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Brief Report

Ageing affects the CD4$^+$ T cell polarization and mucosal tropism induced by TLR2/TLR4-activated dendritic cells

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Abstract

Toll-like receptor (TLR)2 activation induces aldehyde dehydrogenase enzymes in nonmucosal dendritic cells (DCs) enabling them to metabolize vitamin A into all-trans retinoic acid, which induces the expression of mucosal homing molecules (α4β7 and CCR9) in the activated T cells. Recently, we have shown that the simultaneous activation of nonmucosal DCs through TLR2 and TLR4 maintains such capacity while reinforcing the polarization of primed CD4$^+$ T cells towards Th1. Here, we observed that TLR2/TLR4 stimulation of aged DCs leads to the production of less TNFα and more IL-10 and that CD4$^+$ T cells primed by those DCs express lower levels of the mucosal homing receptor CCR9 and produce less type-1 (IFNγ) and more type-2 (IL-4 and IL-13) cytokines. These results emphasize the importance of considering the age-related alterations in DC function when developing novel immunomodulation strategies that rely on the DC-T cell crosstalk through stimulation of pattern recognition receptors.

Keywords: aging, dendritic cells, intestine, toll-like receptor 2, toll-like receptor 4

1. Introduction

Effective subunit vaccines rely not only on the identification of relevant target antigens, but also on the ability to tailor the immune response to the nature of the infectious agent. This requires programming the appropriate protective effector mechanisms while controlling their localization to the pathogen's port of entry or replication sites. Dendritic cells (DCs) play a central role in T cell polarization upon priming and the possibility to influence this process through the activation of pattern recognition receptors (PRRs) on DCs has long been recognized.[1] More recently, DCs were also shown to play a critical role in directing lymphocyte homing to different tissues. For instance, mucosal-associated DCs, unlike DCs from other locations, express aldehyde dehydrogenase (ALDH) enzymes that convert vitamin A into all-trans retinoic acid, which functions as a signal for T cells to express surface molecules involved in mucosal homing.[2] These homing-imprinting properties can also be modulated through PRRs,[3–5] and TLR2 has emerged as a key receptor for the induction of mucosal immunity. Extra-intestinal DCs were shown to acquire gut-specific imprinting properties when stimulated through TLR2/1 by Pam_3CSK_4, but not by signals from other TLRs.[6] Recently, we found that the simultaneous activation of nonmucosal DCs through TLR2 and TLR4 maintains the gut homing instructing capacity while reinforcing the polarization of primed CD4$^+$ T cells towards a Th1 profile,[7] suggesting that the inclusion of TLR2/TLR4 agonists in vaccines and immunotherapy could be explored to promote the gut localization of Th1 cells. These strategies could be particularly impactful to the most vulnerable populations, including the elderly, for whom vaccination and immunotherapies are more necessary and often less effective. Despite the success of several new T cell-dependent vaccines, the decline in vaccine effectivity with age is now well recognized.[8–10] Although the cellular and molecular mechanisms remain unclear, defects in the function of both DCs[11–13] and T cells[14–16] have been identified, including alterations in PRR function in aged DCs.[17,18] However, the changes in DC functions that may affect the tissue homing instructions imprinted on T cells are largely unknown.

In this Brief Report, we present results showing an intrinsic dysfunction of aged DCs to respond to simultaneous activation via TLR2/TLR4, affecting CD4$^+$ T cell polarization and the expression of molecules associated with tissue tropism upon priming. To our knowledge, this is the first time that an age-related alteration in the DC's capacity to imprint signals for mucosal homing receptors on T cells has been described. The results extend our previous findings highlighting the importance of considering age-related changes when developing novel vaccine and immunotherapy strategies that rely on modulation of DC-T cell crosstalk through PRR activation.

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2. Materials and Methods

2.1 Mice and animal procedures

C57BL/6 mice were purchased from IGC (Lisbon, Portugal). OT2.RAG2$^{-/-}$ mice were bred and provided by the LGraça laboratory (GIMM, ULisboa). The mice were maintained at the FMV Animal Facility (FMV, ULisboa) under specific pathogen-free conditions, in cages with sawdust as bedding, in temperature-controlled rooms (22 to 25 °C) with a 12-h light/dark cycle, provided standard diet and water ad libitum. The mice were females aged 5 to 6 mo—OT2.RAG2$^{-/-}$ mice and young groups—or 19 to 20 mo—old groups. Experiments compared cells from young and old mice tested simultaneously. All procedures followed Portuguese (Decreto-Lei 113/2013) and European Commission (Directive 2010/63/EU) guidelines and were approved by FMV's Ethics and Animal Welfare Committee (ORBEA, FMV, ULisboa) and the Portuguese National Agency for Animal Health (DGAV licence number 0421/000/000/2020).

