Age Impacts the Local Immune Response to Tuberculin Skin Challenge in *Mycobacterium Bovis* BCG-vaccinated Baboons

Julia Marianna Scordo
Texas Biomedical Research Institute
Tucker J. Piergallini
Texas Biomedical Research Institute
Nicole D Reuter
The University of Oklahoma Health Sciences Center
Colwyn A. Headley
Texas Biomedical Research Institute
Vida L. Hodara
Texas Biomedical Research Institute
Olga Gonzalez
Texas Biomedical Research Institute
Luis D. Giavedoni
Texas Biomedical Research Institute
James F. Papin
The University of Oklahoma Health Sciences Center
Joanne Turner (joanneturner@txbiomed.org)
Texas Biomedical Research Institute

Research

Keywords: aging, vaccine immunity, tissue recall responses, tuberculin skin test

Posted Date: December 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-118635/v1

Abstract

Background - Individuals over the age of 65 are highly susceptible to infectious diseases, which account for one-third of deaths in this age group. Vaccines are a primary tool to combat infection, yet they are less effective in the elderly population. While many groups have aimed to address this problem by studying vaccine-induced peripheral blood responses in the elderly, work from our lab and others demonstrate that immune responses to vaccination and infectious challenge may differ between tissue sites and the periphery. To improve health outcomes in our aged population, we must study vaccine responses in the tissue. Here we established an *in vivo* delayed-type hypersensitivity model of *Mycobacterium bovis* BCG vaccination and tuberculin skin test (TST) in adult and aged baboons. Vaccination generates BCG-specific immune cells that are recruited to the skin upon tuberculin challenge. We tested short-term recall responses (8 weeks post-vaccination) and long term recall responses (25 weeks post-vaccination) by performing skin punch biopsies around the site of tuberculin injection. In parallel, we determined BCG-induced responses in the peripheral blood of vaccinated animals.

Results - In short term recall responses, we found increased oxidation and decreased production of immune proteins in aged baboon skin at the site of TST challenge, in comparison to adult skin. Differences between adult and aged animals normalized in the long term response to tuberculin. Phenotypic analysis of aged peripheral blood cells found several age-related changes in immune cell populations, independent of BCG vaccination, and no impairment in functional responses. Moreover, aged peripheral blood mononuclear cells had increased migration *in vitro*, suggesting that age-related changes in the tissue *in vivo* impairs aged immune recall responses to antigenic challenge.

Conclusions - These findings highlight the impact of age-associated changes in the local tissue environment in memory recall responses, which may be more broadly applied to the study of other tissues. Moreover, these findings should be considered in future studies aimed at understanding and improving aging immune responses to vaccination and tissue challenge.

Background

By 2060, individuals aged 65 and older will constitute approximately one-fourth of the U.S. population (1). This shift in demographics will have significant financial and public health consequences. As we age, so do many aspects of our immune system resulting in decreased vaccine protective efficacy and increased susceptibility to infection (2, 3). This has been recently highlighted by the increased susceptibility of the elderly to SARS-CoV-2, in addition to other infections, such as influenza and bacterial pneumonia (4, 5). To improve health outcomes in our aging population, we must gain a better understanding of age-associated changes in immune cell and tissue function in the elderly. With increasing age immune cells in the periphery shift to more differentiated CD4 and CD8 T cell subsets with altered function and acquisition of senescence markers (6–9). Additionally, increased systemic levels of inflammation with advanced age contribute to age-related diseases (8, 10). While peripheral blood studies have led to a greater understanding of the immunological aging process, much less is known about the impact of age

on memory responses in tissue compartments. Studies, including those from our lab, have shown that immune responses in the periphery and at the site of infection can differ (11–13). Obtaining elderly tissue samples in humans is limited, necessitating development of a novel model to study aging immune responses specifically in the tissue.

Here we took advantage of the high homology between the baboon and human immune system to establish an *in vivo* tissue model to study immune responses to vaccination (14, 15). We vaccinated adult and aged baboons with *Mycobacterium bovis* BCG (BCG) and utilized the classical delayed-type hypersensitivity (DTH) reaction of the tuberculin skin test (TST) to evaluate antigen-specific recall responses to the skin in timed biopsies (16). Peripheral blood was collected in parallel for the comparative study of phenotypic and functional responses of circulating immune cells. In this model, we tested short-term (ST) recall responses (8 weeks post-vaccination) as well as long term (LT) recall responses (25 weeks post-vaccination).

In the ST recall response, we observed increased oxidation and decreased immune mediator production in aged baboon skin in response to TST, relative to adult skin. These differences normalized between age groups in the LT recall response. Analysis of peripheral blood mononuclear cells (PBMCs) from aged BCG-vaccinated animals showed changes in immune populations and a decreased lymphoid to myeloid ratio, that was a consequence of age and not BCG vaccination. Functionally, PBMC from aged and adult BCG-vaccinated baboons responded similarly to antigen-specific stimulation yet PBMCs from aged baboons had increased migration *in vitro* in response to skin homogenates from both ST and LT challenge time points. Together, these data demonstrate that PBMC function was not reduced in response to BCG-vaccination in aged baboons, but aged skin had blunted immune responses and increased levels of oxidation during the ST TST response. Therefore, in BCG-vaccinated baboons, age-associated changes in the tissue environment impact vaccine-induced recall immune responses to challenge. Overall, these findings highlight the contributions of tissue responses to vaccination and challenge, that may be relevant to other tissues during infection, and should be considered to improve elderly vaccine responses.

Results

Establishing an in vivo model of tuberculin recall response in BCG-vaccinated baboons

To evaluate the impact of age on vaccine-induced recall immunity to the skin, we vaccinated 4 baboons (2 adult and 2 aged, according to age ranges previously described (17)) (See Supplementary Table 1, Additional File 1) with BCG via the intradermal route at a dose of $5–8 \times 10^5$ CFUs (Fig. 1A; See Supplementary Fig. 1A, Additional File 2). We tested ST recall responses to BCG with TST, or 0.9% saline skin injection (control), at 8 weeks post-vaccination (Fig. 1A) by performing two TST and two saline skin injections on the chest of each animal (Fig. 1B). This time period of 8 weeks allows for development of immune memory to BCG (18). Immune recall responses were determined by performing skin punch biopsies surrounding the site of TST or saline injection (Fig. 1B, C) at two time points post-TST (ST 3-day

biopsy, and ST 7-day biopsy) (Fig. 1A). We chose these two time points post-TST for biopsy collection to account for age-related differences in kinetic recall response to TST. In support of this, clinical responses to TST manifest within 3 days of TST; however, maximal cellular infiltration doesn't occur until 7 days (16). Moreover, TST responses in the elderly are commonly delayed or reduced (19, 20). A second challenge was performed 25 weeks after BCG-vaccination to determine LT recall immune responses (Fig. 1A), with two TST and two saline skin injections performed on a different region of the chest. For determination of LT recall responses, LT 3-day biopsies and LT 7-day biopsies post-TST were also obtained (Fig. 1A). Saline injection did not induce any changes in skin biopsies obtained from adult and aged BCG-vaccinated baboons at any time point ST or LT (included in figures as a baseline measure; shown in Fig. 1C, *left*), supporting that the injection procedure did not induce non-specific responses. Additionally, animal weight in both age groups was unchanged throughout the duration of the study (See Supplementary Fig. 1B, Additional File 2).

