Presenter Name: Igor Bychkov

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Primary Theme: Development & Differentiation

Secondary Theme: Ligands & Repertoires

Abstract

γδ and αβ T cells are thought to arise from a common progenitor in the thymus. The specification of these lineage fates is strongly influenced by differences in T cell receptor (TCR) signal strength or duration, with stronger/ more prolonged signals leading to adoption of the γδ fate. The stronger/more prolonged signals instruct adoption of the γδ fate, through marked induction of Id3, which in turn, produces greater reductions in DNA binding by E box family DNA binding proteins (E-proteins). Although it is known that E-proteins play critical roles in regulating the □□/γδ fate decision, neither the transcriptional regulators with which they cooperate nor the molecular targets through which they do so have been defined. To elucidate the molecular processes responsible for γδ-commitment, we performed genome wide analysis of the changes in E protein binding in fetal progenitors that have been induced to adopt the γδ fate in response to strong γδTCR signals. We determined that γδTCR signals induced a striking reduction and remodeling of E protein binding relative to that in uncommitted progenitors. We find that loss of αβ fate potential is linked to the loss of E protein binding at critical fate-determining genes. Moreover, HOMER motif analysis revealed that E protein sites were tightly linked to binding sites of transcriptional regulators TCF1, ETS, Gata, and Runx. Moreover, ATAC-Seq revealed that changes in chromatin accessibility are enriched for occupancy by transcriptional regulators, Relb and Runx2, raising the possibility that they function in concert with E proteins to promote the γδ fate. Indeed, knockdown of Runx2 interfered with the development of γδ T cells in OP9-DL1 stromal cultures. Together, these studies identify Runx2 as a co-factor that collaborates with E proteins in promoting the γδ fate.

Enhancing Immunotherapy in Endometrial Cancer: Targeting PD-1 on γδ T Cells

Presenter Name: Valentina Cazzetta

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Endometrial cancer (EC), the most prevalent gynecological malignancy, has shown a rising incidence and mortality in recent years. Standard treatments primarily involve surgery and/or chemotherapy. However, the identification of tumor escape mechanisms has prompted the development of novel therapeutic strategies, particularly immune checkpoint inhibitors targeting PD-1. In this context, we investigate the role of unconventional gamma delta (γδ) T cells, recognized for their favorable response in various human malignancies, although their role in EC remains unknown.

Notably, a high frequency of Vδ1 T cells correlates with improved overall survival in EC patients. Flow cytometry analysis reveals that Vδ1 is the predominant γδ T cell subset infiltrating EC lesions in both early and advanced stages. Upon in vitro stimulation, these cells preserve their functional activity by producing key cytokines, such as IFN-γ and TNF. However, within the tumor microenvironment, they exhibit increased expression of PD-1, compared to their circulating blood-matched counterparts. Single-cell RNA-sequencing (scRNA-seq) analysis further confirms this upregulation in γδ T cells, exhibiting a progressive increase in PD-1 expression from peritumoral to tumor specimens, in contrast to healthy tissue. Crucially, despite PD-1 upregulation, tumor-infiltrating Vδ1 T cells retain their immune competence, which can be enhanced by PD-1 blockade. Indeed, their cytotoxic response against autologous tumor cell targets significantly increases upon treatment with a specific blocking anti-PD-1 monoclonal antibody compared to controls. Supporting the specific tumor-reactivity of Vδ1 T cells expressing PD-1, we also observed that PD-1+ γδ T cells within the tumor exhibit a more focused γδ repertoire compared with their PD-1 negative counterparts. Consistently, specific activation of Vδ1 T cells upon PD-1 blockade has been revealed by scRNA-seq analysis of EC patients undergoing anti-PD-1 therapy. Remarkably, the presence of Vδ1 T cells already before the treatment serves as a predictor of response to anti-PD-1 therapy.

These findings underscore the potent antitumor role of Vδ1 T cells in EC and highlight their potential as therapeutic targets to enhance current immunotherapeutic strategies.

Gamma-delta T Cell-mediated Tumor Cell Killing and Resistance

Presenter Name: Kok Fei Chan

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

γδ T cells form the first line of immunosurveillance and bridge between the innate and adaptive immune responses. Upon sensing the danger signals, activated γδ T cells mount rapid innate-like immune responses and produce large quantities of cytokines and chemokines to modulate other tumor-infiltrating immune cells for tumor cell killing. Unlike other conventional T cells, activation of γδ T cells is not restricted by major histocompatibility complex (MHC) molecules. Our group has previously defined the molecular aspects of the T-cell receptor (TCR)-dependent activation of Vγ9Vδ2+ T cells by tumors following the presentation of phosphoantigens via BTN2A1/BTN3A1 complexes. High tumor-infiltrating γδ T cells in cancer patients is associated with better survival across 39 cancer types. In a randomized, controlled, double-blinded in vivo experiment we showed that tumor-bearing NSG mice which received activated human γδ T cell adoptive cell transfer had delayed tumor growth and longer survival. Despite γδ T cell protective role, tumor cells can escape γδ T cell-mediated killing through many of the yet to be identified mechanisms. In this study, we further investigated these unknown tumor immune escape mechanisms by performing genome-scale loss-of-function CRISPR screens on human colorectal cancer, acute myeloid leukemia and melanoma cells under Vδ2+ γδ T cell immune pressure. These screens identified different gene candidates in tumor cells including the known genes i.e., BTN2A1 and BTN3A1, that are essential for Vδ2+ γδ T cell-mediated tumor cell killing. The outcome from this study has strengthened our current knowledge of γδ T cell anti-tumor immunity and will allow us to develop an effective γδ T cell-based tumor immunotherapy in the future.

Characterization of γδT Cell Subsets in Nasopharyngeal Carcinoma: Implications for Long-Term Survival After Chemotherapy and Sequential EBV-primed Autologous Adoptive Cell Therapy

Presenter Name: Alice Cheung

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Nasopharyngeal carcinoma (NPC) is a common epithelial cancer in the Southeast Asia region, usually with late-stage diagnosis and a strong association with Epstein-Barr virus (EBV). While gamma-delta T (γδT) cells have previously been correlated with positive NPC prognosis, little is known about the roles and regulations of these cells in NPC patients. Leveraging on longitudinal samples collected from our previous clinical trial (NCT00690872) treating EBV-positive NPC patients with autologous EBV-expanded cytotoxic T lymphocytes (CTLs), we performed scRNA-seq and evaluated the dynamics of γδT cell composition and gene expression in CTL as well as in patient's circulation before and after chemotherapy coupled with adoptive CTL transfer. Our analysis reveals that a Vδ2- predominant circulating γδT compartment can be observed in a subset of long-term survivors (LTS-Ctrl) prior to treatment and negatively correlates with tumour growth. A significant proportion (49.5±12.9%) of these γδT cells were found to be highly activated with an "exhausted CTL" gene expression signature. In contrast, majority of the γδT cells in short-term survivors (STS, 32.1±9.9%) and other LTS with persistent tumour progressions (LTS-Prog, 41.5±19.3%) displayed a "Th17-like" gene expression signature. In vitro priming of patients' baseline PBMC with autologous derived EBV-LCL resulted in an increase in overall representation of γδT cells in CTL specifically in LTS but not in STS patients. Consistent with the well-reported EBV-LCL induced Vδ1+ activation, preferential expansion of the Vδ2- cell subsets were noted in all LTS samples. Interestingly, while the bulk of the expanded γδT cells in CTL of LTS-Ctrl attained a "Th1-like" and "early activated" gene signatures (51.7±14.6% & 38±9.8% respectively), majority of those in CTL of LTS-Prog (43.9±33.9%) were at a much more differentiated cell state ("activated CTL"). Infusion of such Vδ2- enirched autologous CTL into 1 of the LTS-Prog did not change its circulating γδT composition, which maintained to be mostly Vδ2+. Conversely, a persistent Vδ2- predominant circulating γδT compartment was observed up to 7 months post-CTL treatment in 2 of the LTS-Ctrl patients. In summary, our data suggest that less differentiated Vδ2- enriched γδT cells may offer potential therapeutic benefits for NPC patients. Additionally, baseline circulating γδT cell subtype composition may offer prognostic values to autologous EBV-expanded CTL therapies.

Enrichment and Entrapment of Tumor-Reactive Vδ1+ γδT Cells in Primary Microsatellite-Stable Colorectal Cancer

Presenter Name: Dorine De Bont

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

γδ T cells play an important role in cancer immune surveillance and present a promising immunotherapeutic strategy, particularly for tumors with a low mutational load. However, clinical implementation of these cells or their receptors remains challenging, partially due to their functional heterogeneity and diverse T cell receptor repertoire. To address this, we characterized distinct γδ T cell populations across different anatomical compartments, namely peripheral blood (PBMCs), healthy colon tissue intraepithelial lymphocytes (IELs), and tumor infiltrating lymphocytes (TILs) from primary tumor lesions of 29 microsatellite stable colorectal cancer (MSS CRC) patients. Vδ1+ γδ T cell were identified as the predominant subset within primary CRC lesions, with high expression of activation NK co-receptors consistently observed across all colon-resident Vδ1+ γδ T cells. Notably, bulk Vδ1+ γδ TILs released substantial levels of IFNγ in response to CRC cell lines independent of expression of activation NK co-receptor, NKG2D. Similarly, at monoclonal level, Vδ1+ γδ TILs displayed a high frequency of tumor-reactive clones, evidenced by a strong IFNγ release against CRC cell lines, again independent of NKG2D expression implying that the γδTCR derived from Vδ1+ γδTILs is one of the main drivers in tumor recognition. Transfer of the Vδ1+ γδ TIL TCR into αβ T cells confirmed that tumor reactivity is mediated by the γδ-TCR of the Vδ1+ γδ TIL clones as a potent IFNγ release against CRC cell lines was preserved. In conclusion, our findings emphasize the functional diversity and potential of γδ T cells in different tissues and identifies colon-resident Vδ1+ γδ T cells as central mediators of anti-tumor immunity in MSS CRC, supporting their potential use in immunotherapeutic strategies.

Prenatal γδ T Cells determine the inflammatory cytokine shift in aging and inflammation

Presenter Name: Anastasia De Poulpiquet du Halgouet

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Heterogeneity within the γδ T cell compartment has been characterized in terms of Vγ chain usage, tissue residency, and effector capabilities. γδ T cells emerge in distinct developmental waves, with some subsets developing exclusively during fetal life —such as Vγ5$^+$ dendritic epidermal T cells (DETC), which home to and reside in the skin—while others, including Vγ1$^+$ and Vγ4$^+$, develop both pre- and postnatally. While their development appears to follow specific patterns of antigen exposure and expression of determined transcription factors, it remains to be established whether specific functional features are pre-defined by their cellular ontogeny.

To address this question, we developed a fate-mapping system based on the postnatal expression of terminal deoxynucleotidyl transferase (TdT), which results in specific labeling by YFP of post-natal cells. We validated our model showing that all Vγ5$^+$ remained YFP- at any age analyzed, while the postnatal developing Vγ7+ subset was exclusively YFP+.

We next generated a complete fate map across multiple tissues and subsets and further focused our analysis on liver. Within this tissue we identify pre-natal Vγ6$^+$ cells, which account for approximately 30% of all γδ T cells . Vγ1$^+$ and Vγ4$^+$ cells display a mixed pre- and postnatal origin. Functionally, pre-natal Vγ6$^+$ and post-natal Vγ1$^+$ and Vγ4$^+$ appear predetermined, while pre-natal Vγ1$^+$ and Vγ4$^+$ shift from IFN-γ to IL-17 production. This cytokine shift also reflects the overall cytokine switch that occurs during aging, suggesting that prenatal cells are the main drivers of this phenomenon. Liver induced inflammation by Carbon Tetrachloride (CCL4) that models non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH), exacerbates and accelerates this cytokine shift suggesting a key role by pre-natal cells in promoting liver pathogenesis and inflammaging.

Development of EPCR-reactive γδTCR as Bispecific T-cell Engager

Presenter Name: Ben De Wet

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Primary Theme: Ligands & Repertoires

Secondary Theme: Cancer Therapies

Abstract

To overcome HLA-restriction of TCR-based therapies we investigated the developability of a γδTCR bispecific molecule, targeting the monomorphic antigen endothelial protein C receptor, EPCR. We demonstrated that a Vγ4Vδ5 TCR-anti CD3 bispecific engager redirects T cells against 70% of colorectal cell lines and 70% of colorectal organoids that were tested. EPCR expression on targets is required but not sufficient for reactivity, which correlates with post translational antenna fucosylation of EPCR residue N30. Through whole genome CRISPR KO screening we identified glycosylation pathways required for this modification and validated the findings with loss and gain of function experiments. We produced recombinant highly fucosylated EPCR molecules to guide TCR affinity enhancement and obtained Vγ4Vδ5 TCR variants with improved binding kinetics and greater potency. The highest affinity TCR variant was also used as a soluble staining reagent to validate target expression by immunohistochemistry. While binding to critical normal tissues prevented further clinical development of the program, our results pave the way for development of γδTCR bispecific engagers.

Extracellular Matrix Degradation-Driven Accumulation of Cytotoxic Vδ1 T-cells in Modic Type 1 Changes

Presenter Name: Jan Devan

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Primary Theme: Tissues & Inflammation

Secondary Theme: Tissues & Inflammation

Abstract

INTRODUCTION: Modic type 1 changes (MC1) are painful vertebral bone marrow lesions adjacent to degenerated intervertebral discs. Bone marrow inflammation is a hallmark of MC1 lesions, and several reports point to the T cell activation in the tissue.

OBJECTIVE: To investigate the immunological background of MC1.

METHODS: Vertebral bone marrow aspirates were collected from patients with MC1 (n=22, MC1+intra-patient control=8+8, control patients=6) undergoing lumbar spinal fusion, mononuclear cells were isolated and investigated by massively parallel flow cytometry (FC) screening of 360 surface markers followed by machine learning based prediction of their co-expression. T-cell functional properties were analysed with FC: 1.) ex vivo analysis of Granzyme B, 2.) cytokine release after in vitro stimulation with PMA/ionomycin, and 3.) proliferation in response to IL-15. Single-cell RNA-sequencing (scRNA-seq) was performed on enzymatically digested MC1 and intra-patient control biopsies (n=4+4). Extracellular matrix proteins of intervertebral discs (IVDs) were analysed by LC-MS/MS. Cartilage oligomeric matrix protein (COMP) was quantified in bone marrow plasma with ELISA. Finally, MC1-derived mononocytes were treated with COMP and surface IL15 levels were determined by FC.

RESULTS: Immunophenotyping revealed accumulation of Vδ1 T-cells in MC1 compared to control patients (fold change 1.58, fdr<0.01) and intrapatient controls (fold change 1.55, p<0.5). Significant difference in the expression of 73 surface markers on Vδ1 T-cells in MC1 was detected. Most Vδ1 T-cells in MC1 expressed NKp80 (72%), and NKp80 was more expressed on Vδ1 T-cells than on other T-cells (72% vs 23%, p<0,01). NKp80+ Vδ1 cells expressed more Granzyme B (53% vs 16%) and produced more IFNγ (77% vs 25%) and TNF (84% vs 62%) upon stimulation than other T-cells (all p<0.01). Moreover, Vδ1 T-cells expressed higher levels of IL15 receptors than other T-cells, and IL-15 selectively induced their proliferation in vitro (all p<0.01). Bone marrow plasma IL-15 levels correlated with Vδ1 frequency (R2=0.84, P<0.001). ScRNAseq revealed that IL-15 was predominantly expressed by CD16+ monocytes (fold change to 2nd most producing subset is 2.3, p<0.5) and that CD16+ monocytes accumulated in MC1 (fold change 1.3, fdr<0,5). ECM component COMP was enriched in IVDs adjacent to MC1

(p<0.05) and in MC1 bone marrow (fold change 1.2, p<0.05) and COMP induced expression of IL15 by monocytes in vitro.

CONCLUSION: We discovered an extracellular matrix degradation product-driven accumulation of cytotoxic and pro-inflammatory TCR Vδ1 T-cells driven in MC1 lesions. To our knowledge, this is the first comprehensive description of the capacity of extracellular matrix derived DAMPs to induce proliferation of specific γδ T cell population by inducing IL15 expression in monocytes.

Understanding the Fate and Migration Pattern of Tissue-Borne Unconventional T Cells

Presenter Name: Rupak Dey Sarkar

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Primary Theme: Tissues & Inflammation

Secondary Theme: Infection Responses

Abstract

Unconventional T cells (UTCs) comprising of gamma delta T cells, NKT, and MAIT, like DCs form a bridge between the innate and adaptive immune response. UTCs seed non-lymphoid tissues during development in waves and mediate barrier tissue protection via both cytokine and TCR-mediated activation. Parabiosis experiments showed that like Trm or ILCs, UTCs are also tissue-resident in nature. However, in Ataide et al., 2022, we found that tissue-derived UTCs continously migrate via the lymphatic route to locally draining lymph nodes (LNs) where they mediate characteristic immune responses based on the tissue they originate from. For example, skin UTCs have a Th17-like response in skin-draining LNs in mice that is tailored for extracellular pathogen defense. However, the fate of these tissue-derived UTCs once they reach the LNs is unclear as well as why they migrate to the LN to begin with. By using different reporter mice that specifically reports for tissue-derived UTCs, in-vivo lymph node photoconversion techniques and scRNA-seq of UTCs from LNs and blood, I have direct evidence to support the idea that the tissue-derived UTCs leave the LNs, enter the bloodstream and return to the tissue of origin. Additionally, using the photoconversion experiments, I am trying to quantify and model the entire migration pattern and kinetics with our collaborators. These findings challenge the current paradigm that UTC are strictly tissue-resident lymphocytes. I am currently investigating the functional implications of this unexpected migratory pattern by using different genetic mouse models where we can preferentially block/disrupt the migration. Additionally, I'm developing and validating novel mouse models that will allow me to study the function of UTCs as a whole compartment independently of specific subsets (NKT, MAIT and gamma delta T cells). We believe that our results will make an important contribution in understanding UTC-biology and will have direct implications for harnessing this cell type for immunotherapy in humans. Additionally, the presence of tissue-derived UTCs in the blood of humans can be potentially used as a tissue-specific biomarker for diagnosing any tissue-specific inflammation.

Killer Cell Immunoglobulin-like Receptors Delineate Distinct Phenotypes and Functions in Human γδ T Cells

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Primary Theme: Development & Differentiation

Secondary Theme: Infection Responses

Abstract

Killer cell immunoglobulin-like receptors (KIRs) are a diverse family of HLA class I-specific receptors that shape immune responses, primarily through Natural Killer (NK) cells. Beyond NK cells, subsets of T cells, notably γδ T cells, express KIRs, but their roles remain unclear. Here, we demonstrate that KIR^{+}γδ T cells are significantly more abundant in cytomegalovirus (CMV)-seropositive healthy adults, particularly within the Vδ2^{-} subset. These cells exhibit a TEMRA phenotype, are predominantly CD16^{+}CD57^{+}, hallmarks of a highly differentiated state, and express multiple NK cell-associated receptors. KIR+γδ T cells display enhanced cytotoxic potential, with higher levels of granzyme B and perforin than their KIR- counterparts. They demonstrate superior effector responses to PMA-ionomycin stimulation and heightened activity in antibody-dependent cellular cytotoxicity assays. Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) and transcriptional analyses reveal distinct molecular signatures, with KIR+γδ T cells transcriptionally and epigenetically aligning with terminally differentiated effector memory T cells, while KIR- cells retain a naïve-like phenotype. These findings suggest that CMV infection drives the differentiation of γδ T cells into potent, KIR-expressing cytotoxic effectors. This adaptive mechanism may bolster immune defense while maintaining self-tolerance, highlighting a previously unappreciated role for KIRs in shaping γδ T cell immunity.

The γδ T Cell Club: Bringing The Scientific Community Closer Together In The Age Of Zoom

Presenter Name: Matthias Eberl

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Primary Theme: Ligands & Repertoires

Secondary Theme: Development & Differentiation

Abstract

Immunologists are inherently social people because they love to meet other immunologists and talk about immunology (and about other immunologists, constantly). γδ T cell researchers are no exception. As being a minor group within the Immunology field, γδ T cell researchers crave for interactions with like-minded scientists. Which is where the technological solutions being established during the COVID-19 pandemic come into play that have, almost overnight, allowed researchers to hold online meetings and webinars.

We here describe how we set up the virtual ‘γδ T cell Club’, a monthly virtual seminar series that has successfully helped bring the people in our field closer together. During the first 18 months of the webinar series the number of our followers on Twitter/X increased rapidly by >70% to over 3,000; after abandoning Twitter/X in November 2024 and moving the γδ T cell to Bluesky we are now reaching nearly the same number of followers on the new platform. We have already hosted >50 junior and senior researchers from around the world, covering the range of basic, translational and clinical research on γδ T cells in humans and animals, with an average live audience size of 50-100, and further people watching the recordings. We believe that the online format is particularly relevant for people away from the major research hubs and/or who may struggle to attend in-person meetings, and who will thus benefit most from having a chance to participate and contribute to the scientific discourse.

Over the past three years, we have learned immensely about organising, hosting and following up while using Zoom to communicate and promote cutting-edge γδ T cell science globally. We will present a summary of the lessons learned with regard to advertising, diversity, timing and impact – and an outlook of our platform going forward.

Polarisation of naïve CD4+ T cells into T follicular helper cells by human Vγ9Vδ2 T cells

Presenter Name: Kirsty Emery

Kirsty, Emery, Cardiff University
Matthias, Eberl, Cardiff University

Primary Theme: Infection Responses

Secondary Theme: Development & Differentiation

Abstract

Introduction: Human Vγ9Vδ2 T cells respond to a range of infection related molecules, of which the most potent is the microbial metabolite HMB-PP, which is produced by many bacteria. Depending on the microenvironment, activated Vγ9Vδ2 T cells can assume different effector functions, including the capacity to act as professional antigen presenting cells (APCs) that can prime naïve CD4+ and CD8+ T cells. Previous research demonstrated that γδ T cell-primed naïve CD4+ T cells can differentiate into distinct effector subsets, most notably Th1 cells and Th22 cells, as well as IL-10 producing Tr1-like cells. The impact of this plasticity of Vγ9Vδ2 T cells as APCs for the regulation of humoral immunity remains to be investigated.

Aims: This study aims to investigate whether antigen-presenting Vγ9Vδ2 T cells can polarise naïve CD4+ T cells towards a Tfh effector subset, and to elucidate the underlying molecular pathways.

Results: Our initial findings show that LPS-stimulated monocyte-derived dendritic cells are suitable control APCs to induce naïve CD4+ T cells towards a Tfh phenotype characterised by expression of CXCR5, PD-1, ICOS and BCL6. In a screen to identify cytokine combinations that allow HMB-PP activated Vγ9Vδ2 T cells to acquire features associated with APCs (HLA-DR, CD83) and the germinal centre reaction (CXCR5), IL-12, alone or in combination with IL-23 were particularly potent factors in the induction of these surface markers. These polarised Vγ9Vδ2 APCs are now being tested in mixed lymphocyte reactions with allogeneic naïve CD4+ T cells to elucidate which effector Th subsets they may induce, alongside LPS-stimulated monocyte-derived dendritic cells as gold standard.

Conclusion: Human γδ T cells have already been shown to interact with T cells and B cells in secondary lymphoid tissues. The regulation of Tfh cell generation is fundamental for the induction of antibody responses and effective humoral immunity, during natural infections as well as upon vaccination. Numerous studies have shown increased Tfh cell populations positively affect vaccine efficacy. Our research to investigate the capacity of Vγ9Vδ2 T cells to polarise Tfh cells will contribute to our understanding of adaptive immunity in microbial infections but may also help inform the design of novel and improved vaccination strategies.

Dermal Innate-like T cells Mediate Anti-Fungal Immunity through Direct Sensing of Mycoflora-derived Metabolites

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Primary Theme: Tissues & Inflammation

Secondary Theme: Infection Responses

Abstract

IL-17, produced by Type 3 lymphocytes (T3L) Th17, Tgd17, MAIT17, and ILC3 cells, is a critical mediator of anti-fungal immunity. IL-17 production by T3L is regulated by Pattern Recognition Receptors (PRRs), cytokine receptors, or through T cell Receptor (TCR) recognition of antigen. The T3L response to mycoflora is vital for host tolerance and promotion of tissue barrier fitness. Here we show that skin commensal fungus Malassezia furfur is directly recognized by a subset of dermal Tgd17 cells (Vg2+, Raulet Nomenclature), leading to expansion and IL-17 production upon topical association. Mechanistically, Vg2+ Tgd17 cells sensing of M. furfur is independent of canonical MHC molecules, IL-23R, IL-1R, and CARD9-dependent PAMPs. Furthermore, supernatant from M. furfur stimulates specifically Vg2+ Tgd17 cells to produce IL-17 both in vitro and in vivo, suggesting that secreted fungal molecule(s) directly activates Vg2+ Tgd17. cells. The molecular basis of this novel interaction is currently being deciphered. Most critically, mice previously associated with M. furfur are armed with protection against lethal doses of other pathogenic fungal species. This primed protection is strictly dependent on Vg2+ Tgd17 cells as no other T3L subsets can perform the same function. Our studies suggest that a subset of dermal innate-like Tgd17 cells surveil local tissue environment through sensing of fungal-derived metabolites to mediate systemic cross-protection against other pathogens that are controlled by IL-17.

CD8αβ-Expressing γδ T Cells Are Innate Interferon-γ Producers That Develop Perinatally and Expand in IL-7R/STAT5B-driven γδ T Cell Neoplasms

Presenter Name: Gina Fiala

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Primary Theme: Development & Differentiation

Secondary Theme: Infection Responses

Abstract

The non-redundant contributions of γδ T cells to immune responses are often associated with their rapid secretion of interferon-γ (IFNγ). Here, we describe a perinatal thymic wave of innate IFNγ-producing γδ T cells that express CD8αβ heterodimers. CD8αβ-expressing γδ T cells are highly abundant in the perinatal thymus and populate multiple tissues in neonatal mice. Notably, they are the primary γδ T cell source of IFNγ in neonatal lymph nodes. CD8αβ-expressing γδ T cells persist in adult tissues, where they expand in preclinical models of infection and cancer.

In the perinatal thymus, the development of CD8αβ-expressing γδ T cells is supported by low T cell receptor signaling and the cytokines interleukin (IL)-4 and IL-7. Their enhanced expression of IL-7Rα prompted us to analyze two recently established pre-clinical leukemia/lymphoma mouse models driven by constitutive IL-7R-STAT5B signaling; one by IL-7R overexpression and the other through transgenic expression of the most frequent STAT5B oncogenic mutation.

These mouse models of T cell neoplasia demonstrated enhanced thymic development of CD8αβ-expressing γδ T cells and the accumulation of polyclonal CD8αβ+ γδ T cells in the thymus and peripheral lymphoid organs, highlighting their pathological potential under conditions of constitutive IL-7R-STAT5B signaling. Similarly, among pediatric patients with human T cell acute lymphoblastic leukemia, CD8αβ-expressing γδ T cells represent a distinct subset. This study elucidates the normal and malignant development of CD8αβ+ γδ T cells, which are prominent in early life and contribute to innate IFN-γ responses in the contexts of infection and cancer.

γδT Cells Engineered to Secrete B7H3 Targeting Opsonins Show Preclinical Efficacy Against Bone Resident Cancers

Presenter Name: Jonathan Fisher

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Primary Theme: Cancer Therapies

Secondary Theme:

Abstract

Introduction: The innate lymphoid surveillance properties of Vγ9Vδ2 T cells alleviate the need for fully synthetic cytotoxic switches such as chimeric antigen receptors. Antibody dependent cytotoxicity (ADCC) of Vγ9Vδ2 T cells can be harnessed by engineering them to secrete synthetic opsonins, leading to targetable FcγRIII dependent activity. B7H3 is a promising target in solid tumor immunotherapy due to its over-expression in many cancer types and role in both tumor progression and immune evasion. We evaluated the potential of Vγ9Vδ2 T-cells secreting anti-B7H3 opsonins and IL15Ra-IL15 fusion proteins.

Methods: γδT cells were engineered to secrete immune-active payloads including anti-B7H3 scFv-Fc fusion proteins and IL15Ra-IL15 fusion protein. Payload production was determined by ELISA and antigen-specific enhancement determined by ADCC report bio-reporter assays and cytotoxicity against a range of cancer cell lines. Orthotopic models of squamous cell lung carcinoma bone metastasis and patient-derived osteosarcoma were used to probe in-vivo efficacy. Signaling and phenotype in engineered cells was determined using mass cytometry.

Results: B7H3 binders secreted by γδT cells can induce antibody dependent cytotoxicity (ADCC) at sub-nanomolar concentrations, outperforming clinically approved anti-B7H3 antibodies such as enoblituzumab. When combined with zoledronic acid, in-vivo efficacy against intra-tibial models of squamous cell lung carcinoma and patient-derived osteosarcoma models was observed. Computational analysis signaling and phenotype in therapeutic preparations payload-secreting γδT cells demonstrate enhancement of un-engineered bystanders. Product effectiveness is retained after cryopreservation, enabling efficient campaign manufacture of allogeneic therapy.

Conclusion: Payload-secreting γδT differ from conventional cell therapy solutions by acting as a delivery system, retaining enhanced cytotoxicity and conferring this on bystanders. This alternate approach to γδT cell engineering harnesses and enhances their innate cytotoxicity.

CBFb2 Quantity Regulates Tgd17 Cell Development and Age-Dependent Hematopoietic Progenitor Cell Differentiation Trajectory

Presenter Name: Michela Frascoli

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Primary Theme: Development & Differentiation

Secondary Theme:

Abstract

Tgd17 cells acquire their mature phenotype early in thymic development, at the level on DN1d subset, as revealed by their transcriptional profile. A key candidate for regulating this profile is CBFb2, the essential cofactor of RUNX proteins—transcriptional regulators expressed during the earliest stages of mouse embryonic development. The RUNX-CBFβ2 complex has been shown to control the expression of multiple genes that define the Vg2 Tgd17 progenitors, highlighting its critical role in shaping their lineage commitment. Using Cbfb2 heterozygous mice we uncovered crucial insights into how CBFb2 regulates Tgd17 cell generation. Despite a reduction in fetal thymic precursors, neonatal Tgd17 cells persist in haploinsufficient mice, suggesting compensatory mechanisms. Notably, adult bone marrow progenitors, which typically exhibit low efficiency in generating Tgd17 cells, demonstrated enhanced lineage commitment under reduced CBFb2 expression. In contrast, CBFb2 overexpression suppressed this effect, highlighting its dosage-dependent regulation of Tgd17 cell fate. Transcriptomic analyses revealed upregulation of embryonic hematopoiesis-associated genes, including Sox6, in heterozygous lymphoid-primed multipotent progenitors. Furthermore, in utero transplantation of adult haploinsufficient progenitors into E14.5 fetal liver improved the reconstitution of neonatal-origin Tgd17 cells, underscoring their reprogramming potential. These findings establish CBFb2 as a pivotal regulator of Tgd17 cell development and suggest a potential strategy for reprogramming adult progenitors to enhance Tgd17 cell generation for therapeutic applications.

GFI1 as a New Regulator of Adult γδ T Cell Development and IL-17/IFNγ Lineage Decision

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

GFI1, a DNA-binding zinc finger transcription factor, plays a critical role in regulating target gene expression by recruiting histone demethylase 1 (LSD1) and other histone modifiers to specific genomic loci. The GFI1/LSD1 complex is essential for hematopoietic stem- and precursor cells, regulating their commitment to myeloid and lymphoid lineages. Here we report that GFI1 is expressed in γδ T cells from both lymphoid and barrier organs and restricts the cellularity of RORγt+Vγ6+ γδT cells that produce high levels of IL-17A, while supporting the expansion of Vγ1+ and Vγ4+ γδT cells. The absence of GFI1 results in a pronounced bias toward excessive production of γδT17 cells in the thymus, commencing post-birth. We also found that thymic CD4/CD8 double negative (DN) pre-T cells from adult mice lacking GFI1 when cultured on OP9-DL1 cells, give rise predominantly to CD24-CD44+CD45RB- γδT cells, which are known as the γδT17 subtype. These cells likely originate from RORγt+/MAF+ cells that were expanded in the DN1 thymic pre-T cell subset of adult Gfi1 deficient mice. This population, along with other DN1 pre-T cells subsets in Gfi1 deficient mice, exhibit a distinctive γδT17 cell-specific transcriptomic profile. In support of this, we observed that DN2 and DN3 pre-T cells lacking GFI1 preferentially differentiated towards the IFNγ secreting γδT1 subtype, while DN1 cells were more prone to form the γδT17 biased CD24-CD44+CD45RB- γδT cells. Trajectory analysis using sc-RNA-seq data also indicated that lack of GFI1 oriented the differentiation of DN1 pre-T cells toward IL-17+ γδ T cells. Mechanistically, DN1, DN3, and γδ T cells lacking GFI1 show upregulation of the B-ZIP transcription factor MAF, which regulates genes critical for IL-17+ γδ T cells, such as Rorc and Blk that are all induced in Gfi1 deficient cells. We also found that the Maf gene contains several binding sites for GFI1 in its promoter region and ChIP-PCR with thymic DN pre-T cells showed an enrichment for GFI1 in these specific regions. This indicates that GFI1 could repress the expression of Maf in DN cells, limiting the production of γδT17 progenitors in the adult thymus to support the emergence of the other γδ T cell subtypes, essential for an effective innate immune response.

Notch-induced Vγ1.1+ Vδ6.3+ TCRγδ Cells Engage Type 2 Thymo-stromal Crosstalk Associated with Thymic Regeneration

Presenter Name: Miki Gams

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Unconventional TCRγδ and TCRαβ T cell lineages acquire pre-programmed immune effector functions during thymic selection. They often migrate to barrier sites where they function as “poised” responders to tissue stress, infection and cancer. However, some remain and regulate thymus homeostasis. In adult mice, Type 2 polarized natural killer T (NKT2) cells secrete IL4 which promotes thymus regeneration after cytoablative agents trigger acute thymic involution (ATI). IL4 recruits eosinophils (Eos) which interact with thymic innate lymphoid cells (ILC2) and fibroblasts to induce thymus regrowth. However, few NKT cells develop prior to 2 weeks of age. Our work shows that that IL4-producing Vγ1.1+ Vδ6.3+ TCRγδ cells, known as “V6” cells, engage this Type 2 thymo-stromal cross-talk network in the early post-natal (PN) thymus.

Like NKT cells, V6 cells express PLZF, the transcription factor that confers steady-state cytokine secretion, and can adopt Tbet+ Tγ1 or IL4-producing Tγ2 fates. We found that CD4-Cre-induced alterations in Notch signaling significantly altered the balance of these effector subsets in the neonatal thymus. All neonatal TCRγδ lineages were strongly fate mapped by CD4Cre, suggesting that V6 TCRγδ cells are selectively responsive to CD4Cre-induced alterations in Notch signaling.