2.2 DC isolation

Spleens were washed in sterile PBS, cut into pieces in 6 cm petri dishes and incubated in 10 mL of prewarmed RPMI medium (ThermoFisher, #11875093) with DNAse I (Roche, #11284932001; 1 μg/mL) and collagenase IV (Gibco, #17104-019, 200 U/mL) at 37 °C for 30 min with occasional stirring. Splenocytes were then collected by grinding the spleen pieces through sterile 70 μm strainers (Corning, #431751) using a syringe plunge. Filtered splenocytes were centrifuged (300 × *g*, 6 min), resuspended in red cell lysis buffer (Invitrogen, #00-4333-57), 5 mL/spleen, and incubated at room temperature for 5 min. After two washes with magnetic cell sorting (MACS) buffer (1X PBS + 2% FCS + 2 mM EDTA), DCs were isolated by magnetic negative selection using the MojoSort Mouse Pan Dendritic Cell Isolation Kit (Biolegend, #480097) according to the manufacturer's instructions.

2.3 DC stimulation

TLR ligands (Invivogen: P_3CSK_4, #tlrl-pms; MPLA, #tlrl-mpls) were diluted in X-Vivo 15 medium (Lonza, #LONZBE02-060F). Negatively selected DCs were stimulated with the TLR agonists at 1 μg/mL for 24 h in serum-free X-Vivo 15 medium. Cell suspensions were collected and centrifuged (300 × *g*, 8 min). Supernatants were stored at −80 °C for later cytokine evaluation, and pelleted DCs were washed twice with PBS and used for the Aldefluor assay or cocultured with CD4$^+$ T cells.

2.4 ALDH activity

The Aldefluor assay (Stemcell Technology, #01700) was used to assess ALDH activity in DCs stimulated with TLR ligands following the manufacturer's protocol with slight modifications. Cells (10^5/well in 96-well plates) were washed in Aldefluor Assay Buffer, resuspended in 200 μL of the same solution containing Aldefluor Reagent and incubated for 40 min at 37 °C. After washing, cells were stained for immunophenotyping by flow cytometry and resuspended in 0.5 mL Aldefluor Assay Buffer.

2.5 CD4$^+$ isolation for DC cocultures

Spleens from OT2.Rag2$^{-/-}$ mice were washed in sterile PBS and disrupted using a syringe plunger over a moistened 70 μm strainer coupled to a 50 mL tube (Corning, #734-1812). After erythrocyte lysis (Invitrogen, #00-4333-57, 5 min at room temperature), cells were washed in MACS buffer and processed for magnetic separation using the mouse Naïve CD4$^+$ T cell Isolation kit (Miltenyi Biotec, #130-104-453), following the manufacturer's recommendations.

2.6 DC-T cell cocultures

Stimulated DCs were pulsed with MHC class II $Ova_{323-339}$ peptide (Invivogen, #vac-isq; 100 mM) for 2 h at 5% CO_2 and 37 °C, then washed in PBS and cocultured with naïve CD4$^+$ cells from OT2.Rag2$^{-/-}$ mice at a 1:1 ratio (0.5×10^6/mL each) in X-Vivo 15 medium with freshly added retinol (Sigma-Aldrich, #R7632) to a final concentration of 50 nM. Cocultures were incubated for 5 d at 37 °C with 5% CO_2.

2.7 Flow cytometry

Single-cell suspensions from DC stimulations and DC-T cell cocultures were surface stained in PBS with 2% FBS (Gibco, Premium Plus, #A4766801): DCs for 30 min at 4 °C, and cocultures with OT2 cells for 15 min at 37 °C plus 15 min at room temperature. Staining was performed with anti-CD11c (clone N418, eBioscience), anti-MHCII (I-A/I-E) (clone M5/114.15.2, eBioscience), anti-CD19 (clone 1D3, Invitrogen), anti-Thy1.2 (clone 53-2.1, eBioscience), anti-CD8a (clone 53-6.7, Invitrogen), anti-CD4 (clone RM4-5, eBioscience), anti-CD44 (clone IM7, Biolegend), anti-α4β7 integrin (clone DATK32, Invitrogen), anti-CCR9 (clone eBioCW-1.2, Invitrogen), anti-TLR4/CD284-MD2 (clone MTS510, Biolegend), and anti-TLR2/CD282 (clone QA16A01, Biolegend). Cell viability was assessed using a LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Invitrogen, #L34975) to exclude dead cells. If necessary, cells were fixed with the Foxp3/Transcription Factor Staining Buffer Set (eBioscience, #00-5523-00). Flow cytometry was performed on an Attune NxT cytometer (ThermoFisher), and data were analyzed using FlowJo v10 software (TreeStar).

2.8 Cytokine quantification

Cytokine levels in culture supernatants were assessed by ELISA using mouse Ready-Set-Go kits (eBiosciences, #88-7064-88; #88-7105-88; #88-7121-88; #88-7324-88) for IL-6, IL-10, IL-12(p70), TNF-α, or by the Legendplex multiplex assay (Biolegend) for cytokines associated with Th1 (IFNγ), Th2 (IL-4, IL-5, IL-13), Th17 (IL-17A), and Treg (IL-10) in the same sample. Manufacturer's instructions were followed in both cases. The experiment comparing cytokines in cocultures with young *vs* old DCs was performed alongside a comparison between "FLT3L-expanded young DCs" *vs* "bona fide young DCs" reported in our publication describing the TLR2/TLR4 effect on DCs.[7] Therefore, the multiplex assay results from bona fide young DCs in Fig. 1E are also reported there in figure 3C, bottom row—Bona fide DCs—P3C + MPLA group.