Increased oxidation and altered immune mediator production in skin from aged baboons, in response to TST

We evaluated superoxide levels in the skin of BCG-vaccinated adult and aged baboons in response to TST using electron parametric resonance (EPR). In the ST response, we observed increased superoxide in aged skin from 3-day biopsies (Fig. 2A, left). Oxidation in adult skin increased in the ST 7-day biopsy time point reaching levels comparable to that of aged skin (Fig. 2A, right). Higher levels of reduced and total glutathione were found in aged skin from ST 3-day biopsies in response to TST (Fig. 2B & C). Similar to oxidation levels, reduced and total glutathione were higher in adult skin from ST 7-day biopsies, reaching the levels of those observed in the aged skin (Fig. 2D & E). Lastly, we saw no differences in protein carbonyls present in the skin between our age groups in ST biopsies (Fig. 2F). Skin obtained from the LT challenge time point showed a trend increase in superoxide levels and protein carbonyls in aged skin from LT 3-day biopsies (Fig. 2G & H, *3-day*), with oxidation levels decreasing in both age groups in LT 7-day biopsies (Fig. 2G & H, *7-day*). Reduced and total glutathione levels showed a trend increase in adult skin in response to TST in LT 3-day and 7-day biopsies post-TST (Fig. 2I-L), potentially suggesting that aged skin produces less antioxidants to subsequent challenge.

We next evaluated cytokine, chemokine, and growth factors present in aged and adult skin from BCG-vaccinated baboons. Elderly skin has been shown to have higher basal levels of inflammatory cytokines (21), potentially altering cell migration and function at the site of infectious challenge. Contrary to expectations we observed a significant decreased production of cytokines, chemokines, and growth factors in the skin of aged baboons in the ST 3-day biopsies (Fig. 3A). In both age groups, immune protein levels were decreased in ST 7-day biopsies (See Supplementary Fig. 2A, Additional File 2). During the LT recall response, TST-challenged 3-day biopsies from aged skin responded similarly to TST-challenged adult 3-day biopsies, although aged skin had higher levels of IL1β and a fold change increase in several chemokines (Fig. 3B). Overall, the magnitude of LT response in biopsies obtained from all

animals was less than those observed in the ST response to TST, suggesting that recall responses decreased over time (Fig. 3B; See Supplementary Fig. 2B, Additional File 2).

Cell infiltration and skin histological analysis is similar between aged and adult skin

Histological analyses of skin from BCG-vaccinated aged and adult animals was performed to determine the extent and composition of cellular infiltration. In the ST recall response, TST-challenged adult skin from 3-day biopsies had more inflammation in comparison to aged animals supporting the elevated cytokines detected at this same time point (Fig. 4A). Inflammation decreased to comparable levels in ST 7-day biopsies in response to TST (See Supplementary Fig. 3, Additional File 2). We observed equal levels of inflammation in TST-challenged skin from the LT 3-day and 7-day biopsies in our age groups (Fig. 4B; See Supplementary Fig. 4, Additional File 2). In tissue sections from both the ST and LT responses, aged and adult skin had comparable cell infiltration profiles, including lymphocytes, plasma cells, and macrophages (data not shown). Eosinophils were also present in the skin of aged and adult animals from ST and LT biopsies.

Skin from aging individuals has known structural changes including decreased extracellular matrix components and reduced epidermal thickness (21, 22), so we also evaluated structural changes. Adult ST 3-day skin biopsies showed minimal increase in epidermal thickness by visual analysis, which is supported by the slight increase in inflammation in response to TST relative to aged skin (Fig. 4A).

Aged PBMCs have increased migration in response to skin tissue homogenates

To test if advanced age impacts recall responses through changes in cell migration, we tested PBMC chemotaxis in response to mediators present in skin tissue. Adult or aged PBMC migration was evaluated in response to adult skin or aged skin homogenates, respectively, termed homogenous chemotaxis (Fig. 5A). Moreover, heterogeneous chemotaxis was evaluated by testing adult PBMC migration to aged skin and aged PBMC migration to adult skin (Fig. 5B). To account for immune mediators present in resting skin, homogenous and heterogeneous cell migration was also tested in response to 3-day and 7-day biopsies from saline injected skin (Fig. 5A&B, *Adult NaCl & Aged NaCl*). Data are shown as fold-increase in response of PBMC from aged baboons relative to adult. Despite the decreased immune mediators present in TST-challenged aged tissue from ST 3-day biopsies (Fig. 3A), we observed higher levels of aged PBMC migration in response to aged skin tissue (Fig. 5A). This increase of aged PBMC migration was observed regardless of biopsy time point (ST vs LT 3-day and 7-day biopsies). Moreover, aged PBMCs had increased migration in both homogenous and heterogeneous migration assays (Fig. 5A & B) and in response to saline skin tissue, indicating that the PBMC response was increased independent of the tissue-specific milieu. These findings suggest that *in vitro* aged BCG-vaccinated PBMCs have superior capacity to migrate in response to TST biopsy homogenates.

PBMC from aged baboons have functional responses to antigen stimulation

Following our observations of increased migration of PBMC from aged baboons, we tested functional responses of PBMCs from our vaccinated animals in response to *in vitro* stimulation with mycobacterial antigen. In the ST (Fig. 6, *Weeks 8, 8 + 3-day, 8 + 7-day*) and LT (Fig. 6, *Weeks 25, 25 + 3-day, 25 + 7-day*) response to TST, no differences in antigen-specific T cell cytokines (IL2 and IFN-γ) were observed between age groups (Fig. 6A). In the LT response, adult PBMCs had increased CD40L, a marker of T cell activation (Fig. 6B). Also, adult PBMCs had transient increased production of the pro-inflammatory cytokines IL1β, TNF and IL6, although this was from blood collected prior to LT skin challenge and therefore not induced by TST (Fig. 6C). Adult PBMCs from the LT TST response had increased CXCL10 and decreased VEGF, although this was only observed at one time-point and not sustained throughout the LT response (Fig. 6D). At all time points tested, aged and adult PBMC baseline responses (media alone) were below the limit of detection (data not shown). Analysis of plasma cytokine, chemokine, and growth factors throughout the study showed similar results between the age groups (See Supplementary Fig. 5, Additional File 2).