Remarkably, CD4Cre-induced Notch1 gain-of-function (N1-GOFCD4) increased generation of IL4-secreting V6-Tγ2 cells ~50-fold by PN d14. This expansion was accompanied by sudden onset of ATI, as indicated by dramatic loss of thymic cortical regions and near complete loss of CD4+CD8+ (DP) thymocytes. SiglecF+ thymic Eos and ILC2 were greatly increased and medullary stroma were highly expanded and disorganized, suggesting aberrant activation of the regenerative Type 2 thymo-stromal crosstalk axis. Surprisingly, Il4 deficiency only slightly ameliorated these impacts, revealing IL4-independent cross-talk mechanisms. By PN d42, numbers of V6 and Eos declined but not to WT levels. However, DP cellularity was only partially restored and thymic architecture was permanently remodeled. Interestingly, thymic regeneration was less robust and more variable in N1-GOFCD4 males relative to females, revealing a strong sex effect.

Collectively, these data link Notch-induced expansion of V6-Tγ2 cells with induction of ATI and activation of IL4-dependent and independent mechanisms of thymo-stromal crosstalk that promotes thymic regeneration after cytoablative treatments.

Gamma-delta T cell-microglia crosstalk promotes CD8 T cells infiltration into the brain and contributes to AD pathogenesis in APP/PS1 model

Presenter Name: Marilia Garcia de Oliveira

Marilia Garcia de Oliveira, Ana Clara Rosa Guimaraes, Yeseswi Archana Guduri, Patrick da Silva, Toby Black Lanser, Clara Avelar Mendes de Vasconcellos, Joao Oliveira Goes Neno, Rafael Machado Rezende
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Primary Theme: Tissues & Inflammation

Secondary Theme: Ligands & Repertoires

Abstract

Neuroinflammation, a key driver of Alzheimer's disease (AD), contributes to neuronal damage, synaptic dysfunction, amyloid plaques and neurofibrillary tangles deposition. In AD, microglia shift from homeostatic (M0) to a neurodegenerative (MGnD) phenotype, recruiting peripheral immune cells to the brain. Gamma-delta (γδ) T cells play a pivotal role in sustaining neuroinflammation. We found that in a traumatic brain injury (TBI) model, which exhibits amyloid plaque accumulation associated with neuroinflammation, γδ T cell-deficient mice showed fewer plaques and improved cognitive and motor functions. Additionally, Vγ1 and Vγ4 γδ T cells subtypes play opposing roles through the production of TGF-β, and IFN-γ and IL-17, respectively. In the 3xTg AD model, IL-17-producing γδ T cells contribute to pathology. Our scRNA-seq analysis of public datasets shows an enriched IFN-γ and IL-17 signature in γδ T cells from AD patients' PBMCs and hippocampus. We aim to investigate the role of γδ T cells in the APP/PS1 AD model. We found that γδ and CD8 T cells infiltrate the brain, which also exhibited an MGnD phenotype. γδ T cell-deficient APP/PS1 mice improved cognitive performance, reduced amyloid plaque burden, shifted MGnD to M0 phenotype, and reduced CD8 T cells infiltration. Importantly, both Vγ1 and Vγ4 infiltrated the brains of APP/PS1 mice. As our findings suggest, γδ T cells likely play a detrimental role in AD by engaging in a pathological crosstalk with microglia and CD8 T cells.

Expanded Vδ1-γδ (DOT) T cells as a promissing immunotherapy against Glioblastoma

Presenter Name: Coline Gentil

Gabriel Marseres1, Coline Gentil1, Doriane Bomont5, Victor Bigot1, Maxime Courant1, Valerie Prouzet-Mauleon3, Vincent Pitard1,3, Atika Zouine3, Sofia Mensurado4, Claire Larrieu5, Beatrice Turcq3, Bruno Silva-Santos4, Julien Engelhardt2, Lionel Couzi1,2, Thomas Daubon5, Julie Dechanet-Merville1
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Primary Theme: Cancer Therapies

Secondary Theme:

Abstract

Glioblastomas (GBM) are the most frequent and aggressive primary brain malignancies, with very limited therapeutic options and a dismal prognosis. So far, immunotherapies evaluated in clinical trials have failed to provide significant clinical benefit for GBM patients, notably due to the high genetic heterogeneity and immunosuppressive environment of the tumor.

Taking advantage of the combined features of innate and adaptive response of γδ T cells, expressing both TCR receptors and NK receptors, and exhibiting strong cytotoxic characteristics, the recent development of clinical-grade, in-vitro expanded Vδ1 γδ T cells (DOT cell procedure) is currently in clinical trials in Acute Myeloid Leukemia. These characteristics, combined with their specific enrichment in tissues and their association with better survival in different solid cancers, make them of great interest as therapeutic agent, particularly in tumors with low mutational burden or low HLA-I expression, such as GBM.

Here, we aimed at developing a new cell therapy strategy in GBM, using in-vitro expanded Vδ1 T cells (DOT cells).

Initial characterization of γδ T cells isolated from GBM patient samples highlighted Vδ1 and Vδ2 recruitment within tumors with an activated and cytotoxic profile. In-vitro, we showed that DOT cells, used in combination with interleukin (IL)-15, could efficiently recognize and kill commercially available GBM cell lines and spheroids derived from patient glioblastoma stem cells (GSCs). Target cell killing relied on the granule exocytosis pathway and was impacted upon CRISPR-mediated knock-out of γδ TCR and NKG2D molecules suggesting their implication in GBM sensing by DOT cells. Finally, adoptive transfer of DOT cells in patient-derived xenograft mouse models of GBM are showing a delay in tumor growth in preliminary results.

Altogether, our results are providing encouraging results to develop allogeneic use of γδ T cell therapies in GBM patients.

Next Generation Armoring of γδ T CAR T Cells Against Solid Tumors Protects from Multiple Forms of TME Immune Suppression

Presenter Name: Theodore Giavridis

Mitchell Wang, Ivan Reyes-Torres, Maria Guarino, Ashley Thornal, Caroline Hanlon, Eric Johnson, Mark Roberto, Navamallika Reddy, Miao Chen M, Kristen Vogt, Theodoros Giavridis*, Mateusz Legut*
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Primary Theme: Cancer Therapies

Secondary Theme:

Abstract

While cell therapies have achieved remarkable success in hematologic malignancies, their application in solid tumors remains limited due to the immunosuppressive tumor microenvironment (TME). The TME employs multiple, often synergistic, mechanisms to inhibit the efficacy, persistence, and durability of immune cells. Nonetheless, current armoring approaches address individual suppression mechanisms rather than viewing the TME holistically. Additionally, the high cost and logistical challenges of autologous cell therapies further restrict their clinical scalability. γδ T cells offer a promising off-the shelf alternative without the requirement for additional engineering to mitigate alloreactivity. However, their clinical potential in oncology has been constrained by diminished cytotoxicity and limited persistence in vivo. Here, we present a next-generation engineering approach to overcome these limitations and unlock the full therapeutic potential of γδ T cells.

First, we describe the armoring of γδ chimeric antigen receptor (CAR) T cells with lymphotoxin receptor beta (LTBR), derived from a high-pressure multi-suppression assay screen, that protect cells from macrophage-, Treg-, adenosine-, and TGFβ-mediated immunosuppression. Transcriptomic profiling via bulk and single-cell RNA sequencing revealed that LTBR overexpression induces profound reprogramming of CAR T cells, driving them toward highly proliferative, multifunctional, and persistent phenotypes. To validate these findings, we engineered LTBR-armored γδ CAR T cells and evaluated their functionality in vitro and in vivo. In high-stress in vitro models, LTBR expression significantly enhanced γδ CAR T cell cytotoxicity, proliferative capacity, and functional persistence, while also increasing sensitivity to low antigen levels. In ovarian cancer xenograft models, CAR T cells demonstrated superior antitumor activity, prolonged survival, and increased tumor infiltration compared to unmodified controls.

These findings demonstrate that rational engineering of γδ T cells can overcome their inherent limitations, enabling robust resistance to the TME's immunosuppressive pressures. By leveraging LTBR, we have established a framework for developing next-generation, allogeneic cell therapies capable of addressing the unique challenges of solid tumors.

Probing the Microenvironmental Context of γδ T cells in Pancreatic Ductal Adenocarcinoma

Presenter Name: Lucy Goodman

Lucy, Goodman, University of Birmingham
Carrie, Willcox, University of Birmingham
Shivan, Sivakumar, University of Birmingham
Benjamin, Willcox, University of Birmingham

Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a 10% 5-year survival rate that, despite the advent of immunotherapy, has barely improved over the last two decades. Harnessing γδ T cells could conceivably provide a viable avenue for the development of novel immunotherapies against PDAC. While γδ T cells in PDAC have been explored by several studies, there is controversy about their frequency, a lack of subset specific data for infiltrating γδ T cells, and their role in the TME is not properly understood. This project aims to elucidate their frequency, functionality and localisation using a multimodal approach. Preliminary flow cytometry data from fresh PDAC tissue has suggested that both innate-like Vγ9Vδ2 and adaptive-like (eg Vδ1) γδ tumour infiltrating lymphocytes are present, although their frequency is heterogeneous. Consistent with this, single-plex immunohistochemistry (IHC) demonstrated variations in the presence of γδ T cells. Nevertheless, both approaches indicated typically γδ T cells represented <4% of T cells in PDAC, contradicting some previous reports. Multiplex immunofluorescence staining enabled assessment of γδ T cell phenotype, regional localisation, and proximity to other cell types. This highlighted ‘stromal trapping’ of γδ T cells, with only a minority penetrating tumour cell nests. It also enabled imaging of pancreatic ectopic lymphoid structures, likely drivers of local adaptive anti-tumour immune responses, differential formation of which may partly explain heterogeneity in γδ T cell frequency and phenotype. Complementing these approaches, TCR repertoire analysis, of both peripheral and tumour-infiltrating γδ T cells, will provide insights into the potential for an adaptive anti-tumour γδ T cell response in this cancer setting. Going forward, γδ T cell frequency and phenotype will be correlated with survival. In parallel, exploring evidence of γδ T cell anti-tumour reactivity in vitro could provide proof-of-concept data for γδ cellular therapeutic strategies.

γδ T Cells as Gatekeepers of Skin Autoimmunity: Uncovering Their Non-redundant Role in Blistering Diseases

Presenter Name: Gabriela F. Gubert

Gabriela F. Gubert1, Miriam Eckstein1, Fabian Imdahl2, Tobias Krammer2, Sripriya Murthy3, Christian Sadik3, Misa Hirose3 and Martin Vaeth1
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Primary Theme: Tissues & Inflammation

Secondary Theme: Infection Responses

Abstract

Bullous pemphigoid (BP) is a chronic autoimmune skin disease characterized by autoantibodies targeting hemidesmosomal proteins, which impair dermal-epidermal adhesion and trigger subepidermal blister formation. These autoantibodies activate the complement system and promote the recruitment of granulocytes, resulting in inflammation and tissue damage. Relapsing-remitting BP is clinically treated with topical or systemic corticosteroids, but the underlying immunopathology is poorly understood, particularly the role of the adaptive immune system. In this study, we uncovered a crucial and non-redundant role for dermal γδ T cells in the effector phase of BP. Using an established animal model of BP induced by passive transfer of autoantibodies targeting hemidesmosomes, we found that Rag1–/– mice, which lack all lymphocytes, were completely protected from the disease. However, the absence of either B cells or conventional αβ T cells did not prevent BP, indicating the involvement of unconventional lymphocytes. Notably, the ablation of γδ T cells was sufficient to protect mice from BP-induced skin inflammation. Using both constitutive and inducible γδ T cell-deficient mice, we demonstrated that the absence of γδ T cells reduced neutrophil recruitment and prevented tissue damage in the effector phase of BP. Mechanistically, we identified dermal γδ T cells as the predominant source of IL-17A and IL-17F production in the skin following autoantibody-mediated tissue injury. Ablation of IL-17A/F completely protected mice from BP, preventing neutrophil recruitment to the skin. These findings highlight a novel, non-redundant role of dermal γδ T cells in initiating and amplifying autoantibody-mediated skin inflammation. Future work will investigate longitudinal changes in dermal T cell clonality and composition, alongside metabolomic profiling, to further elucidate BP disease progression. A better understanding of how γδ T cells promote autoimmune blistering diseases will pave the way for novel therapeutic strategies that selectively target autoimmune pathology while preserving protective immune responses.

IL-23 Regulates Cytokine Production by γδ T Cells During L. amazonensis Infection

Presenter Name: Herbert Guedes

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Primary Theme: Infection Responses

Secondary Theme: Tissues & Inflammation

Abstract

Leishmaniasis comprises a group of neglected diseases caused by various species of the protozoan genus Leishmania, including Leishmania amazonensis, the etiological agent of diffuse cutaneous leishmaniasis in Brazil. Our group previously demonstrated that Sv129 mice are susceptible to L. amazonensis infection, which is associated with an expansion of IL-17-producing γδ T cells (Dos-Santos, J. S. et al., 2019). Furthermore, C57BL/6 mice deficient in γδ T cells, elastase, or the IL-17 receptor develop smaller lesions, indicating a pathogenic role for IL-17-producing γδ T cells in cutaneous leishmaniasis (Dos-Santos, J. S. et al., 2024). To further investigate the mechanisms underlying IL-17 induction in γδ T cells, we examined the impact of IL-23. C57BL/6 IL-23 knockout (KO) mice were infected, and their infection profile was analyzed during both the peak and chronic phases of the disease in comparison to wild-type (WT) controls. Mice were infected subcutaneously in the right hind paw with 2×10^6 L. amazonensis MHOM/BR/75/Josefa parasites, a strain isolated from a patient. Lesion progression was monitored weekly throughout the experiment using pachymetry. At the peak of infection, IL-23 KO mice exhibited lesion sizes comparable to those of WT mice. However, during the chronic phase, lesion size increased, suggesting heightened inflammation. Parasite load was assessed using a Limiting Dilution Assay (LDA), revealing no significant differences between IL-23 KO and WT mice in the footpad or popliteal draining lymph nodes. Additionally, flow cytometry was performed to evaluate the immunological profile of the mice at both the peak and chronic phases of infection. At the peak of infection, IL-23 KO mice displayed cytokine production levels similar to those of WT mice. However, in the chronic phase, there was an increase in the total number of cells, along with elevated IL-17A- and IFN-γ-producing γδ T cells. These findings suggest that the absence of IL-23 leads to an exacerbated inflammatory environment, likely due to enhanced cytokine production by γδ T cells, which correlates with increased lesion size. Thus, IL-23 plays a regulatory role in suppressing cytokine production in γδ T cells, thereby modulating inflammation during chronic L. amazonensis infection.

Expanded Blood γδT Cells Exhibit Robust Responsiveness, Proliferation and Functionality upon Stimulation

Presenter Name: Johnny Guo

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Vδ1 gamma delta (γδ) T cells, although constituting a minor population in human peripheral blood, serve a crucial function in mucosal tissues as a frontline defense against infections and cellular stress, thanks to their tissue residency and innate sensing capabilities. They are increasingly recognized for their anti-tumor functions in the solid tumor microenvironment, making Vδ1 T cells promising candidates for cancer immunotherapy. Given the low frequency of γδT cells, particularly Vδ1 T cells in peripheral blood, robust expansion is essential before formulating them as a cell therapy product. We have developed an expansion process designed to preferentially expand blood-derived Vδ1 T cells. We sought to compare the phenotype and function of expanded blood γδT cells with those of primary, resting γδT cells from donor-matched peripheral blood. Upon stimulation with plate-coated anti-CD3, anti-Vδ1, or MICA-Fc, the expanded blood γδT cells exhibited elevated expression of several co-stimulatory receptors (CD27, CD28, CD30, NKG2D, DNAM1 & FceRIγ), activation markers (CD38, CD39 & CD5), functional molecules (4-1BB, CD107a, Granzyme B, CXCR3 & CXCR6), and transcription factors (Blimp-1, IRF4, RUNX3, T-bet & TCF1) compared to their primary, resting counterparts. They also showed a significant skewing towards a central memory phenotype, increased viability, and superior functionality. TCR stimulation led to enhanced cytokine production (IFN-γ, IFN-α & TNF-α) and improved cytotoxic activity in expanded γδT cells. Anti-Vδ1 antibody stimulation induced an activation profile in Vδ1 cells closely resembling that of OKT3. Flow cytometry and single cell RNAseq analysis suggest that the enhanced responses of expanded γδT cells to stimulation may be partly due to rewiring of their signaling thresholds and transcriptional profiles during the expansion process. In summary, our in-house expanded blood γδ T cells demonstrate robust responsiveness, proliferation and functionality upon both innate and adaptive stimulation compared to primary γδT cells.

Sustained MAIT and γδ T Cell Leukocytosis in a Healthy Subject

Presenter Name: Mansour Haeryfar

Rashed Rashu, Patrick T. Rudak, S.M. Mansour Haeryfar

Primary Theme: Development & Differentiation

Secondary Theme:

Abstract

Mucosa-associated invariant T (MAIT) and gamma delta (γδ) T cells are innate-like T lymphocytes with remarkable effector and regulatory functions in antimicrobial and anticancer immunity. Here, we report unusually high peripheral blood (PB) MAIT and γδ T cell percentages in an apparently healthy male, but not in his father, siblings or offspring. These cells comprised ~18% and ~25% of total PB T lymphocytes, respectively, and remained stable in frequency over four different visits spanning the last four years. They were also functionally competent as judged by their responsiveness to cognate antigens, mitogens and cytokines. The stimuli tested included 5-OP-RU [5-(2-oxopropylideneamino)-6-D-ribitylaminouracil] (an MR1 ligand that activates MAIT cells), and the bacterial phosphoantigen hydroxymethyl-diphosphomevalonate (HDMAPP) and the self-phosphoantigen isopentenyl pyrophosphate (IPP) that activate γδ T cells. We ruled out the possibility of elevated MR1-restricted γδ T cells in this case. Moreover, as in an age/sex-matched control cohort, the vast majority of MAIT cells in the subject expressed Vα7.2 in their T cell receptor α chain and CD8 as their co-receptor. In addition, they contained large Vβ2+ and Vβ13.2+ subsets. Whole exosome sequencing did not reveal overt genetic changes that may be responsible for MAIT and γδ T cells' overabundance, and RNA sequencing on the subject's and his relatives' peripheral blood mononuclear cells is currently underway.

Cell Type Specific Differences of Butyrophilin 2A1 (BTN2A1) and BTN2A1-Fc in Suppression of Phosphoantigen Induced Activation and in Vg9Vd2 TCR Binding

Presenter Name: Thomas Herrmann

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Primary Theme: Ligands & Repertoires

Secondary Theme: Cancer Responses

Abstract

BTN2A1 homodimers interact with BTN3 heteromers in response to phosphoantigen binding to BTN3A1 forming a Vg9Vd2 TCR activating ligand that engages CDRs, HV4 and framework regions of both TCR chains. Cell surface expressed and soluble BTN2A1 binds Vg9 (GV9) encoded regions of human gd TCRs. It also binds to DC-SIGN (CD209) in a glycosylation- and cell type-specific fashion. We found that over-expression of BTN2A1 in 293T cells (a variant of human embryonic kidney cell line HEK 293) significantly reduces the Vg9Vd2 TCR-mediated PAg response compared to wild type 293T cells. A pivotal role of the BTN2A1 V-domain is indicated by testing chimeric BTN3A1 or BTN3A1-A3JM molecules carrying a BTN2A1 V-domain. Expressing these constructs in 293T cells, resulted in an even more pronounced suppression of the PAg response. Curiously, overexpression of BTN2A1 in RAJI B cell lymphoma cells showed no such effect, suggesting that a dual role of BTN2A1 as an enabler for a Vg9Vd2 T cell response to PAg but also as potential suppressor or terminator of such a response. This Janus-faced function needs to be considered when interpreting BTN2A1 expression data, especially on tumors.

In addition, BTN2A1-Fc constructs showed differences when tested for Vg9Vd2 TCR binding between the cell types used for production. For this purpose, BTN2A1-Fc constructs were immobilized on cell-size beads or bound to 3T3 (murine fibroblasts) or 293T cells expressing the high-affinity IgG receptor (FcγR1A;CD64A). Staining with a goat anti-human IgG Fc specific antibody showed very similar binding of BTN2A1-Fc and CD80-Fc constructs independent from cell line used for production (3T3, 293T, HEK 293, ExpiCHO-S) whereas only BTN2A1-Fc protein produced in ExpiCHO-S cells bound Vg9Vd2 TCR tetramers. This finding hints at posttranslational modifications that modulate the TCR binding of BTN2A1-Fc. This cell type-specific difference is of interest beyond Vg9Vd2 T cell activation as BTN-Fc and BTNL-Fc constructs are commonly used to search for BTN- and BTNL ligands as well as to test for co-stimulatory and co-inhibitory properties and most commonly they are produced in HEK293 cells. Importantly, the cell type-specific differences in TCR binding were confined to the BTN2A1-Fc constructs as membrane-expressed BTN2A1 bound Vg9Vd2 TCR tetramers independent of the origin of the expressing cell line.

Regulation of γδ TCR Signal Strength by the SLAM/SAP Signaling Pathway

Presenter Name: Brianna Hilton

Brianna M., Hilton, University of Vermont
Oliver, Dienz, University of Vermont
Remi, Savard, University of Vermont
Jonathan E., Boyson, University of Vermont

Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Gamma delta (γδ) T cells are innate-like T cells that shape the developing adaptive immune response through their ability to rapidly produce large amounts of cytokines such as IL-17 (γδT17) and IFN-γ (γδT1). Unlike conventional αβ T cells, γδ T cells acquire their effector function during thymic development through mechanisms that remain unclear. Previously, we reported that the SLAM/SAP signaling pathway regulates both thymic γδT17 and γδT1 development. Here, we investigated the effect of the SLAM/SAP signaling pathway on γδ TCR signal strength during thymic development. Using Nur77-GFP and CD5 expression as surrogate markers of γδ TCR signal strength in in vitro stimulation assays, we found that anti-SlamF6, but not anti-SlamF1, augmented thymic γδ T cell Nur77-GFP expression, suggesting the ability to modulate TCR signal strength is mediated by only a subset of SLAM family receptors. Initial ex vivo characterization of CD5 and Nur77-GFP expression on thymic γδ T cells revealed broad expression of CD5 among thymic γδ T cells, while Nur77-GFP expression was primarily restricted to immature CD25+ post-selection and CD73+ γδ T cells. In addition, Nur77-GFP expression was significantly higher in Vγ1 vs Vγ4 or Vγ6 T cells, consistent with a model in which γδ T cell subsets experience quantitative differences in TCR signal strength during development. Interestingly, ex vivo comparison of E.17 thymic γδ T cell CD5 and Nur77-GFP expression between B6.Nur77GFP and B6.Nur77GFPSAP-/- mice revealed that SAP-deficient γδ T cells exhibited both decreased CD5 expression in immature CD24+73- γδ T cells, and increased Nur77-GFP in immature signaled CD24+73+ γδ T cells. A comparison of Nur77-GFP expression between TCR-stimulated B6.Nur77GFP and B6.Nur77GFPSAP-/- thymic γ T cells confirmed that SAP-deficient γδ T cells expressed significantly more Nur77-GFP than their wild-type counterparts. Altogether, these data suggest that the SLAM/SAP signaling pathway can modulate thymic γδ T cell TCR signal strength, but that it may utilize fundamentally different modes of action in cells destined for the γδT17 and γδT1 effector fate.

Mechanistic Investigations of TLR4 Adjuvant Effects on γ9δ2 T Cells

Presenter Name: Kathleen Holloway

Kathleen Holloway Saint Louis University, Mei Xia Saint Louis University, Christopher Fox The Access to Advanced Health Institute, Nikolai Petrovsky Vaxine, Darrick Carter PAI Life Sciences, Daniel Hoft Saint Louis University

Primary Theme: Ligands & Repertoires

Secondary Theme: Infection Responses

Abstract

γδ T cells provide protective effects against infectious diseases and cancer. We reported that the current vaccine for Tuberculosis (TB), BCG, induces memory γδ T cells. Those memory T cells can be expanded by the Mycobacterium tuberculosis antigen 6-O-methylglucose lipopolysaccharide (mGLP) to inhibit intracellular mycobacterial growth. MGLP also induced protection in a non-human primate model of TB. However, γδ T cell in vitro and in vivo expansion requires IL-2. IL-2 has multiple drawbacks for adjuvant use, including preferential induction of Tregs and autoimmune stimulation, requiring the identification of safer adjuvants that promote γδ expansion. We recently demonstrated that the synthetic TLR4 adjuvant, Glucopyranosyl Lipid A (GLA), promotes expansion of mGLP and phosphoantigen reactive γδ T cells. In vitro expansion assays were initially performed using PBMCs with and without dendritic cells stimulated with antigen and either IL-2 or novel adjuvants. The absolute numbers of expanded effector γδ T cells were calculated using flow cytometry after 7 days of culture and novel adjuvant responses compared to IL-2. We report the optimal cellular requirements for GLA to promote γδ T cell expansion include dendritic cells and monocytes. These results have broad implications for development of vaccines targeting γδ T cells and suggest mGLP combined with the adjuvant GLA may activate and expand γδ T cells protective against TB in humans.

A Novel Autochthonous Model of KrasG12D/Stk11 Loss-Driven Lung Adenocarcinoma for the Study of γδ T Cell Activation and Function During Tumor Initiation and Resolution

Presenter Name: Katherine Horrigan

Katherine J Horrigan, University of Vermont
Joshua M Ragusa, University of Vermont
Somen K Mistri, University of Vermont
Oliver Dienz, University of Vermont
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Primary Theme: Cancer Responses

Secondary Theme:

Abstract

γδ T cells play critical roles in tumor immunity and can both promote tumor growth/metastasis and efficiently kill tumor cells. These dual functions of γδ T cells have been ascribed to different functional subsets, with γδT1 cells associated with anti-tumor immunity, and γδT17 cells associated with tumor growth and metastasis. While this dual functional role is well-accepted, the factors that regulate the balance of γδT1 vs. γδT17 in the tumor microenvironment is unclear, as are the effects of these γδ T cell subsets in shaping the tumor-immune microenvironment. To explore these questions, we have developed a novel autochthonous mouse model lung adenocarcinoma in which KrasG12D is expressed in lung club cells with or without a co-mutation in the tumor suppressor gene Stk11. We found that KrasG12D/Stk11fl exhibited significantly greater tumor burden and mortality than KrasG12D mice. Whole-lung luminex analysis revealed significant changes in a number of cytokines, chemokines, and growth factors in KrasG12D/Stk11fl, including amphiregulin, G-CSF, IL-25, IL-9, IL-33, IL-6, IL-27, and IL-23. Increased tumor burden in KrasG12D/Stk11fl mice was associated with a preferential expansion of lung Vγ4 and Vγ6 γδT17 cells, CD4+Foxp3+ Tregs, neutrophils, and DCs. scCITEseq w/ V(D)J profiling revealed the expansion of a Vγ6 γδT17 subset enriched in Il2ra, Areg, and Ctla4 in KrasG12D/Stk11fl lungs. Interestingly, V(D)J profiling suggested that within the Vγ4 subset, the Vδ5, but not the Vδ4-expressing γδ T cells exhibited an activated phenotype. Finally, scCITEseq analysis on the tdTomato+ lung epithelial cells revealed that KrasG12D/Stk11fl tumor cells were themselves enriched in immune-relevant growth factor, cytokine, and chemokine transcripts including Areg, Il33, and Cxcl16. Taken together, these data suggest that the increased tumor burden and mortality resulting from tumor-specific KrasG12D/Stk11 loss is associated with the concomitant expansion of CD4+ Tregs and specific γδT17 subsets with immunosuppressive potential. These data suggest a model whereby γδT17 may contribute to tumor pathogenesis through suppression of the tumor-specific adaptive immune response.

Common Variable Immunodeficiency as a Model to Understand Gamma Delta T Cell Biology

Presenter Name: Lauren Howson

Samantha Chan, Royal Melbourne Hospital
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Primary Theme: Ligands & Repertoires

Secondary Theme: Infection Responses

Abstract

Studying γδ T cells in the context of primary immunodeficiency can offer valuable insights into the factors that regulate their role in host immunity. Common variable immunodeficiency (CVID) is a primary immunodeficiency disorder characterized by impaired antibody responses, leading to increased susceptibility to infections. Although it is a well characterised immune disorder, the function and phenotype of γδ T cells in the context of CVID has not been explored.

In this study, we performed TCR repertoire analysis, immune profiling, and functional assays of γδ T cells in 23 CVID patients. Among these, 34% had identified inborn errors in immunity (5 NFKB1, 1 NFKB2, 1 TCF3), and 5 patients were confirmed to have CMV viremia. Our results show that Vδ1+ γδ T cells in CVID patients are expanded, activated, functional, and exhibit clonal TCR repertoire focusing. This expansion mirrors the clonal repertoire and inverted Vδ1/Vδ2 ratio typically seen in elderly healthy individuals.

Moreover, we demonstrate that γδ T cells in CVID function independently of humoral immunity and are not impaired by insufficient NF-κB signaling. Notably, persistent CMV viremia exacerbates the clonal expansion and activation of Vδ1+ γδ T cells in CVID patients. Despite the dysregulated humoral response and, in some cases, defective NF-κB signaling, γδ T cells remain functional, clonally expanded, and actively contribute to immune defense.

Our findings provide crucial insights into γδ T cell biology and reveal intact anti-CMV responses in CVID patients, highlighting γδ T cells as promising targets for therapeutic interventions in this cohort.

Peritumoral Adipocyte-secreted Leukotriene B4 Induces NKG2A+γδ T cells Exhibiting Anti-tumor Immunity in Breast Cancer

Presenter Name: Xiaoxiao Hu

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Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Adipose tissue (AT) content in breasts, as an integral component of the tumor microenvironment (TME), is linked to breast cancer (BC) development. As for human γδ T cells, it is reported that innate-like Vδ1 cells are associated with a positive prognosis of BC in healthy women breasts. Clinically, on the set of tumorigenesis, early-stage BC rarely invades AT, suggesting the presence of a specialized immune response compartment at the tumor margin. However, heterogeneity of γδ T cells and their roles within breast AT from BC patients remain unclear, and the crosstalk between adipocytes and immune cells within TME is insufficiently explored.

In our study, by using single-cell RNA sequencing, we revealed that γδ T cells, particularly NKG2A+γδ T cells, were highly enriched in peritumoral adipose (PA) and exhibited the strongest cytotoxic activity with better prognosis. Additionally, we observed that mobilization of NKG2A+γδ T cells varied across BC molecular classifications, influenced by tumor invasiveness. Notably, in patients with BMI>24 kg/m2, NKG2A+γδ T cells decreased. In vitro patient-derived tumor organoid-immune cell co-culture model further proved the potent cell killing ability of NKG2A+γδ T cells isolated from PA.

To explore the interplay between immune cells and other stromal cells within AT microenvironment, we co-cultured adipocytes and T cells. Interestingly, peritumoral adipocytes specifically increased NKG2A expressions on γδ T cells, instead of αβ T cells. Surprisingly, we found that peritumoral adipocytes predominantly expressed high levels of 5-LOX pathway to produce leukotriene B4 (LTB4). Metabolite LTB4 subsequently combined LTB4 receptors to activate NKG2A+γδ T cells via the STAT1 pathway, enhancing their anti-tumor immunity.

Taken together, our study highlights the crucial roles of NKG2A+γδ T cells and the interplay between γδ T cells and adipocytes within PA microenvironment, showing potential implications for adoptive cellular therapy and adipose tissue transplantation in BC patients.

Selective In Vivo Depletion of CD3 Bright Gamma Delta T Cells Prevents Acute Vascularized Composite Allotransplantation Rejection in Swine

Presenter Name: Christene Huang

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Primary Theme: Tissues & Inflammation

Secondary Theme: Tissues & Inflammation

Abstract

Diphtheria toxin based recombinant CD3-immunotoxin fusion proteins consist of single chain variable fragments of CD3 monoclonal antibodies linked to the translocation and catalytic domains of truncated diphtheria toxin. Replacement of the diphtheria toxin binding domain with CD3 antibody binding fragments results in effective in vivo targeting and depletion of CD3+ lymphocytes. Our data in the miniature swine model demonstrate that CD3-immunotoxin preferentially depletes gamma delta T cells which are shown to express the highest level of surface CD3 among all T cell subpopulations. We observe greater peripheral blood depletion levels among the gamma delta T cell population and prolonged depletion compared with conventional T cells and T regulatory cells. Although most T cell subpopulations rapidly recover following treatment, we observe selective prolonged depletion of the gamma delta T cells in our miniature swine transplantation models with no noticeable adverse effects long term in these animals. Acute rejection is a common complication of clinical vascularized composite allotransplantation (VCA), with an incidence of greater than 80% in hand and face transplantation. In the highly immunogenic skin containing porcine myofasciocutaneous VCA model, we observe accelerated acute rejection with histological evidence on biopsies taken on day 6 even when recipients are administered standard clinical triple immunosuppression therapy. Histology of day 6 biopsies shows inflammation in the dermis surrounding the superficial vascular plexus, extending into the epidermis and accompanied by single keratinocyte necrosis (grade 3A out of 4 rejection using a modification of the Banff VCA rejection scale). Immunohistochemistry reveals increased dermal and epidermal gamma delta T cells surrounding the superficial vascular plexus and at sites of keratinocyte injury. Recent data in this model demonstrate that the addition of CD3-immunotoxin as induction therapy starting immediately prior to transplantation results in complete avoidance of acute rejection. The day 6 biopsy data for these swine recipients show a near absence of infiltrating cells at the superficial

vascular plexus and epidermis (grade 0 out of 4). CD3-immunotoxin mediated depletion of CD3 bright gamma-delta T cells may provide a unique strategy to prevent acute VCA rejection and to treat any clinical disorder involving dysregulated gamma-delta T cell activation.

Expansion and Differentiation of Activated Gamma Delta T Cells in Response to In Utero Cytomegalovirus Infection

Presenter Name: Riana Hunter

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Primary Theme: Infection Responses

Secondary Theme: Development & Differentiation

Abstract

Congenital cytomegalovirus (cCMV) is a leading cause of developmental defects in newborns. As the first T cells to develop in the mammalian lineage, γδ T cells are poised to defend the neonate against such an in utero infection. Indeed, cCMV-infected infants display an expansion of γδ T cells, and at least one germline-encoded CMV-reactive TCR has been reported. We set out to thoroughly characterize the γδ T cell response to congenital CMV-infection by analyzing cord blood mononuclear cells collected from a birth cohort of cCMV-infected and cCMV-uninfected neonates living in Eastern Uganda. We designed and utilized a 28-color spectral flow cytometry panel to examine the phenotype and expansion of fetal γδ T cells in response to in utero CMV exposure. We found several γδ T cell subsets expand and adopt a terminally effector-differentiated state in cCMV+ neonates, consistent with existing reports. This Type 1-like phenotype was associated with increased expression of CD8, CX3CR1, and cytolytic mediators granzyme B and perforin. Conversely, in prioritizing this phenotype, γδ T cells from cCMV+ neonates less frequently assumed Type 2 and Type 3 programs and rather exhibited increased expression of NK-like markers CD16 and CD94, but not CD56. Unsupervised clustering of our flow cytometry data confirmed these differentiated effector populations and revealed further insights regarding chemokine and co-receptor expression. Our results provide further evidence to suggest that fetal γδ T cells effectively differentiate to assume a Type 1-like cytolytic effector immune program in response to congenital CMV infection. Future studies will build on these findings to elucidate the mechanisms by which γδ T cells contribute to anti-viral immunity that may be harnessed for therapeutic strategies in the developing fetus and early life.