2.9 Quantification and statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0. Data are presented as mean ± standard error of the mean (s.e.m.) of three replicates. Group differences between young and old mice were assessed using unpaired two-tailed Student's t-test, with significance ranked as follows: ns, not significant; $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

3. Results

We investigated how aging affects DCs' ability to induce signals for mucosal tropism while at the same time influencing the polarization of primed CD4$^+$ T lymphocytes. DCs from aged vs young animals were stimulated via TLR2/TLR4, using the TLR2 agonist Pam_3CSK_4 and the TLR4 agonist MPLA, and cocultured with

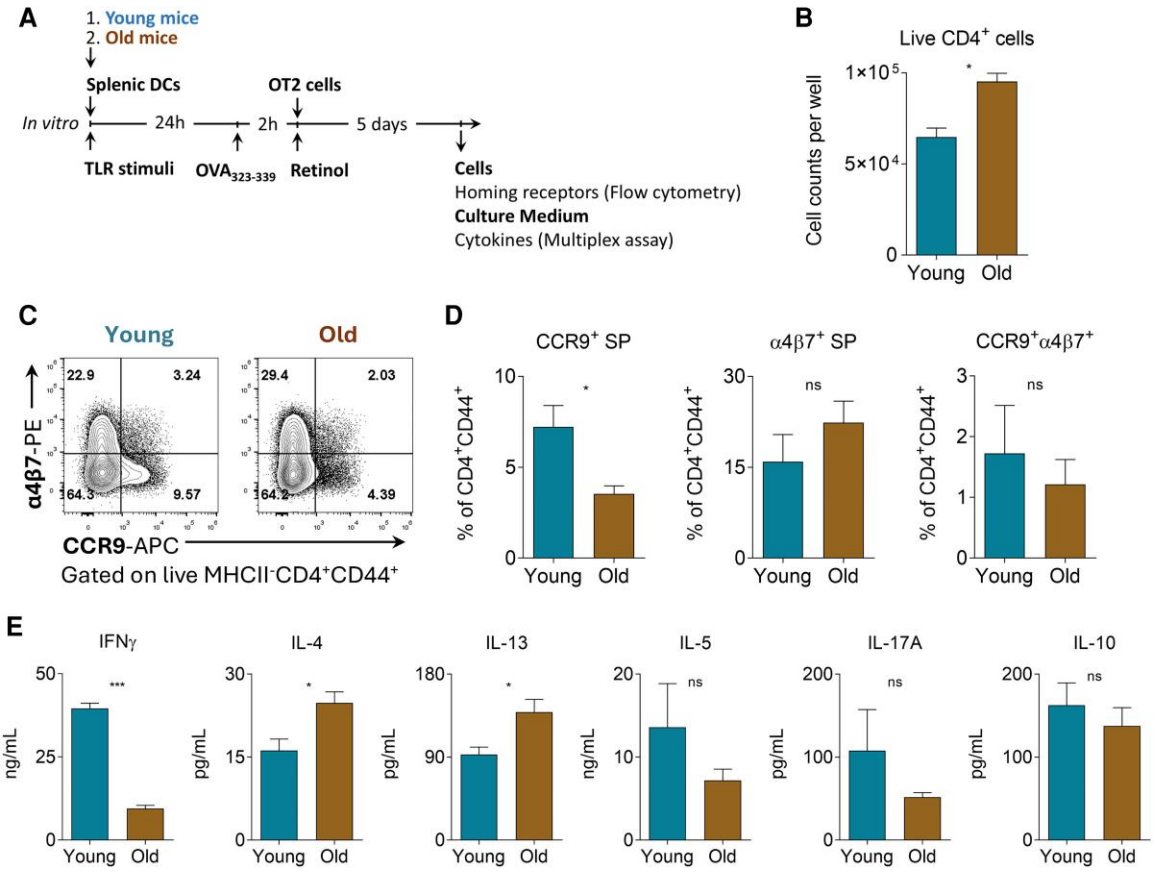

Fig. 1. CD4$^+$ T cells primed by TLR2/TLR4-activated DCs from old animals express lower levels of the gut homing receptor CCR9 and produce less type 1- and more type 2-cytokines. Experimental design of the in vitro DC-T cell coculture system (A). DCs isolated by MACS negative selection from the spleens of young and old C57BL/6 mice were stimulated with the TLR2 agonist Pam_3CSK_4 together with the TLR4 agonist MPLA. After 24 h, the DCs were loaded with MHC class II OVA peptide and cocultured with OT2 cells in the presence of retinol. After 5 d, the culture medium and cells were collected and analyzed by flow cytometry and multiplex assay. Absolute quantification of live CD4$^+$ cells at the end of coculture by flow cytometry (B). Representative contour plots (C) and quantification (D) of gut homing receptors (CCR9 and α4β7; SP—single positive) surface expression on activated OT2 cells (MHCII$^-$CD4$^+$ CD44$^+$) analyzed by flow cytometry. Quantification of the cytokines IFNγ, IL-4, IL-13, IL-5, IL-17A, and IL-10 in the culture medium by multiplex assay (E). Note that the unit for the IFNγ graph is ng/mL, whereas for the remaining cytokines it is pg/mL. Data are presented as mean ± s.e.m. and analyzed by Student's *t*-test: $^*P < 0.05$, $^{***}P < 0.001$; ns, not significant.