Age-associated changes are observed in PBMCs from aged baboons

We evaluated PBMCs from aged and adult BCG-vaccinated baboons using flow cytometry to better characterize cellular phenotypes in the blood that might factor into the increased cell recruitment seen in the migration assays (See Supplementary Fig. 6, Additional File 2). We observed a decreased lymphoid to myeloid ratio in aged baboons (Fig. 7A), and increased NK, NKT and γδ T cell populations in aged animals (Fig. 7B). Many of these age-associated changes in peripheral cell populations are consistent with published literature in humans (23–25), with the exception of γδ T cells that are decreased in frequency in elderly humans (26). Of note, changes in PBMC populations were independent of BCG vaccination. Overall, other cell populations, including monocytes, plasmacytoid dendritic cells, and T and B cells, were unaltered between age groups throughout the course of the study (Fig. 7C & D).

With increasing age, memory T cell populations shift to a more differentiated state (7–9) and the naïve T cell pool decrease (27). We observed a slight increase of CD4 T central memory (Tcm) and decreased CD4 T effector memory (Tem) cells in aged baboons, although these differences were present prior to and not further altered by BCG vaccination (Fig. 8A). No changes in naïve CD4 T cells or CD8 memory populations were observed between aged and adult baboons (Fig. 8A & B). CD25 (IL-2Rα) expression, important for T cell memory development and recall responses to antigen (28), was moderately, but not significantly, higher on CD4 and CD8 T cells from aged baboons during the ST or LT response time points (Fig. 8C & D).

We also evaluated expression of chemokine receptors expression, which are important for T cell recruitment to the skin (16, 29–31). CD4 and CD8 T cells from aged baboons had slightly higher levels of

CCR4 expression during the ST response (Fig. 9A & B), and a trend toward higher CCR5 expression was observed on aged CD8 T cells during the LT response (Fig. 9A). Also, CD8 T cells from aged baboons had slight increases in CXCR3 expression throughout the ST and LT responses (Fig. 9A), although all noted differences failed to reach significance. These findings are supported by studies in old mice, which showed higher chemokine receptor expression on aged T cells (32). Lastly, we evaluated PD1 expression, a checkpoint molecule involved in inhibiting T cell migration to tissues, including the skin (33). There were slight increases in PD-1 on both CD4 and CD8 Tem populations (See Supplementary Fig. 7A&B, Additional File 2), as well as CD8 Tcm, in adult cells (See Supplementary Fig. 7D, Additional File 2), although no differences were statistically significant. Together, less PD-1 and more skin homing receptor expression on T cells from aged baboons may contribute to increased cell migration. While no major changes were observed, our findings increase the available data on immune cell changes in baboons (adult and aged), and further catalogue antibodies that can be used in the baboon model (34, 35).

Discussion

It is well accepted that age-associated changes in the immune system enhance infection susceptibility and decrease vaccine responsiveness (2, 36, 37). We, and others, have shown that systemic immune responses and immunity at the site of infection may differ (11–13, 38). To test this, we vaccinated baboons with BCG and performed TST, allowing for *in vivo* evaluation of peripheral blood and tissue immune responses to antigenic challenge. We tested both ST and LT responses to TST in the tissue of BCG-vaccinated aged and adult animals. We found that skin from aged baboons had increased oxidation and reduced cytokines, chemokines, and growth factors in the ST response to TST. The magnitude of immune response in both age groups was decreased, as well as differences lost between age groups, in the LT response to TST. Despite changes observed early in the skin, PBMCs from aged baboons had increased migration *in vitro* to skin tissue homogenates. We found age-associated changes in immune cells in the peripheral blood of vaccinated aged and adult baboons; however, no significant alterations were driven by BCG-vaccination. Moreover, aged PBMCs from BCG-vaccinated animals behaved functionally similar to their adult counterparts in response to antigen stimulation. These findings show that, in our model, age-associated changes in the skin tissue reduce responses of aged animals to antigen challenge, despite no impairment in their peripheral immune responses.

Work from our labs has shown that the elderly lung environment is pro-oxidative and inflammatory, leading to increased susceptibility to *Mycobacterium tuberculosis* (*M.tb*) infection (39–41). Moreover, cutaneous infections are more commonly observed in older individuals due to age-related changes in the skin, such as decreases in structural integrity and cellularity and increases in reactive oxygen species (21, 42–44). In line with these findings, we found higher levels of superoxide in the skin of aged baboons in response to TST in both ST and LT 3-day biopsies. In 7-day biopsies, oxidation levels in adult tissue increased to levels equivalent to those found in TST-challenged aged skin. This suggests that older animals mount an early, enhanced oxidative response not observed in adult animals, which may impair the generation of local immune responses to antigen challenge. In support of this, we observed that aged skin had significantly lower immune mediator production in ST 3-day biopsies, including chemokines and

growth factors necessary for cell migration to and function within the tissue. We also found that TST-challenged aged skin had decreased production of IL-6 and IL-1β in ST 3-day biopsies, suggesting that inflammatory responses in aged animals are impaired. Histological analysis supported this, showing slightly increased epidermal thickness and inflammation in adult skin tissue from ST 3-day biopsies. Published studies in humans show that aged skin responses to challenge are impaired due to early (within 6 hours) or non-specific (sterile) sources of inflammation (45, 46). While we did not test aged skin responses 6 hours after TST, we found no differences in aged skin responses to saline, which suggests that non-specific responses are not contributing to the impaired immunity we observe in TST-challenged aged skin. Other studies have shown that elderly skin responses are delayed relative to their adult counterparts (19). We included 7-day biopsies in both the ST and LT challenges to test whether kinetic differences would impact tissue immune responses to TST. Interestingly, we found that aged and adult BCG-vaccinated animals had decreased skin responses in ST and LT 7-day biopsies, supporting that delayed recall in aged animals is not driving impaired tissue immunity in our model.

Protective immune responses to tissue challenge require cell migration into the tissue, as well recruited and resident cells within the tissue that are functional (16, 47). Having observed impairment in immune responses in TST-challenged aged skin, we tested the ability of PBMCS from aged and adult BCG-vaccinated animals to migrate *in vitro* to skin tissue homogenates. Interestingly, aged PBMCs had greater migration than adult PBMCs, regardless of the source of the tissue (aged, adult) or type of skin challenge (saline or TST). This may suggest that aged PBMCs have intrinsically higher levels of migration, although we observed only slightly higher levels of skin homing receptor expression of these cells. While these studies were performed using PBMCs as the input cell source, and thus a mixture of cell types, it is important to note that our immune cell phenotyping found no significant changes in major cell populations. Future studies will evaluate the phenotype of those cells migrating. It is also possible that a yet unidentified chemoattractant is driving this enhanced migration, although that is less likely as it would need to be found in both adult and aged skin, and at both basal levels and in response to TST. A more likely explanation is that age-associated changes in the tissue structure *in vivo* impacts cell migration that is not detected using an *in vitro* cell assay that requires tissue disruption. We evaluated collagen and elastin bundles, structural components of the skin known to be altered with age (21); however, we did not find any major changes between age groups (data not shown). Future studies will focus on more detailed analyses of skin structural changes as a contributor to decreased tissue responses in aged animals.