MicroRNA-Mediated Post-Transcriptional Regulation of Peripheral γδ T Cell Effector Functions

Presenter Name: Daniel Inácio

Daniel Inácio1, Tiago Amado1, Daniel Sobral2, Francisco Enguita3, Carolina Cunha1, Anita Q. Gomes1,4* and Bruno Silva-Santos1,3
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Primary Theme: Development & differentiation

Secondary Theme: Infection Responses

Abstract

γδ T cells are unconventional lymphocytes able to rapidly produce large quantities of interleukin-17A (IL-17A) or interferon-γ (IFNγ), which often have major effects on the pathophysiology of diseases such as cancer or infections. Yet, we do not fully understand how the thymic-derived effector γδ T cell subsets making either IL-17A (γδ17 cells) or IFNγ (γδIFN cells) are activated and function in peripheral tissues. Here, we established an Il17a-GFP:Ifng-YFP double-reporter mouse strain to analyse at unprecedented depth the microRNA (miRNA) transcriptomes of pure γδ17 versus γδIFN cell populations from peripheral lymph nodes, and uncover new mechanisms of miRNA-mediated post-transcriptional regulation of γδ T cell effector functions. We found 103 differentially expressed miRNAs between γδ17 and γδIFN cells, from which we selected 10 candidates likely to target members of the transcriptional networks underlying IL-17A of IFN-γ production, to test their effects on γδ T cell differentiation or effector functions. Retroviral gain-of-function approaches in vitro highlighted a plethora of context-dependent miRNA-specific effects on the differentiation or expansion of γδ17 or γδIFN cells, and respective cytokine production. Furthermore, a detailed analysis of each candidate miRNA expression throughout ontogeny showed that in some cases it was imprinted in the thymus, whereas in others it was peripherally induced. This led to the observation that miR-181a-5p, highly expressed in early thymic γδ T cell subsets, favours thymic γδ17 cell commitment, while conversely promoting peripheral γδIFN activation and proliferation in response to TCR stimulation in vitro and upon malaria infection in vivo. On the contrary, we found miR-128-3p to act as a peripheral regulator of IFN-γ, preventing aberrant IFN-γ production upon TCR stimulation. These findings unravel a new layer of regulation of γδ T cell effector functions, which we are further characterizing with additional molecular assays.

Skin gd T Cell Subsets Have Distinct Functions in Alopecia Areata

Presenter Name: Julie Jameson

Eliana Solis, Nashea Lampkins, Natalie Canto, Julie Jameson. California State University San Marcos

Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

Alopecia areata is an autoimmune disease of the skin characterized by nonscarring, patchy hair loss across the scalp, affecting roughly 1.7% of the global population. Genetic susceptibility and environmental factors contribute to the onset of alopecia areata. Cytotoxic CD8+ T cells infiltrate the intrafollicular region of the hair follicle and degrade immune privilege in an IFN-g dependent manner. Our lab and others have shown that gd T cells are also increased in the skin of mice and humans during alopecia areata, however less is known about their exact role in disease progression. Through reanalysis of publicly available single cell RNA sequencing data obtained from mouse and human skin, we identified upregulated genes in gd T cells from alopecia areata skin. Dermal Vg6Vd1 T cells upregulate IL17a similar to responses in psoriasis, whereas dermal Vg4Vd1 T cells upregulate genes associated with antigen presentation (H2-Ab1, H2-Aa, H2-Eb1, CD74). Epidermal gd T cells upregulate genes induced by interferon-g including transcription factor STAT1 and Ly6a, a protein regulated by STAT1. We validated the upregulation of STAT1 regulated proteins by skin gd T cells from C3H/HeJ mice with alopecia areata using immunofluorescent microscopy. In humans we found that Vd1 T cells respond to stress in alopecia areata by upregulating heat shock response genes (HSP90AA1, HSPA1A, HSPA1B, DNAJB1) likely as a self-protective response. Vd1 T cells also upregulate CXCL8 and CCL4 to recruit neutrophils, macrophages and T cells and IL-17-associated genes (NEAT1). Our findings show involvement of gd T cells in inflammatory responses associated with alopecia areata disease pathogenesis making them interesting targets for therapeutics.

Infiltrating PD-1+ γδ T Cells Rely On the Key Transcription Factor BATF for Anti-tumor Immunity in Human Colorectal Cancer

Presenter Name: Jiahuan Jiang

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Primary Theme: Cancer Therapies

Secondary Theme: Ligands & Repertoires

Abstract

γδ T cells, a pivotal lymphocyte subset in colorectal cancer (CRC), exhibit marked heterogeneity and functional plasticity. Importantly, PD-1+ γδ T cells display higher responses to immune checkpoint inhibitors (ICIs) than CD8+ T cells, underscoring their potential as a specialized subset in CRC immunotherapy1. Additionally, the majority of mismatch repair-deficient (dMMR) CRCs, as opposed to mismatch repair-proficient (pMMR) CRCs show sensitivity to ICI treatment. However, the fundamental functions and mechanisms of tumor-infiltrating PD-1$^+$ γδ T cells and their interaction with ICIs remain unresolved. In this study, we identified that tumor-derived PD-1$^+$ γδ T cells were highly enriched and associated with better patient prognosis. These cells expressed higher levels of basic leucine zipper ATF-like transcription factor (BATF), which plays a crucial role in preventing PD-1$^+$ γδ T exhaustion and activating the cell's glycolysis for strong anti-tumor effects. Interestingly, PD-1+ γδ T cells with elevated BATF can recruit IgG1+ B cells via the produced CXCL13 to promote tertiary lymphoid structures (TLS) formation, in addition to secrete activating cytokines. Notably, PD-1 monoclonal antibodies can sustain high BATF expression of PD-1$^+$ γδ T cells, enhancing their anti-exhaustion capabilities. Our findings highlight the essential roles of PD-1+ γδ T cells in TLS-driven anti-tumor immunity and provide novel avenues especially for targeting dMMR CRC.

BCG Vaccination Leads to Expansion of Cytotoxic TRDV1 Gamma Delta T Cells in Infants

Presenter Name: Dylan Kain

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Primary Theme: Infection Responses

Secondary Theme:

Abstract

Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains the leading infectious disease cause of death worldwide and a novel efficacious vaccine is urgently needed. gd T cells are rapidly cytotoxic to Mtb, enriched in human airways where Mtb first causes infection and have been shown to be important in defense against TB in animal models. We postulate that they are uniquely poised to defend against mycobacterial infection.

To further explore their response to mycobacteria in humans we utilized samples from South African infants who either received BCG-vaccination at birth or who had vaccination delayed until after blood was collected at 9-weeks of age. These cells were then sorter for gd T cells and single-cell gene-expression and TCR-sequencing was then performed. gd T cells from vaccinated infants had significant upregulation of interferon-stimulated and cytotoxicity genes following vaccination. gd T cells from vaccinated infants also showed significant expansions of TRDV1 clonotypes compared to unvaccinated infants. These expanded clonotypes had upregulation of cytotoxicity and effector genes and downregulation of naïve-associated genes. Expanded clonotype TCRs were then expressed in reporter cell lines to demonstrate that these TCRs are reactive to both Mtb lysate and BCG.

This findings suggest that gd T cells respond to BCG vaccination to upregulate genes that suggest they may be more cytotoxic and inflammatory upon secondary exposure. Furthermore, we demonstrate clonotypic expansion to BCG antigens in TRDV1 gd T cells, with associated genetic changes suggesting they may be more effective on secondary pathogen encounter. This provides support for targeting gd T cells in future TB vaccine development.

Human Skin Vd1 T Cells Comprise An Unique Lymphocyte Compartment With Multifaceted, Manipulable Therapeutic Potentials

Presenter Name: Shraddha Kamdar

Shraddha Kamdar1,2*, Daniel Davies,1,2*, Josephine Eum1,2, Fernanda Kyle-Cezar1,2 and Adrian C. Hayday1,2
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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Adoptive T cell therapies (ACT) have shown impressive efficacy targeting haematological cancers but extrapolating this to solid tumours has proved challenging. Given abundant evidence that solid tumours can be naturally targeted by tissue-intrinsic gd T cell subsets in a non-MHC-restricted, neoantigen-independent fashion, we have characterised human skin Vd1+ cells, establishing their differences from both blood Vd1+ cells and co-localised, skin CD8+ ab T cells, which are also centrally implicated in cancer control. Conspicuous differences were the cells' tissue-resident traits (versus blood Vd1 cells) and their innateness (versus skin CD8 T cells). As a step toward ACT, we also show that expanding skin Vd1+ cells in culture further enriched for cytolytic tumour-targeting and innate immune potentials, which together with tissue-homing / residency traits, could be enhanced by TGF-b, often considered detrimental to immunotherapy. Collectively, these results identify capacities by which local gdT cells may naturally target solid tumours in TGF-b rich environments and demonstrate the practicality of capturing those capacities with therapeutic intent.

Characterization of repertoire and functions of naives gamma-delta T cells responding to CMV

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Primary Theme: Infection Responses

Secondary Theme: Infection Responses

Abstract

Cytomegalovirus (CMV) infection is a major concern in immunocompromised individuals, particularly in transplantation settings. While the role of memory γδ T cells in CMV immunity is well established, the characteristics and functions of naïve γδ T cells in response to CMV remain largely unknown. Getting insights on these very first steps of gd T cell response would be instrumental to understand their natural response to viruses. This study aimed to investigate the phenotype, activation mechanisms, and functional responses of naïve Vδ2-negative γδ T cells in CMV-negative individuals.

We analyzed the in vitro activation kinetics of naïve γδ T cells compared to naïve ab CD8 T cells and memory γδ T cells.Naïve γδ T cells can rapidly produce IFN-γ in response to CMV-infected cells, but not to HSV-infected cells, although with lower intensity than memory γδ T cells, but much faster than naïve CD8ab T cells. This activation is independent of classical TCR, NKG2D, or LFA1 recognition but involves both membrane contact and soluble factors, particularly type I interferons. Moreover, single-cell transcriptomic analyses revealed a distinct signature of naïve γδ T cells upon CMV exposure, suggesting an early and broad innate response.

Our findings highlight a potential innate-like role of a CMV-specific naïve γδ T cell repertoire and suggest a transition from innate to adaptive features during their response to the virus. Understanding these mechanisms could provide new insights into the immune response against CMV and contribute to the development of novel immunotherapeutic strategies.

Metabolic Regulation of Interleukin-17-producing γδ T cells under Inflammation

Presenter Name: Yu-San Kao

Yu-San Kao and Lydia Lynch
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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

The immune system protects our body by eliminating infection via recognizing non-self-antigens on the pathogen. Psoriasis is induced by over-reactive immune cells attacking the patient's body without infection. Scientists identified that the leading cause of psoriasis is driven by specialized immune cells, type 17 T cells. Among type 17 T cells, γδT17 amplifies the inflammation by responding to psoriatic inflammation without recognizing auto-antigens from the patient's body. Therefore, understanding how psoriatic inflammation turns these γδT17 pathogenic can help us to control or cure the diseases. This project aims to elucidate how to target pathogenic γδT17 to provide more insights into psoriasis therapy. Metabolic reprogramming determines γδ T cell fate during thymic development (Lopes et al., 2021). γδT17 cells have higher lipid storage than their IFN-γ-producing counterpart (Lopes et al., 2021). Combining high-throughput techniques, including sequencing, proteomics, and stable isotope tracing, we demonstrated that inflammatory conditions caused γδT17 cells to perform metabolic reprogramming to boost lipid metabolism (Kao et al., in press). Interestingly, γδT17 cells did not burn the lipid droplets as the primary energy source through fatty acid oxidation under psoriatic conditions but rather increased the lipid droplet accumulation (Kao et al., in press). Therefore, it is unclear why γδT17 cells accumulate lipid droplets under homeostasis and inflammation. Therefore, we aim to investigate the roles of lipid droplets in γδT17 cells by super-resolution microscopy and innovative microenvironment-mapping methods to provide spatial resolution. Our study reveals the underappreciated function of lipid droplets in γδT17 cells, which could open new avenues for regulating inflammation.

Lopes N, McIntyre C, Martin S, Raverdeau M, Sumaria N, Kohlgruber AC, Fiala GJ, Agudelo LZ, Dyck L, Kane H, Douglas A, Cunningham S, Prendeville H, Loftus R, Carmody C, Pierre P, Kellis M, Brenner M, Argüello RJ, Silva-Santos B, Pennington DJ, Lynch L. Distinct metabolic programs established in the thymus control effector functions of γδ T cell subsets in tumor microenvironments. Nat Immunol. 2021 22,179-192. doi: 10.1038/s41590-020-00848-3.

Kao YS*, Lauterbach M, Lopez Krol A, Distler U, Godoy GJ, Klein M, Argüello RJ, Boukhallouk F, Vallejo Fuente S, Braband KL, Nurbekova A, Romero M, Mamareli P, Silva L, Damasceno LEA, Rampoldi F, Berod L, Lynch L, Hiller K, Sparwasser T*. Metabolic reprogramming of interleukin-17-producing γδ T cells promotes ACC1-mediated de novo lipogenesis under psoriatic conditions. Nat Metab. In press

Comparative Functional Insights into Human and Alpaca BTN-Vγ9Vδ2 TCR Interactions

Presenter Name: Mohindar Murugesh Karunakaran

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Primary Theme: Ligands & Repertoires

Secondary Theme: Development & Differentiation

Abstract

Introduction: Vγ9Vδ2 T cells constitute the major proportion of circulating human γδ T cells. They recognize phosphorylated metabolites (phosphoantigens – PAg) of isoprenoid syntheses like IPP and HMBPP. Their activation depends on the butyrophilin (BTN)3 family and BTN2A1. BTN3 family comprises BTN3A1, BTN3A2, and BTN3A3, where the BTN3 complex involving PAg-bound BTN3A1 and BTN3A2/ BTN3A3 interact with BTN2A1 for optimal T cell activation. However, molecular insights on TCR recognition of BTN3 remain unclear.

Objective: To elucidate the interaction dynamics between the TCR and the IgV domain of BTN3, as well as to unravel the mechanistic principles underlying juxtamembrane (JM) region-mediated modulation of phosphoantigen (PAg) sensing by the TCR, through a comparative analysis of human and alpaca counterparts.

Methods: 293T cells deficient for BTN3A and/or BTN2 family members were reconstituted with combinations of BTN3A/BTN2A1 family members, chimaeras and mutants and tested for their capacity to stimulate Vγ9Vδ2 TCR transduced 53/4 murine responder cells in the presence of HMBPP. 293T cell transductants were also tested for cellular distribution of BTN-fusion proteins by FACS, confocal microscopy, and FRET

Results and Conclusions: Reconstitution of BTN-deficient 293T cells with various BTN proteins demonstrated the pivotal role of the juxtamembrane (JM) region in modulating cytoplasmic domain interactions among BTN proteins. Co-expression studies of human and alpaca BTN proteins revealed significant differences in the ability of alpaca and human TCRs to recognize BTN proteins from either species. Furthermore, the generation of a series of BTN mutants from humans and alpacas is expected to identify critical features required for cytoplasmic domain interactions and TCR sensing conserved between the two species. These findings will provide detailed insights into the molecular interactions essential for TCR recognition of BTN proteins, potentially distinguishing between the resting and activated states of BTN proteins as detected by TCR engagement.

Identifying Allergen-Specific Transcriptional and TCR Signatures in Human γδT Cells

Presenter Name: Kendall Kearns

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Primary Theme: Tissues & Inflammation

Secondary Theme: Ligands & Repertoires

Abstract

Gamma-delta T (γδT) cells are becoming a population of particular immunological interest as they are positioned at the interface of innate and adaptive immunity and are involved in the initiation and regulation of many immunopathologies. However, the role of γδT cells in immune responses to common allergens is still being unraveled. We utilized a novel Activation-Induced Marker (AIM) assay to detect allergen-reactive γδT cells from blood samples that were exposed to mouse and cockroach extracts. Using single-cell transcriptomics to characterize the transcriptional landscapes and TCR repertoires of allergen-reactive γδT cells, we identified both shared and allergen-specific γδT cell activation patterns and gene expression profiles. Mouse extract primarily stimulated Vδ2 cells whereas cockroach extract activated both Vδ1 and Vδ2 subsets. The analysis also revealed allergen-specific clusters with distinct functional signatures, including enhanced inflammatory responses and cytotoxic effector functions in mouse-specific γδT cells and natural killer cell-mediated immunity and IFNγ signaling in cockroach-specific populations. Comparing allergic and non-allergic cohorts also highlighted differences in gene expression and TCR repertoires, including higher IFNG expression in Vδ2 cells in cockroach-allergic compared to non-allergic individuals, suggesting that there are phenotypic and functional differences that are associated with allergen responses in γδT cells. These findings provide insight into the heterogeneity and functionality of allergen-reactive γδT cells, laying the groundwork for understanding their role in allergic diseases and exploring potential therapeutic applications.

Gene-Environment Interactions: the Potential of Altered Diets to Promote Intestinal Inflammation by Dysregulating Intestinal γδ Cells.

Presenter Name: Vivien Kohlhaas

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

γδ T cell enrichment in the intestinal epithelium is well documented in mice and humans. The cells' development and steady-state phenotypes are regulated by poorly understood, B7-like, butyrophilin-like (BTNL) proteins [human BTNL3+8; mouse Btnl1+6] expressed specifically by gut epithelial cells. Thus, mice lacking the Btnl1-4-6 locus have greatly reduced numbers of Vγ7+ intraepithelial lymphocytes (IELs), while persons homozygous for a hypomorphic allele (BTNL8*3) carry fewer Vγ4+ IELs and are at increased risk of severe Crohn's disease, a form of inflammatory bowel disease (IBD) (PMID: 37708268). Consistent with this, rodent Vγ7+ IELs and human Vγ4+ IELs have been implicated in maintaining tissue integrity via a TCR-dependent process known as "normality sensing" (PMID: 35165446).

Several environmental risk factors for IBD are also known, including high fat diet (HFD). Given that γδ T cells regulate dietary adaptation (PMID: 33737460), we hypothesised that HFD might act in part by dysregulating the BTNL-γδ IEL axis, limiting the maintenance of the epithelial barrier, and contributing to epithelial erosion a pathognomonic feature of IBD.

To test this hypothesis, we established a series of dietary challenges, including HFD, high-carb, and high-protein diets, and examined their impact on γδ IEL in wild-type and Btnl deficient mice. Our results provide evidence of genetic environmental interactions in that HFD dysregulates the intestinal γδ IEL compartment, with reduced availability of mature Vγ7+ IELs that are partly replaced by immature cells, phenocopying a major trait of Btnl-deficient mice. Furthermore, the remaining γδ IELs show diet-dependent differences in their immunophenotypic characteristics, which collectively indicate altered function and activity. The pathological implications of this, and whether dietary regulation affects γδ IEL by acting directly through the Btnl axis are now being examined, with a view to actionable targets.

Ligand Identification for an Invariant Vδ1+ γδ T Cell Receptor in the Context of Congenital CMV Infection

Presenter Name: Rixa Köhn

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Primary Theme: Ligands & Repertoires

Secondary Theme: Infection Responses

Abstract

γδ T cells are not restricted to conventional peptide:MHC recognition. A concept for antigen recognition of γδ TCR is missing and only very few and diverse ligands are known. Here, we investigate the so far unknown cognate ligand(s) of a public human Vγ8Vδ1+ TCR that clonally expanded among in utero CMV-infected neonates (γδTCR_DV). Therefore, we use a JE6.1-NFKB-GFP Jurkat reporter cell line, transduced with γδTCR_DV, and tetramerized soluble ectopic domains of γδTCR_DV to screen for reactivity against various cell lines. Among all tested cells, the fibroblast cell lines HT1080 and BJ5ta elicited the highest levels of GFP-signaling in γδTCR_DV+ reporter cells. Interestingly, activation of Jurkat cells was not restricted to human cell lines and was not affected by the CMV infection status, suggesting that the ligand(s) of γδTCR_DV are CMV-induced but not CMV-encoded. Following an unbiased CRISPR/Cas9 genome-wide knockout screen, we sorted and cloned HT1080 target cells that showed loss of binding to γδTCR_DV tetramers. We are analyzing these γδTCR_DV- HT1080 clones alongside bioinformatic analysis of gene expression datasets to identify potential candidate genes encoding the ligand(s). Our results suggest that γδTCR_DV is responsive to a conserved, non-CMV-specific ligand(s), requiring further validation. This study could further shed light on the complex ligand recognition patterns of γδ T cells and provide evidence how adaptive-like γδ T cells recognize stress-induced self-antigens rather than pathogen-specific antigens.

Listeria-Activated γδ T cells are Uniquely Cytotoxic and Durable

Presenter Name: Jonathan Kotula

Ed Lemmens, Laguna Biotherapeutics, Inc. San Francisco, CA; Preethi Thattai Ragunathan, University of California, Berkeley; Daniel A. Portnoy, University of California, Berkeley, Laguna Biotherapeutics, Inc. San Francisco, CA; and Jonathan Kotula, Laguna Biotherapeutics, Inc. San Francisco, CA

Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

There is broad agreement that increasing the number of endogenous **γδ** T cells leads to beneficial outcomes in both oncology and infectious disease indications. The method of **γδ** T cell activation and expansion impacts the rate and durability of expansion to the cytotoxic phenotype. We have found that attenuated Listeria monocytogenes, the natural target of **γδ** T cells, induce robust **γδ** T cell expansion and activation with very favorable therapeutic potential. We will present supporting in vitro and in vivo data, combined with results from previous clinical studies, demonstrating that strains of attenuated Listeria induce specific proliferation and durable activation of endogenous **γδ** T cells in cancer patients.

Our in vitro and in vivo results using attenuated Listeria compare favorably with other approaches such as zoledronate and HMBPP. These results demonstrate that **γδ** T cells activated by attenuated Listeria are more active, kill more target cells, are more durable, and are less exhausted than **γδ** T cells activated by other methods. Our ex vivo results combined with published clinical studies support that Listeria-activated **γδ** T cells can target and kill leukemic blasts in patients and can be used in concert with existing bispecific T cell engagers to direct expanded and activated **γδ** T cells to specific tumors for durable cytotoxicity against cancer cells. Together, our results support that attenuated Listeria are the foundation of a safe, effective, and re-dosable method to activate and expand endogenous **γδ** T cells for significant therapeutic outcomes.

CD16+ T Cells, Predominantly γδ T Cells, Exhibit Enhanced Antibody-Dependent Cell-Mediated Cytotoxicity and Improve the Therapeutic Response in Diffuse Large B-Cell Lymphoma

Presenter Name: Nadja Küchler

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common form of aggressive non-Hodgkin lymphoma (NHL). The first-line therapy for DLBCL consists of combined immuno-chemotherapy based on rituximab, an anti-CD20 antibody, and chemotherapy. Rituximab binds Fc-gamma receptor+ (e.g. CD16+) cytotoxic immune cells and CD20+ B cells, inducing cell death through antibody-dependent cell-mediated cytotoxicity (ADCC). CD16+ natural killer (NK) cells represent the major cytotoxic effector population and their ADCC is well characterized. Despite the use of combined immuno-chemotherapy, 30-40% of patients relapse, underscoring the need for improved therapeutic strategies.

A recent study by Zöphel et al. (2024) demonstrated that a high proportion of CD16+ T cells at diagnosis is associated with superior progression-free survival over the first 24 months after treatment. In contrast to CD16+ NK cells, the amount of CD16+ T cells does not undergo significant reduction in peripheral blood during treatment, suggesting increased chemoresistance and highlighting the importance of this T cell population during therapy.

To elucidate the phenotype and function of this specific T cell population, CD16+ T cells from eight healthy donors and four DLBCL patients were sorted and analyzed by flow cytometry and cytotoxicity assays. The analysis showed that the majority of CD16+ T cells were γδ T cells mainly lacking CD4 and CD8 expression (double negative, DN). A significant positive correlation was observed between the number of CD16+ DN γδ T cells and the capacity for ADCC against the DLBCL cell line TMD8 ($p=0.028$). In addition, single-cell analysis revealed that ADCC by sorted CD16+ T cells mainly induces apoptosis in TMD8 cells. Notably, CD16+ T cells from DLBCL patients eliminated the target cells faster than their CD16+ NK cells. To obtain further phenotypic information about CD16+ T cells, frozen PBMC from DLBCL patients and healthy controls will be analyzed by CyTOF with 42 markers.

Based on these findings, therapeutic administration of in vitro/in vivo expanded γδ T cells to DLBCL patients undergoing rituximab-based therapies could improve survival outcomes. To evaluate the potential benefit of this approach, we will compare the ADCC capacity of expanded γδ T cells and CD16+ expanded γδ T cells with that of expanded NK cells and CD16+ expanded NK cells in the context of rituximab-based treatment.

Spatial Distribution of Tγδ Lymphocytes and their γδ1 and γδ2 Subsets in Whole Tissue Samples Using Immunohistochemistry-Based Techniques: Elaboration of Protocol for Further Anticancer Immunotherapies

Presenter Name: Piotr Kupczyk

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Tγδ cells are a rare subpopulation of T lymphocytes with unique biological functions; and protocols for their spatial identification in whole tissue samples are still under development. Tγδ cells consist of two main subpopulations: Tγδ1 (identified in most mucosa-like tissues) and Tγδ2 (present in whole blood circulation, constituting about 0.5% to 5% of total leukocytes). Tγδ cells may demonstrate different transcriptomic and proteomic signatures depending on their interactions with other cells and the tissue microenvironment.

In contrast to conventional T cells with αβ TCR chains, Tγδ cells are activated in a non-MHC manner, making their use in allogenic immunotherapies against cancer highly desirable. Single-cell RNA sequencing and proteomic data analyses have contributed to significant progress in decoding their molecular and immunological signatures. These analyses have confirmed that Tγδ cells and Tαβ cells, especially CD8 T and NK cells, share common genes identified within tumor-infiltrating lymphocytes (TILs). The potential of TILs is highly variable within human cancers. In glioblastoma multiforme (GBM), the most lethal brain human cancer, the tumor microenvironment (TME) creates an immunosuppressive milieu that strongly inhibits TILs. This low immunotherapy response and rapid immune escape classify GBM as a "cold tumor".

The role of Tγδ cells as immunotherapeutic agents in GBM has not been completely ruled out, but further analyses using microscopy-based techniques are needed. To better understand the potential of Tγδ cells, we have developed an immunohistochemistry (IHC) protocol on formalin-fixed paraffin-embedded (FFPE) samples from control tissues (lymph nodes, tonsils, melanoma) and GBM patients. For protein detection, we use a set of commercially available antibodies that recognize the pan-δ chain of the TCR receptor as well as their subsets (δ1 and δ2) and CD3, which marks all T lymphocytes. Additionally, Tγδ cells and their subsets isolated from the peripheral blood of healthy donors, expanded and embedded as cytoblocks, were used as

internal controls. All slides underwent scanning using a panoramic histopathological imaging system.

The results of our research indicate that we have developed an effective protocol for the detection of Tγδ cells and their subsets. This protocol could serve as an auxiliary tool in studies assessing the infiltration potential and spatial distribution of Tγδ cells in normal and neoplastic tissues such us GBM.

Spatial Distribution and Identification of γδ T Lymphocytes in the Skin Tissue of Patients with Hidradenitis Suppurativa: Possible Role of the Cells in the Molecular and Immunological Background of the Disease

Presenter Name: Piotr Kupczyk

Piotr Kupczyk1, Piotr Krajewski2, Pawel Karpinski3, Mateusz Speruda1, Danuta Nowicka-Suszko2, Grzegorz Chodaczek4, Alina Jankowska-Konsur2.
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Primary Theme: Tissues & Inflammation

Abstract

Hidradenitis Suppurativa (HS) is a chronic proinflammatory skin disease with an unexplained molecular and immunological background. The main susceptibility factors for the disease include a combination of genetic predisposition, endocrine, immune, and environmental factors such as obesity and smoking. HS is a debilitating condition characterized, among other things, by disordered interactions between keratinocytes and fibroblasts, which further exacerbate the inflammatory milieu. The histopathological hallmark and diagnostic factor in HS are epithelialized-like tunnels, which serve as indicators of an intensified and tissue-destructive inflammatory process involving both innate and adaptive immune responses. HS creates a unique proinflammatory environment that releases cytokines and chemokines, recruiting various immune cells.

In the last decade, there has been significant progress in understanding the immunopathology of the disease, including the cytotoxic NK and CD8 T cells, that share many featutres with biologically unique Tγδ cells. Tγδ are rare T cell subset, which role in HS etiopathology is completly unknown. Hovewer, single-cell RNA sequencing and proteomic results indicate on Tγδ cells as important drivers of inflammation in HS. Two main subsets of Tγδ cells have been identified: Tγδ1, which resides in mucosal tissues, and Tγδ2, which circulates in whole blood leukocytes, with a prevalence ranging from 0.5% to 5% of total leukocytes. Tγδ cells are activated under various stress factors (infection, inflammation, cancer transformation) and can adopt different phenotypes and secrete various cytokines depending on the tissue microenvironment.

Tγδ cells have never been investigated in HS, and we present preliminary results regarding their spatial identification and distribution. Using immunohistochemistry (IHC) and an antibody recognizing all infiltrating CD3 T lymphocytes, along with an antibody against the pan-δ chain of the TCR receptor, we detected Tγδ cells in formalin-fixed paraffin-embedded (FFPE) samples from HS patients and healthy controls. Our study results demonstrate that Tγδ cells are present in higher amounts in perilesional and lesional skin of HS patients compared to healthy skin. These findings are further supported by the analysis of single-cell RNA-seq data. In conclusion,

Tγδ cells may be a new potential drivers of the inflammatory milieu in HS, and their roles in immunological background of disease should be further elucidated.

Humanized Immune System Mice Faithfully Recapitulate the Ontogeny and Function of Human γδ T Cells

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Primary Theme: Development & Differentiation

Secondary Theme: Ligands & Repertoires

Abstract

Human and mouse gamma delta T cells (γδ T cells) greatly differ in terms of their ontogeny, T cell receptor (TCR) repertoire and functionality. Humanized immune system (HIS) mice offer unique opportunities to address human-specific questions in vivo. Yet, little is known about γδ T cell development in these next-generation models.

In this study, we investigated the γδ T cell lineage in NBSGW mice (NOD,B6.PrkdcscidIl2rγ-/-KitW41/W41) reconstituted at birth with CD34+ hematopoietic stem and progenitor cells (HSPCs) isolated from human fetal liver.

Using flow cytometry and single cell transcriptomics coupled with CITEseq and TCR repertoire analysis, we found that human γδ T cells were generated de novo in the thymus of the HIS mice, where they followed progressive maturation steps and underwent strong effector programming with type 1, type 1-3 and type 2 mature clusters. As observed in human thymus, these distinct functional γδ thymocyte subsets were associated with different TCR features.

Human γδ T cells populated peripheral organs and were circulating in the blood of the mice. They were biased towards type 1-cytotoxic effector functions, as seen in humans: they produced large amounts of cytotoxic mediators (granzymes A and B, perforin and the human-restricted granulysin) and IFNγ. Large clonal expansions (Vδ1 and Vδ3 γδ T cells) were observed in the peripheral tissues, like in humans.

Remarkably, the human-specific innate-like Vγ9Vδ2 T cells were readily detected both in thymus and peripheral organs. Their repertoire was characterized by an enrichment of the public

CALWEVQELGKKIKVF CDR3γ sequence and by an enrichment of a hydrophobic amino-acid at the 5th position of the CDR3δ, both associated with phosphoantigen reactivity. Accordingly, these Vγ9Vδ2 T cells exhibited responsiveness to phosphoantigen both in vitro and in vivo, with a robust activation not observed in other T cell subsets.

Our findings demonstrate that HIS mice can recapitulate the ontogeny and function of human γδ T cells, making them a powerful tool for studying human γδ T cell biology and evaluating novel immunotherapies targeting human γδ T cells in vivo.

INB-100: Phase I Trial of Allogeneic Ex-Vivo Expanded/Activated γδT Cell Infusion Following Haploidentical Bone Marrow Transplantation and Post-Transplant Cyclophosphamide

Presenter Name: Lawrence Lamb

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Background: Gamma-delta (γδ) T cells engage cancer cells via innate sensing of multiple ligands, such as DNAM-1 and NKG2DL, but do not initiate GvHD. In this trial (NCT03533816), we examined whether a single infusion of allogeneic, ex vivo expanded and activated γδ T cells (EAGD) in older leukemia patients receiving haploidentical bone marrow transplant (BMT) and reduced intensity conditioning (RIC) could improve progression free (PFS) and overall (OS) survival with manageable safety.

Methods: Adults with newly diagnosed or recurrent ALL, CML, AML, or MDS undergoing Haplo/Cy BMT following RIC received an infusion of EAGD cells within 7 days of neutrophil engraftment. Primary endpoints include dose-limiting toxicities (DLT), grade (G) 3-4 adverse events (AE's), with secondary endpoints of PFS and OS. Lymphocyte recovery and cytokine levels were tracked for one year.

Results: 23 patients were enrolled and 16 were treated as of January 17, 2025; 4 at dose level (DL)1 of 1x106 cells/kg and 12 at DL2 of 3x106 cells/kg. Patients were 50% male, median age 68, 62.5% AML. Typical AEs related to transplant observed, but possible EAGD related toxicities include maculopapular rash, aGVHD and cGvHD. No DLTs, neurotoxicity (ICANS), cytokine release syndrome (CRS) or treatment related deaths were reported. At median follow-up of 18.8 months (m), 15.5m median duration of morphologic CR was observed with 12/16 subjects remaining in CR. One patient died relapse-free at 15.5m of idiopathic pulmonary syndrome (IPS) deemed unrelated to treatment, 2 patients with double allele TP53 mutations (an MDS/MPN overlap and an ALL subject) relapsed at 12.4m and14.7m respectively. In AML patients, median CR is now 20.1 months with 100% PFS to-date. Three high-risk AML patients remain relapse free beyond 3 years, including 2 with trisomy 8 and del 7 with IDH and/or FLT-3 mutations. Circulating γδ T cell expansion and persistence is observed in dose-dependent manner in DL1 and DL2 patients through 365d post-BMT vs. Control haplo/PTCy patients ($p = <0.05$).

Summary: EAGD cells demonstrated a manageable safety profile. Durable PFS ≥ 12mos was achieved in 100% of AML patients comparing favorably with historically expected 1-year PFS for HaploCy BMT of ~51%. Enhanced survival and dose-dependent post-BMT expansion of circulating γδ T cells replicates previous observational findings showing γδ T cell - associated protection against relapse.

Generating CAR γδ T cells for Effective Targeting of B Lymphomas

Presenter Name: Marilee Larrivée

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Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

Chimeric Antigen Receptor (CAR) T-cell therapy has attracted growing interest in the treatment of various malignancies and represents the most advanced technology to date. This approach involves the use of genetically engineered immune cells expressing a surface receptor, called CAR, that specifically detects tumor-associated antigens. Here, we generate polyclonal CAR γδ T cells, as these cells do not require patient-donor compatibility and are ideal candidates due to their natural antigen polyspecificity. The cells are modified to express a B lymphoma-specific CAR, featuring an anti-CD22 camelid single-domain nanobody, a 4-1BB costimulatory domain, and a CD3ζ activation domain. Using a recently developed packaging and producer line of 293SF-3F6 cells, we have produced and stocked the lentiviral vector for this construct. Lentiviral titration was performed on HEK 293A cells and validated by flow cytometry. γδ T cells were then transduced with the CAR lentivirus at different multiplicities of infection. Analyses using flow cytometry showed a CAR transduction efficacy of over 30%, with all γδ T cell subsets being efficiently transduced. This transduction rate exceeds the clinical threshold for CAR therapy, representing a significant milestone in our research. The CD22 CAR polyclonal γδ T cell product will be tested in vitro against both wild-type and CD22-negative Ramos B cells to assess its specificity to B lymphoma. It will then be tested against a wider range of B lymphoma lines. Analyses will be performed using flow cytometry and the IncuCyte Live-Cell Analysis System to examine function over longer co-culture periods.