naïve ovalbumin (OVA)-specific CD4$^+$ (OT2) cells in the presence of retinol (Fig. 1A). By flow cytometry, we observed that T cells proliferated more (Fig. 1B) when activated in culture with aged DCs and that the induction of CCR9 was reduced in T cells primed by aged DCs stimulated via TLR2/TLR4 when compared with DCs from young animals (Fig. 1C and D).

We then quantified cytokines in the medium of the cocultures using a multiplex assay, testing Th1 (IFNγ), Th2 (IL-4, IL-5, IL-13), Th17 (IL-17A), and regulatory (IL-10)-related cytokines (Fig. 1E). While no differences were observed for the IL-5, IL-17A, and IL-10 cytokines, we found a striking reduction of IFNγ and increased IL-4 and IL-13 levels in cocultures with aged DCs stimulated via TLR2/TLR4. These results indicate a skew toward Th2 or a less-polarized Th1 differentiation. To clarify whether these alterations were specific to the double stimulation via TLR2/TLR4, we assessed the same parameters in DCs activated via TLR2 alone and TLR4 alone (Fig. 2). In both cases, CCR9 expression was not reduced (Fig. 2A), whereas a reduction of IFNγ and IL-13 production was observed for TLR2 activation and a reduction of IL-4 for TLR4 activation (Fig. 2B). Therefore, our results suggest that the defect in aged DCs in the priming of Th1 responses with a concomitant defect in the induction of mucosal tropism receptors, specifically CCR9, is characteristic of double stimulation by TLR2 plus TLR4.

To investigate if the observed differences resulted from altered TLR expression in aged DCs, we evaluated the expression of TLRs in DCs of young vs aged animals by flow cytometry. We established a panel to test TLR2 and TLR4/MD2 surface expression in mouse DCs and performed an antibody titration that showed the expected staining pattern for the anti-TLR2 antibody and a poor staining for the anti-TLR4 antibody (not shown). We then tested the TLR expression in *ex vivo* DCs after enrichment by MACS (Fig. 3A and B) and found no differences in the percentage of positive cells and fluorescence intensity of cell staining (mean fluorescence intensity [MFI]) between DCs from young and aged animals (Fig. 3B).

To functionally evaluate the activity of ALDHs in splenic DCs stimulated via TLR2/TLR4, we incubated MACS-sorted DCs from young vs old animals with Pam_3CSK_4 plus MPLA and performed the Aldefluor assay. In this assay, cells are incubated with a fluorescent nontoxic ALDH substrate, which diffuses into viable cells and when metabolized is retained inside the cell, leading to a fluorescence intensity proportional to the ALDH enzymatic activity. No differences were found in the ALDHs activity of DCs from old vs young animals under TLR2/TLR4 combined stimulation (Fig. 3C and D).

Finally, we looked at whether cytokine production by DCs in our system was altered by aging. Since the aged immune system