Findings from our group and others show that aging alters mucosal tissues (38, 40, 41, 48–50), resulting in increased susceptibility to infections. Moreover, age-associated changes in lymph node architecture impact immune responses (51). These findings underscore the need to account for the contribution of the local milieu on cellular phenotype and function in immune responses to challenge. It is important that studies are designed to evaluate both peripheral and tissue responses to vaccination and/or infection to identify similarities and differences in these responses. Here we have developed a novel *in vivo* model with high human relevance to interrogate these research questions. Limitations of this study included the modest age group difference between our adult and aged animals and the sample size. Despite these

limitations, we observed robust tissue changes between our age groups and recapitulated age-related changes in peripheral immune cell populations found in humans (23–25). Also, we performed extensive immune phenotyping of baboon cells, which establishes panels for future use in this model.

Generation of immune memory following vaccination is critical for conferring protection against subsequent challenge. In the aged population, immune memory is reduced or impaired, requiring new approaches to improve clinical outcomes such as the use of boosters (36, 51). While boosters are frequently recommended for aged individuals, they are far less immunogenic and may require additional doses in comparison to their adult counterparts (36). In our study, we found decreased immune responses in aged skin in response to TST in the ST response. We would expect that use of a second dose of BCG in our study would improve recall responses in aged BCG-vaccinated animals. Interestingly, both age groups had reduced immune responses in the LT response, suggesting the ability of animals to mount recall responses diminished over time independent of age. The waning LT responses observed in our adult animals may be due to their age. It is important to note that in our study, adult animals were defined as less than 12 years old; however, other aging baboon studies define adult animals as younger (52).

Conclusion

Our findings demonstrate that age-related changes in the skin tissue (increased oxidation, decreased immune proteins, and decreased inflammation) blunt early immune responses to antigenic challenge at the tissue site. This suggests that studying the impact of increased age on tissue immune responses in response to vaccination or infection may be a more useful approach to understand immunity in the elderly.

Methods

Animal Procedures

Studies were conducted in four (two adult and two aged) baboons from the conventional colony at The University of Oklahoma Health Sciences Center (OUHSC) [Figure 1]. All animals were housed in Animal Biosafety Level 1 (ABSL1) facilities at OUHSC for the duration of the study. All procedures were approved by the University Institutional Animal Care and Use Committee at OUHSC and the OUHSC Biosafety Committee. Animals were vaccinated with *Mycobacterium bovis* BCG (Pasteur strain, ATCC, Manassas, VA) via the intradermal route in the upper arm at a dose of 5×10^5 CFUs. Skin tests were performed on animals at the times indicated in Fig. 1 by injecting 100 µL of tuberculin (Colorado Serum Company, Denver, CO) containing 5 tuberculin units of purified protein derivative (PPD). Saline injections (100 µL of 0.9% NaCl, Baxter International Inc., Deerfield, IL) were performed in animals to serve as negative controls. All skin tests were performed in the chest skin of animals according to the diagram in Fig. 1. All animals had positive TST responses in the study as determined by visual observation (Fig. 1C). At 72 hours and 7-days post-skin test challenge, 8 mm skin punch biopsies (ThermoFisher Scientific, Waltham, MA) were

obtained from the sites of tuberculin and saline injection (schematic in Fig. 1). All biopsies were shipped overnight to Texas Biomedical Research Institute (Texas Biomed) for downstream processing. Blood was collected in sodium heparin-vacutainers according to the timeline in Fig. 1 and shipped overnight to Texas Biomed for processing. Blood was also obtained for serum collection (in serum-separating tubes [SST] vacutainers), both performed at OUHSC. All procedures were performed under anesthesia (10 mg/kg Ketamine and 0.05–0.5 mg/kg Acepromazine, Covetrus, Portland, ME), and included monitoring of weight, body temperature, heart rate, respiration rate and capillary refill time.

Preparation and storage of skin punch biopsies

Immediately following collection at OUHSC, each 8 mm skin biopsy was cut in half using a pathology blade and biopsy halves were stored in 10% neutral buffered formalin (ThermoFisher Scientific), snap-frozen in liquid nitrogen, or prepared for electron paramagnetic resonance (EPR) analysis. Remaining skin tissue was banked for future analysis. For EPR analysis, skin biopsies were further divided into two pieces, weighed, and incubated with the following: 1) 400 μM CMH-hydrochloride (Enzo Life Sciences, Farmingdale, NY) or 2) 400 μM CMH + 500 nM rotenone (MP Biomedicals, Santa Ana, CA) + 100 μM antimycin A from Streptomyces sp. (MilliporeSigma, Burlington, MA) (CMH + RA) for 30 minutes at 37 °C. After 30-minute incubation, CMH or CMH + RA solution was removed from the tissue and stored in a cryovial. Both tissue and CMH solutions were snap-frozen in liquid nitrogen. Biopsies were shipped on dry ice (snap-frozen tissues) or at room temperature (tissues in formalin) to Texas Biomed for downstream processing.

Preparation of Tissue Homogenates from Skin Biopsies

Skin punch biopsies (snap-frozen in liquid nitrogen) were homogenized in Lysing Matrix D tubes (MP Biomedicals) in Tissue Extraction Reagent I (ThermoFisher Scientific) with cOmplete Mini Protease Inhibitor Cocktail (MilliporeSigma). Protein content was determined by Pierce BCA protein assay kit (ThermoFisher Scientific), according to kit instructions.

Luminex Assay Measurement of Immune Mediators

Immune mediators were measured in cell supernatants, plasma, and tissue homogenates using the following Luminex assays cross-reactive with baboons: NHP XL Cytokine for detection of CCL5, CCL20, CD40 Ligand, CXCL10, GM-CSF, IL1β, IL2, IL6, PD-L1, TNFα, VEGF, and IL8, and Human XL Cytokine for detection of CCL2, CXCL10, IL8, GM-CSF, IL1β, IL-1ra, IL6, and VEGF (R&D Systems, Minneapolis, MN), according to manufacturer's instructions. For detection of IFN-γ and IL2, monkey interferon-gamma (IFN-γ) ELISA kit and monkey IL-2 ELISA kit (Mabtech, Inc., Cincinnati, OH) were performed, according to kit instructions. For tissue homogenates, analyte levels were normalized to protein content in homogenates (pg analyte per μg protein), as determined by BCA assay.

Measurement of Tissue Oxidation Levels

For superoxide determination by EPR, CMH and CMH + RA tissue supernatants were thawed and loaded into a quartz cell. Superoxide levels in the samples were determined by EPR. EPR spectra were obtained

on the Bruker EMXnano ESR system (Bruker Corporation, MA, USA) using the following parameters: Frequency, 9.636541 GHz; Center Field, 3435.30 G; Modulation Amplitude, 2.000 G; Power 0.3162 mW; Conversion Time of 40.00 ms; Time Constant of 1.28 ms; Sweep Width of 100.0 G; Receiver Gain at 40 dB; and 1 total number of scans. Samples were baseline corrected relative to CMH only (control). Area under the curve for each sample was then determined.

Protein carbonyls in skin tissue homogenates were determined using a Oxiselect Protein Carbonyl ELISA kit (Cell Biolabs, Inc., San Diego, CA), according to kit instructions. Carbonyl levels were normalized to protein content in homogenates, as determined by BCA assay.