Use of γδ T cells Armed With Monoclonal Antibodies as a Novel Cancer Cell Therapy.

Presenter Name: Marion Lenain

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Human γδ T cells are involved in the anti-tumor response of many solid and hematological cancers (e.g., myeloma, lymphoma, melanoma, breast, colon, lung, ovarian and prostate). Their anti-tumor properties are based on a direct cytotoxic activity against tumor cells and their ability to stimulate the biological functions of other immune cells, which are necessary for the initiation and establishment of an effective anti-tumor immune response. Unlike αβ T cells, γδ T cells: (i) display a potent MHC-independent reactivity against a broad panel of tumors, (ii) show limited if any alloreactivity. This supports why γδ T cells are considered as highly attractive therapeutic targets for anti-tumor immunotherapies. Currently, many clinical trials are ongoing based on various strategies such as the use of bispecific antibodies targeting both tumor cells and γδ T cells, CAR γδ T cells, γδ T cells-activating molecules. Here, we propose a new innovative strategy of cell therapies based on the ability of γδ T cells to express low affinity receptor of IgG Fc portion (FcγRIII or CD16) and to be armed with monoclonal antibodies (mAbs) targeting specifically tumor cells. Indeed, several studies, including our own, have shown that γδ T cells can express CD16 and similar to NK cells, CD16+ γδ T cells are able to perform antibody-dependent cellular cytotoxicity (ADCC). We hypothesized that γδ T cells could be "armed" by binding of CD16 with monoclonal antibodies selected to recognize tumor-specific antigens and lyse tumor cells via their ADCC capability. Our first results show that the expression of CD16 on γδ T cells differs from donor to donor and also with differentiation status. Second, we have shown that γδ T cells have cytotoxic activity against cancer cells in 2D culture models. We are currently working on the development of 3D models of different ovarian cancer lineages. Our preliminary results in 3D models showed that: 1- IL-2 and IL-15 increase the infiltration of γδ T cells of into spheres, and 2- the activation of γδ T cells by simultaneous TCR and CD16 recruitment has an additive effect on their cytotoxic activity against tumor cells. These findings are very interesting and require further investigation.

Transcriptional and TCR Profiling Reveals an Expansion of Cytotoxic, Differentiated γδ T Cells in Response to in utero CMV Infection

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Primary Theme: Infection Responses

Secondary Theme: Development & Differentiation

Abstract

Gamma delta T cells (γδTs) are the first T cells to develop during gestation in humans and possess many features that make them uniquely suited to protection of the fetus and infant. Indeed, it has been shown that γδTs can respond to viral infection in utero, including congenital CMV (cCMV) infection. Previous studies have found that γδ T cells in the human fetus are transcriptionally pre-programmed for effector function and can be rapidly activated to produce IFNγ and granzymes upon stimulation, and furthermore that cCMV-infected infants possess an expansion of cells with CMV-reactive TCRs. However, studies pairing the functional programming of fetal γδ T with specific TCR information in cCMV-infected neonates are lacking. We performed flow cytometry and paired single-cell RNA and TCR sequencing on cord blood mononuclear cells collected from cCMV-infected and uninfected neonates in Eastern Uganda to examine how in utero infection with cCMV influences the phenotype, gene expression, TCR repertoire development, and effector functions of γδT cells in the fetus. Agreeing with prior data in the field, we see that multiple subsets of γδTs expand in cCMV+ neonates, and furthermore show that these cells are more differentiated and activated, with decreased CD27 expression and increased CD16 and HLA-DR expression. In addition, γδ T cells from cCMV+ neonates are more cytotoxic and proliferative. We found that the overall TCR repertoires of cCMV+ are more focused than their cCMV- counterparts, but not more public, and display an enrichment of cells with fetal-like TCR characteristics, including shorter CDR3 lengths and few N additions, as identified by prior studies of the human fetal thymus. Interestingly, although the overall repertoires of cCMV+ subjects are not more public, the TCRs that are clonally expanded in these neonates are more likely to be public. In addition to their publicity, these expanded clonotypes show other striking differences compared to rare clonotypes, with more cytotoxic effector transcriptional programming and with TCRs possessing fetal-like characteristics. Given the potential for γδTs to mediate early life immunity, these findings may have implications for developing immunotherapeutic interventions against congenital infections.

The Role of TCR Signaling in Human γδ T Cell Selection

Presenter Name: Tzu-Chieh Liao

Tzu-Chieh (Jackie) Liao1, Mayuri Viswanathan2, David Wiest3, Juan-Carlos Zúñiga-Pflücker4, Erin Adams2, Maria Ciofani1

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Primary Theme: Development & Differentiation

Abstract

γδ T cells are enriched in epithelial tissues and play critical roles in pathogen defense, wound healing, tissue homeostasis, autoimmunity, and cancer. While their peripheral functions are well characterized, the thymic signals governing human γδ T cell lineage commitment remain poorly understood. A key question is how TCR signaling strength influences transcriptional programs that drive γδ T cell fate. We hypothesize that stronger TCR signaling promotes γδ lineage differentiation over αβ commitment. To test this, we computationally reanalyzed single-cell RNA sequencing data from human pediatric thymocytes, revealing that γδ thymocytes exhibit significantly higher activation of immediate early genes compared to αβ thymocytes, indicative of heightened TCR signaling. Chromatin accessibility analysis identified AP-1 motifs as predominant in newly formed γδ thymocytes, consistent with regulon analysis showing active AP-1 transcriptional networks throughout γδ T cell differentiation. To experimentally examine γδ lineage commitment, we harnessed an in vitro T cell differentiation system in which CD34+ early T cell progenitors (ETPs) were co-cultured with OP9-DL4 cells. By day 24, differentiation yielded CD4+CD8+ double-positive cells, αβTCR+ T cells, and γδ T cells, including a substantial fraction of Vδ1+ T cells. Notably, both total γδ and Vδ1+ cells matured and acquired a TBET+ type 1 effector profile. Tetramer staining confirmed that Vδ1+ cells specifically engaged CD1d-sulfatide, their cognate ligand. Importantly, trends in CD5 and CD73 expression, which are both correlated with TCR signal strength, suggest that Vδ1+ γδ T cells experience stronger TCR signaling compared to αβ T cells. Our findings support a model in which the role of TCR signal strength in γδ versus αβ lineage selection can be effectively studied in vitro. To further dissect thymocyte fate decisions, we plan to transduce ETPs with TCRs of defined signaling strengths, including CD1d-sulfatide-recognizing DP10.7 TCRs paired with Cγ1 or Cγ2 domain, and systematically evaluate their impact on γδ and αβ commitment, effector function, and epigenetic regulation. These insights will deepen our understanding of human γδ T cell development and inform the design of γδ T cell-based immunotherapies.

NEMO Exon 5 Skipping Triggers Systemic Autoinflammation, Cell Death Pathway Activation, and γδ T Cell Expansion

Presenter Name: Bin Lin

Bin Lin, PhD1; Adriana A. de Jesus, MD, PhD1; Keith Kauffman2, Dana Kahle, BS1; Eric Karlins, MS3; Colton McNinch, PhD3; Sara Alehashemi, MD, MPH1; Sophia Park, BS1; Elizabeth Morgan, BS1; Christopher Friend, BS1, Farzana Bhuyan, PhD1; Kader Cetin Gedik, MD1; Kat Uss, RN, BSN1; Sofia Torreggiani, MD1; Andre Rastegar, BA1; Jacob Mitchell, BSc1; Amanda Chen, BS1; Taylor Newbolt, BS2; Andrea C. Bohrer22, Ehydel Castro22, Eric Hanson, MD4; Amer Khojah, MD5; Eveline Wu, MD, MSc6; Christiaan Scott, MD, MBChB7; Timothy Ronan Leahy, MD, PhD8; Emma J. MacDermott, MD8; Orla G. Killeen, MD8; Chyi-Chia Richard Lee, MD, PhD9; Thaschawee Arkachaisri, MD10; Zoran Gucev, MD11; Kathryn Cook, DO12; Vafa Mammadova, MD13; Gulnara Nasrullayeva, MD13; Mariana Marques, MD14; Abigail Bosk, MD15; Seza Ozen, MD16; Abigail Lang, MD5; Brian Nolan, MD5; Scott Canna, MD17; Maude Tusseau18, Emilie Chopin18; Guilain Boursier19; Danielle Fink, MS20; Douglas Kuhns, PhD20; Alexandre Belot, MD, PhD18; Katrin D. Mayer-Barber22; Timothy Moran, MD, PhD6; Andrew Oler, PhD3; Daniel L. Barber, PhD2 & Raphaela Goldbach-Mansky, MD, MHS1

Primary Theme: Tissues & Inflammation

Abstract

Mechanisms leading to organ inflammation, particularly those studied in murine models of TNF-induced cell death, remain elusive. Here we investigated 21 patients (16 female & 5 male, mosaic) with genetically confirmed NEMO (NF-□B essential modulator, encoded by IKBKG) Deleted Exon 5 Autoinflammatory Syndrome (NEMO-NDAS). These patients presented with clinical features of systemic inflammation, leaky gut, portal hypertension, liver and skin inflammation, and highly elevated serum levels of IFN□ and IL-12p40, distinguishing NEMO-NDAS from other autoinflammatory diseases. Interestingly, stimulation of NEMO-NDAS patients' cells did not lead to increased cytokine production in response to various immune stimuli including Toll-like receptor ligands, IFN□, and IFN□, suggesting an indirect mechanism. To explore the pathogenesis, we generated a U937 cell line with NEMO exon 5 skipping by CRISPR editing. Similar to patients' cells, the mutant U937 cells exhibited partially impaired immune responses but were highly susceptible to TNF induced cell death, which was exaggerated by IFN□ and other cell stressors. Peripheral blood analysis of clinically active NEMO-NDAS patients revealed significant expansions of □□ T cells, constituting 10-30% of the peripheral blood T cells. The □□ T cells were activated (granzyme B+, PD-1+) and produced IFN□ upon PMA stimulation. Notably, the activated □□ T cells (PD-1+) predominantly expressed wildtype, rather than mutant NEMO, in both female and male patients. These results led to the hypothesis that wildtype □□ T cells may be activated and expanded by dysregulated IKBKG/NEMO mutant tissue cells, exacerbating systemic inflammation and, in extreme cases, inducing tissue cell death through IFN□□production. In conclusion, our study identifies NEMO-NDAS as a novel inflammatory phenotype that relies on both mutant and wild-type NEMO expression, thus expanding the spectrum of NEMO-associated diseases.

This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Disease, National Institutes of Health.

Harnessing Engineered γ9δ2TCR T Cells and Bispecific T cell Engagers to Target the Dysregulated Metabolism of Tumors

Presenter Name: Jairo Lommen

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Primary Theme: Cancer Therapies

Secondary Theme: Ligands & Repertoires

Abstract

Dysregulated metabolism is a hallmark of many cancers, contributing to tumor growth and resistance to treatment. Recent advancements in immunotherapy have led to novel approaches that target metabolic alterations in tumors. By harnessing engineered γ9δ2TCR T cells and bispecific T cell engagers, we aim to specifically target the dysregulated mevalonate pathway within tumors, offering a potential strategy to overcome the current limitations in treatment. Vγ9Vδ2T cells have served as inspiration for many different types of immunotherapies, but to date many of these strategies fall short in clinical trials. One of the reasons of these disappointing clinical outcomes can be related to the low affinity of natural γ9δ2TCRs for the BTN2A1-BTN3A-phosphoantigen complex. Therefore, we hypothesized that increasing the affinity of Vγ9Vδ2TCRs using protein engineering could enable for an effective tumor targeting of the Vγ9Vδ2 TCR. To achieve this, a large mutagenesis screen, using Vγ9Vδ2TCR-antiCD3 T Cell Engagers, revealed potency enhancing mutations in both the γ9 chain and the δ2 chain. Based on predicted regions of the Vγ9Vδ2 TCR that are required for TCR activation, the screening was performed on the γ9 chain interface, which interacts with BTN2A1 and the CDR1, CDR2 and HV4 of the δ2 chain. Herein we discovered mutations leading to an over tenfold increase in potency, both as single mutation and in combination, affecting both the γ9 and the δ2 chain. Engineered T cells expressing these novel affinity enhanced γ9δ2TCRs demonstrated a significant enhanced tumor control in a multiple myeloma xenograft model, outperforming the engineered T cells expressing a natural γ9δ2TCR. These findings highlight the potential of affinity-matured γ9δ2TCRs to advance next-generation γ9δ2TCR-based immunotherapies for cancer treatment.

Universal Expansion and Superior Antitumor Reactivity of Tumor Infiltrating γδ T Cells Support Their Utility for Adoptive Cell Therapy

Presenter Name: Michael Lotze

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Background: Tumor infiltrating lymphocyte (TIL) therapies utilize αβ T cell receptor (TCR) expressing cells, recognizing major histocompatibility complex (MHC) restricted peptide antigen. However, αβ TIL are limited by immune escape through neoantigen editing and MHC loss. γδ TCR expressing cells are an evolutionarily conserved lymphocyte subset that demonstrate MHC-independent recognition of non-peptide metabolites and stress antigens.

Methods: To consider the therapeutic utility of tumor infiltrating γδ T cells, we prospectively evaluated expanded αβ and γδ TIL from patients with solid tumors. Expanded TIL were characterized by multispectral flow cytometry and TCR sequencing. Tumor-specific reactivity of matched TIL cultures was assessed with cancer cell lines and primary tumor digests. The relative abundance and prognostic role of individual γδ TIL populations was also evaluated across the 20 most prevalent human cancers within the Cancer Genome Atlas (TCGA).

Results: Despite sparse infiltration, γδ TIL could be negatively selected from bulk TIL cultures and rapidly expanded with combinations of γ common chain cytokines from multiple tumor types including metastatic melanoma (n=7), kidney cancer (n=7), peritoneal surface malignancies (n=18), and pancreatic cancer (n=6). Compared to expanded αβ TIL, γδ TIL possess a tissue resident effector memory phenotype with lower PD-1 exhaustion and higher expression of CD137 and the natural cytotoxicity receptors NKG2D and NKp46. Moreover, γδ TIL display superior TCR and NKG2D dependent autologous tumor Th1 cytokine production and cytotoxicity that is positively associated with Vδ1 abundance (r=0.66, p=0.001) and minimal IL-10 and IL-17A secretion. In fact, 88% of γδ TIL cultures showed tumor-specific reactivity greater than a threshold associated with clinical TIL therapy response, compared to 50% of αβ TIL cultures (p=0.004). Despite a largely private Vδ repertoire, γδ TIL were capable of recognizing allogeneic tumors. Within the TCGA, Vδ1 T cells demonstrated a high relative abundance in both immunologically hot (lung and renal cell carcinomas) and cold (pancreatic and breast carcinomas) tumors. Patients with increased Vδ1 expression demonstrated a significant survival advantage across most indications.

Conclusions: These findings highlight the therapeutic potential of adoptive cell therapy with γδ TIL that warrants further clinical development in patients with advanced cancer.

Recognition of MR1-antigen Complexes by TCR Vγ9Vδ2

Presenter Name: José Pedro Loureiro

José Pedro Loureiro, Alessandro Vacchini, Giuliano Berloffa, Jan Devan, Vladimir Nosi, Benedikt Meyer, Mike Recher, Lucia Mori and Gennaro De Libero

Primary Theme: Ligands & Repertoires

Secondary Theme: Tissues & Inflammation

Abstract

The activation of TCR Vγ9Vδ2-expressing cells depends on an innate-like TCR-mediated mechanism that involves the butyrophilin 3A1, 3A2, and 2A1 molecules as well as phospho-antigens, without the participation of classical antigen-presenting molecules. It is unknown whether TCR Vγ9Vδ2 cells also recognize complexes of antigens and MR1 molecules in an adaptive-like manner.

Here, we identify MR1-autoreactive cells that express the TCR Vγ9Vδ2. This MR1-restricted response depends on both antigen and CDR3δ region but is independent of butyrophilin 3A1.

TCR gene transfer reconstituted MR1-antigen recognition and engineered TCR Vγ9Vδ2 tetramers interacted with soluble MR1-antigen complexes. Adding MR1 binding molecules in antigen competition experiments reduced TCR binding, revealing the importance of the antigen. Additionally, the K43A MR1 mutant, which presents a different repertoire of antigens than the wild-type MR1 (MR1*01), triggered a stronger response from select T cells, highlighting the significance of the cognate interaction between TCR and MR1-antigen complexes.

These cells are present in healthy individuals at low frequencies and are predominantly CD8+ or CD4-CD8 double negative. We also studied a patient with autoimmune symptoms and TCR γδ lymphocytosis, finding that approximately 10% of circulating T cells were MR1-self-reactive and expressed TCR Vγ9Vδ2. These cells released pro-inflammatory cytokines, suggesting a potential role in disease pathogenesis.

Thus, MR1-self-antigen complexes can interact with certain TCR Vγ9Vδ2, promoting complete cell activation and potentially contributing to physiological and pathological immune responses.

Gamma-Delta and Alpha-Beta Intraepithelial Lymphocytes in Cancer Immunosurveillance: It Takes Two to Tango

Presenter Name: Federico Lupo

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Primary Theme: Cancer Responses

Secondary Theme: Tissues & Inflammation

Abstract

Intestinal tissue contains two tissue-resident populations of intraepithelial lymphocytes (IEL) that express CD8αα and either αβ or γδ T cell receptors (TCR). Both αβIEL and γδIEL are maintained by epithelial-derived molecules such as IL-15, while γδIEL specifically rely on butyrophilin-like molecules. αβIEL and γδIEL protect the gut from infection, but their roles in tumor initiation and progression are not fully understood. To explore this question, we used a mouse model of tubular adenomas that combines loss of the tumor suppressor Apc with oncogenic Kras specifically in intestinal epithelial cells: Vil1-CreERT2;ApcF/+;KrasLSL-G12D/+ (VAK) mice. About 70 days after Cre induction by tamoxifen, these mice succumb to small intestine and colonic tumors. VAK mice were crossed with Tcrb or Tcrd knockout mice to generate tumor-bearing mice lacking either αβ or γδ T cells, including IEL. At the humane endpoint, VAK mice deficient for either αβIEL or γδIEL showed no differences in survival and tumor number compared to controls. However, VAK mice lacking both αβIEL and γδIEL (VAK;Tcrb—/—; Tcrd—/—) exhibited reduced survival and increased tumor number compared to T cell-proficient VAK mice, suggesting that both populations are redundant during cancer immunosurveillance. To examine how tumorigenesis affects IEL phenotype, we analyzed IEL from tumor-bearing VAK and tumor-free control mice by flow cytometry. 50 days post tamoxifen injection, αβIEL, γδIEL, and ILC1 populations from the small intestine (SI) and colon of VAK mice were significantly decreased compared to control. Whereas we failed to detect changes in the expression of cytotoxic intracellular molecules such as granzyme B or TNF, CD8β+ αβIEL and Vγ1+ γδIEL in the SI showed increased surface expression of the cytolytic receptor NKG2D. Interestingly, ILC1 cells, almost entirely NKG2D+, were the only IEL subset significantly decreased in both SI and colon, and as early as 25 days post tumorigenesis. To investigate whether NKG2D-expressing cells regulate tumor initiation, VAK mice were crossed with Klrk—/— mice, which lack NKG2D expression. Compared to VAK, NKG2D-deficient VAK mice had increased tumor numbers 50 days after tamoxifen injection, suggesting that NKG2D participates in cancer immunosurveillance. Current efforts are focused on understanding how adenomas affect IEL survival, identifying the most anti-tumorigenic IEL subsets, and determining whether IEL can directly kill tumor cells through NKG2D.

The Toxic Relationship: the Synapse Between Gamma Delta T Cells and Glioblastoma Cells

Presenter Name: Justyna Mączyńska

Justyna Mączyńska1, Agnieszka Chwastek1, Agnieszka Szyposzyńska1, Piotr Kupczyk2, Grzegorz Chodaczek1
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Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Glioblastoma multiforme (GBM) is one of the most aggressive forms of brain cancer, capable of evading immune surveillance. Ongoing efforts in anti-GBM immunotherapy focus on finding more efficient effector cells insensitive to tumor microenvironment suppression. Among the immune cells, gamma delta (γδ) T cells hold promise in oncological therapeutic applications, yet their mechanisms of action remain incompletely understood. Lymphocyte cytotoxic functions require cell activation and several activatory receptors have been described in this process, including T cell receptor (TCR) and NKG2D.

This study investigates the role of the γδ TCR in anti-GBM responses and immune synapse formation – an organized multimolecular interface essential for T cell activation and cancer cell elimination. To assess γδ TCR engagement, we expanded and compared effector functions of two blood-derived γδ T cell subsets (Vδ1 and Vδ2) against primary patient-derived and established GBM cell lines. The γδ T cell phenotype was verified by flow cytometry and γδ T cell cytotoxicity toward GBM was assessed by LDH viability assay. Direct interactions between γδ T cells and GBM cells were analyzed by confocal imaging. TCR activation was visualized by immunofluorescent staining of the phosphorylated CD3ζ chain, while CD3 cross-linking with an anti-CD3 antibody served as a positive control.

Our data show that both γδ T cell subtypes can destroy GBM cells, but effectiveness varied between T cell and cancer cell lines. Immunofluorescence imaging revealed phospho-CD3ζ clusters exclusively at intercellular contact sites, confirming immune synapse formation. Vδ2 cells exhibited a higher frequency of phospho-CD3ζ clusters than Vδ1 cells, suggesting greater specificity of Vδ2 TCR to GBM antigens and enhanced activation of Vδ2 subset. These differences highlight the importance of studying direct cell-to-cell contacts and the need to explore additional activation receptors in γδ T cell-mediated cytotoxicity.

Our study provides novel insights into the mechanisms underlying γδ T cell activation in the context of GBM. These findings underscore the potential of γδ TCR-targeted strategies to enhance γδ T cell-based immunotherapies for GBM treatment. Future studies should further investigate how γδ TCR signaling can be optimized to improve anti-tumor responses.

This work is supported by the National Science Centre (Poland) grant No 2019/35/B/NZ6/03748.

Gamma-Delta T cells Regulate Tumour-Associated Macrophages and Metastasis in Pancreatic Cancer.

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Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with a five-year survival rate of around 5%, where 80% of patients present with metastases on diagnosis. Metastasis is driven by inflammation, which potentiates the invasive and migratory behaviour of pancreatic cancer cells. Recent reports have shown that IL-17-producing gamma delta T cells are crucial for PDAC progression in transplantable mouse models, and they constitute a large proportion of tumour-infiltrating lymphocytes within human PDAC. However, the mechanisms by which IL-17-producing gamma delta T cells function in PDAC are largely unknown. To investigate this, we used the KrasG12D; Trp53R172H; Pdx1-Cre (KPC) mouse model. We found that gamma delta T cells are absent in normal pancreas but are relatively abundant in PDAC tumours. In gamma delta T cell-deficient KPC mice (KPC;Tcrd-/-), we found reduced incidence of liver metastasis and observed a striking reduction of F4/80+ macrophages within the PDAC tumours. We also observed a decreased metastatic incidence in the livers of KPC;iCCR-/- mice which are deficient in monocyte recruitment to tissues. These results indicate that within the PDAC microenvironment, gamma delta T cells, monocytes, and macrophages conspire to promote metastasis. Ongoing efforts aim to understand the mechanisms connecting these cell types, and to examine this crosstalk in the context of metastatic PDAC.

Cytomegalovirus Infection Remodels the γδ T Cell Repertoire Compartment

Presenter Name: Ellie Mann

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

Human γδ T cells are an important component of the anti-viral T cell immune response. However, it remains unclear how the γδ T cell compartment is shaped by viral infection. In this study, we explored the γδ T cell response to both primary and latent human cytomegalovirus (HCMV) infection in kidney and lung transplant patients. We used spectral flow cytometry, TCR sequencing and single cell multiomic analysis to investigate the long-term shaping of the γδ T cell compartment and the impact of HCMV on the γδ receptor repertoire.

Blood was drawn weekly from lung/kidney transplant patients before surgery and up to 5 years post-transplant, with which peripheral blood mononuclear cells (PBMCs) were isolated and CMV titer was assessed. In primary HCMV infection in kidney transplant patients over 5 years of longitudinal sampling, defined a major expansion of Vδ2neg γδ T cells (predominantly Vδ1+ T cells) post primary infection. These cells exhibited a distinct Granzyme A/B+ Tbet+ Eomes+ CX3CR1+ CD45RA+ CD127- CD27- γδ T effector population. A year after infection, γδ TCRs recruited into this effector population were highly diverse but by 5-years focused clonotypes emerged, with a preferential enrichment of Vδ1 TCR chains and paired with expanded Vγ2, 3 and 5.

In a solid organ transplant setting, the γδ T cell repertoire is shaped not only by the nature of the transplant itself, but also upon infection with CMV. This viral challenge drives acute changes and repertoire restructuring of γδ T cell compartment, fostering a predominance towards effector-like T cell phenotypes.

Overcoming Speed Bumps on the Road to Cancer Treatment: The Bi-Specific Antibody Mosunetuzumab and Immune Checkpoint Inhibitors Are Paving the Way for γδ T Cells

Presenter Name: Lothar Marischen

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Primary Theme: Cancer Therapies

Abstract

Immunotherapy has been firmly established as an additional option for cancer treatment. Aside from the transplantation of stem cells and the involvement of patient's own immune system, the adoptive transfer of allogeneic immune cells provides another treatment opportunity. γδ T cells are MHC-independently activated and ideally do not provoke a graft-vs-host reaction. Therefore, they are appropriate candidates for allogeneic transplantation. Since former studies with adoptively transferred γδ T cells revealed heterogenous results, in-vitro studies were carried out to improve the killing efficiency of γδ T cells towards malignant B cell lines by the application of therapeutic antibodies or immune checkpoint inhibitors. Co-cultures of Vγ9Vδ2 T cell lines and B cells from lymphoma cell lines, from the blood of healthy donors or CLL patients were incubated with the bi-specific antibody mosunetuzumab or the mono-specific antibody obinutuzumab. Expression of CD107 and IFN-γ by γδ T cells were strongly increased due to mosunetuzumab. As could be shown by life cell imaging and flow cytometry, mosunetuzumab initiated a higher killing efficiency than obinutuzumab. Since it was tempting to speculate, whether the application of immune checkpoint inhibitors could further improve the killing efficiency, we analyzed the expression of TIM-3 and TIGIT on γδ T cell lines – and their ligands' expression on B cell lines from different origins. While TIGIT was readily detectable, TIM-3 expression could be shown on several, but not all, γδ T cell lines. TIM-3 could be blocked successfully. Preliminary data indicated an increase of the killing efficiency and of the expression of CD69 and IFN-γ, when αTIM-3 and mosunetuzumab were applicated together. In conclusion, the combination of γδ T cells and mosunetuzumab can be regarded as a promising therapeutic option for the treatment of hematological cancers. Possible impacts by immune checkpoint inhibitors are still under investigation.

Decoding the Functional Vδ1 T cell-Tumor Cell Interactome by Integrating Proximity Labeling and Genetic Screening

Presenter Name: Gabriel Marseres

Gabriel Marsères1, Zowi Huinen1, Adriaan de Groot1, Cristina Trueba Sanchez2, Pauline Bos1, Marta Rosseger1, Kelly Stecker2, Maarten Altelaar2, Daniel Peeper1.
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Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Vδ1+ γδ T cells have emerged as attractive targets for cancer immunotherapies owing to their unique properties. They are MHC-unrestricted allowing for allogeneic strategies, infiltrate solid tumors, and can bypass tumor resistance enabled by antigen loss. However, our understanding of how Vδ1 T cells recognize and kill tumor cells remains limited, precluding their efficient use for immunotherapy.

To decipher the Vδ1 T cell-tumor cell interactome, we used two complementary approaches. First, we developed an in vitro proximity labeling pipeline, by expressing the TurboID enzyme at the surface of tumor cells, to biotinylate Vδ1 T cell surface proteins present at the immune synapse with tumor cells. Second, to identify key regulators of tumor cell susceptibility to Vδ1+ γδ T attack, we performed genome-wide CRISPR knockout and activation screens under Vδ1 T cell pressure.

Mass spectrometry analysis of biotinylated Vδ1 T cell proteins revealed an interactome comprising adhesion molecules, stimulatory and inhibitory receptors, cytotoxic effectors, as well as surface ligands trogocytosed from tumor cells following interaction. This comprehensive map provides a valuable resource to investigate and target Vδ1 T cell receptors involved in tumor recognition.

Complementing these findings, in vitro CRISPR screens in two tumor cell lines uncovered multiple regulators of their interaction with Vδ1 T cells and subsequent killing. These hits were validated by single-gene knockout and/or activation. Notably, disruption of multiple components of the neddylation and ubiquitination pathways sensitized tumor cells to Vδ1 T cell-mediated killing. This sensitization was associated with an upregulation of NK receptor ligands and death receptors at the surface of tumor cells. Additionally, we identified several surface molecules acting as tumor immune checkpoints conferring resistance to Vδ1 T cells upon activation. Their knockout or blockade sensitized tumor cells to Vδ1 T cell killing across multiple in vitro models.

Together, proximity labeling and CRISPR screening provide a powerful platform to decipher Vδ1 T cell interactions with tumor cells. Our study notably identified several tumor-intrinsic regulators amenable to therapeutic intervention to enhance Vδ1 T cell-based immunotherapies.

CD39+ γδ IELs Restrain CD8αβ IEL Pro-inflammatory Potential Through Adenosinergic Signaling

Presenter Name: Irving Martinez Vargas

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Primary Theme: Tissues & Inflammation

Abstract

Loss of immunological tolerance, or the imbalance between pro-inflammatory and regulatory immune responses, is thought to contribute to the initiation of Crohn's disease (CD). Intraepithelial lymphocytes (IEL) are located within the epithelial barrier with different subsets exhibiting regulatory and/or cytotoxic properties. Cytotoxic CD8αβ IELs contribute to the development of spontaneous CD-like ileitis in TNFΔARE/+ mice, yet it remains unclear if crosstalk between IEL subsets functions to restrain CD8αβ IEL inflammatory potential. IELs expressing the γδ T cell receptor (γδ IEL) express CD39, an ectonucleotidase that converts extracellular adenosine triphosphate (eATP) into adenosine, which suppresses immune cell activation via adenosine 2A receptor (A2AR) signaling. We recently reported a 30% reduction in CD39+ γδ IELs across all Vγ subsets in 4-6-week-old TNFΔARE/+ mice, before the onset of ileal inflammation (p=0.001). Concomitant with the initiation of ileitis in TNFΔARE/+ mice at 8 weeks of age, we observed a 20% increase in the frequency of pro-inflammatory CD8αβ IELs (p=0.01) that was sustained throughout disease progression. Based on this, we hypothesized that adenosine generation by CD39+ γδ IELs restrains CD8αβ IEL activation. As expected, increased CD8αβ IEL proliferation and pro-inflammatory effector function coincide with the onset of ileitis in TNFΔARE/+ mice. Using in vitro suppression assays, we found that CD39+ γδ IELs suppress TNF production by CD8αβ IELs by 31% (p=0.03), with inhibition requiring CD39 enzymatic activity. Moreover, 70% of CD8αβ IELs express A2AR compared to 40% of CD8+ splenocytes suggesting that the local microenvironment may enhance CD8αβ IEL responsiveness to adenosine. In vitro treatment with the A2AR agonist, NECA, led to a 20% reduction in TNF+ CD8+ TCRαβ IELs (p=0.01) that could be reversed with an A2AR antagonist. Together, our findings show that CD39+ γδ IELs contribute to the generation of adenosine to suppress the pro-inflammatory potential of CD8αβ IELs via A2AR activation. We posit that the early loss of CD39+ γδ IELs and impaired adenosinergic signaling in CD8αβ IELs may drive the initiation of inflammation in CD-like ileitis.

Assessing the metabolic alignment between adaptive-like γδ and αβ T cells

Presenter Name: Eva McCabe

Eva McCabe, Taher E. Taher, Carrie R. Willcox and Benjamin E. Willcox

Primary Theme: Development & Differentiation

Abstract

Although human γδ T cells have historically been regarded as innate-like lymphocytes, recent clonotypic, phenotypic and transcriptional data indicate several subsets exhibit an adaptive-like biology broadly analogous to CD8 T cells. However, whether this alignment extends to the metabolic changes observed between naïve and antigen-experienced conventional αβ T cell subsets is unclear. Here we employed metabolic, mitochondrial and imaging approaches to probe the metabolic profile of naïve and effector Vδ1 and CD8 αβ T cells.

Firstly, we used the flow cytometric-based Single Cell ENergetIc metabolism by profiling Translation inHibition (SCENITH) assay to assess dependence of different adaptive subsets on distinct metabolic pathways at a single cell level. Vδ1 Tnaive and Teffector subsets appeared to broadly match CD8 Tnaive and CD8 Temra subsets, respectively. Tnaive subsets were enriched for amino acid and fatty acid oxidation, and exhibited high mitochondrial dependence, coupled with low glucose dependence. In contrast, both Vδ1 Teffector and CD8 Temra subsets were glycolytically focussed, with high glucose dependence, coupled with reduced mitochrondrial metabolism and lower amino acid/fatty acid oxidation. In alignment with this, GLUT1 was enriched on Vδ1 Teffector and CD8 Temra cells versus their naïve counterparts, and was further increased in expression upon TCR stimulation, suggesting an analogous role in glucose transport. Mitochondrial mass (assessed by Mitotracker) was increased similarly in both Vδ1 Teffector and CD8 Temra cells relative to Tnaive subsets, with high mitochondrial polarisation observed (via TMRE) in a subset of cells in both effector compartments. Current efforts include assessment of nutrient transporter expression across different subsets, and use of transmission electron microscopy to assess intracellular organelle morphology. Overall these results suggest that a common metabolic programme underpins adaptive-like Vδ1 and CD8 αβ T cell compartments. This involves Tnaive subsets dominated by mitochondrial oxidative phosphorylation, which upon clonal amplification and differentiation undergo metabolic reprogramming, resulting in glycolytically focussed antigen-experienced effector cells, with potential for futher metabolic changes upon subsequent antigenic stimulation.