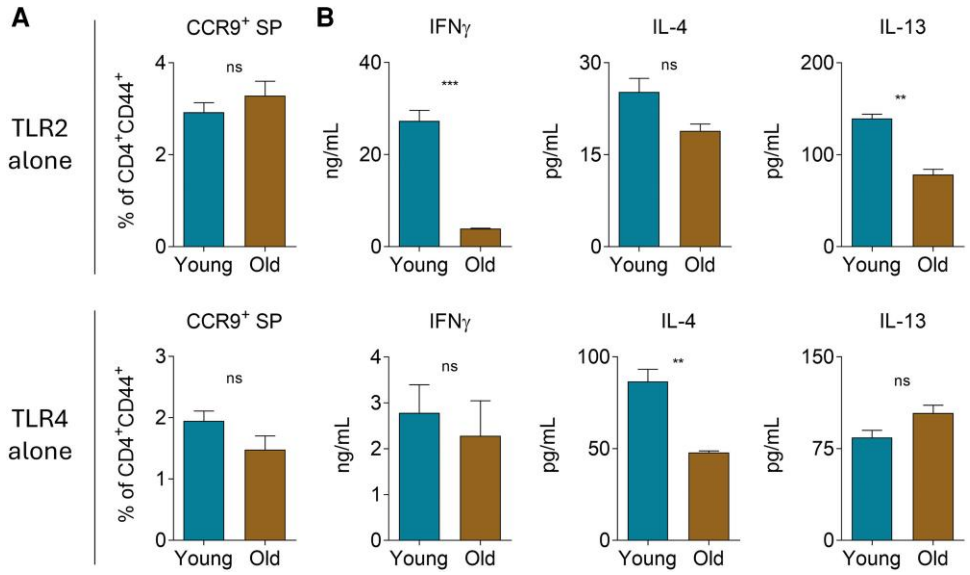

Fig. 2. The defect of aged DCs in priming Th1 responses with a concomitant defect in inducing mucosal tropism is specific to double stimulation by TLR2 plus TLR4. DCs isolated by MACS negative selection from the spleens of young and old C57BL/6 mice were stimulated with the TLR2 agonist Pam_3CSK_4 alone (top row) or with the TLR4 agonist MPLA alone (bottom row). After 24 h, the DCs were loaded with MHC class II OVA peptide and cocultured with OT2 cells in the presence of retinol. After 5 d, the culture medium and cells were collected and analyzed by flow cytometry and multiplex assay. Quantification of the surface expression of the gut homing receptor CCR9 (SP) on activated OT2 cells ($MHCII^-CD4^+$ $CD44^+$) analyzed by flow cytometry (A). Quantification of the cytokines IFNγ, IL-4 and IL-13 in the culture medium by multiplex assay (B). Note that the unit for the IFNγ graph is ng/mL, whereas for the remaining cytokines it is pg/mL. Data are presented as mean ± s.e.m. and analyzed by Student's *t*-test: $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant.

is often characterized by an inflammatory landscape under homeostatic conditions, we quantified the pro-inflammatory cytokines TNFα and IL-6 in supernatants of unstimulated DC cultures, but observed no differences between aged and young DCs (Fig. 3E). We then measured by ELISA a set of cytokines relevant for T cell activation and polarization upon TLR2/TLR4 stimulation (Fig. 3F). Under these activation conditions, we found a slight reduction in TNFα and a strong increase in IL-10 secretion by DCs from aged animals compared with cells from young animals (Fig. 3F).

4. Discussion

Our results show that age influences the response of DCs to dual stimulation via TLR2/TLR4, as well as the subsequent polarization and induction of tissue homing receptors on $CD4^+$ T cells primed by these DCs. In our system, the effects on the primed T cells—reduction in CCR9 expression and pronounced decline in IFNγ production—could not be attributed to diminished TLR2 expression levels on aged DCs. This finding aligns with studies quantifying TLR expression on aged conventional DCs, which show stable TLR expression with age,[19] including TLR2 and TLR4 in both human monocyte-derived and conventional DCs[20,21] and in mouse bone marrow-derived DCs.[19]

Regardless of surface expression levels, the functional responses of TLRs have been shown to be altered in some circumstances in aged animals, as was the case in the present study. However, the results reported in the literature regarding the cytokine response of aged DCs to PRR agonists are conflicting.[19–23] In our study, using bona fide mouse splenic DCs, we observed no difference in IL-6 production in aged DCs stimulated via TLR2/TLR4, but TNFα levels were slightly reduced. However, the most notable alteration was an exceptional elevation in IL-10 production by aged DCs. In older individuals, lower IL-10 levels together with higher IFNγ and granzyme B activity determined upon *ex vivo* restimulation of peripheral blood mononuclear cells have been shown to be a good correlate of protection for an influenza vaccine,[24] suggesting a possible role for IL-10 in the reduced vaccine response in the elderly. In fact, IL-10 levels in human serum[25] and mouse lymphoid and nonlymphoid tissues[26] are increased in aging individuals and IL-10-producing Tfh cells have been shown to be a likely important IL-10 source in aged mice.[26] However, the contribution of DCs to this overall increase has not been documented and the effect of aging on the IL-10 response of antigen-presenting cells when stimulated by pathogens or pathogen-derived molecules remains unclear, with both increases[27,28] and reductions[29] in IL-10 production reported. These variations may reflect differences in the animal species and antigen-presenting cell models used, and the fact that the signaling pathways downstream of the different PRRs studied, including TLR2 and TLR4, are not fully coincident. Interestingly, the functional defects in aged DCs in our system are prominent specifically upon combined TLR2/TLR4 stimulation, while responses to individual agonists show different patterns of alteration. This suggests that the age-related dysfunction may lie not merely in the capacity to respond to a single TLR, but rather in the integration, synergistic crosstalk, or sustained coordination of signals from these distinct pathways. Several potential cellular mechanisms could underpin this loss of coordination in aged DCs. For instance, alterations of the PI3K/AKT–MAPK axis, a regulator of TLR signaling,[30] has been reported in aged DCs.[21] Furthermore, age-associated metabolic reprogramming and oxidative stress[31] or epigenetic modifications, such as altered histone methylation patterns affecting promoter accessibility,[32] might differentially impact the DCs' capacity to mount a coordinated response to multiligand stimuli vs singular agonists.