GSH and total glutathione (reduced and oxidized) were determined in skin tissue homogenates using a GSH/GSSG Ratio Detection Assay kit (Abcam, Cambridge, MA), per kit instructions. Glutathione levels were normalized to protein content in homogenates, as determined by BCA assay.

Collection of plasma from whole blood

Blood in sodium-heparin vacutainers was centrifuged at 200 x g for 15 minutes at room temperature. Plasma was collected and stored at -80 °C.

Isolation of peripheral blood mononuclear cells (PBMCs) from whole blood

After plasma collection, blood was diluted with 1X PBS. Lymphocyte Separation Media (Corning Life Sciences, Tewksbury, MA) was slowly dispensed underneath the blood, followed by centrifugation at 950 x g for 20 minutes at room temperature with disconnected brake. After centrifugation, the interface containing PBMCs was transferred to a new tube, washed once with 1X PBS, and red blood cells were lysed (freshly prepared lysis solution containing 0.15M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2-EDTA). Cells were washed twice to remove lysis solution and re-suspended in complete medium: 1X RPMI 1640 supplemented with 25 mM HEPES (MilliporeSigma), 10% heat-inactivated fetal bovine serum (Atlas Biologicals, Fort Collins, CO), 1% HyClone, 1% L-glutamine, and 1% MEM Non-Essential Amino Acids (all from ThermoFisher Scientific).

PBMC Stimulation

For cell stimulations, PBMCs were plated at a final concentration of 250,000 c/well. Cells were stimulated for up to 5 days in the presence of media or *Mycobacterium tuberculosis* culture filtrate protein (CFP), which contains BCG cross-reactive antigens (BEI Resources, Manassas, VA). For 24–28 hour incubations, CFP was used at a concentration of 20 µg/mL. For 5 day incubations, CFP was used at a concentration of 10 µg/mL. Supernatants were collected at the end of the incubation and stored at -80 °C.

Freezing and thawing of PBMCs

To prepare PBMCs for freezing, cells were re-suspended at a concentration of 10×10^6 c/mL in freezing medium: 85% heat-inactivated FBS + 10% DMSO + 5% of glucose 45% solution (MilliporeSigma).

Cryovials containing 1 mL cell suspension were transferred to a pre-chilled Mr. Frosty and stored at -80 °C for no longer than 48 hours. Vials were then transferred to liquid nitrogen for long-term storage. To thaw PBMCs, pre-warmed complete medium was supplemented with 0.2 µl/ml of Benzonase HC (MilliporeSigma). Cryovials containing frozen PBMCs were quickly thawed in a 37 °C water bath. After thaw, 1 mL cell medium + 0.2 µl/ml Benzonase was added to each cryovial and the volume was transferred to a 15 mL conical tube. Cells were centrifuged at 250 x g for 7 minutes at room temperature. Cells were then re-suspended in complete cell medium and counted for downstream analyses.

Cell staining for flow cytometry

Flow cytometric analysis was performed on whole blood and thawed PBMCs according to the following two methods: 1) 150 µL whole blood was stained with antibody cocktails (See Supplementary Table 2, Additional File 1) prepared in deficient RPMI (dRPMI; RPMI-1640 supplemented with HEPES and 1 g/L sodium azide [ThermoFisher Scientific]) with 10% heat-inactivated FBS (dRPMI + FBS) for 15 minutes at room temperature. After antibody incubation, red blood cells were lysed with freshly prepared lysis solution as described, and cells were washed once with 1X PBS. Cells were then washed twice with dRPMI + FBS. After the final wash, cells were fixed with 2% paraformaldehyde (ThermoFisher Scientific) for 15 minutes at room temperature in the dark; and 2) Thawed PBMCs were re-suspended at a concentration of 10×10^6 c/mL and 100 µL was added to a 96-well plate. Cells were washed once with 1X PBS and stained with Zombie Aqua Fixable Viability Kit (1:1000; Biolegend, San Diego, CA) for 15 minutes at room temperature in the dark. After the 15-minute incubation, cells were washed once with dRPMI + FBS and stained with antibody cocktails (See Supplementary Table 2, Additional File 1) prepared in dRPMI + FBS for 20 minutes at 4 °C. After antibody staining, cells were washed three times and fixed with 2% PFA for 15 minutes at room temperature in the dark. All stained and fixed cells were suspended in dRPMI + FBS and stored at 4 °C until analyzed using a Beckman Coulter CyAn or BD FACS Symphony flow cytometer. At least 50,000 events were counted and analyzed using FlowJo Version 10.7.0 Software.

PBMC Migration to Skin Tissue Homogenates

PBMC migration was determined using the CytoSelect 96-Well Cell Migration Assay (5 µM, Fluorometric Format [Cell Biolabs, Inc]), according to manufacturer's instructions. Briefly, skin tissue homogenates with known protein content according to BCA assay were prepared at 50 µg protein in a final volume of 150 µL serum-free medium (1X RPMI 1640 supplemented with 25 mM HEPES, 1% HyClone, 1% L-glutamine, and 1% MEM Non-Essential Amino Acids). Homogenates were added to the bottom chamber of the cell migration plate according to homogenous (Adult to Adult; Aged to Aged) or heterogeneous (Adult to Aged; Aged to Adult) experimental design. Then 500,000 PBMCs in serum-free media were added to the top chamber of the cell migration plate and incubated for 6 hours at 37 °C. At the end of the incubation period, cell detachment solution was added to the cell harvesting plate. Next, media in the top chamber wells from cell migration plate containing non-migrating cells was discarded and the cell migration top chamber plate was inserted into the cell harvesting tray for 30 minutes at 37 °C to detach cells. The bottom chambers of the cell migration plate were set aside. After the cell detachment incubation, 75 µL of detachment media (from harvesting plate) and 75 µL of media (from bottom chambers of the cell

migration plate) was added to a clear 96-well plate. Lysis buffer and dye solution (from kit) was added to each well of the clear 96-well plate and incubated for 20 minutes at room temperature. After the incubation, 150 µL from the clear 96-well plate was moved to a clear bottom, black plate and fluorescent was read in a plate reader with a 485 nm/ 538 nm filter and 530 nm cutoff.

Histological Analysis of Skin Tissue

Skin biopsies fixed in 10% neutral buffered formalin were paraffin-embedded, sectioned at 4 µm thickness, stained with hematoxylin and eosin using standard methods, and evaluated by a board-certified veterinary pathologist at Texas Biomed. For percent affected inflammation, the following scores were used for grading: 1 = < 10%, 2 = < 25%, 3 = < 50%, 4 = < 75%, 5 = > 75%. Paraffin-embedded skin biopsies were also processed for trichrome elastin staining per standard methods, and similarly evaluated. Tissue images were viewed using Image Scope x64 software.