Sphingolipids Differentially Regulate γδ17 and γδIFN Cell Proliferation and Effector Functions

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Primary Theme: Development & Differentiation

Secondary Theme: Cancer Therapies

Abstract

Cellular metabolism is essential for regulating immune cell activation and function. Less is known about gd T cell metabolic requirements, compared to adaptive CD4 and CD8 T cells. We recently found that different subsets of gd T cells have distinct metabolic needs, with lipid metabolism as a key feature of IL-17-producing (γδ17) compared to their IFNg-producing (γδIFN) counterparts. gd T cells hold significant promise for immunotherapy in cancer, however, they can be either anti-tumor (γδIFN) or pro-tumor (γδ17). We propose the differences in lipid metabolic requirements between γδ T cell subsets could be targeted to promote a pro-tumor and suppress an anti-tumor response. Using newly developed single cell lipidomics technology, we analyzed lipid species in activated γδ17 and γδIFN cells. This identified de novo sphingolipid synthesis as a key pathway in in both subsets, but the downstream fate of ceramide was a major distinction between γδ17 and γδIFN cells. Functional experiments targeting enzymes in the sphingolipid pathway, revealed that different sphingolipids are required for optimal proliferation in γδ17 compared with γδIFN cells. Further, targeting sphingolipid formation in gdIFN cells caused a reduction in cytotoxic marker expression and impaired their ability to kill tumor cells. This highlights the importance of sphingolipids for promoting γδ T cell anti-tumor function. Mechanistically, we found that sphingolipids are required for upregulating or maintaining cytokine receptor expression following activation. These results provide new insights into the lipid metabolic requirements of γδ T cells, with specific sphingolipids regulating the function of either γδ17 or γδIFN cells, which could be beneficial in targeting γδ T cells for cancer immunotherapy.

Deciphering γδ T cells in Spondyloarthropathies

Presenter Name: Anja Meyer

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

In humans, the presence of IL-23R+RORγt+ γδ T cells in the enthesis suggests their involvement in the local inflammatory processes driving AS. Vδ2+ T cells are present at the human enthesis and express RORγt and CCR6, demonstrating their Th17-like phenotype (γδ17 T cells). Importantly, Vδ2 but not Vδ1 γδ T cells, display a transcriptional profile (IL-23R, RORC, and CCR6), characterizing tissue residency of this cell subtype. In murine models driven by IL-23, a distinct group of RORγt+CD4−CD8−IL-23R+ entheseal-resident γδ T cells drive enthesitis and appear responsible for joint swelling and osteoproliferative signature, due to their production of IL-22 and IL-17. Furthermore, tissue-resident Vγ6, but not Vγ1 and Vγ4, γδ T cells, accumulate in the enthesis of various mouse joints. Tissue-resident RORγt+ γδ T cells from a parabiosis mouse model show open chromatin regions encoding for Syndecan-1 (SDC-1), a transmembrane heparan sulfate proteoglycan that also showed involvement in murine models of arthritis. Therefore, SDC-1 could also play a role in γδ T cells in SpA. Current murine models, like hydrodynamic injection of IL-23 minicircle DNA or transgenic overexpression of IL-23, have provided valuable insights into the mechanisms underlying γδ T cell-mediated inflammation in SpA. Nevertheless, these models are harmful, and novel models are needed.

We have established a novel IL-23-driven rAAV-induced murine model to tackle the much needed knowledge gaps. The IL-23-rAAV leads to an overexpression of IL-23 followed by arthritis induction starting as early as day 12 post rAAV injection. Further, the frequency of γδ T cells among the CD3 population in lymph nodes (pooled from axillary, brachial, and popliteal lymph nodes) is significantly increased after IL-23-rAAV injection.

Understanding the mechanisms by which γδ T cells contribute to AS pathogenesis is crucial for the development of targeted therapies. Efforts to modulate γδ T cell activity or block specific signaling pathways involved in their activation hold promise for alleviating disease symptoms and improving patient outcomes. Further research, both in human cohorts and animal models, is needed to fully unravel the complexity of γδ T cell involvement in SpA and to translate these findings into effective novel therapeutic strategies.

Characterization of Tumor-Infiltrating γδ TCR Repertoires in Human Solid Tumors Using a Low-Input, Sorting-Free Approach

Presenter Name: Joanna Mikulak

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Primary Theme: Ligands & Repertoires

Secondary Theme: Cancer Responses

Abstract

γδ T cells play a crucial role in tumor immunity, yet their tumor-infiltrating TCR-repertoire remains poorly characterized across different cancer types. Additionally, the relationship between γδ TCR repertoires in the blood and tumor compartments remains largely unexplored, limiting our understanding on γδ T cell trafficking, tumor-specific responses, and their therapeutic potential. A major challenge in studying the γδ TCR repertoire in human solid tumors is the low abundance of γδ T cells in the tumor microenvironment. Here, we describe a low-cell-input method that enables direct γδ TCR characterization from bulk tumor samples without the need for cell sorting or enrichment. This approach allows efficient analysis despite limited cellular material, overcomes technical barriers associated with γδ TCR amplification in tumors and provides a more comprehensive view of γδ T cell diversity across different malignancies, offering new insights into their role in cancer immunity.

Insights in the Molecular Mechanisms of Proximal γδ TCR Activation

Presenter Name: Susana Minguet

Marina Zintchenko, Caroline Schwenzel, Hannah Blumtritt, Gina Fiala, Anna-Maria Schaffer, Nadine Wossner and Susana Minguet

Primary Theme: Ligands & Repertoires

Secondary Theme:

Abstract

The broad range of antigens and binding topologies of the γδTCR suggests unique assembly and signal transduction properties. Both γδTCRs and αβTCRs associate with the signaling CD3 chains (CD3γ, CD3δ, CD3ε, and CD3ζ) to form a functional complex. In contrast to the αβTCRs, the γδTCRs constant domains are not tightly associated with the extracellular CD3 domains, allowing for increased flexibility. Some γδTCRs exhibit a monomeric (e.g. Vγ9Vδ2) assembly while and others display a dimeric assembly (e.g. Vγ5Vδ1). How γδTCR signaling is molecular initiated is unknow.

The most intuitive model to explain how the αβTCR is activated suggest that binding to the antigenic peptide presented by MHC is accompanied by the recruitment of the coreceptors CD4 or CD8 bound to the kinase Lck. Lck will, in turn, phosphorylate the CD3-ITAMs to initiate signaling cascades. However, this model has been recently challenged by animal models expressing Lck molecules that cannot bind to the CD4 or CD8 coreceptors, demonstrating that co-receptor-bound Lck seems to be dispensable for the initial phosphorylation of the αβTCR. This suggests the existence of additional mechanisms allowing Lck proximity to the TCR to initiate signaling. γδ T cells lack CD4 expression and only a minor population expresses CD8. We hypothesize that there is a common mechanism to phosphorylate the γδTCRs and αβTCRs independently of CD4 or CD8.

We have discovered a novel motif in the CD3ε cytoplasmic tail (RK motif) that directly binds to the SH3 domain of Lck prior to any receptor phosphorylation to initiate signaling (Hartl et al., 2020). Now, we investigate whether this mechanism might explain how proximal γδTCR signaling is initiated. To this end, we have expressed three model γδTCRs (Vγ3Vδ1, Vγ4Vδ5 and Vγ9Vδ2) in Jurkat reporter cell lines as wild type receptors, or loss of function mutants unable to recruit LCK to CD3ε. Our results show that γδTCRs unable to recruit Lck exhibit reduced signaling (calcium influx, NFAT activation and expression of activation markers), when stimulated with their natural ligands. Furthermore, we have generated a knock-in mouse model by mutating the RK motif in the CD3ε cytoplasmic tail (RKAA mice). Our preliminary results show equal numbers of γδT cells in the thymus, spleen and lymph nodes of the homozygous RKAA mice when compared to wild type littermates. In lymph nodes, CD24-CD44+CD45RB- cells primed to produce IL17, in which Lck is not expressed, were enriched in the homozygous RKAA mice. In contrast, IFNγ-producing γδT cells (CD24-CD44+/-CD45RB+, expressing Lck) were unchanged. These data were confirmed by in vitro stimulations with phorbol 12-myristate 13-acetate (PMA) and ionomycin. scRNA analysis are running to deeply characterize γδTCR repertoire and developmental trajectories.

ACTallo: An engineered γδ T Cell therapy platform for adoptive cell therapies

Presenter Name: Nina Moeker

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Adoptive cell therapies such as chimeric antigen receptor (CAR) based or T cell receptor (TCR) based therapies are promising anti-cancer approaches. Autologous CAR-T therapies have shown remarkable success against hematological malignancies while autologous TCR-T therapies such as ACTengine IMA203 targeting Preferentially Expressed Antigen in Melanoma (PRAME) have demonstrated positive responses in heavily pretreated patients with solid tumors in clinical trials. As a next generation strategy to supply cell therapy products to patients without the requirement for personalized manufacturing by using healthy donor derived material, we present the development of an allogeneic, off-the-shelf adoptive cell therapy platform, ACTallo.

ACTallo is based on ex-vivo, feeder-free expanded, multiplex-engineered γ9δ2 T cells. gδ T cells possess several desirable characteristics for an allogeneic product including their HLA independence, thus avoiding graft-vs-host disease, intrinsic anti-tumor functionality, and correlation with favorable disease prognosis. Through our systematically developed manufacturing process, including highly efficient CRISPR/Cas mediated genome editing, we can introduce several kilobases of cargo simultaneously that could confer 1) targeting capabilities through a TCR, 2) cloaking to avoid acute graft rejection and, 3) armoring to counteract the suppressive tumor microenvironment, and support γδ T cell proliferation and persistence.

As a proof-of-concept for our platform, we have developed a multi-edited product candidate targeting a proprietary peptide target and incorporating cloaking approaches to avoid host vs graft responses. The product candidate shows specific cytotoxic activity against target-positive tumor cell lines with low levels of target peptide expression in vitro, indicative of its strong potency. In vitro experiments also show reduced rejection of the engineered cells by HLA-mismatched T cells, and NK cells providing validation for the cloaking strategy. In vivo, a single dose of the engineered γδ T cells induced complete remission in a cell line derived xenograft tumor mouse model and the T cells persisted for several weeks. These data, together with the versatility of the engineering strategy, demonstrate the potential of ACTallo as a promising platform for TCR-T cell therapies with the option for future exploration in CAR-T cell therapies.

Regulation of Gut γδ T Cell Activation by Innate and Adaptive TCR Engagement

Presenter Name: Leticia Monin Aldama

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Primary Theme: Tissues & Inflammation

Secondary Theme: Ligands & Repertoires

Abstract

Barrier sites are constantly challenged by microbial and environmental threats. Central to their defence and repair are intraepithelial γδ T cells. Mouse and human gut intraepithelial lymphocytes (IEL) predominantly express TCR chains Vγ7 and Vγ4, respectively, reflecting their selection by Butyrophilin-like (Btnl) proteins expressed by healthy enterocytes or colonocytes. Btnls engage germline-encoded TCR-Vγ-chain residues, eliciting nonclonal, innate-like responses that contrast with powerful adaptive responses triggered by stress-induced antigens, such as the MHC-I-like protein T22. What underpins this difference? Here, we investigate how the γδ TCR signals distinct responses to innate and adaptive ligands, appropriate to intestinal physiology.

Compared to α-CD3 stimulation, Btnl1+6 engagement induced TCR-proximal signalling but did not initiate Akt and mTOR phosphorylation, and showed significantly reduced protein translation and cell proliferation. To directly compare the recognition of innate and adaptive ligands, we next engineered retrogenic mice expressing a Vγ7 γδ TCR that recognises both Btnl1+6 and T22. These retrogenic γδ T cells closely mirrored naturally occurring IEL in tissue-tropism and immunophenotype.

Using this model, we dissected the signalling pathways, activation dynamics, and transcriptional changes associated with Btnl and T22-mediated activation. We found that Btnl and T22 induced common signalling pathways, with the responses to both muted relative to anti-CD3. Nonetheless, T22 engagement increased co-stimulatory receptor expression facilitating responses in contexts in which γδ T cells would see stress-antigens. Through studies with mutant mice, we identified FceRIγ and PI3K as major mediators of IEL signalling. Of note, however, mice expressing constitutively active PI3K did not show increased responsiveness to Btnl1+6, suggesting that gut γδ IEL may be repressed by inositol phosphatases. Indeed, inhibitor studies targeting protein and lipid phosphatases indicated that SHIP-2 plays a critical role in limiting IEL activation.

In conclusion, our findings demonstrate that IEL can engage their TCR in distinct ways, leading to qualitatively and quantitatively different outcomes. This intricate signaling network is tightly regulated by lipid phosphatases, providing insights into gut γδ T cell biology and implications for therapeutic interventions.

Acute, Not Chronic, Exposure to IL-15 Leads to Optimal Anti-tumor Function of Gamma Delta T cell Products Through the Jak/STAT/c-Myc Signaling Axis

Presenter Name: Allyson Moore

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Gamma 9 delta 2 T cells (G9D2 T cells) are an attractive substrate for cancer cell therapies, but clinical results have been modest. Engineering G9D2 T cells with tumor-specific synthetic antigen receptors can enhance their therapeutic potential, but better understanding of G9D2 T cell biology is still required to maximize efficacy. High proliferative capacity upon repeated stimulation is an important feature of alpha-beta T cell-based therapies. There is a paucity of data on the proliferative capacity of G9D2 T cells post-manufacturing. Therefore, we explored the requirements for proliferation of engineered G9D2 T cells in the context of glioblastoma and multiple myeloma using multiple synthetic antigen receptor designs, targets, and nutrient conditions. The net result of these studies revealed that target antigen, antigen binding domain and tumor class all influence the proliferative response. The addition of rhIL-15 at the time of antigen stimulation mitigated this variability and led to robust proliferation, regardless of antigen, tumor target or nutrient conditions. IL-15 rendered the T cells more metabolically flexible and drove upregulation of c-myc and E2F transcriptional pathways to push the cells across the G1/S barrier. This effect of IL-15 was wholly dependent upon Jak/STAT signaling and independent of Akt signaling. The importance of having IL-15 present at time of G9D2 T cell:tumour engagement was most apparent upon recursive stimulation. G9D2 T cells were unable to proliferate following a second round of tumor cell stimulus unless IL-15 was present at the time of secondary exposure to antigen despite a robust antigen-dependent activation signal. It was also observed that G9D2 T cells lost their ability to effectively adhere to tumour cells after one round of tumour engagement in the absence of IL-15. Introduction of various IL-15 transgene configurations into the G9D2 T cells increased their ability to survive in the absence of exogenous cytokine support but also resulted in impaired proliferative capacity, possibly due to STAT5-mediated exhaustion resulting from chronic exposure to IL-15. Further investigation revealed that optimal proliferation required acute exposure to IL-15 at the time of tumor contact. We are presently working on engineering strategies to enable transient and robust STAT5 signaling to maximize the anti-tumor potential of G9D2 T cells. This work was supported by the Samuel Foundation, ORF, Mitacs and the LLSC.

Multiomic and Metabolic Profiling of Human Tissue-Associated γδ T Cell Repertoires

Presenter Name: Daniela Moreno Vicencio

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Primary Theme: Tissues & Inflammation

Secondary Theme: Ligands & Repertoires

Abstract

Mouse models have been pivotal in studying γδ T cell biology, particularly regarding their tissue-restricted T cell receptor (TCR) repertoire selection during development and their pre-programmed effector functions. However, translating these findings to human γδ T cells remains challenging due to the absence of direct murine counterparts and the lack of clear tissue-associated TCR repertoire restrictions in humans. The most studied human γδ T cell subset, Vδ2+Vγ9+, is mainly found in blood, and rapidly respond to PAg via BTN2A1/3A1, mediating their effector responses. In contrast, Vδ1+ T cells, though present in blood at low levels, are enriched in solid tissues and have been shown to undergo clonal expansion with a transition from a naïve to effector-like phenotype. However, how these γδ T cell subsets organise in human tissues remains poorly understood.

Here, we aimed to comprehensively characterize γδ T cell subsets across multiple human tissues, including the spleen, bone marrow, gut, and lymph nodes, and compare them to the blood. We performed high-parameter immunophenotyping, metabolic spectral flow cytometry and single-cell multiomic transcriptome and linked-TCR sequencing. We successfully mapped Vδ1+, Vδ2+, and Vδ3+ γδ T cells at the single-cell level. Our findings reveal that tissue-associated Vδ1+ T cells exhibit distinct transcriptional and functional profiles compared to their circulating counterparts, highlighting tissue-specific organ-dependent adaptation and potential adaptive-like homing properties. Moreover, Vδ2+Vγ9+ T cells also exhibit a degree of clonal expansion in the tissues. Furthermore, metabolic profiling uncovered distinct metabolic pathways usage among the distinct γδ T cell subsets, correlating with their innate-like or adaptive-like immune functions, further refining our understanding of γδ T cell specialisation in human tissues.

These findings add to our understanding of human γδ T cell's biology, uncovering novel tissue-associated properties and functional diversity. This work lays a foundation for advancing our understanding of tissue-specific immunity.

Live Listeria Vaccine Stimulates Long-lived Immunity by Vγ2Vδ2 T Cells

Presenter Name: Craig Morita

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Primary Theme: Infection Responses

Secondary Theme: Cancer Therapies

Abstract

Human γδ T cells expressing Vγ2Vδ2 TCRs monitor foreign- and self-isoprenoid metabolites to mediate immunity to microbes and tumors. Direct immunization of cancer patients with either bisphosphonates or prenyl pyrophosphates, such as bromohydrin pyrophosphate, and IL-2, expands Vγ2Vδ2 cells but they rapidly contract, terminally differentiate, and lose responsiveness. Monkeys immunized with zoledronic acid also lose responsiveness. To overcome these problems, we sought to develop a live bacterial vaccine for Vγ2Vδ2 cells that would mimic a natural microbial infection. Listeria monocytogenes (Lm) were attenuated by actA deletion but had a mutant PrfA protein (G155S) that slightly increased their virulence. Immunization of 2 rhesus macaques with these attenuated Lm expanded Vγ2Vδ2 cells to high levels (60% and 30% of CD3 T cells) after the 2nd dose. A 4th immunization given 2.4 year later similarly expanded Vγ2Vδ2 cells (82% and 60%) as did a 5th immunization given after 6 years (59% and 42%). Between immunizations, Vγ2Vδ2 cells decreased to baseline levels (<5%). No apparent toxicity was noted with the monkeys exhibiting normal blood counts and chemistries without elevations in liver transaminases. Thus, Vγ2Vδ2 T cell-responsiveness was maintained through five immunizations over a 6 year period. Vγ2Vδ2 cells retained central memory phenotypes, expressed inflammatory chemokine receptors, and produced IFN-γ and TNF-α without induction of anergy. Expression of mutant PrfA (G155S) was critical because immunization with ΔactA Lm with wild-type PrfA resulted in minimal Vγ2Vδ2 T cell expansions. Similarly, despite high HMBPP levels, immunization with ΔlytB ΔactA prfAwt Lm also resulted in minimal expansions. Thus, Lm stimulation of Vγ2Vδ2 T cells is dependent on their relative virulence rather than their HMBPP levels. Unlike zoledronic acid or HMBPP, immunization with ΔactA prfAG155S Lm induces large expansions of Vγ2Vδ2 T cells that retain central memory phenotypes and responsiveness after multiple immunizations over at least 6 years. This vaccine could be used to augment immunity to a variety of microbial pathogens that produce HMBPP or to expand Vγ2Vδ2 T cells prior to cancer immunotherapy with the anti-BTN3 mAb, ICT01, or with bispecific antibodies that have Vγ2Vδ2-specific T cell engagers.

Adoptive cell therapy using human skin resident gamma delta T cells for the treatment of cutaneous squamous cell carcinoma

Presenter Name: Giorgia Nasi

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Gamma delta (γδ) T cells play important roles in the surveillance of cellular stress, tumors, and infection, that help maintain tissue integrity and modulate adaptive responses to these stimuli. γδ T cells can recognize malignant cells via surface molecules and display killing activity upon activation. γδ T cells respond to a variety of solid and hematological tumors in vitro and in in vivo xenograft models, and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. In clinical trials, adoptive transfer of ex vivo expanded γδ T cells lead to temporary tumor regression and increased survival of leukemia patients. Hence, γδ T cells are promising candidates for anti-tumor immune therapeutic approaches. Due to technical difficulties to isolate enough γδ T cells from human skin, most studies on cutaneous γδ T cell biology were focused on murine skin resident γδ T cells. Here we are using novel methodologies to expand functional cutaneous γδ T cells ex vivo. Upon adoptive transfer of these cells into mice that have received skin tumors, we can study their migration, maintenance, and phenotypic adaptation in vivo. Specifically, we have established a cutaneous squamous cell carcinoma (SCC) xenograft mouse model in which the grafted SCC tissue resembles tumors of patients macroscopically and microscopically. In this model, γδ T cells engrafted and are maintained in the tumor tissue. Crucially, these cells displayed an activated and cytotoxic phenotype after isolation from the tumor mass. This model enables in depth and mechanistic studies of the biology of cutaneous γδ T cells in the tumor microenvironment. Additionally, the in vivo tumor model can be utilized to study the therapeutic potential of γδ T cells in cutaneous carcinomas, paving the way to novel anti-tumor treatments.

The impact of ER stress on γδ T cells in the tumor microenvironment

Presenter Name: Giorgia Nasi

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Primary Theme: Cancer Responses

Secondary Theme: Tissues & Inflammation

Abstract

Gamma delta T cells (γδ T cells) are unconventional T lymphocytes with a role in the immune surveillance of cellular stress. γδ T cells respond to a wide range of tumors and tumor infiltrating γδ are the most significant favorable prognostic immune population among 39 cancer types. However, a suppressive tumor microenvironment (TME) can impair γδ function, and the mechanisms are not fully understood. Thus, we aim to elucidate cellular communication between tumor cells and tissue resident γδ T cells to provide a basis for the development of effective anti-tumor therapies. The TME is often characterized by increased endoplasmic reticulum (ER) stress, a cellular response to an accumulation of un-/misfolded proteins in the ER lumen that can be promoted by several environmental insults (e.g. hypoxia, glucose deprivation). To restore cell homeostasis, the ER activates pathways collectively known as unfolded protein response (UPR), and an enhanced UPR correlates with poor clinical outcomes in cancer patients. Crucially, the UPR can support tumor growth by regulating immune cell function. We hypothesize that ER stress is a mechanism used by tumor cells to dysregulate the activation and function of tumor-infiltrating cutaneous γδ T cells. To this end we induced ER stress pharmacologically in primary human cutaneous squamous cell carcinoma cell lines using thapsigargin and treated cutaneous γδ T cells with the conditioned medium of these cells. This induced the upregulation of activation and cytotoxic markers and concomitantly reduced the expression of NK cell receptors (NKRs) and the production of pro-inflammatory cytokines by γδ T cells. These findings support the hypothesis that cutaneous γδ T cells respond to ER stress within the TME and this modulates their activation and function. Our findings will provide important new insights into the immune surveillance and anti-tumor function of cutaneous γδ T cells under ER stress and help optimize their future clinical applications for cancer therapies.

γδ T cell and macrophage interplay within the ER stress tumor microenvironment

Presenter Name: Giorgia Nasi

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Primary Theme: Cancer Responses

Secondary Theme: Tissues & Inflammation

Abstract

γδ T cells play a dual role in tumor immunity, with both anti-tumor and pro-tumorigenic functions, depending on how they are influenced by the tumor microenvironment. The mechanisms behind this functional contradiction are not fully understood. Endoplasmic reticulum (ER) stress, caused by the accumulation of misfolded proteins, is known to promote tumor growth and alter immune cell functions within the tumor microenvironment. When γδ T cells were exposed to conditioned media from ER-stressed cutaneous squamous cell carcinoma (cSCC) cells, they upregulated genes involved in monocyte recruitment and macrophage differentiation. Additionally, these γδ T cells released higher levels of IL-4 and IL-10, cytokines known to induce M2 macrophage polarization, a key characteristic of tumor-associated macrophages (TAMs) that support tumor growth and angiogenesis. Given that ER stress is a common feature of the tumor microenvironment, we hypothesize that ER stress alters γδ T cell function, which in turn promotes the differentiation of macrophages toward a pro-tumorigenic M2 phenotype. No studies have yet explored the interaction between γδ T cells and macrophages in the ER stress tumor microenvironment. This study aims to investigate how ER stress-conditioned γδ T cells influence macrophage differentiation and polarization toward either the M1 or M2 phenotype. The findings will provide new insights into how γδ T cells regulate macrophage anti-tumor responses under ER stress, potentially revealing new targets for cancer immunotherapy.

The Impact of Disrupting the BTNL- γδ T cell Axis, with implications for Crohn's Disease

Presenter Name: Ambra Natalini

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

The development of mouse and human intestinal TCRγδ+ intraepithelial lymphocytes depends on organ-specific butyrophilin-like (BTNL) molecules expressed by mature healthy epithelial cells. Interestingly, genetic variants of BTNL have been associated with Crohn's disease (CD) severity and post-COVID childhood MIS-C. In this context we hypothesise that common, idiopathic cases of such diseases reflect an underlying disruption of the BTNL-γδ+ IEL axis, thus impairing barrier integrity. To test this, we now define the impact of acute and constitutive BTNL deletion on healthy mouse and human gut tissues, including γδ IEL, and then ask whether such signatures are evident in CD patients. To this end, we compare the scRNASeq phenotypes of healthy colon, including γδ IEL, from donors with or without variant alleles of BTNL3+8, cross-referencing to the impacts of constitutive and acute BTNL deletion in mice, as measured by transcriptomics and flow cytometry. The aggregate results are then compared with publicly available scRNAseq datasets of CD patients.

The constitutive deletion of mouse and human BTNLs leads to reduced Vγ7+/Vγ4+ cells with atypical phenotypes phenocopying immature cells in mice (CD122lo, Thy1+, TIGIT−, Lag3−, CD8αα−, CD5+, CD24+), with likewise significant increases of CD5+ expression on Vγ4+ cells in humans: of note, this phenocopies that of Vγ4+ cells in inflamed IBD tissues. Acute Btnl1 loss in mice did not immediately deplete Vγ7+ cell numbers but did rapidly alter the cells' phenotype and transcriptomes, with acquisition of a gene signature reported for disrupted immunosurveillance in the skin, namely downregulated Tnfrsf9, Xcl1, Ptpn6), upregulated Klrk1 (encodes NKG2D), and decreased expression of CD69 and TIGIT, which is likewise decreased by IBD-associated cytokines. Concurrent transcriptomic changes in epithelial, stromal and other immune compartments after BTNL deletion will be also presented.

These results are important to establish whether or not disruption of the BTNL-γδ+ IEL axis alone is sufficient to induce early biomarkers of gut inflammation, consistent with BTNL-deficiency pre-disposing toward penetrating CD. Such a result should offer actionable targets en route to new therapies.

Development and Preclinical Validation of a γδ TCR-Engineered Cell Product for Pan-Cancer Targeting

Presenter Name: Hayley Nault

Hayley Nault, Princess Margaret Cancer Centre, University Health Network & Department of Immunology, University of Toronto
Scott Lien, Princess Margaret Cancer Centre, University Health Network & Department of Immunology, University of Toronto
Pamela Ohashi, Princess Margaret Cancer Centre, University Health Network & Department of Immunology, University of Toronto

Primary Theme: Cancer Therapies

Secondary Theme: Ligands & Repertoires

Abstract

Engineered adoptive cell therapies such as CAR-T and TCR-T cells have revolutionized cancer treatment. However, significant challenges remain, including the lack of suitable and targetable tumour antigens, antigen escape, and limited patient accessibility. γδ TCR-engineered T cells offer the potential to overcome these barriers due to their ability to recognize upregulated stress ligands in a major histocompatibility complex (MHC)-independent manner. Recently, our lab has characterized T cells expressing a clonal γδ TCR that were significantly expanded in a Merkel cell carcinoma (MCC) patient who demonstrated a complete response to anti-PD-1 therapy. When cloned and expressed in Jurkats, this γδ TCR demonstrated significant reactivity to a wide variety of cancer cell lines including MCC, lung, melanoma, breast and myeloma, but not to healthy cells. Transwell assays demonstrated that this activity is dependent on cell-cell contact. Furthermore, CRISPR knockout experiments confirmed its reactivity is MHC-independent and does not recognize any currently known γδ TCR ligands, thus positioning it as a potentially novel, pan-cancer therapy.

To advance this γδ TCR towards clinical application, we have optimized co-culture conditions with human lung tumour organoid models, demonstrating the specific upregulation of activation markers such as CD69 when Jurkats are cultured with these patient-derived cells. Additionally, we have refined lentiviral transduction protocols to efficiently introduce this γδ TCR into primary T cells, thus enabling significant, specific killing against lung and MCC cell lines at low effector to target ratios. To further support clinical translation, strategies to identify its ligand are currently ongoing, including the use of whole-genome CRISPR screens in target cell lines to identify genes and pathways essential for killing. This HLA-independent γδ TCR represents an innovative strategy for treating a more diverse patient population and overcoming current challenges of adoptive cell therapy.

Drug Screening Identifies Dasatinib as a Src/Abl Inhibitor of IL-23 Signaling, IL-17 Production, and Imiquimod-Induced Skin Inflammation

Presenter Name: Maria N. Navarro

Valle-Pastor MJ, Cayuela I, Santiago J, Pastor-Fernández G, Oliva A, Traba J and Navarro MN.

Primary Theme: Ligands & Repertoires

Secondary Theme: Tissues & Inflammation

Abstract

IL-23 signalling plays a pivotal role in the production of IL-17 by γδ17 and Th17 T cells, which are critical drivers of inflammatory and autoimmune diseases. Pharmacological inhibition of IL-23-induced IL-17 production may offer therapeutic potential for IL-23-mediated diseases such as psoriasis. We developed long-term IL-7 cultures of γδ17 T cells (γδ17 T-LTICs) that retained hallmark features of γδ17 T cells, to perform a drug screening of 88 FDA-approved compounds. This screening identified Src/Abl inhibitors Dasatinib and Bosutinib as potent inhibitors of IL-23-induced IL-17 production. Their efficacy was further validated in vitro and in a psoriasis-like skin inflammation model using the Imiquimod (IMQ) mouse model, where intraperitoneal and topical administration of Dasatinib reduced IL-17 production, leukocyte infiltration, and epidermal thickness. Mechanistically, we found that Dasatinib and Bosutinib selectively inhibited IL-23-induced mTORC1 activation in γδ17 T cells without affecting STAT3 phosphorylation. Functional studies using shRNAs targeting the Src kinase Blk demonstrated its essential role in IL-23-induced IL-17 production and mTORC1 activation. Our findings highlight Dasatinib and Bosutinib as promising therapeutic candidates for IL-23-mediated diseases. These results underscore the potential of targeting Src family kinases, particularly Blk, to modulate type 3 immune responses in inflammatory diseases.

Single-cell transcriptomics identifies merocytic dendritic cells (McDc) as potential modulator of γδ T-cell activity in colorectal cancer tumor microenvironment

Presenter Name: Jeremy Wee Kiat Ng

Jeremy Wee Kiat, Ng, Singapore General Hospital

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Primary Theme: Tissues & Inflammation

Secondary Theme: Cancer Responses

Abstract

Gamma-delta T-cells (γδT cells) have been associated with improved survival outcomes in various solid malignancies, including colorectal cancer (CRC). Studies have identified key effector types in the CRC tumor microenvironment (TME). However, little is known about the CRC TME determinants of γδT cell infiltration. To address this gap, we performed an in-silico analysis of publicly available single-cell RNA-seq (scRNA-seq) dataset from CRC patients. 10 published scRNA-seq datasets from 169 patients were curated for integration and analysis. Integration of datasets was performed using RPCA method. Following integration, cells were annotated using scGate. γδT cells were identified among T/NK cells using a gene module score comprising of TRGC1, TRDC and CD3 quadruple complex (CD3D/E/G/Z). Cell-cell interaction was identified using CellChat. The Wilcoxon test with a p-value cutoff of 0.05 was used to determine statistical significance of continuous variables. 489,858 cells were retained for downstream analysis following adaptive quality control to remove poor quality cells. Across all datasets, 21,928 γδT cells (4.48% of cells) were identified in patients with frequencies ranging from 0.2488% to 17.79% (3.96 □2.88%). Composition analysis of patients with □5% of γδT -cells (γδThigh) revealed significant depletion of plasma cells in the TME (4.82□5.12% vs. 9.04□10.5%, p-value = 0.025). In keeping with the reported anti-tumoral function of γδT cells, cell-cell interaction analysis showed robust interactions between γδT cells and epithelial cells (77 samples). Interestingly, we also observed robust γδT cell- merocytic dendritic cells (McDc) interactions (63 and 35 samples as source and receiver respectively). The most frequent ligand-receptor interactions between McDc and γδT cell were was the immunosuppressive MIF-CD74 signaling axis. In conclusion, applying cell-cell interaction analysis applied across a large cohort of 169 patients revealed potential interactions between γδT cells and McDc in the CRC. The robustness of γδT cell-McDc interactions involving the immunosuppressive MIF signaling pathway suggests a potential role of McDc in regulating γδT cell activity in the CRC TME. Further work is ongoing to dissect how this interaction can shape the γδ T-cell effector subtypes. Additionally, we aim to validate these findings from high-resolution spatial transcriptomics.

Inflammatory Gamma Delta T Cell Responses are a Hallmark of Unstable Atherosclerosis

Presenter Name: Jonathan Noonan

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Primary Theme: Tissues & Inflammation

Secondary Theme: Ligands & Repertoires

Abstract

Atherosclerosis is a chronic inflammatory disease characterised by the formation of lipid-laden plaques within arteries. The rupture of these plaques leads to myocardial infarctions and ischaemic strokes, which are leading causes of death globally. While recognised as an inflammatory pathology, the immune pathways driving plaque destabilization and rupture remain poorly understood, limiting therapeutic advancement. To date, few studies have assessed gamma delta (gd) T cells in atherosclerosis, and there is a near-complete absence of data from human disease. To address this, we have performed integrated flow cytometry, single cell RNA-sequencing (scRNA-seq) and histological assessments of gdT cells in both murine and human atherosclerosis.

Firstly, we identified that gdT cells are a key source of detrimental pro-inflammatory cytokines in atherosclerotic vessels, including IL-17A and TNF, and are significantly enriched within unstable versus stable plaques. Differential gene expression analysis of scRNA-seq data demonstrated overlapping transcriptional signatures between mouse and human plaque gdT cells, including strong evidence of conserved activation, oxidative stress, altered metabolism, reduced survival signalling, and cytotoxicity. T cell receptor (TCR) usage in both mouse and human gdT cells was biased. In mice, most gdT cells expressed an invariant Vg6Vd1 receptor, or Vg4. In humans, Vd1 TCRs dominated above all others. gdT cells in both mice and humans also displayed evidence of tissue residency, including heightened expression of genes such as CD69, BHLHE40, CXCR6, and VIM. Critically, genetic knockout of gdT cells significantly blunted unstable plaque development in mice, proving they directly contribute to disease progression.

In summary, investigations of gdT cell immunology in atherosclerosis, a major driver of global mortality, are limited. We now provide evidence that gdT cells are highly activated, pro-inflammatory and cytotoxic in the atherosclerotic plaques of both mice and humans. Most critically, using a unique mouse model, we identify that they contribute directly to the unstable plaque development which ultimately underpins most myocardial infarctions and ischaemic

strokes. These observations introduce gdT cells as a novel and previously unrecognized hallmark of atherosclerotic disease, highlighting new avenues for tailored interventions in the future.