The observed marked decrease in IFNγ and increase in IL-4 and IL-13 in our study is possibly related with the enhanced DC production of IL-10, as it cannot be attributed to alterations in IL-12, which

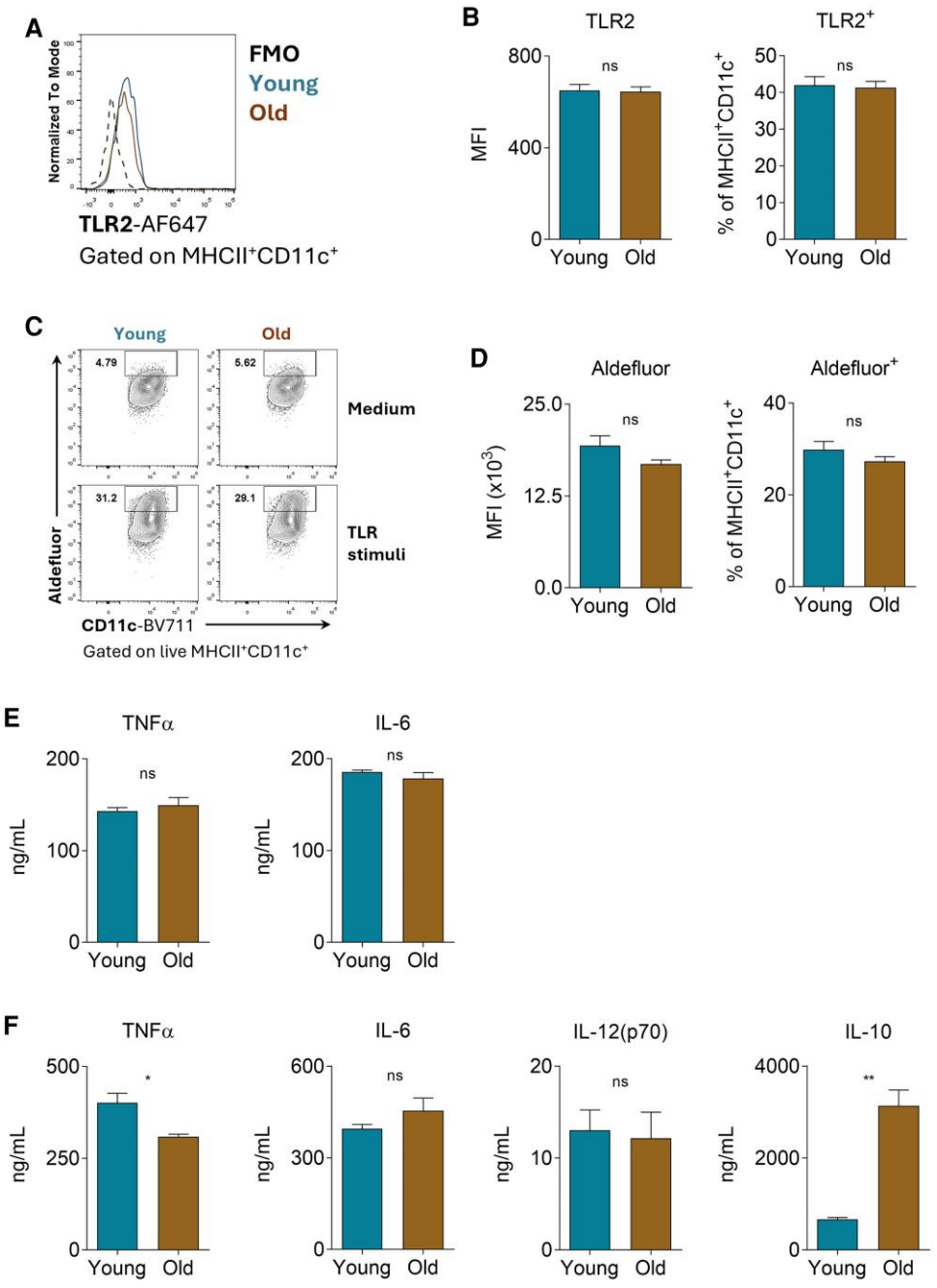

Fig. 3. The alterations in DC functions from old animals are not due to reduced TLR expression or reduced ALDH activity by the stimulated DCs, but the cytokine profile is altered. DCs, isolated by MACS negative selection from spleens of young and old C57BL/6 mice, were collected and analyzed by flow cytometry. (A) Representative histograms with fully stained (solid lines) and fluorescence minus one (FMO) control (dashed line) and (B) quantification of TLR2 MFI and frequency of TLR2 positive DCs (MHCII$^+$ CD11c$^+$ cells) analyzed by flow cytometry. DCs, isolated by MACS negative selection from spleens of young and old C57BL/6 mice, were cultured unstimulated (Medium) or stimulated with the TLR2 agonist Pam_3CSK_4 and the TLR4 agonist MPLA for 24 h. Representative contour plots (C) and quantification (D) of the Aldefluor reaction (MFI and frequency of positive) in cultured DCs (gated on MHCII$^+$ CD11c$^+$ cells) analyzed by flow cytometry. Quantification of the cytokines TNFα and IL-6 by ELISA in supernatants of DCs cultured unstimulated (E) or the cytokines TNFα, IL-6, IL-12(p70) and IL-10 upon TLR2/TLR4 stimulation (F). Data are expressed as mean ± s.e.m. and analyzed by Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$; ns, not significant.

was unaffected. This is consistent with other studies that have not observed changes in IL-12 production by TLR-stimulated aged DCs,[19] while others have reported reduced IL-12 levels.[20,23,32]

In our system, CD4$^+$ T cells cocultured with stimulated aged DCs have shown reduced CCR9 expression. Decreased CCR9 expression on CD4$^+$ T cells from aged mice compared with those from young mice upon retinoic acid treatment has been reported previously,[33] as well as defective functions of retinoic acid-treated DCs.[34] However, to our knowledge, this is the first report of an age-related defect in the DC imprinting of mucosal homing receptors on T cells. Factors other than ALDH activity in splenic DCs may explain this effect, since the results of the Aldefluor assay suggest that this enzymatic function is preserved in aged DCs upon TLR2/TLR4 stimulation.