Statistical Analyses

Data analysis, graphing, and statistical analysis was performed using GraphPad Prism version 7–9 (La Jolla, CA). For statistical analysis the following were used as described in the figure legends: One-way ANOVA and Tukey's post hoc correction for multiple-testing and Student's *t* test for testing of means between two groups. Statistical differences between groups were reported significant when the p-value is less than or equal to 0.05. The data are presented in mean ± SEM for n = 2 animals per age group.

Declarations

Availability of data and materials:

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Ethics Declarations:

Animal Studies Approval: These studies were performed in compliance with and with approval from the University Institutional Animal Care and Use Committee at OUHSC and the OUHSC Biosafety Committee (Protocol #19-044-SFIU).

Consent for publication:

Not applicable

Competing interests:

The authors declare that they have no competing interests.

Funding:

Research reported in this publication was supported byNational Institute of Aging (NIA) of the National Institutes of Health under award number P01-AG051428 to JT and NIH/NIA NRSA T32-AG021890 to JMS. Start-up funds to JT supported components of this study.

Authors' contributions:

JMS and JT designed the experiments. JMS, TJP, CAH, VLH, and OG performed the experiments. NR performed the veterinary procedures, under supervision of JFP. JMS collected and analyzed data, and wrote the paper. JT and LDG reviewed and provided feedback on the paper. All authors read and approved the final manuscript.

Acknowledgements:

We would like to acknowledge Dr. Ron Banks and the veterinary team at The University of Oklahoma Health Sciences Center for performing the animal studies. We would also like to acknowledge the Texas Biomed Biology Core for their assistance with flow cytometry panel design and data collection for flow cytometry and Luminex assays. Lastly, we acknowledge the Clinical Pathology Department at Texas Biomed for their assistance with processing samples and performing histological analyses.

References

1. Mather M. Fact Sheet: Aging in the United States Washington, D.C.: Population Reference Bureau; 2016 [Available from: https://www.prb.org/aging-unitedstates-fact-sheet/.
2. Kline KA, Bowdish DM. Infection in an aging population. Curr Opin Microbiol. 2016;29:63-7.
3. Pinti M, Appay V, Campisi J, Frasca D, Fulop T, Sauce D, et al. Aging of the immune system: Focus on inflammation and vaccination. European journal of immunology. 2016;46(10):2286-301.
4. CDC. Coronavirus Disease 2019 (COVID-19): Older Adults: Centers for Disease Control and Prevention; 2020 [updated 8/16/2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/older-adults.html.
5. Meyer KC. Aging. Proc Am Thorac Soc. 2005;2(5):433-9.
6. Qin L, Jing X, Qiu Z, Cao W, Jiao Y, Routy JP, et al. Aging of immune system: Immune signature from peripheral blood lymphocyte subsets in 1068 healthy adults. Aging (Albany NY). 2016;8(5):848-59.
7. Goronzy JJ, Weyand CM. Successful and Maladaptive T Cell Aging. Immunity. 2017;46(3):364-78.

8. Nikolich-Zugich J. The twilight of immunity: emerging concepts in aging of the immune system. Nature Immunology. 2018;19(1):10-9.
9. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. J Clin Invest. 2013;123(3):958-65.
10. Man MQ, Elias PM. Could Inflammaging and Its Sequelae Be Prevented or Mitigated? Clin Interv Aging. 2019;14:2301-4.
11. Brighenti S, Andersson J. Local immune responses in human tuberculosis: learning from the site of infection. J Infect Dis. 2012;205 Suppl 2:S316-24.
12. Ault R, Dwivedi V, Koivisto E, Nagy J, Miller K, Nagendran K, et al. Altered monocyte phenotypes but not impaired peripheral T cell immunity may explain susceptibility of the elderly to develop tuberculosis. Experimental gerontology. 2018;111:35-44.
13. Vukmanovic-Stejic M, Sandhu D, Seidel JA, Patel N, Sobande TO, Agius E, et al. The Characterization of Varicella Zoster Virus-Specific T Cells in Skin and Blood during Aging. J Invest Dermatol. 2015;135(7):1752-62.
14. Magden ER, Nehete BP, Chitta S, Williams LE, Simmons JH, Abee CR, et al. Comparative analysis of cellular immune responses in conventional and SPF Baboons (Papio spp.). bioRxiv. 2018:455881.
15. Cox LA, Comuzzie AG, Havill LM, Karere GM, Spradling KD, Mahaney MC, et al. Baboons as a model to study genetics and epigenetics of human disease. ILAR J. 2013;54(2):106-21.
16. Vukmanovic-Stejic M, Reed JR, Lacy KE, Rustin MH, Akbar AN. Mantoux Test as a model for a secondary immune response in humans. Immunology letters. 2006;107(2):93-101.
17. Dick EJ, Jr., Owston MA, David JM, Sharp RM, Rouse S, Hubbard GB. Mortality in captive baboons (Papio spp.): a-23-year study. Journal of medical primatology. 2014;43(3):169-96.
18. Covian C, Fernandez-Fierro A, Retamal-Diaz A, Diaz FE, Vasquez AE, Lay MK, et al. BCG-Induced Cross-Protection and Development of Trained Immunity: Implication for Vaccine Design. Front Immunol. 2019;10:2806.
19. Nayak S, Acharjya B. Mantoux test and its interpretation. Indian Dermatol Online J. 2012;3(1):2-6.
20. Dorken E, Grzybowski S, Allen EA. Significance of the Tuberculin Test in the Elderly. Chest. 1987;92(2):237-40.
21. Chambers ES, Vukmanovic-Stejic M. Skin barrier immunity and ageing. Immunology. 2020;160(2):116-25.
22. Zhang S, Duan E. Fighting against Skin Aging: The Way from Bench to Bedside. Cell Transplant. 2018;27(5):729-38.
23. Valiathan R, Ashman M, Asthana D. Effects of Ageing on the Immune System: Infants to Elderly. Scand J Immunol. 2016;83(4):255-66.
24. Camous X, Pera A, Solana R, Larbi A. NK cells in healthy aging and age-associated diseases. J Biomed Biotechnol. 2012;2012:195956.