Characterization of Tumor-Infiltrating γδ T Cells in a Novel AVATAR-Model for Personalized Immunotherapy

Presenter Name: Hans-Heinrich Oberg

Hans-Heinrich Oberg, Institute of Immunology, University Medical Center Kiel, Germany; Matthias Peipp, Division of Antibody-based Immunotherapy, University Medical Center Kiel & Christian-Albrechts University to Kiel, Germany; Dirk Bauerschlag, Clinic and Polyclinic for Gynaecology and Reproductive Medicine, University Medical Center Jena, Germany; Daniela Wesch, Institute of Immunology, University Medical Center Kiel, Germany

Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

An infiltration of different γδ T-cell subsets and correlations with patient outcome have been demonstrated in epithelial and solid tumors, including pancreatic and ovarian cancer. Recently, we described an abundance of Vδ1 and Vδ3 tumor-infiltrating γδ T cells (γδ TIL) in pancreatic and ovarian tumors, while Vγ9Vδ2-expressing γδ TIL are reduced but still present.

To improve γδ T-cell-based immunotherapy, we designed γδ T Cell Engagers (γδ TCE) such as bispecific antibodies targeting different γδ T-cell subsets to defined tumor-associated antigens, and we developed a novel predictive patient-derived AVATAR-Model to better understand the functional properties of γδ T-cell subsets at the tumor site. Our AVATAR-Model decipher cytotoxicity and proliferation of γδ TIL subsets by selectively activating them by γδ TCE within the complete human freshly isolated tumor tissue, including an immunosuppressive tumor-microenvironment (TME), and without separation and labeling of cells.

Our results revealed that the cytotoxic activity of γδ TIL subsets against ovarian tumor cells of 16 tested patients depends on the stage of disease, the tumor burden and the presence of regulatory T cells. Interestingly, the γδ TIL cytotoxicity is independent of the differential upregulation of check-point molecules on the γδ TIL subsets and their absolute cell number. The latter is obvious since the assay depicts the influence of a physiological effector/target ratio. The assay reflects better the individual situation in the human tumor tissue than separated co-cultured cell populations with an experimental adapted effector/target ratio did.

In conclusion, the novel preclinical AVATAR-Model allows a cost-effective, short-time and personalized measurement of the capacity of patient's γδ TIL to lyse tumor cells, and thereby predicts information about a possible cancer treatment in just a few days. Moreover, the method can replenish mouse models based on its benefit to depict a complete human autologous model and the possibility to analyze a lot of different tumor enities and a high tumor diversity.

Modulation of Gamma Delta T-cell Phenotype and Effector Potential Using Biomaterials

Presenter Name: Favour Obuseh

Favour Omafuvwe Obuseh, Harvard-MIT Health Science and Technology, Michelle Chang, Harvard School of Engineering and Applied Science, David J Mooney, Harvard School of Engineering and Applied Science

Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

The variable success of γδ T cell-based therapies in vivo and in clinical trials highlights the need to understand how mechanical forces influence their cytotoxic function, as their activation and effector responses may be altered when transitioning from viscous blood to tissues with varying mechanical properties. We hypothesized that a 3D collagen biomaterial system, designed to mimic in vivo mechanical properties, would modulate γδ T cell behavior by providing physiologically relevant mechanical stimulus. In this study, we activated γδ T cells and then encapsulated them in a collagen biomaterial system, then we compared their outcomes to γδ T cells which were cultured in 2D plate for the same duration. We also explored how properties of tissues such as viscoelasticity and stiffness affected the outcome of the encapsulated cells. Flow cytometry, cytokine production, and tumor cytotoxicity were examined for cells encapsulated in the 3D hydrogel system vs non encapsulated (2D culture). Our results showed that 3D encapsulation and culture of γδ T cells after activation, led to a decrease in markers associated with traditional terminal differentiation of T-cells (CD27 and CD45RA), and increased expression of markers associated with activation, tumor cytotoxicity, and tissue persistence (NKG2D, FAS, and PD1) for both Vδ1 and Vδ2 γδ T cells. Cytokine profile assays showed increased production of IL5, GMCSF, IL13, CCL3, CCL5, and IFNY in 3D culture conditions. Cytotoxicity studies showed an increase in killing by the encapsulated cells in the context of K562 cells and improved killing against HCT116 cells. When exploring stiffness and viscoelasticity, we saw that more elastic gel conditions led to an increase in expression of markers associated with activation and cytotoxicity, and that increased stiffness also had a similar effect. Our findings reveal new insights into how γδ T cells integrate mechanical signals to fine-tune immune responses by modulating their phenotype and activation within 3D gels, demonstrating that the mechanical properties of 3D environments have significant implications for γδ T cell-based cancer immunotherapy

Development of Epidermal γδ-T-Cells and Langerhans Cells

Presenter Name: David Obwegs

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Epithelial differentiation and barrier formation are essential steps to shield the organism from the environment upon birth. Therefore, epithelial barriers need to undergo a rapid differentiation process influenced by perinatal factors such as microbiota, which the fetus is spontaneously exposed to after birth. Epithelial barriers, including epidermis and oral mucosa, harbor specialized subsets of resident immune cells: the Langerhans cells (LC) and dendritic epidermal T cells (DETC). LC constitute a distinct immune cell type with features of macrophages and dendritic cells while DETC are specialized γδ T cells only found in the murine epithelial tissues. Both LC and DETC contribute to the immune defense and tissue homeostasis of epithelial barriers throughout life. In the mouse, resident DETC and LC colonize the epidermis and oral mucosa during embryonic development followed by their rapid differentiation in the early postnatal phase. Hence, this perinatal phase represents a crucial time window where multiple factors can influence DETC and LC differentiation, thereby contributing to the establishment of a healthy epithelial barrier. To date, the molecular crosstalk between DETC and LC that supports the idea of their dependence on each other for their mutual differentiation remains elusive. Furthermore, the impact of the sudden exposure to a changing environment, such as colonization with microbiota, on this dynamic process is not well understood. In this project we unravel how DETC and LC differentiate after reaching the epidermis and how external factors, such as microbial colonization, shape this process. Specifically, we aim to characterize the differentiation dynamics of DETC and LC within the epidermis, to understand the role of interactions between DETC and LC in driving their mutual differentiation and to decipher the role of microbial colonization during DETC and LC differentiation and adaptation.

Human Breast Milk γδ T cells Exhibit Unique Characteristics Compared to Blood Circulating Counterparts

Presenter Name: Maria Papadopoulou

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Breastfeeding offers significant immunological benefits to both mother and child. Breast milk contains a range of immunoactive components, including a relatively high proportion of γδ T cells. γδ T cells are unconventional T cells that express a γ and a δ chain in their TCR and are not dependent on MHC for antigen recognition. The contribution of γδ T cells to lactation immunity is not known. Here, we extensively studied the γδ T cell populations in a cohort consisting of paired blood and milk samples at different lactation time points, including early milk (colostrum) and mature milk (a few weeks postpartum). We phenotyped γδ T cells using flow cytometry and performed single-cell sequencing (RNA-seq, TCR-seq, and CITE-seq). Compared to their blood counterparts, milk γδ T cells showed elevated levels of tissue-residency markers, showed differences at the TCR level (e.g. higher Vδ1 usage), and exhibited a transitional differentiation status that was associated with multiple T cell activation pathways. Thus, γδ T cells in breast milk are distinct from their peripheral blood counterparts, likely contributing to local immunosurveillance during lactation.

Throttling the PI3K/mTOR Axis During Gamma-Delta T Cell Manufacturing Yields Products with Enhanced Therapeutic Properties

Presenter Name: Lisa Papara

Lisa, Papara, McMaster University
Allyson, Moore, McMaster University
Bojana, Bojovic, McMaster University
Joanne, Hammill, McMaster University
Nickolas, Serniuck, McMaster University
Christopher, Baker, McMaster University
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Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

Gamma 9 delta 2 T cells (G9D2 T cells) are an attractive substrate for cancer cell therapies, but clinical results have been modest. Engineering G9D2 T cells with tumor-specific synthetic antigen receptors can enhance their therapeutic potential, but better understanding of G9D2 T cell biology is still required to maximize efficacy. The PI3K/mTORC1 signaling axis plays an important role in the differentiation of conventional alpha beta T cells where disruption of this signaling axis using pharmacologic intervention during manufacturing results in a more potent anti-cancer product. Therefore, we assessed the impact of PI3K inhibitors (CAL-101 and Duvelisib) and an mTORC1 inhibitor (Rapamycin) on G9D2 T cell manufacturing and function. G9D2T cells were engineered with synthetic antigen receptors directed against BCMA, CD133 and Robo-1 and functional assessments were made in the context of myeloma and glioblastoma. Inhibitor concentrations were titrated to suppress cell yield by approx. 50%, although growth suppression varied from batch-to-batch. CAL-101 or Duvelisib treatment enriched for cells with memory-like features and reduced CD56 expression. Rapamycin treatment yielded a distinct phenotype characterized by reduced CD28 expression and a marked loss in CD16 expression. CAL-101 and Duvelisib-treated G9D2 T cells displayed enhanced mitochondrial respiration while Rapamycin-treated cells shifted towards glycolytic metabolism, indicating that while PI3K inhibition promotes fatty acid oxidation, asymmetric perturbance of the PI3K-mTOR axis promotes glycolysis presumably via mTORC2. Although throttling of PI3K signaling and mTORC1 signaling had different biological impacts on the cell product, both strategies imbued some attractive properties. PI3K inhibition had a modest impact on G9D2 T cell effector function in vitro but improved survival in cytokine-free conditions and yielded a modest improvement in therapeutic efficacy in a myeloma xenograft model. Rapamycin treatment enriched for G9D2 T cells expressing the synthetic receptors and improved survival in cytokine-free conditions in vitro. Further, rapamycin-treated engineered-G9D2 T cells displayed enhanced expansion following exposure to tumor cells, which was amplified by the presence of IL-15. Thus, throttling PI3K signaling or mTORC1 signaling can benefit engineered G9D2 T cell products, albeit via distinct biological mechanisms. This work was supported by the Samuel Foundation, ORF, Mitacs and LLSC.

Restriction of innate Tγδ17 cell plasticity by an AP-1 regulatory axis

Presenter Name: Morgan Parker

Morgan E. Parker 1, 2*, Naren U. Mehta 1,2*, Tzu-Chieh Liao 1, 2, William H. Tomaszewski 1, Stephanie A. Snyder 1,2, Julia Busch 1,2, and Maria Ciofani 1, 2, 3
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Primary Theme: Development & Differentiation

Secondary Theme: Infection Responses

Abstract

Type 3 lymphocytes are marked by the expression of RORγt, and include Th17 cells, ILC3s, and IL-17-producing γδ T (Tγδ17) cells. A feature of type 3 lymphocytes is their high degree of effector plasticity that contributes to both immunity to infection and autoimmunity. While several studies have reported the generation of multifunctional IFNγ+ IL-17A+ Tγδ17 cells in inflammatory settings, the nature, context-dependence, and regulatory control of Tγδ17 cell plasticity remains to be defined. Towards this goal, we used type 3 fate-mapping mice in combination with single cell ATAC+RNA-seq multiome epigenomic and gene expression profiling to track and characterize Tγδ17 cell plasticity at the cellular and molecular level. During homeostasis, Tγδ17 cell effector identity was stable across tissues, including for intestinal T-bet+ Tγδ17 cells that restrained IFNγ production. Notably, oral Salmonella typhimurium infection induced type 1 effector conversion of intestinal Tγδ17 cells into IFNγ single producers selectively in the innate-like Vγ6+ subset. Vγ6+ Tγδ17 cell plasticity was characterized by a loss of IL-17A production and an intermediate downregulation of RORγt expression, along with a global shift from a type 3 to type 1 effector expression program. Additionally, expression of the coinhibitory receptor TIM-3 selectively marked converted ex-Tγδ17 cells with enhanced type 1 functionality. An integrated analysis of gene expression, chromatin accessibility, transcription factor motif enrichment, and gene regulatory networks that were altered along the single cell Vγ6+ Tγδ17 type 1 conversion trajectory revealed the dominant activity of an AP-1 regulatory axis in this process. We validated novel roles for AP-1 factors JunB and Fosl2 in restricting intestinal Tγδ17 cell plasticity by stabilizing the expression of type 3 effectors (RORγt and IL-17A) and repressing the expression of type 1 effectors (T-bet or IFNγ). Furthermore, genetic downmodulation of RORγt expression was sufficient for de-repression of IFNγ expression in Tγδ17 cells, thus identifying the regulation of RORγt as a critical node in the AP-1 network governing Vγ6+ Tγδ17 cell effector plasticity. Finally, conditional Fosl2 deletion in Il17a+ fate-mapped cells resulted in reduced S. typhimurium burden, through enhanced type 1 plasticity by Vγ6+ Tγδ17 cells and Th17 cells, underscoring the functional significance of type 3-to-1 lymphocyte plasticity.

N-glycosylation Controls thymic γδ17 T-Cell Development By Regulating TCRγδ Signal Strength

Presenter Name: Rúben Pinheiro

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Primary Theme: Development & Differentiation

Secondary Theme:

Abstract

γδ T-cells differentiate in developmentally programmed thymic waves, and present a remarkable tropism for many peripheral tissues, where they play key roles in tissue homeostasis and surveillance. Several of these properties are acquired during thymic development, and hence unveiling the underlying molecular mechanisms is essential to understand their functional potential in the periphery. Glycosylation is one of the most common post-translational modifications in eukaryotes, involved in numerous aspects of the immune system; however, its impact on thymic γδ T-cell development and biology remains mostly unexplored. Here, we used single-cell RNA-sequencing to investigate the transcriptional programs employed by γδ thymocytes during their development in the newborn thymus. This analysis revealed that IL-17-producing γδ (γδ17) thymocytes are substantially enriched in genes associated with the N-glycosylation pathway when compared to their IFNγδ-producing γδ (γδIFN) counterparts. Mechanistically, we show that N-glycosylation is regulated in a TCR-dependent manner where strong TCR signaling leads to the downregulation of this pathway in developing γδ thymocytes. As a result, embryonic γδIFN thymocytes display a greater content in less complex N-glycans than thymic γδ17 cells. Critically, in the Rag1creMgat1fl/fl mouse model, where lymphocytes lack the early glycan-branching enzyme GnT-I, we observed a severe impairment in the differentiation of thymic γδ17 (but not γδIFN) T-cells in both embryonic and adult thymus, which associated with a defect in the expression of the γδ17 lineage master transcriptional regulator, RORγδt. Lastly, we propose that N-glycosylation decreases the strength of TCRγδ signaling perceived during γδ T-cell differentiation, since incubation of wild-type immature γδ thymocytes with the N-glycosylation inhibitor kifunensine during TCR stimulation drastically augments the expression of TCR downstream targets (e.g. Nur77) and associated cell surface activation markers (e.g. CD69, CD73 and CD44). In sum, our data suggests that N-glycosylation is a key determinant of the effector differentiation of γδ thymocytes by tuning TCRγδ signal strength and adjusting it to the developmental requirements of γδ17 T-cells.

Mevalonate pathway is important for pro-inflammatory function of Vdelta2 T cells

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Primary Theme: Cancer Responses

Secondary Theme: Tissues & Inflammation

Abstract

Mevalonate pathway, which leads to isoprenoid synthesis, is an essential metabolic pathway for proper functioning of eukaryotic cells. Widely prescribed drugs like statins and bisphosphonates, such as zoledronate inhibit specific enzymes in the pathway. The clinical relevance of the pathway manifests in autoinflammatory disorder: hyper IgD syndrome (HIDS) caused by a mutation of mevalonate kinase. Interestingly, an intermediate metabolite: isopentenyl pyrophosphate of the pathway is recognized by V delta 2 (Vd2) T cells as a phosphoantigen. Vd2 T cells are important players in anti-cancer surveillance and infection clearance. We used peripheral blood mononuclear cell (PBMC) cultures from healthy donors, HIDS patients and patients on statin therapy to study the role of mevalonate pathway in Vd2 T cell function.

We show that incubation of PBMCs from healthy donors with various mevalonate pathway inhibitors, including statins and zoledronate, results in impaired TNFa and IFNg secretion by Vd2 T cells. Furthermore, the ex vivo analysis of Vd2 T cells from HIDS snd patients on statin therapy showed a similar phenotype confirming our in vitro findings. RNAseq and ATACseq analysis revealed transcriptomic but not epigenetic reprogramming of Vd2 T cells upon mevalonate pathway inhibition.

Using various downstream inhibitors of the mevalonate pathway we revealed that protein prenylation is important for the proinflammatory function of Vd2 T cells. WesternBlot experiments and Pamgene kinase activity analysis confirmed that mevalonate pathway

inhibition in Vd2 T cells leads to the decrease of prenylated GTPases and showed that indeed JNK and P38 signaling pathways downstream GTPase signaling is hindered, resulting in the compromised effector function of Vd2 T cells. Our study reveals the importance of mevalonate pathway in the proper functioning of Vd2 T cells and points to potential factors for the improvement of therapeutic interventions.

Clonal Tracing of γδ and αβ T Cells Before, During, and After Cytomegalovirus (CMV) Reactivation in Transplant Patients

Presenter Name: Immo Prinz

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

CMV infection is typically harmless in immunocompetent individuals, but poses serious risks to immunocompromised patients. We follow the immune response to CMV reactivation of individual γδ and αβ T cell clones of the reconstituted immune system of patients after hematopoietic stem cell transplantation. Longitudinal blood samples were collected from 8 patients after HSCT and scRNA and scTCR sequencing of sorted γδ and αβ T cells were performed. Using individual CDR3 sequences, we tracked the activation and differentiation of γδ and αβ T cell clones during the course of infection projected onto a two-dimensional reduction. A sequential reconstitution of T cell subsets was observed, with γδ T cells appearing first, followed by CD8 and CD4 T cells. Consistent with previous reports, we identified more public TCR gamma and more private TCR delta chain repertoires. Vδ1+ T cells expanded in response to CMV reactivation, reducing clonal diversity, although not in all patients. Clonally expanded cells showed a phenotypic shift from cytotoxic memory to a cytotoxic or antiviral Temra phenotype, whereas innate Vγ9JPVδ2 T cells showed only bystander activation during CMV reactivation.

The observed interpatient heterogeneity of Vδ1+ T cell clones responding to CMV or CMV-induced antigens with clonal expansion and phenotypic shifts further supports the adaptive-like properties of this γδ T cell subset.

Integrative Single-Cell and Computational Analysis Reveals Ligands Modulating Human Gamma Delta T Cell Phenotypes in Colorectal Cancer

Presenter Name: Ran Ran

Ran Ran, Case Western Reserve University

Douglas Brubaker, Case Western Reserve University

Primary Theme: Cancer Responses

Secondary Theme: Development & Differentiation

Abstract

γδ T cells, a unique subset of T cells enriched in the gut epithelium, play a crucial role in maintaining colon epithelial health and contributing to the anti-tumoral response against colorectal cancer. Using publicly available single-cell transcriptomic data, we developed a comprehensive gene expression atlas of γδ T cells in human colorectal cancer (CRC) in our previous work and confirmed their anti-tumoral activity across various subtypes. This work lays a foundation for further exploring the immunotherapeutic potential of γδ T cells. To investigate how specific ligands in the tumor microenvironment shape γδ T cell phenotypes and how their activation can be enhanced, we first inferred the condition-specific regulatory relationships between transcription factors (TFs) and their target genes in γδ T cells using SCENIC, based on our integrated data. We then incorporated the inferred gene regulatory network (GRN) into the known network and applied the Personalized PageRank-based algorithm NicheNet to link differentially expressed genes in each γδ T cell subset to their potential upstream ligands. SCENIC predicted 13,200+ novel TF-target interactions in human CRC γδ T cells, and their integration into the known network did not compromise the model's ability to predict perturbed ligands in reference data. Our analysis predicted that ligands such as CCN6, PD-L1, CDHR2, and LGALS9 contribute to the exhaustion of CRC γδ T cells, while IL-1B, IL-4, IL-15, and CD40LG are predicted to enhance γδ T cell activation—although this activation is also under the influence from suppressive ligands including IL-10 and PD-L1. Using the annotated CRC whole-tissue scRNA-seq reference dataset from Joanito et al. (2022), we further assessed the enrichment of top γδ T cell phenotype-shifting ligands across various cell types within the CRC microenvironment. Our findings indicate that in both MSS and MSI-H CRC, monocytes/classical dendritic cells (McDCs) have the highest potential to promote γδ T cells' transition from a less activated, TRM-like state to a more activated, effector-like state. In contrast, fibroblasts are predicted to exert the strongest influence in polarizing γδ T cells toward exhaustion.

Antibody-like Recognition of a γδ T Cell Receptor Towards a Foreign Antigen.

Presenter Name: Liam Rashleigh

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Primary Theme: Ligands & Repertoires

Secondary Theme: Infection Responses

Abstract

Across lymphocyte lineages, the antigen recognition principles of B cells and αβ T cells have been well described. Conversely, γδ T cell antigen recognition mechanisms remain partially defined. Notably, γδ T cells - by way of their specificity conferring receptor (γδTCR) – are thought to directly bind a broad range of proteinaceous antigens via unknown mechanisms. A known γδ T cell and B cell antigen is phycoerythrin, a light harvesting protein from rhodophytes and cyanobacteria. Here we probed human γδTCR reactivity to phycoerythrin, in which a Vδ1Vγ5 TCR bound with high affinity to induce proximal signalling and cellular activation. We determined the cryo-electron microscopy (cryo-EM) structure of the γδTCR-phycoerythrin immune complex (~1.9 Å) to reveal δ-chain aromatics that enabled binding. This represents the first structural example of a γδ TCR recognising a non-self-antigen directly. For direct comparisons to other lymphocyte receptors, we then determined the cryo-EM structures of an antibody fragment and an αβ TCR in complex with phycoerythrin (2.3Å and 3.4Å, respectively). The lymphocyte receptor-phycoerythrin complexes revealed the convergent use of apical aromatics in the complementarity determining region 3 loops to mediate contacts with a common epitope. Structural and compositional analyses of known ligand-reactive γδTCRs revealed multiple antibody-like characteristics, including an enrichment of apical aromatics. Our findings reveal further facets of antigen recognition by the γδTCR and further reveal its distinctiveness amongst lymphocyte receptors.

Role of CD39 on γδ T Cell-based Therapy in Follicular Lymphoma

Presenter Name: Léa Rimailho

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

T lymphocytes, particularly γδ T cells, play a crucial role in anti-tumor immunity and are promising candidates for cancer immunotherapy (Mensurado et al 2023; Rimailho et al 2023). γδ T cells also express Fc receptor CD16, enabling antibody-dependent cellular cytotoxicity, particularly interesting in non-Hodgkin's lymphomas (NHL), where Vγ9Vδ2 T cells contribute to the anti-cancer response (Braza et al 2011; Rossi et al 2018). Additionally, γδ T cells express CD39 (Gruenbacher et al 2016), involved in the adenosine pathway (Young et al 2014), playing a key role in immune evasion, particularly in NHL (Laurent et al 2015). Our study aimed to better understand the role of CD39-expressing γδ T cells in immunotherapy for follicular lymphoma (FL).

Long-term γδ T cell cultures were established from fresh or thawed PBMCs. Using multiparametric flow cytometry analyses, these cultures were fully characterized phenotypically based on the expression of CD16, CD39 among other immune checkpoint (ICP), and functionally based on their capacity to lyse 3D FL cultures directly or in presence of anti-CD20 antibody treatment.

Regardless of their origin, γδ T cells could be efficiently expanded with BrHPP/IL2 when their initial frequency (CD3+/γ9+) was superior to 0.7%. The expression of CD16, CD39 and ICPs as well as their anti-lymphoma activity, varied among donors. The role of CD39 was investigated using a blocking anti-CD39 antibody or by sorting CD39- γδ T cells. In both cases, CD39 inhibition enhanced both direct and anti-CD20-mediated cytotoxicity, as evidenced by increased B-cell depletion and granzyme B secretion. These results were validated in Patient-Derived Lymphoma Spheroids (PDLS), well-characterized preclinical FL models (Faria et al 2023; Dobano-Lopez et al 2024).

Altogether, our study showed that: i) fresh or thawed PBMC can be used to establish γδ T cells, ii) γδ T cells co-cultured with 3D FL cultures are functional; iii) CD39 blockade enhanced their capacity to kill FL cells directly or in a context of immunotherapy. This work opens new perspectives, not only for the establishment and the use of fully characterized γδ T cells that can

be selected for specific questions in a context of immunotherapy but also for considering CD39 as an ICP in FL. Future investigations with preclinical relevant models and clinical trials will therefore demonstrate the benefit for FL patients management to combine anti-CD20 (mono or bispecific) to anti-CD39 antibodies.

Fetal Type-1 γδ T-Cell Responses at Single-Cell Resolution During In Utero Viral Infection

Presenter Name: Guillem SÁNCHEZ

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Primary Theme: Infection Responses

Secondary Theme: Tissue immunology

Abstract

γδ T cells are the first T cells to develop in the human fetus and they contain already programmed effector subsets in utero. Human congenital cytomegalovirus (CMV) is the most common congenital intrauterine infection with potential life-long consequences for the child. In this study, we investigated the fetal blood γδ T cell response of mid-gestation fetuses to CMV infection with single cell technologies (gene expression, TCR, cell surface protein) to obtain a deeper insight into the γδ T cell response to a viral infection in utero.

Upon CMV infection, the expanded and differentiated fetal γδ T cells showed a distinct cluster exhibiting type-1 immune function with elevated expressions of genes associated with cytotoxicity, natural killer (NK) cell function and IFNγ, suggesting a strong antiviral response. Pre-existing type-2 and type-3 γδ subsets appear not to react upon CMV infection. Single-cell T cell receptor (TCR) repertoire analysis showed predominant clonotypes identified both public (shared) and private (individual-specific) γ and δ CDR3 sequences associated with antiviral effector functions in CMV infected subjects.

Parallel investigations into other immune cell populations including αβ T cells, B cells, and myeloid compartments are underway to provide a comprehensive understanding of the immune landscape during congenital CMV infection.

Human Invariant γδ T Cells Populate Fetal Peripheral Organs to Provide Developmentally Restricted and Site-Specific Immunity

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

In contrast to the development of γδ T cells in the post-natal thymus, human fetal γδ thymocytes are committed to either a type 1, a type 3 or a type 2-like effector fate and possess invariant public TCRs. However, it is not clear what fate is of these different thymic effector types in the human fetal immune system. Therefore, we investigated fetal γδ T cell biology in lymphoid (blood, mesenteric lymph node and spleen) and non-lymphoid (liver, lung, small intestine and skin) tissues by coupled single cell (sc) RNA/TCR sequencing and multiparametric flow cytometry.

Thymic effector γδ T cells colonized peripheral organs, where they underwent differentiation, which coincided with the emergence of tissue-specific cell states. Of note, phosphoantigen-reactive Vγ9Vδ2 T cells were differentially enriched among the effector modules in the distinct peripheral tissues. Interestingly, certain TCR sequences were more prone to be associated with particular transcriptomic profiles and niche-specific CDR3 motifs were identified. Finally, the presence of these TCR sequences and defined effector profiles were exclusively present in tissues during the fetal period, suggesting ontogenically restricted roles for γδ T cells.

Thus, human fetal γδ T cell cells appear to acquire tissue-specific specialization both at TCR and functional level. This knowledge provides a basis to study the role of these cells in human early life immunity and physiology.

Evolution of Human γδ T cells: Has Plasmodium Infection impacted γδ TCR selection?

Presenter Name: Rosie Sanders

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

Plasmodium infection is one of the most impactful evolutionary forces in human history, driving the emergence of numerous, well-characterised mutations that confer protection against malaria. While γδ T cells are highly responsive to Plasmodium infection, the evolutionary significance of this relationship is poorly understood. We used flow cytometry, computational analyses and TCR sequencing, to (i) understand whether selection by Plasmodium has impacted γδ T-cell receptor (TCR) evolution, and (ii) investigate how such evolution may have impacted the γδ T cell responses that are observed in vivo.

To explore 43 million years of γδ TCR evolution, non-human primate (NHP) genomes were analysed to identify homologs of human γδ TCR genes in NHPs with varied Plasmodium exposure. Our analysis revealed potential purifying selection acting on malaria-exposed primate TCR genes, particularly TRGV9 (TCR Vγ9), which may reflect the importance of Vγ9+ T cells during malarial infection.

To investigate dynamic changes in the γδ TCR repertoire during Plasmodium infection in vivo, we analysed samples from a human longitudinal cohort from a malaria endemic region of Mali. We utilised samples from individuals with recurrent P. falciparum infection over the course of four years, during the malaria-free dry season. Using high-dimensional flow cytometry, TCR sequencing and single cell RNA sequencing, we observed a significant expansion of γδ T cells, particularly Vδ1+ γδ T cells in endemically exposed Malian children and adults. Moreover, in these individuals, changes in marker-expression in Vδ2+ T cells exhibited signs of dysfunction. This indicates a potential shift towards a Vδ1+ response in place of Vδ2+ T cells due to repeated P. falciparum exposure.

In identifying γδ TCR genes under selection, we hope to develop insight into γδ T cell subsets associated with functional malaria-protection. These findings begin to piece together the critical role of γδ T cells in malaria immunity and provide evidence of evolutionary adaption driven by Plasmodium infection.

Temporary Depletion And Re-Emergence Of Gamma-Delta T Cells Supports An Anti-Tumor Response In A Model For Colorectal Cancer

Presenter Name: André Santos

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Primary Theme: Cancer Responses

Secondary Theme: Tissues & Inflammation

Abstract

γδ T cells constitute a major immune population in the colon, where they are critical for maintaining intestinal homeostasis through immune-epithelial crosstalk. These cells prevent mucosal barrier disruption by microbes and detect transformed malignant cells. However, γδ T cells are a heterogeneous group whose functions are determined by the variable region of the TCRγ chain that they express. Vγ7+ γδ T cells predominate within the intraepithelial compartment, whereas IL-17-producing Vγ4+ and Vγ6+ cells reside in the lamina propria. The specific roles of these subsets in colorectal cancer remain poorly understood, with conflicting evidence suggesting both pro- and anti-tumor effects.

Using the TCRdGDL mouse model, we investigated the effects of different γδ T cell depletion regimens. Weekly depletion resulted in a complete loss of γδ T cells, whereas intermittent depletion allowed for the recovery of anti-tumor Vγ1+ and Vγ7+ subsets. Importantly, weekly γδ T-cell depletion was associated with increased tumor number and burden, whereas intermittent depletion led to the establishment of anti-tumor γδ T-cell subsets within the tumor microenvironment. These findings underscore the critical role of γδ T cells in protecting against colorectal cancer and highlight the therapeutic potential of targeting specific subsets to enhance anti-tumor immunity.

These results provide a foundation for future research to elucidate the mechanisms that drive γδ T cell-mediated tumor recognition and killing, and to develop strategies to selectively enhance anti-tumor γδ T cell subsets as a novel therapeutic approach for colorectal cancer.

The impact of the tumor micromilieu on gamma delta T cell function in squamous cell carcinoma in Epidermolysis bullosa

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Primary Theme: Cancer Responses

Secondary Theme: Tissues & Inflammation

Abstract

Recessive Dystrophic epidermolysis bullosa (RDEB) is an inherited skin disorder, characterized by mucocutaneous fragility and blister formation upon minimal trauma. Almost all RDEB patients will develop highly aggressive cutaneous squamous cell carcinomas (SCC) before reaching 45 years of age. SCC in RDEB patients not only develop earlier, they also have higher metastasis rates compared to SCC in patients without RDEB. Since there is no effective therapy available as of yet, SCC are the main cause of premature death in RDEB patients. The pathomechanisms are still largely unknown but may be linked to immune cell dysregulation and hyperinflammation, which may render anti-tumor immunity ineffective. Here we analyzed gamma delta (γδ) T cells, anti-tumor cells which constituted the most significant favorable prognostic immune population among 39 human cancer types when present in the tumor infiltrate. Whereas γδ T cell function has been studied in various skin cancer types, their role in regulating the growth of the uniquely aggressive SCC in RDEB patients has not been investigated. We discovered that circulating γδ T cells from RDEB patients have a reduced expression of cytotoxic molecules including granzyme B and granulysin compared to those of healthy donors. Moreover, we found that tumor infiltrating γδ T cells have a heterogenous phenotype, consisting of cytotoxic but also tissue resident and wound healing γδ T cells, which might lack anti-tumor function. Furthermore, the altered cytokine milieu associated with RDEB, might drive phenotypical and functional changes of γδ T cells in RDEB SCC. This study will provide insights on the mechanisms causing dysfunction of γδ T cells in RDEB patients with SCC and contribute to developing therapies against the highly aggressive SCC in RDEB patients.

Arming γ9δ2TCR-based Immunotherapies with Overexpression of CCR5 Increases T-cell Migration and thereby Therapeutic Efficacy

Presenter Name: Caroline Schwenzel

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

αβT cells engineered to express a γδTCR (TEGs) combine the proliferative capacity and efficient cytotoxicity of αβT cells with targeting highly prevalent natural γ9δ2TCR ligands on malignant cells independent of Major histocompatibility class I (MHC I) presented antigens. As sufficient infiltration of adoptive T cell therapies to the tumor site is a limiting factor for their therapeutic efficacy, this study aims to explore strategies to improve infiltration of γ9δ2TCR-based immunotherapies towards the tumor.

We developed biomimetic pre-clinical 3D co-culture models which model the extracellular matrix dense tumor site, including stromal cells, and mimic the infiltration of T cells towards the tumor by separating the tumor site and the T cell compartment with a trans well. In this study, we target both hematological as well as solid patient-derived cancers with BTN2/3-targeting TEGs and simultaneously analyze migration, tumor targeting and phenotypic changes in T cells in a quantitative and qualitative way.

Using long-term 3D in vitro co-culture models, we found that infiltration of BTN2/3-targeting TEGs towards the tumor was limited; particularly in the CD8+ T lymphocyte compartment. Chemokines including CCL4 were found to be essential for the migration of adoptive γ9δ2TCR-based immunotherapies, with blocking of these chemokines showing a reduction in migration

and tumor-specific killing. Furthermore, overexpressing the corresponding chemokine receptor CCR5 in tumor-reactive TEGs significantly improved infiltration capacity and tumor targeting. Using 3D live video imaging and single cell tracking revealed insights into migration dynamics of different T cell subsets towards the tumor.

This study highlights the importance of improving the infiltration of γ9δ2TCR-based immunotherapies as increased infiltration can boost the therapeutic efficacy and thereby improve clinical outcomes, particularly in the treatment of solid malignancies. Our results argue for the potential of using CCR5 overexpression in CD8+ TEGs as we show their improved migration to the tumor site and consequentially increased target-specific killing.