Since in our coculture system, only the DCs are from old animals, the decreased imprinting of the gut homing receptor CCR9 could be due to an aged environment created in the coculture by the DCs. This could be due to the altered cytokine environment or due to changes in antigen presentation, which are known to occur in old DCs.[17] It is known that the antigen dose has an impact on the expression of homing receptors,[35] therefore altered TCR signals transmitted by the aged DCs could contribute to the observed effect. It will also be important in the future to investigate whether an aging environment can influence the sensitivity and signaling of T cells in response to retinoic acid in vivo. For example, changes in the expression levels of retinoic acid receptors, retinoid X receptors and their binding to retinoic acid responsive elements[36] should be evaluated.

Our results emphasize the importance of considering age-related alterations in DC functions when developing vaccine or immunotherapy strategies that rely on the modulation of DC-T cell crosstalk through PRR activation.

Author contributions

A.P.B. and A.L. conceptualized the study. S.Z., I.L.S.D., M.T.R., A.C.M., D.S., S.N., and A.P.B. performed research. S.Z., M.F., I.L.S.D., S.N., L.G., A.L., and A.P.B. contributed to designing research, analyzing data, and writing the paper. All authors read and approved the final manuscript.

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Conflicts of interest. The authors declare no conflict of interest

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Manicassamy S, Pulendran B. Modulation of adaptive immunity with toll-like receptors. *Semin Immunol*. 2009:21:185–193. https://doi.org/10.1016/j.smim.2009.05.005
2. Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol*. 2008:9:981–987. https://doi.org/10.1038/ni.f.208
3. Ng SC, Kamm MA, Stagg AJ, Knight SC. Intestinal dendritic cells: their role in bacterial recognition, lymphocyte homing, and intestinal inflammation. *Inflamm Bowel Dis*. 2010:16:1787–1807. https://doi.org/10.1002/ibd.21247
4. Manicassamy S, *et al*. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. *Nat Med*. 2009:15:401–409. https://doi.org/10.1038/nm.1925
5. Stock A, Napolitani G, Cerundolo V. Intestinal DC in migrational imprinting of immune cells. *Immunol Cell Biol*. 2013:91:240–249. https://doi.org/10.1038/icb.2012.73
6. Wang S, *et al*. MyD88-dependent TLR1/2 signals educate dendritic cells with gut-specific imprinting properties. *J Immunol*. 2011:187:141–150. https://doi.org/10.4049/jimmunol.1003740
7. Zúquete S, *et al*. Combined TLR2/TLR4 activation equip non-mucosal dendritic cells to prime Th1 cells with gut tropism. *iScience*. 2024:27:111232. https://doi.org/10.1016/j.isci.2024.111232
8. Lee JL, Linterman MA. Mechanisms underpinning poor antibody responses to vaccines in ageing. *Immunol Lett*. 2022:241:1–14. https://doi.org/10.1016/j.imlet.2021.11.001
9. Chen J, Deng JC, Goldstein DR. How aging impacts vaccine efficacy: known molecular and cellular mechanisms and future directions. *Trends Mol Med*. 2022:28:1100–1111. https://doi.org/10.1016/j.molmed.2022.09.008
10. Hou Y, *et al*. Insights into vaccines for elderly individuals: from the impacts of immunosenescence to delivery strategies. *NPJ Vaccines*. 2024:9:1–13. https://doi.org/10.1038/s41541-024-00874-4
11. Stebegg M, *et al*. Rejuvenating conventional dendritic cells and T follicular helper cell formation after vaccination. *Elife*. 2020:9:e52473. https://doi.org/10.7554/eLife.52473
12. Agrawal S, Gollapudi S, Gupta S, Agrawal A. Dendritic cells from the elderly display an intrinsic defect in the production of IL-10 in response to lithium chloride. *Exp Gerontol*. 2013:48:1285–1292. https://doi.org/10.1016/j.exger.2013.08.006
13. Wong C, Goldstein DR. Impact of aging on antigen presentation cell function of dendritic cells. *Curr Opin Immunol*. 2013:25:535–541. https://doi.org/10.1016/j.coi.2013.05.016
14. Bryl E, Witkowski JM. Decreased proliferative capability of CD4(+) cells of elderly people is associated with faster loss of activation-related antigens and accumulation of regulatory T cells. *Exp Gerontol*. 2004:39:587–595. https://doi.org/10.1016/j.exger.2003.10.029
15. Lorenzo EC, Bartley JM, Haynes L. The impact of aging on CD4+ T cell responses to influenza infection. *Biogerontology*. 2018:19:437–446. https://doi.org/10.1007/s10522-018-9754-8
16. Lanna A, *et al*. A sestrin-dependent Erk–Jnk–p38 MAPK activation complex inhibits immunity during aging. *Nat Immunol*. 2017:18:354–363. https://doi.org/10.1038/ni.3665
17. Agrawal A, Gupta S. Impact of aging on dendritic cell functions in humans. *Ageing Res Rev*. 2011:10:336–345. https://doi.org/10.1016/j.arr.2010.06.004
18. Metcalf TU, *et al*. Global analyses revealed age-related alterations in innate immune responses after stimulation of pathogen recognition receptors. *Aging Cell*. 2015:14:421–432. https://doi.org/10.1111/acel.12320
19. Tesar BM, *et al*. Murine myeloid dendritic cell-dependent toll-like receptor immunity is preserved with aging. *Aging Cell*. 2006:5:473–486. https://doi.org/10.1111/j.1474-9726.2006.00245.x
20. Panda A, *et al*. Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. *J Immunol*. 2010:184:2518–2527. https://doi.org/10.4049/jimmunol.0901022
21. Agrawal A, *et al*. Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signaling pathway. *J Immunol*. 2007:178:6912–6922. https://doi.org/10.4049/jimmunol.178.11.6912
22. Pereira LF, de Souza APD, Borges TJ, Bonorino C. Impaired in vivo CD4+ T cell expansion and differentiation in aged mice is not solely due to T cell defects: decreased stimulation by aged dendritic cells. *Mech Ageing Dev*. 2011:132:187–194. https://doi.org/10.1016/j.mad.2011.03.005
23. Della Bella S, *et al*. Peripheral blood dendritic cells and monocytes are differently regulated in the elderly. *Clin Immunol*. 2007:122:220–228. https://doi.org/10.1016/j.clim.2006.09.012