25. Chen Y-J, Liao H-F. NK/NKT Cells and Aging. International Journal of Gerontology. 2007;1(2):65-76.
26. Colonna-Romano G, Potestio M, Aquino A, Candore G, Lio D, Caruso C. Gamma/delta T lymphocytes are affected in the elderly. Experimental gerontology. 2002;37(2-3):205-11.
27. Li M, Yao D, Zeng X, Kasakovski D, Zhang Y, Chen S, et al. Age related human T cell subset evolution and senescence. Immunity & Ageing. 2019;16(1):24.
28. Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity. 2010;33(2):153-65.
29. de Nadai P, Chenivesse C, Gilet J, Porte H, Vorng H, Chang Y, et al. CCR5 usage by CCL5 induces a selective leukocyte recruitment in human skin xenografts in vivo. J Invest Dermatol. 2006;126(9):2057-64.
30. Vangelista L, Vento S. The Expanding Therapeutic Perspective of CCR5 Blockade. Front Immunol. 2017;8:1981.
31. Kuo PT, Zeng Z, Salim N, Mattarollo S, Wells JW, Leggatt GR. The Role of CXCR3 and Its Chemokine Ligands in Skin Disease and Cancer. Front Med (Lausanne). 2018;5:271.
32. Mo R, Chen J, Han Y, Bueno-Cannizares C, Misek DE, Lescure PA, et al. T Cell Chemokine Receptor Expression in Aging. The Journal of Immunology. 2003;170(2):895-904.
33. Pena-Cruz V, McDonough SM, Diaz-Griffero F, Crum CP, Carrasco RD, Freeman GJ. PD-1 on immature and PD-1 ligands on migratory human Langerhans cells regulate antigen-presenting cell activity. J Invest Dermatol. 2010;130(9):2222-30.
34. Giavedoni LD, Schlabritz-Loutsevitch N, Hodara VL, Parodi LM, Hubbard GB, Dudley DJ, et al. Phenotypic changes associated with advancing gestation in maternal and fetal baboon lymphocytes. Journal of Reproductive Immunology. 2004;64(1):121-32.
35. Obregon-Perko V, Hodara VL, Parodi LM, Giavedoni LD. Baboon CD8 T cells suppress SIVmac infection in CD4 T cells through contact-dependent production of MIP-1α, MIP-1β, and RANTES. Cytokine. 2018;111:408-19.
36. Ciabattini A, Nardini C, Santoro F, Garagnani P, Franceschi C, Medaglini D. Vaccination in the elderly: The challenge of immune changes with aging. Seminars in immunology. 2018;40:83-94.
37. Oh S-J, Lee JK, Shin OS. Aging and the Immune System: the Impact of Immunosenescence on Viral Infection, Immunity and Vaccine Immunogenicity. Immune Netw. 2019;19(6):e37-e.
38. Dock J, Ramirez CM, Hultin L, Hausner MA, Hultin P, Elliott J, et al. Distinct aging profiles of CD8+ T cells in blood versus gastrointestinal mucosal compartments. PLoS One. 2017;12(8):e0182498.
39. Moliva JI, Rajaram MV, Sidiki S, Sasindran SJ, Guirado E, Pan XJ, et al. Molecular composition of the alveolar lining fluid in the aging lung. Age (Dordr). 2014;36(3):9633.
40. Lafuse WP, Rajaram MVS, Wu Q, Moliva JI, Torrelles JB, Turner J, et al. Identification of an Increased Alveolar Macrophage Subpopulation in Old Mice That Displays Unique Inflammatory Characteristics and Is Permissive to Mycobacterium tuberculosis Infection. The Journal of Immunology. 2019:ji1900495.

41. Moliva JI, Duncan MA, Olmo-Fontánez A, Akhter A, Arnett E, Scordo JM, et al. The Lung Mucosa Environment in the Elderly Increases Host Susceptibility to Mycobacterium tuberculosis Infection. The Journal of Infectious Diseases. 2019.
42. Farage MA, Miller KW, Elsner P, Maibach HI. Functional and physiological characteristics of the aging skin. Aging Clin Exp Res. 2008;20(3):195-200.
43. Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K. Oxidative stress in aging human skin. Biomolecules. 2015;5(2):545-89.
44. Vukmanovic-Stejic M, Rustin MH, Nikolich-Zugich J, Akbar AN. Immune responses in the skin in old age. Curr Opin Immunol. 2011;23(4):525-31.
45. Vukmanovic-Stejic M, Chambers ES, Suarez-Farinas M, Sandhu D, Fuentes-Duculan J, Patel N, et al. Enhancement of cutaneous immunity during aging by blocking p38 mitogen-activated protein (MAP) kinase-induced inflammation. J Allergy Clin Immunol. 2018;142(3):844-56.
46. Chambers ES, Akbar AN. Can blocking inflammation enhance immunity during aging? J Allergy Clin Immunol. 2020;145(5):1323-31.
47. Vohr H-W. Delayed-Type Hypersensitivity. In: Vohr H-W, editor. Encyclopedic Reference of Immunotoxicology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2005. p. 192-4.
48. El Chakhtoura NG, Bonomo RA, Jump RLP. Influence of Aging and Environment on Presentation of Infection in Older Adults. Infect Dis Clin North Am. 2017;31(4):593-608.
49. Vukmanovic-Stejic M, Sandhu D, Sobande TO, Agius E, Lacy KE, Riddell N, et al. Varicella zoster-specific CD4+Foxp3+ T cells accumulate after cutaneous antigen challenge in humans. J Immunol. 2013;190(3):977-86.
50. Vukmanovic-Stejic M, Agius E, Booth N, Dunne PJ, Lacy KE, Reed JR, et al. The kinetics of CD4+Foxp3+ T cell accumulation during a human cutaneous antigen-specific memory response in vivo. The Journal of clinical investigation. 2008;118(11):3639-50.
51. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. Immun Ageing. 2019;16:25.
52. Bronikowski AM, Alberts SC, Altmann J, Packer C, Carey KD, Tatar M. The aging baboon: Comparative demography in a non-human primate. Proceedings of the National Academy of Sciences. 2002;99(14):9591.