Oncolytic Vaccinia Virus Encoding An Agonist Anti-Butyrophilin Antibody Efficiently Activates Antitumor Properties Of Human Vγ9Vδ2 T Cells In Vitro And In Vivo

Presenter Name: Emmanuel SCOTET

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Oncolytic virotherapy is an attractive and emerging cancer therapeutic approach, which relies on the use of viruses that are able to selectively replicate in and destroy tumor cells while leaving normal cells undamaged. However, in some cases, a fraction of tumor cells can escape oncolysis. This fostered the development of improved therapies based on the administration of modified viruses as well as combination with adoptive transfer of immune effectors, such as human γδ T cells, to fully eradicate these resistant tumor cells. First, we sought to characterize how human Vγ9Vδ2 T cells would react to tumor cells infected with different oncolytic viruses. A modest activation of Vγ9Vδ2 T lymphocytes was measured, only after infection of breast cancer cells by an oncolytic measles virus, through a non-TCR pathway. In order to boost anti-tumor reactivity following viral infections, we inserted into the genomes of oncolytic Vaccinia Virus and Vesicular Stomatitis Virus full-length or shortened sequences (scFv) of an agonist anti-butyrophilin antibody, which had been described to induce the antigenic activation of Vγ9Vδ2 T cells. We demonstrate that tumor cells infected by these recombinant oncolytic viruses can efficiently produce both integral or scFv functional antibodies that can bind to tumor cells and further induce a strong antigenic activation of Vγ9Vδ2 T cells in vitro. This was confirmed in an in vivo model where a combination of an armed oncolytic vaccinia virus with adoptively transferred human Vγ9Vδ2 T cells was far more efficient than either of the two treatments used alone to control tumor growth. Our study shows that oncolytic viruses are appropriate vectors to produce agonist butyrophilin antibodies into solid tumors and to trigger the reactivity of human Vγ9Vδ2 T lymphocytes.

Identification of Novel γδ T Cell Populations in Malaria-exposed African Individuals

Presenter Name: Leon Separautzki

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Primary Theme: Development & Differentiation

Secondary Theme: Infection Responses

Abstract

Human γδ T cells are divided into innate-like and adaptive-like subsets with distinct γδ T cell immunobiology. Research on adaptive-like γδ T cells using single cell approaches revealed different naïve and effector differentiation states in peripheral blood and other tissues. We demonstrated that adaptive-like γδ T cell subsets co-expressing CD38 and PD1 are activated and oligoclonal following asexual blood stage infection with P. falciparum in African individuals. To further explore unknown differentiation/maturation states of γδ T cells and their heterogeneity in malaria-exposed African individuals, we analyzed single-cell RNA, TCR, and CITE-seq data combined. Enriched γδ T cells from peripheral blood of 17 West African individuals revealed known and unknown differentiation/maturation states in both innate- and adaptive-like γδ T cells. Based on the transcriptome and surface protein expression (CITE-seq) of approx. 40’000 cells, we identified nine γδ T cell clusters with unique patterns. Analysis of the γδ T cell clusters revealed innate- and adaptive-like populations with features of naïve, immature and effector T cells. We further found that some innate- and adaptive-like γδ T cells exhibit unique differentiation/maturation states, suggesting specialized functions such as antigen-presentation and immune cell recruitment. Another γδ T cell population expressed transcripts and surface markers associated with memory and tissue residency, including a unique combination of integrins and proteins enabling tissue-homing. These data suggest that innate- and adaptive-like γδ T cells might have specialized functions and tissue-homing capacities. Interestingly, the adaptive naïve and effector γδ T cell populations differed between individuals from rural and urban areas of West Africa, further suggesting that environmental conditions may influence the γδ T cell compartment. Controlled human malaria infections in urban African individuals resulted in an increase of blood circulating naïve γδ T cells with genes involved in IFN-responses. The reduction in adaptive-like effector γδ T cells suggests alterations induced by P. falciparum infection and the contribution of distinct γδ T cell populations in malaria. The current analysis of TCR sequencing data will provide further information about TCR clonotypes involved. Overall, the heterogeneity of γδ T cells with unique characteristics could influence the functions and immunobiology of γδ T cells in health and disease.

Generating a Universal Gamma Delta T Cell Therapy Using Covalent Immune Recruiters

Presenter Name: Nickolas Serniuck

Nickolas Serniuck, McMaster University
Ally Moore, McMaster University
Dr. Joanne Hammill, McMaster University
Dr. Anthony Rullo, McMaster University
Dr. Jonathan Bramson, McMaster University

Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

Engineered T-cells have proven to be a highly promising tool in the treatment of cancer, but the personalized nature of current engineered T cell therapies results in high cost and long wait times due to manufacturing logistics. Off-the-shelf allogeneic cell therapies offer a solution to these challenges as they can be manufactured en masse and be available on demand. Gamma 9 delta 2 T cells (G9D2 T cells) are an attractive substrate for off-the-shelf cancer cell therapies; however, the clinical outcomes of G9D2 T cell therapies have been modest. Engineering G9D2 T cells with tumor-specific synthetic antigen receptors can enhance their therapeutic potential, but conventional synthetic antigen receptors are limited to 1-2 antigen targets, necessitating the generation of multiple G9D2 T cell products to address the broad spectrum of potential tumor targets. We propose to engineer G9D2 T cells with a universal synthetic antigen receptor which can be programmed on-demand with molecular adapters that direct the T cells against discrete targets. Using this strategy, only a single engineered G9D2 T cell product is required. To this end, we have developed universal synthetic antigen receptors (SARs) that can be programmed with molecular adapters that covalently attach to the universal synthetic antigen receptor, termed CIRs (Serniuck et al, Mol. Ther. Oncol. 2024). The modular nature of this platform has allowed us to address tumor heterogeneity through the development of adapters for a broad range of tumor ligands using small molecules, peptides, and nanobodies. We have engineered human G9D2 T cells to express three different universal SARs: 4-1BB and CD28-based second generation chimeric antigen receptors and a chimeric version of KIR2DS2 (KIR-CAR). In vitro studies have confirmed that CIRs can functionalize all SARs and trigger activation in an antigen-dependent manner. The KIR-CAR yielded the most robust functional attributes (cytotoxicity, proliferation). We have also determined that covalent ligation via the CIRs is required for optimal performance of T cells. We are presently examining the potential enhancements of the KIR-CAR through modifications to the DAP12 component. In summary, covalent programming of G9D2 T cells engineered with a universal synthetic antigen receptor offers a potential off-the-shelf universal T cell therapy with capacity for on-demand targeting. This work was supported by the Samuel Family Foundation, the CRC program, and CIHR.

Is BTN3A1 A Regulator of αβ T Cell Responses?

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Primary Theme: Ligands & Repertoires

Secondary Theme: Infection Responses

Abstract

The butyrophilin (BTN) family of proteins are emerging as key mediators of human γδ T cell biology. However, a recent study reported that BTN member 3A1 (BTN3A1) may also suppress human CD4+ and CD8+ αβ T cell responses through engagement with CD45RO, positioning BTN3A1 as a potential immunotherapeutic target. Separately, HIV-specific CD8+ T cells from people living with HIV (PLWH) on suppressive antiretroviral therapy (ART) exhibit exhaustion phenotype, which impedes immune-mediated clearance of the HIV reservoir. We therefore aimed to clarify the potential immunosuppressive role of BTN3A1 towards CD4+ and CD8+ αβ T cells in vitro and the potential therapeutical benefit of targeting BTN3A proteins to reinvigorate HIV-specific CD8+ T cell responses. Using a redirected presentation assay, we were unable to demonstrate BTN3A1-mediated suppression of CD4+ and CD8+ αβ T cell activation, proliferation, cytokine secretion or cytotoxicity. In line with this, treatment of peripheral blood mononuclear cells (PBMCs) from PLWH on suppressive ART with anti-BTN3A neutralising mAbs failed to reinvigorate HIV-specific CD8+ T cell cytotoxicity or proliferative capacity. Lastly, using tetramers of BTN3A1 or CD45RO ectodomains, we did not observe BTN3A1 tetramer labelling of CD45RO+ cells within human PBMCs, or CD45RO ectodomain tetramer labelling of BTN3A1 overexpressing cells. Collectively, we were unable to demonstrate the immunosuppressive role of BTN3A1 towards CD4+ and CD8+ αβ T cell function, nor a BTN3A1 and CD45RO interaction, as previously reported. Whilst the reasons for this discrepancy are unclear, further studies will be required to establish whether targeting BTNs with agonist or antagonist mAbs will confer any therapeutic benefit for αβ T cell responses in infection, cancer and other diseases.

Beneath the Waves, an Ocean Churns: ART-suppressed HIV infection and Aging Stir up Deep Sea Creatures (Latent Herpesviruses) which Spur Waves of gd T Cell Red Tides of distinct Vd1+ and Vd2+ subsets

Presenter Name: Erika Smith-Mahoney

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Primary Theme: Infection Responses

Secondary Theme: Development & Differentiation

Abstract

Herpesviruses, including CMV and EBV, are found in more than 90% of the world's adult population and while directly connected to cancers and now believed to be the causative agent of multiple sclerosis, the frequencies of reactivation events and impacts on health span of immunocompetent people are largely unknown. Vd1+ gd T cells clonally expand in response to CMV viremia and malaria, implicating these unique T cells in anti-microbial responses. We have previously reported an enrichment of discrete TIGIT+ 'EMRA' Vd1+ gd T cell subsets distinguishable by CD8, CD16, CD38, and CD56 in people with ART-suppressed HIV infection (PWH) and with aging, similar to the gd T subsets found to be clonally expanded by CMV viremia. Therefore, we hypothesized that shifts in the Vd1:Vd2 ratio, alterations in the circulating gd T cell compartment, and the chronic inflammation found with PWH and with aging are in fact driven by 'silent' herpesviruses reactivation events. To test our hypothesis, we measured the frequencies of IFN-g secreting cells in peripheral blood in response to CMV, EBV, VZV, HHV6, and HSV-1/2 peptide pools to quantify the magnitude of herpesvirus T cell memory as an indirect assessment of a history of reactivation/prolonged viremia. We next compared the size of these T cell responses with gd T cell subset distributions, measured using a newly optimized 35-color spectral flow cytometry panel. Distinct subsets of 'EMRA' Vd1+ and Vg9-Vd2+ gd T cells defined via expression of CD8, CD56, CD16, and CD57 selectively tracked with memory T cell responses to CMV, HSV-1/2, HHV-6, and VZV among participants of our HIV and Aging cohort. We also measured canonical T-cell lineage defining transcription factors and cytokine secretion profiles of the total gd T cell compartment using additional flow cytometry panels. CD8+/- Vd1+ 'EMRA' cells with diffuse expression of CD56 and CD57 produced granzyme B, IFNg, and TNFa and also expressed T-bet or GATA3. Assessment of Vd2+ factions demonstrated that the majority of Vd2+ produce both IFNg and TNFa and express T-bet or RORgT. In sum, these results illuminate potential herpesvirus-driven clonal expansion and differentiation of 'adaptive' gd T cell effectors that we predict contribute to the progression and exacerbation of chronic inflammation with ART-suppressed HIV infection and aging that shorten the health spans in these populations.

Butyrophillin-like Molecules Bind a Broad Repertoire of γδ TCRs

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Primary Theme: Ligands & Repertoires

Secondary Theme:

Abstract

γδ T cells have been demonstrated to be important mediators of stress surveillance particularly in epithelial barrier tissues such as the mucosal lining of the gut. In these tissues, γδ intraepithelial lymphocytes (IELs) aid in the healing and maintenance of host homeostasis. Unlike conventional αβ T cells, relatively less is known about the ligand repertoire that may trigger and attract γδ IELs to tissues. While γδ T cells can adopt a similar convention for activation, they are uninhibited by a restriction to MHC-mediated recognition of antigens. The γδ T cell receptor has repeatedly demonstrated the capacity for recognising a large repertoire of ligands, with one of the most remarkable being those of the butyrophillin (BTN) family of molecules. BTN2A1 and BTN3A1 are known mediators of intracellular phosphoantigen recognition, binding and activating Vγ9Vδ2+ T cells that are abundant in the peripheral blood. However, the role of other butyrophillins in γδ T cell activation is still relatively unknown. We investigated the role of BTNL3 and BTNL8, close relatives of BTN2A1 and BTN3A1 in γδ TCR binding. There is an accumulating amount of evidence of the importance of BTNL3 and BTNL8 in maintaining the homeostatic state of the gut, particularly in individuals with enteropathies including inflammatory bowel disease (IBD) such as ulcerative colitis (UC), celiac disease, and colorectal cancer. In these same enteropathies, dysregulation of BTNL3 and BTNL8 is becoming a promising prognostic marker for disease severity. Our surface plasmon resonance (SPR) experiments have shown that previously held dogmas regarding BTNL3 restriction to Vγ4+ γδ TCRs should perhaps be expanded to also include a larger array of TCRs. We have additionally obtained x-ray crystallography structures of the extracellular domains of both BTNL3 and BTNL8 and further size exclusion chromatograhy small-angle x-ray scattering (SEC-SAXS) experiment that bring to the light the overall morphology of the BTNL3 IgV-γδ TCR complex. Thus, our research aids in better understanding what mechanisms of the BTNL3/BTNL8 axis can be harnessed in γδ T cell-based immunotherapies that may aid in reducing the disease burden of enteropathies in humans.

Role of Aryl Hydrocarbon Receptor in Regulating Inflammatory Potential and Wound Healing Functions of Dendritic Epidermal T Cells

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

The aryl hydrocarbon receptor (AHR) is crucial for dendritic epidermal T cells (DETC), a subset of Vγ5Vδ1 T cells in the mouse epidermis. These cells seed the skin around birth and contribute to skin homeostasis, immune surveillance, and wound healing by secreting cytokines, chemokines, or growth factors. Our previous studies showed that AHR is essential for DETC expansion in neonatal skin. In young AHR-deficient (AHR-KO) mice, inflammatory pathways were upregulated in DETCs. To assess AHR's role in adult DETCs, we deleted AHR in 8–10-week-old mice using a tamoxifen-inducible DETC-specific AHR deletion model. AHR was deleted in up to 90% of DETCs within three weeks after tamoxifen-injection. Unlike in systemic AHR-KO mice, DETC-specific AHR deletion did not impair skin barrier integrity (measured by transepidermal water loss, TEWL). RNA sequencing of these “adult” AHR-KO DETCs and wild-type (WT) DETCs from the dorsal skin did not reveal an increased inflammatory gene signature as in the prepubertal systemic AHR-KO mice. However, genes related to wound healing such as Vegfd and Angptl4 were negatively regulated compared to WT-DETCs. Ticrr or Wfdc3 were also significantly downregulated, indicating impaired proliferation and wound healing ability. In contrast, significantly upregulated Wfdc3 was detected in the AHR-KO DETC whose dorsal skin had been mechanically stressed. Other genes related to proliferation or wound healing showed no significant changes, suggesting a delayed activation of wound healing, possibly compensating for the initial deficits. Similarly, we found compensation in the migration of WT- and AHR-KO DETC towards a freshly punched wound in the ears. On the first day, fewer AHR-deficient DETCs than WT-DETCs were detectable near the wound edge, but within 48 hours the number of AHR-deficient DETCs equaled that of the WT. We deleted AHR from a DETC cell line, 7-17, and tested parameters related to proliferation and metabolism in vitro. Compared to AHR-proficient cells, the AHR-KO 7-17 cells had a reduced Warburg effect after CD3/CD28 stimulation, an incapacity to round up, as well as lower levels of the chemokines CCL3 and CCL4, and the cytokines IFN-γ and IL-1β. These changes suggest that the AHR influences both the activation of DETC and their ability to coordinate immune responses. Our results emphasize the role of AHR in DETC homeostasis, inflammation and wound healing.

How Maternal Infection Shapes the Development of Gamma Delta T Cells with Early Life Ontogeny

Presenter Name: Yuelin Song

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

Vertebrates undergo layered hematopoiesis, with certain immune cells generated exclusively before birth. While bone marrow replenishes immune cells in adulthood, subsets of γδT cells, such as Vγ5 dendritic epidermal T cells (DETCs) and Vγ6 γδT17 cells, arise only in the embryonic thymus. Environmental stress during early lineage specification can alter differentiation trajectories, and if not replaced by new progenitors, may leave long-term impacts. We hypothesize that early life-derived γδT cells are vulnerable to maternal environmental programming, impacting long-term health by "mis-using" the restricted developmental window. To test this, we infected pregnant mice with influenza B virus (IBV) and fostered newborns to healthy dams. Despite no vertical transmission, neonates from IBV-infected dams (mIBV) had lower birth weights and altered thymic γδT cell profiles. Multiple innate immune signaling and developmental pathways were significantly altered in all mIBV γδT cell subsets sorted for RNA-seq, including genes related to cell proliferation and migration, effector function, TCR repertoire regulation, and γδNKT development. mIBV newborn thymus retained a significant population of Vγ5δ1 (Vγ5+17D1+) cells, phenotypically distinct from thymic Vγ5+, 17D1+ (Vγ6+) single positives, or skin DETCs. Interestingly, no decrease of DETCs is observed in mIBV newborn epidermis, suggesting thymic γδT cell dysregulation, rather than delayed DETC migration. γδT cells have been shown to be important in epithelial repair and tissue homeostasis. To assess functional consequences of gestational infection, we transferred neonatal thymic γδT cells from mIBV and mPBS pups into TCRδ-/- mice with bleomycin-induced lung fibrosis, where γδT cells provide protective effects. Mice receiving mIBV γδT cells lost more weight than those receiving mPBS γδT cells. Upon bleomycin treatment, Vγ6+ γδT cells are the most expanded γδT cells and produce IL-17A, indicating their functional relevance. Adult mIBV offspring exhibited higher susceptibility to bleomycin-induced weight loss, which could be attribute to altered Vγ6+ cells early thymic development. To test cell intrinsic effects, we will transfer Vγ6+ cells from mIBV and mPBS adult lungs to TCRδ-/- mice and examine their phenotype and function. We will also investigate single-cell TCR repertoire, transcriptional and epigenetic profiles in adult offspring to further define the impact of maternal infection on offspring immune development.

A 10-Year Longitudinal Single-Cell Multiomics Study Reveals the Phenotypic and Clonotypic Dynamics of Vδ2-negative γδ T Cells in Repeated Malaria Infections

Presenter Name: Zheng Song

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

γδ T cells, especially the Vδ2-negative populations, are involved in the immune response to repeated Plasmodium falciparum infections in humans. However, the underlying mechanisms of such responses in naturally recurring malaria infections and their impact on γδ T cell phenotypes remain poorly understood. Here, we employed ultra-high-throughput combinatorial barcoding-based single-cell RNA sequencing, single-cell αβ TCR sequencing, and in-house single-cell γδ TCR sequencing to profile the phenotypes and clonotypes of γδ T cells in a 10-year longitudinal cohort including four Malian children (aged 4 to 5 years at the time of enrollment) exposed to intense seasonal malaria transmission. From 96 PBMC samples, collected at two annual cross-sectional blood-draws and shortly after febrile malaria episodes, we recovered 381,638 CD3+ T cells, which comprised 38,392 γδ T cells. We found that the Vδ2-negative γδ T cells were enriched in all of the Malian subjects, contrasting to the predominant Vδ2+ T cell population observed in donors from Western countries. Specifically, Vδ1+ and Vδ3+ T cells displayed markers associated with cytotoxicity (GNLY, GZMB, and CX3CR1) and underwent clonal expansion during acute malaria infection in early childhood. Following repeated infections, a substantial proportion of these cells exhibited regulatory (CTLA4, IL2RA) and exhaustion (PDCD1, HAVCR2, and IL10) phenotypes. Together, our findings reveal the dynamic expansion and phenotypic adaptation of γδ T cells in naturally recurring malaria infections.

Diverse Functional Outcomes Drive Resident Memory γδ T Cell Responsiveness to Diverse Intestinal Pathogens

Presenter Name: Julio Souza dos Santos

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Primary Theme: Infection Responses

Secondary Theme: Tissues & Inflammation

Abstract

The immune response to foodborne pathogens involves a complex interplay between innate and adaptive immunity. γδ T cells bridge these immune compartments, yet the mechanisms underlying their memory responses remain incompletely understood. Memory Vγ4Vδ1 T cells induced by foodborne Listeria monocytogenes (Lm) infection, produce IL-17A and IFNγ in response to diverse bacteria. To understand the induction of memory γδ T cell function, we stimulated bulk mesenteric lymph node (MLN) cells from Lm-immune mice with live or heat-killed (HK) Lm for 6 or 24 hours. After 6 hours, Vγ4 T cells produced IL-17A in response to live, but not HKLm. Neither stimulus induced IFNγ at 6 hours. Rapid induction of IL-17A mediated by live Lm occurred independent of accessory cells and was inducible by soluble factors produced by replicating Lm. Collectively, this data suggests that rapid IL-17A induction represents an innate-like l response of memory Vγ4 T cells through direct sensing of pathogen products. In contrast to this innate like-IL-17A response, a robust multifunctional response of IFNγ and IL-17A was observed at 24 hours for both live and HKLm. The delayed response suggests that antigen processing and presentation pathways may contribute to adaptive Vγ4 T cell functions. Co-culturing MLN from Batf3-/- mice, which lack conventional Type 1 DCs (cDC1), altered the functional profile from IFNγ+ and IFNγ+ IL-17A+ to predominantly an IL-17A+ response. We also observed that MHC-II inaccessibility reduced both IFNγ-producing and IFNγ/IL-17A co-producing cells, demonstrating that MHC-II expression by cDC1 contributes to adaptive Vγ4 T cell responses. IL-12/23p40 blockade reduced both IFNγ and IL-17A production while IL-23p19 neutralization only modestly impacted IL-17A. These results suggest that IL-12, rather than IL-23, is the primary driver of IFNγ/IL-17A co-production. γδTCR signaling is essential for γδ T cell expansion elicited by Lm. Our data demonstrate that Calcineurin/NFAT and PKC-θ are crucial for the delayed adaptive functions, with PI3K contributing to the multifunctional IL-17A and IFNγ response to HKLm. These findings reveal a bifurcation in memory γδ T cell responses, with a rapid, innate-like IL-17A production and a delayed, antigen-dependent adaptive function. cDC1, MHC-II, and IL-12 shape these responses, while activation can occur through both TCR-dependent and TCR-independent mechanisms. These insights clarify γδ T cell immunity and its uses in therapy.

Identification of Tumor-Reactive $\gamma\delta$ T Cell Receptors to treat Multiple Myeloma

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

Multiple myeloma (MM) is a cancer of plasma cells and is often fatal. T cells play a role in controlling tumor growth, with $\gamma\delta$ T cells being particularly important as their presence is strongly correlated with longer patient survival. However, exploiting the anti-tumor properties of these cells has been a challenge. A major barrier to discovering γδ TCRs suitable for clinical therapy is the difficulty in screening for them in a high-throughput fashion. To solve this problem, we developed a machine-learning approach that can efficiently identify tumor-reactive $\gamma\delta$ TCRs with therapeutic potential by analyzing single-cell sequencing data. We performed single-cell RNA and TCR sequencing of bone marrow samples from 22 MM patients and identified 769 unique γδ TCRs. We selected 135 unique γδ TCRs from 7 MM patients and developed a screening assay to assess the anti-tumor reactivity of these TCRs. By comparing the sequencing data of T cells that were tumor-reactive vs. non-reactive in this assay, we generated a signature of tumor-reactive γδ T cells that would allow for their identification using scRNA sequencing data alone. We used this data to train an algorithm which proved to be highly accurate in identifying anti-tumor γδ TCRs in a separate validation cohort of MM samples. To date, we have identified over 70 validated tumor-reactive γδ TCRs, many of which are not MHC-restricted and do not recognize known γδ ligands. Engineered expression of these γδ TCRs by primary T cells

confers upon them the ability to specifically recognize and kill MM cells in vitro, suggesting that such modified cells could be made into a viable cell-therapy product. Finally, we demonstrate that the presence of tumor-reactive γδ T cells can serve as a predictive biomarker for response to biological therapy. In conclusion, we have successfully generated a prediction algorithm to accurately identify tumor-reactive γδ TCRs. The application of this algorithm to massive single-cell sequencing datasets can selectively pinpoint the most effective γδ TCRs, opening the way to novel treatment strategies for MM and potentially other cancers.

Zooming in on the immune synapse between Delta One T cells and tumour cells

Presenter Name: Georgia Stevens

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Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Immunological synapse formation between a T cell and its tumour cell target is necessary for T cell-mediated cytotoxicity. Whereas the receptors and ligands involved in aB T cell: tumour cell synapses have been well characterised, much less in known about the equivalent process for γδ T cells. These recognise tumour cells in a MHC-independent manner, using for instance NK cell receptors (NKRs) to sense conserved stress signals prevalent in transformed cells. This applies to Delta One T (DOT) cells, a cancer immunotherapy product based on Vδ1+ γδ T-cells expressing enhanced levels of the NKRs, DNAM-1, NKG2D and NKp30. How these may (or not) participate in γδ T cell: tumour cell synapses remains to be established. In the present study, we use imaging flow cytometry to perform quantitative analysis of receptor clustering in the synapse to elucidate the molecular architecture of immune synapses between DOT cells and acute myeloid leukaemia (AML) cells. The analysed receptors include DNAM-1 and NKG2D, as well as CD3 and Vδ1 of the TCR complex. Additionally, we identified and characterised involvement of these receptors within synapse interactions of Vδ1+ cells with CD3+ or CD3- cells within DOT:AML co-cultures, and compared the findings with expanded Vδ2+ T cells. This analysis will now be performed with DOT cell-resistant versus susceptible AML cell lines, aiming to provide key insights on how productive immune synapses are structured. Taken together, we expect this study to provide new mechanistic insight into tumour targeting by DOT cells which may inform the design of DOT cell-based clinical trials.

BCG and MMR Vaccines Induce Innate Immune Memory in Human Gamma Delta T cells

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

Immunological memory, traditionally associated with adaptive immune cells, has recently been linked to innate immune cells, a phenomenon termed "trained immunity." In our study, we investigate how live attenuated vaccines: Bacillus Calmette-Guérin (BCG) and Measles-Mumps-Rubella (MMR) induce trained immunity in human γδ T cells, highlighting their cross-protective

effects. We show that the BCG vaccine, known to mediate trained immunity in myeloid cells, enhances γδ T cell function, particularly by increasing perforin production and promoting higher cytokine release such as TNF and IFN-γ in vaccinated individuals upon heterologous challenges compared to non-vaccinated ones. In BCG-vaccinated individuals, γδ T cells displayed adaptive memory traits alongside trained immunity characteristics, suggesting their ability to bridge both immune memory responses. Transcriptomic analysis revealed a subset of γδ T cells expressing high levels of IFN-γ and discovered a close interaction between monocytes and γδ T cells associated with interferon response for the formation of trained immunity. Similarly, the MMR vaccine induced systemic inflammatory changes in human subjects, characterized by an increase in circulating myeloid cells and notable transcriptional shifts in γδ T cells. A metabolic shift in γδ T cells, including reduced glycolytic capacity and increased mitochondrial dependency, was observed in individuals post-MMR re-vaccination. The γδ T cells of the re-vaccinated individuals demonstrated enhanced cytokine production, resembling the trained immunity phenotype observed in classical monocytes. Overall, our findings suggest that both BCG and MMR vaccines promote trained immunity in γδ T cells and underscoring their potential dual role in both adaptive and innate immunity. Our findings contribute to the understanding of how live vaccines induce broad, cross-protective immunity beyond their target pathogens.

Amphiregulin-Producing γδ T Cells Drive Colorectal Cancer Initiation and Growth

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Primary Theme: Cancer Responses

Abstract

Gamma delta (γδ) T cells in the intestine consist of several distinct subsets characterized by expression of different T cell receptors and opposing functions in colorectal cancer (CRC). In mice, Vγ4+ and Vγ6+ subsets can exert pro-tumourigenic effects by IL-17A secretion, while Vγ7+ protect against cancer by sensing mutated epithelial cells. Whether γδ T cell subsets promote CRC progression by mechanisms independent from IL-17A is unknown. Here, we utilised a genetically engineered mouse model, Villin1-CreERT2;KrasG12D/+;Trp53F/F;Rosa26Nicd1/+ (KPN) mice, which represents the Consensus Molecular Subtype (CMS) 4 subgroup of CRC and succumbs to intestinal and colonic adenocarcinomas with high rates of metastasis to the liver. In the KPN model, we found that primary tumour growth is associated with an expansion of Vγ4+ and Vγ6+ subsets and a reduction in Vγ7+ cells. We crossed KPN mice with γδ T cell-deficient mice, and observed extended survival of tumour-bearing mice lacking γδ T cells, highlighting the importance of pro-tumourigenic γδ T cells in this model. Further analysis indicated that γδ T cells promoted KPN tumour initation. To determine whether γδ T cells are also necessary to support growth of established tumours, organoids derived from KPN adenocarcinomas were transplanted into both wild-type and γδ T cell-deficient mice. KPN organoids grew in wild-type mice, but failed to efficiently grow in γδ T cell-deficient mice; whereas Villin1-CreERT3;ApcF/F;KrasG12D/+;Trp53F/F;Tgfbr1F/F (AKPT) organoids grew in both mouse lines. Gene expression analysis comparing KPN and AKPT organoids revealed elevated levels of the IL-17 receptor (IL-17RA) and epidermal growth factor receptor (EGFR) in KPN organoids. Phenotypic analysis of Vγ4+ and Vγ6+ subsets in KPN tumours showed that these cells up-regulate ligands to these receptors, IL-17A and amphiregulin (AREG), suggesting the involvement of these two pathways in KPN tumour progression. In vitro and in vivo experiments ruled out a role for the IL-17 pathway in this model. By contrast, the AREG-EGFR pathway was important for KPN growth, because AREG stimulated MAPK and PI3K/AKT signaling in KPN cancer cells, AREG induced proliferation of KPN organoids, and EGFR inhibitors suppressed KPN tumour growth in mice. These data establish an IL-17-independent function of γδ T cells that express AREG to drive progression of CRC. Current efforts are focused on characterizing AREG-expressing γδ T cells in human CRC.

Human splenic γδ T cells are localised for MHC-independent immunosurveillance of the erythroid niche and are enriched in active malaria infection

Presenter Name: Taher Taher

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Primary Theme: Infection Responses

Secondary Theme: Tissues & Inflammation

Abstract

γδ T-cells make important contributions to tissue immunosurveillance and anti-pathogen immunity, however mechanistic understanding is hampered by a lack of robust approaches for imaging them in tissues. We combined immunohistochemistry (IHC) and multiplex immunofluorescence (mIF) with single cell RNAseq (scRNAseq) and TCR repertoire analysis, and applied it to human spleen. IHC revealed that relative to other tissues, γδ T cells were enriched in the spleen. Moreover, unlike conventional splenic αβ T cells, splenic γδ T cells were localised exclusively in the red pulp, adjacent to erythrocytes and splenic macrophages. mIF panels indicated a predominant CD27negTCF7negTbet+GrzBneg 'tissue effector' phenotype distinct from γδ T cells in peripheral blood and other lymphoid organs.

Assessment of scRNAseq data defined a clear CD3+TRDC+ splenic γδ T cell compartment in all individuals. Cell assignment confirmed splenic γδ T cells adopted an effector phenotype. Single cell γδ TCR repertoire assessment indicated the presence of diverse γδ T cell subsets, including a dominant Vδ1+ subset, and also Vγ9negVδ2+, Vδ2negVδ1neg, and Vγ9+Vδ2+ T cells. Substantial clonotypic expansion was evident in adaptive-like (ie non-Vγ9+Vδ2+) T cells. The relative proportions of γδ T cell subsets varied substantially between individuals.

These results suggested human γδ T cells occupy a distinct anatomical niche in the spleen, adjacent to the major MHC-negative erythroid compartment. Consistent with this, IHC comparisons indicated enhanced numbers of red pulp γδ T cells in spleens from Plasmodium falciparum-infected and Plasmodium vivax-infected spleens versus control uninfected spleens, with evidence of increased clustering in the infected scenario. Current directions include assessment of γδ T cell activation markers, and direct imaging of γδ T cells in relation to malaria infected erythroid cells. Overall these results indicate a multifaceted immunosurveillance role for splenic γδ T cells, involving both innate-like and adaptive-like subsets, including potentially in detection and phagocytosis of aged, defective, or infected red blood cells.

Development of Innate-Immune-Cell-Based Immunotherapy for Adult T-cell Leukemia–Lymphoma

Presenter Name: Yoshimasa Tanaka

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Therapies

Abstract

γδ T cells and natural killer (NK) cells have attracted much attention as promising effector cell subsets for adoptive transfer for use in the treatment of malignant and infectious diseases, because they exhibit potent cytotoxic activity against a variety of malignant tumors, as well as virus-infected cells, in a major histocompatibility complex (MHC)-unrestricted manner. In addition, γδ T cells and NK cells express a high level of CD16, a receptor required for antibody-dependent cellular cytotoxicity. Adult T-cell leukemia-lymphoma (ATL) is caused by human T-lymphotropic virus type I (HTLV-1) and is characterized by the proliferation of malignant peripheral CD4+ T cells. Although several treatments, such as chemotherapy, monoclonal antibodies, and allogeneic hematopoietic stem cell transplantation, are currently available, their efficacy is limited. In order to develop alternative therapeutic modalities, we considered the possibility of infusion therapy harnessing γδ T cells and NK cells expanded using a novel nitrogen-containing bisphosphonate prodrug (PTA) and interleukin (IL)-2/IL-18, and we examined the efficacy of the cell-based therapy for ATL in vitro. Peripheral blood samples were collected from 55 patients with ATL and peripheral blood mononuclear cells (PBMCs) were stimulated with PTA and IL-2/IL-18 for 11 days to expand γδ T cells and NK cells. To expand NK cells alone, CD3+ T-cell-depleted PBMCs were cultured with IL-2/IL-18 for 10 days. Subsequently, the expanded cells were examined for cytotoxicity against ATL cell lines in vitro. The proportion of γδ T cells in PBMCs was markedly low in elderly ATL patients. The median expansion rate of the γδ T cells was 1998-fold, and it was 12-fold for the NK cells, indicating that γδ T cells derived from ATL patients were efficiently expanded ex vivo, irrespective of aging and HTLV-1 infection status. Anti-CCR4 antibodies enhanced the cytotoxic activity of the γδ T cells and NK cells against HTLV-1-infected CCR4-expressing CD4+T cells in an antibody concentration-dependent manner. Taken together, the adoptive transfer of γδ T cells and NK cells expanded with PTA/IL-2/IL-18 is a promising alternative therapy for ATL.

Multimodal Profiling of Phosphomycoketide-Specific Vδ1 T Cells in BCG-Vaccinated South African Infants

Presenter Name: Victoria Tappen

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Primary Theme: Ligands & Repertoires

Secondary Theme: Infection Responses

Abstract

The waxy cell wall of Mycobacterium tuberculosis (Mtb) contains a variety of immunogenic lipids, including phosphomycoketides (PM), which are recognized by Vδ1+ T cells via the CD1c antigen presentation pathway. PM is also found in the Bacillus Calmette-Guérin (BCG) vaccine, administered at birth to protect against disseminated TB in childhood. To dissect the molecular basis of CD1c-PM recognition, we analyzed γδ T cell repertoires from BCG-vaccinated South African 10-week-old infants (n=6). This cohort enabled us to characterize Vδ1 responses following in vivo PM exposure and before age-related repertoire focusing.

We used CD1c-PM tetramers to isolate CD1c-PM-specific T cells directly ex vivo and performed sort-based single-cell RNA sequencing to define paired TCRγδ chains, analyze TCRγ constant region allele usage, and determine protein and transcript expression of select phenotypic markers. CD1c-PM+ cells were predominantly Vδ1+ (52 ± 21%). Strikingly, these cells comprised a significant fraction (3.5 ± 1.4%) of the overall Vδ2- repertoire.