24. McElhaney JE, Kuchel GA, Zhou X, Swain SL, Haynes L. T-cell immunity to influenza in older adults: a pathophysiological framework for development of more effective vaccines. *Front Immunol.* 2016:7:41. https://doi.org/10.3389/fimmu.2016.00041
25. Lustig A, *et al.* Telomere shortening, inflammatory cytokines, and anti-cytomegalovirus antibody follow distinct age-associated trajectories in humans. *Front Immunol.* 2017:8:1027. https://doi.org/10.3389/fimmu.2017.01027
26. Almanan M, *et al.* IL-10–producing Tfh cells accumulate with age and link inflammation with age-related immune suppression. *Sci Adv.* 2020:6:eabb0806. https://doi.org/10.1126/sciadv.abb0806
27. Chiu B-C, Stolberg VR, Freeman CM, Chensue SW. Mononuclear phagocyte-derived interleukin-10 suppresses the innate pulmonary granuloma cytokine response in aged mice. *Am J Pathol.* 2007:171:829–837. https://doi.org/10.2353/ajpath.2007.061122
28. Chiu B-C, Stolberg VR, Chensue SW. Mononuclear phagocyte-derived IL-10 suppresses the innate IL-12/IFN-gamma axis in lung-challenged aged mice. *J Immunol.* 2008:181:3156–3166. https://doi.org/10.4049/jimmunol.181.5.3156
29. Prakash S, *et al.* Dendritic cells from aged subjects display enhanced inflammatory responses to Chlamydophila pneumoniae. *Mediators Inflamm.* 2014:2014:1–11. https://doi.org/10.1155/2014/436438
30. Fukao T, Koyasu S. PI3K and negative regulation of TLR signaling. *Trends Immunol.* 2003:24:358–363. https://doi.org/10.1016/S1471-4906(03)00139-X
31. Ginefra P, Hope HC, Lorusso G, D'Amelio P, Vannini N. The immunometabolic roots of aging. *Curr Opin Immunol.* 2024:91:102498. https://doi.org/10.1016/j.coi.2024.102498
32. El Mezayen R, El Gazzar M, Myer R, High KP. Aging-dependent upregulation of IL-23p19 gene expression in dendritic cells is associated with differential transcription factor binding and histone modifications. *Aging Cell.* 2009:8:553–565. https://doi.org/10.1111/j.1474-9726.2009.00502.x
33. Park J, *et al.* Attenuation of migration properties of CD4+ T cells from aged mice correlates with decrease in chemokine receptor expression, response to retinoic acid, and RALDH expression compared to young mice. *Biosci Biotechnol Biochem.* 2014:78:976–980. https://doi.org/10.1080/09168451.2014.910099
34. Agrawal S, Ganguly S, Tran A, Sundaram P, Agrawal A. Retinoic acid treated human dendritic cells induce T regulatory cells via the expression of CD141 and GARP which is impaired with age. *Aging (Albany NY).* 2016:8:1223–1235. https://doi.org/10.18632/aging.100973
35. Svensson M, *et al.* Retinoic acid receptor signaling levels and antigen dose regulate gut homing receptor expression on CD8+ T cells. *Mucosal Immunol.* 2008:1:38–48. https://doi.org/10.1038/mi.2007.4
36. Bono MR, *et al.* Retinoic acid as a modulator of T cell immunity. *Nutrients.* 2016:8:1–15. https://doi.org/10.3390/nu8060349