Comparing CD1c-PM+ (n=458) and CD1c-PM- (n=440) γδ+ T cells revealed that CD1c-PM+ γδ T cells preferentially displayed a cytotoxic Temra phenotype (perforin+ CD45RA+ CD27-). These T cells also showed evidence of clonal expansion in three infants, with some clones comprising ~10% of the CD1c-PM+ repertoire. CD1c-PM+ cells preferentially utilized TRDV1, TRDJ1, TRGV5/8, and TRGJ2 gene segments, with CDR3γ regions remaining near germline, while CDR3δ regions were longer and incorporated multiple TRDD gene segments. Analysis using TCRdist3 revealed a distinct “YWIGR” CDR3δ hydrophobic motif that is partially encoded by the germline TRDD3 region. This motif was also present in published CD1c-PM+ γδ TCRs, indicating a potentially conserved mechanism of CD1c-PM recognition.

Our study provides the first in-depth characterization of CD1c-PM+ γδ T cells in BCG-vaccinated infants, revealing evidence of clonal expansion, cytotoxic phenotype, and biased TCR features. Future studies will further define the TCR determinants of CD1c-PM binding and assess their impact on vaccine-induced protection against Mtb.

WC1 Functions as a PRR and Co-receptor in the Gamma Delta T cell response to Leptospira and Mycobacterium spp.

Presenter Name: Janice Telfer

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Primary Theme: Infection Responses

Secondary Theme: Ligands & Repertoires

Abstract

OBJECTIVE: Gamma delta T cells are a crucial component of the immune response to a number of increasingly relevant and largely zoonotic pathogens to which efficacious vaccination is lacking. In ruminants and swine, gamma delta T cells represent a major population of peripheral blood and epithelial tissue-resident lymphocytes. Upon activation, gamma delta T cells elicit a variety of effector functions and play an indispensable role of orchestrating the downstream immune response. WC1genes are expressed as a multigenic array on gamma delta T cells in ruminants and swine, and are encoded in the genome of most animal species in varying numbers. WC1 is a member of the Scavenger Receptor Cysteine Rich (SRCR) family, and are homologous to CD163A and CD163c-alpha in humans. In cattle , there are 13 WC1 genes (WC1-1 to WC1-13), each comprised of 6-11 SRCR domains that selectively bind bacterial antigens in a manner analogous to a pattern recognition receptor (PRR). Individual WC1 SRCR domains can be characterized across species with the alphabet designations a-d. WC1 proteins function as a hybrid PRR and co-receptor for the gamma delta TCR, in that they encode specificity of response, co-localize in the immune synapse, and potentiate gamma delta T cell activation. We hypothesize that the WC1 genes across species have co-evolved with pathogens, and thus sought to characterize their diversity and ligand-binding potential.

RESULTS: Unlike the WC1 multigenic arrays in cattle, sheep, goats and swine, there is a single WC1 6-SRCR domain gene in dogs and cats. We confirmed that selected WC1 SRCR domains from cattle, swine, cats and dogs are capable of directly binding to fractionated and whole fixed Leptospira spp or Mycobacterium spp. Surprisingly, the subset and number of SRCR domains from different WC1 proteins that bind to Leptospira or to Mycobacterium are non-overlapping. Transcription of the WC1 that binds to Mycobacterium is upregulated in response to Mycobacterium infection and shows genetic variation in the open reading frame, suggesting that a divergence in function happened soon after the WC1 gene array expansion and is still ongoing.

CONCLUSIONS: Selected WC1 SRCR domains from cattle, swine, dogs and cats bind to Leptospira spp and Mycobacterium spp, supporting the hypothesis that they have co-evolved with important pathogens as a hybrid co-receptor and pattern recognition receptor on gamma delta T cells.

Investigating Gut Microbiota Determinants of IL-17-producing gdT Cells During Early Life

Presenter Name: Bavanitha Thurairajah

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Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

Interleukin-17 producing gd (gd17) T cells are a thymically-imprinted subset of innate-like T cells implicated in host defense against infection at barrier sites but also pathological conditions such as colorectal cancer. Although gd17 T cells seed peripheral tissues during embryogenesis, the impact of early-life events, such as microbial colonization of the intestine, on the activation and function of these cells remains to be determined. Here we use diverse gnotobiotic approaches in mice to examine the impact of the gut microbiota on intestinal gd17 T cell dynamics across the lifespan. We demonstrate that colonic gd17 T cells exhibit spontaneous secretion of IL-17 and IL-22 just prior to weaning, a time point coinciding with significant intestinal growth and robust expansion of the commensal microbiota. Using germ-free mice, we establish that the gut microbiota is critical for this early-life response and that fetal-derived vg6+ gd T cells are the primary source of these cytokines. Further, we show developmental activation of gd17 T cells selectively occurs within the colonic lamina propria, requires IL-1 receptor signals and subsides with age. However, this response is not age-dependent as colonization of germ-free mice with a diverse microbial community during adulthood recapitulates early-life gd17 T cell activation. Reconstitution experiments using the feces of mice from diverse vivariums indicate that microbial composition, not simply colonization per se, determines the magnitude of colonic gd17 T cell activation. Collectively, our results provide new insight into mechanisms regulating intestinal gd17 T cells and set the stage for identifying specific microbial signals that shape the long-term function of this important innate-like T cell subset.

Human Double-Negative T Cells Exhibit a Unique Cellular Composition and Favourable Genetic Signature for Cancer Therapy

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Primary Theme: Cancer Therapies

Abstract

Allogeneic CD3+CD4-CD8- double-negative T-cell (DNT) therapy has emerged as a novel, off-the-shelf cellular treatment with clinical feasibility, safety, and promising efficacy in a phase I clinical trial to treat high-risk patients with acute myeloid leukemia. However, the biology of DNTs is less well characterized, and how DNT therapy distinguishes from conventional γδ T-cell therapy remains unclear. Collectively, this hinders our ability to bolster DNT functionalities in cancer therapy. Here, we performed single-cell RNA sequencing with in vitro and in vivo functional analysis on DNTs. As a significant proportion of DNTs express Vγ9Vδ2 (Vδ2) TCR chain, we compared DNTs with donor-matched conventional Vδ2 T cells expanded with zoledronic acid.

Despite a shared Vδ2 expression between cellular products, we identified unique cellular compositions in DNTs that contribute to distinct transcriptional and cellular communication patterns relative to the donor-matched Vδ2 T cells. This includes higher expression of genes identified in chimeric antigen receptor-expressing DNTs that persisted in patients with durable cancer-remission from three independent clinical trials. Vδ2- DNTs exhibited strong persistence characteristics, and their presence promoted the cytotoxic capabilities of Vδ2+ DNTs in repeated stimulation assays. The unique genetic signature and diverse cellular interactions in DNTs resulted in a better overall ex vivo expansion, prolonged persistence, and superior anti-leukemic activity compared to Vδ2 T cells in vitro and in vivo. These results highlight the distinct transcriptional, cellular, and functional profile of human DNTs and suggest a desirable transcriptional combination for optimal cellular therapies to treat cancer.

Characterisation of γδ T Cell Subtypes in the Human Postnatal Thymus

Presenter Name: Jolien Van Hulle

Jolien Van Hulle* (Department of Diagnostic Sciences, Ghent University, Belgium), Lena Boehme* (Department of Diagnostic Sciences, Ghent University, Belgium), Yana Van Droogenbroek (Department of Diagnostic Sciences, Ghent University, Belgium), Imke Velghe (Department of Diagnostic Sciences, Ghent University, Belgium), Lukas Minack (Department of Diagnostic Sciences, Ghent University, Belgium), Tom Putteman (Department of Diagnostic Sciences, Ghent University, Belgium), Ellen De Meester (Laboratory of Pharmaceutical Biotechnology, Ghent University, Belgium), Filip Van Nieuwerburgh (Laboratory of Pharmaceutical Biotechnology, Ghent University, Belgium & Cancer Research Institute Ghent, Belgium), Tom Taghon (Department of Diagnostic Sciences, Ghent University, Belgium & Cancer Research Institute Ghent, Belgium)

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Primary Theme: Development & Differentiation

Secondary Theme:

Abstract

γδ T cells originate from bone-marrow derived progenitors that differentiate in the thymus. In mice, different effector types have been shown to develop in the thymus through distinct lineage programs. While the same classification of effector types does not apply to human γδ T cells, some effector programming has been observed in the human fetal thymus. However, evidence supporting similar γδ T cell effector programming in the human postnatal thymus remains limited and the intrathymic differentiation of γδ T cells generally remains poorly understood.

To tackle this, we first generated an extensive single-cell CITE-seq data set by profiling γδTCR+ thymocytes from multiple pediatric donors using a customised 166-plex antibody panel. In-depth analysis of the nonVγ9Vδ2 γδTCR+ thymocyte subset revealed the presence of different γδ T cell subtypes in the postnatal thymus with distinct surface marker profiles that showed transcription of specific cytokines and cytolytic enzymes. However, whether these subsets represent intrathymic differentiation stages or if these are independent γδ T cell subtypes with unique molecular programmes remains unclear.

To now address these questions, we employed computational analyses on the CITE-seq data set in combination with flow cytometry to better characterise these novel γδ T cell subsets. We conducted trajectory analysis and fate predictions to investigate the developmental relationships between the different γδ T cell subsets and their connection to immature γδ T cell precursors. To further dissect the transcriptional programmes associated with these stages and identify the underlying transcription factors, we performed gene regulatory network analysis. Additionally, we assessed the expression of genes associated with distinct effector programs within these subsets. Finally, using surface marker data, we designed an antibody panel to screen for these cells in ex vivo thymus samples, which can also be applied in future in vitro differentiation studies.

In summary, in-depth profiling of intrathymic γδ T cell subsets using CITE-seq has allowed us to distinguish previously unreported γδ T cell subsets in the human postnatal thymus. These findings provide a foundation for their further characterisation with respect to their development, origin, function, and tissue distribution through computational and experimental approaches.

Critical Real-time dependence of Human Vδ1+ T cells on TCRγδ

Presenter Name: Nicolas Veland

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Primary Theme: Cancer Responses

Secondary Theme: Cancer Therapies

Abstract

Human γδ cells are particularly attractive for off-the-shelf cancer immunotherapies, because their antitumor activity is independent of MHC restriction and neoantigen burden and combines killing mechanisms of CD8+ γδ T T cells and NK cells. Moreover, the Vδ1+ T cell subset is enriched in epithelial tissues in which multiple cancers form, with high numbers of tissue-resident Vδ1+ T cells being associated with better outcomes in several cancers1,2.

Given their translational potential, a fundamental question needs answering: what is the role of the γδ TCR? While it is the cells' defining feature, many of their properties have been attributed to innate activation via NK receptors (NKRs) and cytokines. Here we address this question. Although protocols exist for expanding human blood Vδ1+ cells, they require reiterative TCR stimulation, which inevitably blurs the issue. Hence, to seek a TCR-independent means of establishing Vδ1+ cells, we examined HTLV1-immortalised blood Vδ1+ cell lines. By using CRISPR, we acutely deleted the TRDV1 gene, with flow cytometry confirming Vδ1 TCR loss and pronounced CD3 downregulation. Bulk RNA-sequencing revealed that over 1300 genes were differentially expressed in TRDV1 KO cells compared to non-targeted controls at steady-state. The TCR-dependent gene expression signature included several Killer-cell immunoglobulin-like receptor (KIR) genes, together with cytotoxic granzyme genes and other genes encoding regulatory surface molecules such as TIGIT, 4-1BB, CRTAM and CD83, all of which collectively contribute to cytolytic responses. These changes were then validated functionally. In contrast, upregulated genes were associated with fatty acid metabolism, oxidative phosphorylation and multiple cytokines associated with wound-healing, which were also reportedly upregulated by nutrient deprivation. These data show a striking real-time dependence on the TCR of Vδ1 cells, even those immortalised by virus.

This approach is now being applied to epithelial-resident Vδ1 and γδ T cells using our TCR-independent methods for large-scale cultivation of primary skin-derived T cells3. Our data provide fundamental new insights into human γδ T cell biology and should inform the refinement of clinical γδT cell modalities.

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T Cell Receptor γδ is a Real-time Determinant of Unique, Innate-like Tissue Immune Surveillance

Presenter Name: Nicolas Veland

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Primary Theme: Development & Differentiation

Secondary Theme: Tissues & Inflammation

Abstract

γδ T cells compose a largely conserved third lineage of lymphocytes with adaptive potential owing to their expression of highly diverse T cell receptors (TCRs). Nonetheless, γδ T cells have overt innate responses, leaving the importance of the TCR unresolved. By acutely ablating the TCR in tissue-intrinsic γδ T cells, we show a real-time requirement for the TCR to maintain core components of the cells' phenotype, at both transcriptional and post-transcriptional levels. Intriguing and largely unexpected modes of TCR-dependent regulation emerged from the comparison of gene and protein expression and chromatin accessibility. Acute loss of the TCR-dependent state was associated with physiologic dysregulation, and although TCR-ablated cells remained competent to respond to challenges in vitro, they seemed totally compromised in vivo in their responses to a variety of innate challenges. This profound, real-time dependence on the TCR clearly distinguishes γδ T cells from innate lymphocytes with implications for immunosurveillance and immunotherapy.

Gamma Delta T Cells Under Fire: How Pregnancy Complications Rewrite Maternal and Fetal Immunity

Presenter Name: Ziqing Wang

Ziqing Wang1, Paulina Lukomska1, Tao Yang1, Aavani Sindhu2, Madeleine Wagner2, Ralf Schild3, Anke Katharina Bergmann2, Christine Morfeld3, Sarina Ravens1
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Primary Theme: Tissues & Inflammation

Secondary Theme: Infection Responses

Abstract

Preeclampsia (PE) and gestational diabetes mellitus (GDM) are pregnancy complications that disrupt maternal health and fetal development, while increasing lifelong metabolic risks for both mother and child. Gamma delta (γδ) T cells, an immune cell subset that predominantly develops in the fetal thymus and that is enriched at the decidua, can have pro- and anti-inflammatory functions. However, their response to pregnancy complications remains poorly understood.

Using single-cell RNA sequencing and flow cytometry, we profiled decidual γδ T cells from pregnancies affected by GDM (n = 4), PE (n = 3), and healthy pregnant women (n = 5), uncovering functional and phenotypic shifts. In GDM, decidual γδ T cells exhibited heightened cytotoxicity alongside mitochondrial dysfunction, signaling immune stress. Notably, CD16+GZMB+ Vδ2 T cells are enriched in GDM, highlighting a hyper-cytotoxic γδ T cell subset potentially contributing to local immune imbalance. In PE, decidual Vδ2 T cells exhibited a distinct proinflammatory signature, reinforcing the link between γδ T cell dysfunction and placental inflammation.

Beyond the maternal compartment, fetal-derived γδ T cells in the cord blood of the offspring from adverse pregnancies showed increased frequencies of granzyme B+ and CD56+ Vδ2 T cells. This suggests that maternal immune dysregulation during pregnancy also imprints on the fetal immune system, potentially predisposing offspring to immune and metabolic disorders later in life.

Our findings indicate a potential role of γδ T cells in immune dysregulation during adverse pregnancies, and imply that modulating maternal-fetal immune crosstalk could improve pregnancy outcomes and long-term offspring health.

HuTCR-T1: A Humanized γδ TCR Mouse Model for Immunological Research and Therapeutic Advancements

Presenter Name: Wei Weng

Ellen Chen, Husheem Michael, Wei He, Wei Weng

Ingenious Targeting Laboratory, Inc. 761-80 Coates Ave Holbrook, NY 11741

Primary Theme: Cancer Therapies

Secondary Theme: Infection Responses

Abstract

The development of humanized gamma delta (γδ) T cell receptor (TCR) mice represents a significant advancement in immunological research, enabling the study of human γδ T cell biology in a physiologically relevant model. Our laboratory utilized inGenious' TruHumanization technology, incorporating a large bacterial artificial chromosome (BAC) genomic DNA platform (>100 kb) to generate humanized γδ TCR mice through precise gene replacement. The murine TCR γ and δ loci were systematically replaced with their human counterparts to ensure appropriate signaling while maintaining functional integrity. Specifically, the humanized TCR γ locus was engineered by replacing the mouse Vγ1 (TRGV1) and J4 segments with human Vγ9-11 and J segments fused to murine C4 regions. Similarly, the humanized TCR δ locus was generated by substituting the mouse TRDV4, D, and J segments with human Vδ1-Vδ8, D, and J segments, fused to a murine C region. The successful replacement led to highly diverse repertoires of human γδ TCRs, which were confirmed across multiple tissues. Our research showed that human TCR repertoires undergo focusing events upon pathogen challenges, similar to what is observed in human patients. Introducing human γδ TCR into mice induced metabolomic changes, aligning mouse metabolites more closely with those of humans. The γδ T cells isolated from T1 mice were able to release anticancer cytokines (IFN-γ, TNF-α, IL-17) when interacting with human cancer cell lines. Notably, our T1 mice showed a stronger immune response, leading to better antibody production than wild-type (WT) mice. Furthermore, we reconstructed the interaction between human butyrophilin (BTNAs) and human Vγ9Vδ2 cells within this model, further validating its clinical relevance. Overall, the development of γδ HuTCR-T1 mice represents a key advancement in bridging experimental studies and clinical applications, offering insights into human immune responses and therapeutic opportunities, particularly in areas where human γδ T cells are relevant.

γδ T cells are the prime anti-tumoral T cells in neuroblastoma derived TIL products

Presenter Name: Monika Wolkers

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Primary Theme: Cancer Therapies

Secondary Theme: Cancer Responses

Abstract

High-risk pediatric neuroblastoma patients have a dismal survival rate despite intensive treatment regimens. New treatment options are thus required. Even though HLA expression in neuroblastoma is low and immune cell infiltrates limited, the presence of tumor infiltrating lymphocytes (TILs) is indicative for better patient survival. Here, we show that most tumor lesions contain viable immune cell infiltrates with high percentages of CD3+ T cells. We therefore expanded the TILs and tested their anti-tumoral activity. With sufficient starting material, TIL expansion was equally efficient as for adult solid tumors. However, whereas TIL products from adult tumors almost exclusively contained αβ T cells, in neuroblastoma-derived TILs γδ T cells expanded with similar efficacy as αβ T cells. Importantly, the anti-tumor responses in response to autologous tumor digest primarily originated from (Vδ1- and Vδ3-expressing) γδ T cells, and not from αβ T cells. In conclusion, this finding creates a window of opportunity for immunotherapy for neuroblastoma patients, with γδ T cells as potential prime responders.

Chemical Synthesis of Mycobacterial Glycolipids (mGLP) for Advanced Immunotherapeutic Applications

Presenter Name: Mei Xia

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Primary Theme: Infection Responses

Secondary Theme: Tissues & Inflammation

Abstract

Tuberculosis (TB) remains a major global health challenge, exacerbated by the TB-HIV coinfection and the emergence of multidrug-resistant TB, necessitating the development of more effective vaccines and therapeutics beyond the bacillus Calmette-Guérin (BCG) vaccine and current first-line treatments. γδ T cells, which bridge innate and adaptive immunity, play a pivotal role in mucosal defense, rapidly responding to TB infection and BCG vaccination. Notably, Mycobacterium-expanded γδ T cells represent a distinct subset characterized by an oligoclonal repertoire of T cell receptor (TCR) sequences that are highly effective in recognizing and inhibiting intracellular Mycobacterium tuberculosis (Mtb).

Our prior studies identified a novel Mtb antigen, O-methyl-glucose-containing lipopolysaccharides (mGLP), which uniquely induce a subset of γδ T cells protective against mycobacteria. Structural analysis of native mGLP revealed acyl (acetyl and isobutyryl) modifications on the non-reducing end Glc residues, critical for its biological activity. Limitations related to the biohazard of purifying sufficient amounts of native mGLP prompted us to develop a synthetic alternative for detailed structure-activity relationship (SAR) studies.

Here, we report the chemical synthesis of a tetrasaccharide mimicking the non-reducing end of mGLP, incorporating its native acetyl, isobutyl, and methyl modifications. Using advanced nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry, we confirmed the identity and purity of the synthetic mGLP. This synthetic molecule demonstrated robust γδ T cell activation leading to generation of γδ T cells able to inhibit intracellular Mtb. Furthermore, we identified two γδ TCR pairings shared between mGLP- and Mtb-antigen-reactive γδ T cells, aligning with clonotypes that consistently expand following BCG vaccination in humans.

Using these TCR sequences, we engineered γδ T cells expressing defined TCRs to elucidate the molecular mechanisms of mGLP antigen recognition and presentation. These engineered γδ T cells were responsive to both synthetic and native mGLP, confirming the bioactivity of the synthesized molecule.

Our findings establish proof-of-concept for the synthesis of mGLP fragments with defined acylation patterns, enabling downstream biological studies. Synthetic mGLP retains the immunological activity of its natural counterpart and represents a promising candidate for the immunotherapeutic treatment of drug-sensitive and multidrug-resistant TB, as well as potential incorporation into next-generation prophylactic TB vaccines.

γδ T Cells From the Very Young to the Very Old and Their Role in Viral Defense

Presenter Name: Tao Yang

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Primary Theme: Development & Differentiation

Secondary Theme: Infection Responses

Abstract

Immune aging is associated with increased autoimmunity, susceptibility to infections and cancer, and a diminished response to vaccinations. Recent single-cell analyses of peripheral blood mononuclear cells (PBMC) have provided valuable insights into the age-related changes of multiple immune lineages, while γδ T cells remain underexplored due to their low abundance in peripheral blood.

Here, by integrating public (Leon-Lara et al. JEM 2024; Cramer et al. Nat Communi 2024; Wang et al. Nat Immunol 2025; Tan et al. Sci Immunol 2021; Terekhova et al. Immunity 2023) and newly generated datasets, we profiled 144.513 γδ T cells from 389 healthy individuals from birth to over 90 years old. γδ T cells show decreased mitochondrial activity with increasing age, which has been described as a hallmark of aging for other immune cells. We identified three major compartments: 1) Naïve Vδ1+ T cell subsets (CCR9hi, CCR9lowSOX4hi, and CCR9lowSOX4int), which progressively lose early differentiation genes with age; 2) CCR4+ or CCR6+ Vδ2 T cell cluster that decline with age; and 3) Vδ1+/Vδ2+ GZMA+ effectors, further classified into GZMKhi (K+) and GZMBhiGZMK/low (B+) subsets. Compared to K+ effectors, B+ effectors express higher levels of cytotoxic genes such as PRF1, GNLY, and NKG7. Notably, Vδ2+ K+ effectors exist since birth, while Vδ2+ B+ effectors emerge postnatally and stabilize after two years.

This reference map allowed us to further explore γδ T cell function in viral infections. For this, we performed a differential gene analysis of γδ T cells from an individual with acute Cytomegalovirus (CMV) infection (Deseke et al. JEM 2022), adults with HBV infections (Zhang et al. Gut 2023), and a newly generated dataset of adult individuals with chronic HIV-1 infection. Upon viral infection, γδ T cells exhibited an enrichment of granzyme-expressing effectors, predominantly Vδ1^{+} T cells. We identified an antiviral program in γδ T cells, including activation markers (e.g., CD38, HLA-DRA, and CD160); type I and type II interferon-related genes; and IFTM1, IFTM2, IFTM3, which inhibit viral replication and thus describe underlying mechanisms of the anti-viral response of γδ T cells.

In sum, our study reveals transcriptional profiles and potential functionalities of γδ T cells from the very young to the elderly, providing a valuable transcriptomic resource for future understanding of γδ T cells in aging and viral infections.

IL-17A-Producing γδ T cells Govern Thyroid Hormone Excess-Mediated Metabolic Complications

Presenter Name: Hyon-Seung Yi

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Ho Yeop Lee, Chungnam National University College of Medicine
Alfin Mohammad Abdillah, Chungnam National University College of Medicine

Primary Theme: Tissues & Inflammation

Secondary Theme: Development & Differentiation

Abstract

Thyrotoxicosis, caused by excessive thyroid hormone production, disrupts energy metabolism, muscle function, and bone integrity. While its effects on musculoskeletal health are well established, the underlying immunometabolic mechanisms remain poorly understood.

To address this, we conducted flow cytometric and single-cell transcriptomic analyses on peripheral blood samples from Graves' disease (GD) patients. Additionally, we assessed muscle and bone mass and function to investigate the link between immune alterations and musculoskeletal health in thyrotoxicosis. We generated a thyrotoxicosis mouse model and analyzed skeletal muscle and bone using electron microscopy and micro-CT. Immune profiling of bone marrow, liver, and adipose tissue was performed, and plasma metabolomics and fatty acid flux assays were utilized to examine the systemic metabolic effects of thyroid hormone excess on γδ T cells.

Our results demonstrated reduced skeletal muscle function in thyrotoxic mice, accompanied by mitochondrial swelling in muscle fibers. Micro-CT and von Kossa staining revealed significant bone loss in both cortical and trabecular compartments due to increased bone resorption. Immune profiling showed a marked expansion of IL-17A-producing γδ T cells in both thyrotoxicosis models and GD patients. Single-cell transcriptomic analysis further identified increased expression of inflammation-related genes in γδ T cells and monocytes. Thyroid hormone excess increased the vδ1:vδ2 T cell ratio, characterized by an expansion of pro-inflammatory vδ1 T cells, which exhibited elevated thyroid hormone receptor expression.

In vitro, triiodothyronine treatment promoted IL-17A production in γδ T cells by activating glycolysis, increasing ACLY expression, and enhancing histone acetylation. Given that lipolysis is a key feature of thyrotoxicosis, we observed increased fat mobilization, muscle dysfunction, and bone loss in thyrotoxic mice. Notably, these changes were absent in TCRδ-deficient mice. Furthermore, IL-17A neutralization mitigated muscle weakness and bone loss, highlighting its pathological role.

Collectively, our findings implicate IL-17A-producing γδ T cells as critical mediators of lipid dysregulation, muscle dysfunction, and bone loss in thyrotoxicosis. These results suggest IL-17A as a potential therapeutic target for mitigating the adverse metabolic and musculoskeletal consequences of excessive thyroid hormone exposure.

Modulation of γδ T Cell Function by BTK Inhibitors: Implications for Immunotherapy

Presenter Name: Michal Zarobkiewicz

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Primary Theme: Cancer Therapies

Secondary Theme: Ligands & Repertoires

Abstract

γδ T cells represent a small yet functionally significant subset (0.5–5%) of peripheral blood T cells, playing a crucial role in pathogen defense and anticancer immunity. While Bruton's tyrosine kinase (BTK) inhibitors have revolutionized the treatment of B-cell malignancies, their effects on non-B cells, including γδ T cells, remain insufficiently explored. Given BTK expression in various immune cell types, understanding its impact on γδ T cell function is essential for optimizing immunotherapeutic strategies.

In this study, peripheral blood mononuclear cells were isolated from buffy coats, and selective γδ T cell expansion was induced using zoledronic acid and IL-2. After 14 days, γδ T cells were exposed to BTK inhibitors (ibrutinib, acalabrutinib, or zanubrutinib) for 48 hours. To assess activation and cytokine production, cells were stimulated with HMBPP or the CLL-derived Duller cell line, followed by flow cytometric analysis. The cytotoxic potential of γδ T cells was evaluated using a CD107a degranulation assay with Duller cells as the target.

The results indicate that BTK inhibitors modulate γδ T cell activity in a stimulation-specific manner. Ibrutinib significantly reduced IFN-γ and TNF-α production in response to HMBPP but not to Duller cells, suggesting that TCR-independent pathways may compensate for BTK inhibition. While all inhibitors diminished early activation (as indicated by reduced CD69 expression), late-stage activation remained unaffected. Notably, next-generation inhibitors demonstrated a reduced inhibitory effect, likely due to their increased BTK selectivity. No significant differences were observed for cytotoxicity.

These findings highlight the nuanced impact of BTK inhibition on γδ T cells, suggesting that its effects may be partially reversible and pathway-dependent. From a therapeutic perspective, integrating BTK inhibitors with γδ T cell expansion strategies could offer a promising avenue for enhancing immunotherapy outcomes.

Study was funded by the DKMS Poland Research Grant [2023-2025]

Super-mbIL15 Significantly Enhances the Antitumor Efficacy of CAR-γδ T Cells

Presenter Name: Lin Zhang

Lin Zhang, Tsinghua University; Yinqiang Sui, Tsinghua University; WeiWei Ma, Tsinghua University; Yonghui Zhang, Tsinghua University

Primary Theme: Cancer Therapies

Secondary Theme: Ligands & Repertoires

Abstract

IL-15, a multifunctional cytokine known to boost the immune activity of T cells, NK cells, and other immune cells, has been extensively studied in antitumor therapy. In cell therapy, IL-15 is often co-expressed with immune cells to enhance their function, typically in two forms: secretory and membrane-bound. While the former has a short half-life and uncontrolled tissue distribution, leading to limitations in efficacy and potential toxicity, the membrane-bound form (mbIL15) links IL-15 and IL-15Rα via a linker and anchors the complex to the cell membrane through the transmembrane domain of IL-15Rα. This approach has demonstrated promising results in various studies.

In this study, we optimized the structure of IL-15/IL-15Rα and designed super-mbIL15(membrane-bound IL-15/IL-15Rα complex), which significantly improved the antitumor activity of CAR-γδ T. We incorporated the super-mbIL15 structure into CD19/B7H3-CAR constructs. Cellular-level experiments revealed that super-mbIL15 did not affect the expansion or transduction efficiency of CAR-γδT compared to unmodified or mbIL15-modified cells. However, super-mbIL15 exhibited higher pSTAT5 expression levels, indicating enhanced IL-15 signaling. Under cytokine-free conditions, super-mbIL15 better sustained the proliferation of CAR-γδT, and exhibited more durable cytotoxicity and higher levels of IFNγ in vitro assays. In vivo studies using CDX animal models further confirmed the superior antitumor efficacy of super-mbIL15, with enhanced peripheral expansion and tissue infiltration of CAR-γδT.

In conclusion, this study introduces an optimized super-mbIL15 structure that significantly enhances the persistence, expansion, and antitumor activity of CAR-γδT, offering substantial potential for clinical applications in cancer immunotherapy.

Leveraging the Potential of Vδ3+ γδT Cells Through Bispecific Antibodies

Presenter Name: Tina Zhang

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Primary Theme: Cancer Therapies

Secondary Theme: Development & Differentiation

Abstract

In humans, γδT cells are typically divided into three dominant subsets defined by their T cell receptor delta chain usage, namely, the Vδ1, Vδ2 and Vδ3+ γδT cell populations. While Vδ1 and Vδ2 subsets have been well characterised as functionally distinct populations, far less is known about the biological features and roles of Vδ3+ γδT cells. This is largely due to a lack of readily available reagents to isolate these cells. To address this, we have developed a monoclonal antibody (mAb) that specifically bind Vδ3+ T cell receptors. Although Vδ3+ γδT cells are typically less frequent than Vδ1 and Vδ2+ γδT cells in healthy blood, they have the capacity for robust in vitro expansion in response to TCR stimulation.

Utilising a combination of flow cytometry and single cell RNA sequencing, we demonstrate that Vδ3+ γδT cells phenotypically align with Vδ1+ γδT cells and are distinct to Vδ2+ γδT cells. In addition, their phenotype of high granzyme A, B and granulysin expression at steady state indicate that they may possess cytotoxic potential. To test this, we engineered the anti-Vδ3 mAb into a bispecific antibody directing Vδ3+ γδT cells towards the model tumour antigen Her2. In vitro expanded Vδ3+ γδT cells efficiently killed Her2+ breast tumour cell lines with comparable efficiency to Vδ2+ γδT cells when activated by the Vδ3+ γδTCR bispecific antibody and outcompeted a CD3-targetting bispecific control.

Together, we show that Vδ3+ γδT cells can be phenotypically and functionally similar to Vδ1+ γδT cells and we have developed bispecific antibodies that specifically harness the functionality of this subset. Considering the emerging interest and utility of Vδ1+ γδT in cancer immunotherapy, this study highlights the potential of Vδ3+ γδT cells as a novel immunotherapeutic target.

Integrative Analysis of Single-cell Datasets Reveals Disease-specific γδ T Cells Phenotypes

Presenter Name: Yu Zhang

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Primary Theme: Tissues & Inflammation

Secondary Theme: Cancer Responses

Abstract

T cells play a crucial role in liver diseases, exhibiting features of both innate and adaptive immunity. However, their phenotypic and clonotypic diversity across liver diseases remains underexplored. In this study, we profiled a total number of > 20,000 γδ T cells in liver tissue samples derived from patients with alcoholic liver disease and colorectal cancer with liver metastasis using single-cell transcriptomic data generated from Parse Biosciences and 10x Genomics platforms. Additionally, we identified and re-analyzed hepatic γδ T cells in public scRNA-seq datasets from 500 patients across various liver conditions, including healthy individuals, primary sclerosing cholangitis, hepatitis B virus infection, and liver cancer.

With over 45,000 γδ T cells analyzed from both PBMC and 55,000 from liver tissues, we identified disease-specific γδ T cell subsets. Clonal expansion and T cell receptor (TCR) repertoire analysis revealed antigen-specific responses and dynamic clonal changes associated with disease progression. Our findings suggest that γδ T cells undergo clonotypic expansion and differentiation, giving rise to distinct peripheral and liver-resident memory subsets.

Unlocking the Potential of γδ CAR-T Cells: Tailoring CAR Designs to Exploit the Unique Properties of γδ T Cells

Presenter Name: Marina Zintchenko

Marina Zintchenko, Susana Minguet

Primary Theme: Cancer Therapies

Secondary Theme:

Abstract

γδ T cells are unconventional T cells that play a key role in immune responses against infections and cancers, with promising potential in immunotherapy. Unlike αβ T cells, γδ T cells recognize transformed cells through MHC-independent mechanisms, sensing stress-induced molecules and phosphoantigens on tumor cells. This unique ability makes them ideal candidates for off-the-shelf immunotherapies without the risk of graft-versus-host disease.

While FDA-approved CAR T cell therapies targeting CD19 have shown success in B-cell malignancies, their reliance on autologous αβ T cells poses challenges. Conventional CAR designs combine the binding potential of antibodies with the activation potential of the CD3ζ chain. Our recent study demonstrated that alternative CAR designs incorporating CD3δ, CD3ε or CD3γ cytoplasmic tails enhance αβ CAR T cell function in vivo, with BBδ CAR exhibiting superior anti-tumor efficacy (Velasco et al., 2023).

In this study, we investigated novel CAR designs to determine whether γδ T cell activation and anti-tumor functionality can be enhanced by subtype-specific CAR engineering. We optimized human γδ T cell expansion and CAR transduction protocols to evaluate CAR performance in three different bulk γδ T cell populations, each protocol with a define enrichment of Vd2, Vd1 or Vd2-/Vd1- cells. Our results demonstrate that the expansion and transduction protocol do not impact the functionality of the CAR T cells. Contrary to our initial hypothesis, we observed that the CAR design primarily dictates anti-tumoral functions across different T cell subtypes. CAR constructs emerged as the dominant factor in determining cell activation and anti-tumor efficacy. This finding implies that well-designed CARs can potentially overcome intrinsic limitations of specific T cell subsets, providing a more universal approach to enhancing anti-tumor functionality.

By comparing various CAR constructs across different T cell subsets, our study contributes to unlocking the full anti-tumor potential of γδ T cell-based immunotherapies. These findings have significant implications for the development of more effective and versatile CAR T cell therapies in cancer treatment.