Figures

Fig. 1

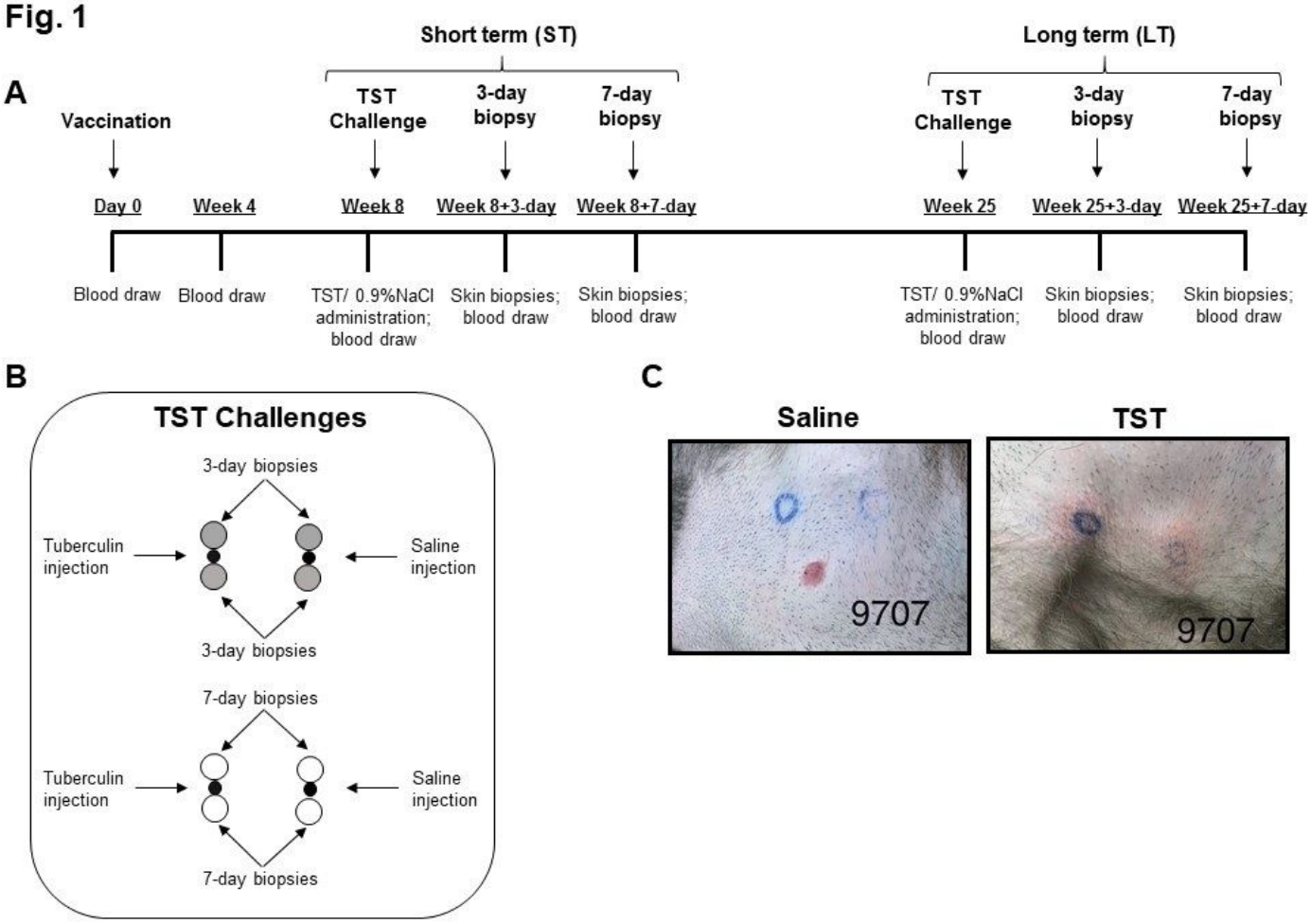

Figure 1

Design for an in vivo model of tuberculin recall response in Mycobacterium bovis BCG-vaccinated baboons. (A) Study timeline: adult and aged baboons were BCG-vaccinated and challenged with tuberculin (TST) or saline (NaCl) for determination of the short term (ST) recall response. Skin biopsies were performed 3 days and 7 days post-challenge. Long term (LT) responses were tested by challenge and biopsies performed at week 25, week 25+3 days, and week 25+7 days, as indicated. Peripheral blood was collected at the times indicated for determination of systemic responses in vaccinated baboons. (B) Schematic of TST challenges and biopsy sites on the chest of vaccinated baboons. Saline injection sites served as the control for antigen-specific responses. (C) Representative images of saline (left) and TST (right) injection sites, indicated by blue circle, on the chest of vaccinated baboons, showing positive responses to TST.

Fig. 2

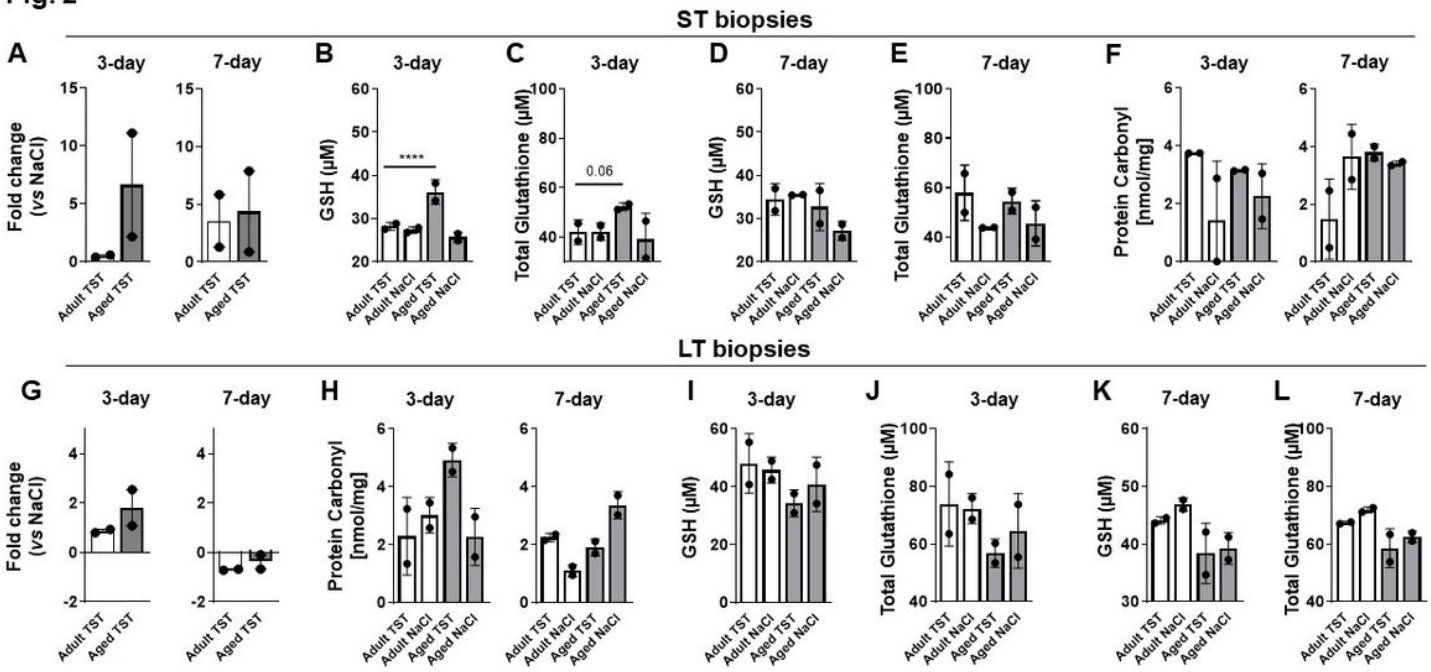

Figure 2

Higher oxidation levels in aged baboon skin in response to TST. (A) Levels of superoxide detected by EPR in adult (A) and aged (O) baboon skin tissue. Shown is the fold change of superoxide in TST biopsies (TST) vs saline (NaCl) biopsies from ST 3-day (left) and 7-day (right) biopsies. (B) Reduced glutathione (GSH) and (C) total glutathione levels in ST 3-day biopsies, determined by ELISA. (D) GSH and (E) total glutathione levels in ST 7-day biopsies. (F) Protein carbonyl levels in ST 3-day (left) and 7-day (right) biopsies, determined by ELISA. (G) Superoxide in baboon skin tissue in response to TST (fold change vs saline) from LT 3-day (left) and 7-day (right) biopsies. (H) Protein carbonyl levels in LT 3-day (left) and 7-day (right) biopsies. (I) GSH and (J) total glutathione levels in LT 3-day biopsies. (K) GSH and (L) total glutathione levels in LT 7-day biopsies. One-way ANOVA post-Tukey analyses Adult TST vs Aged TST, ****$p<0.0001$.

Fig. 3

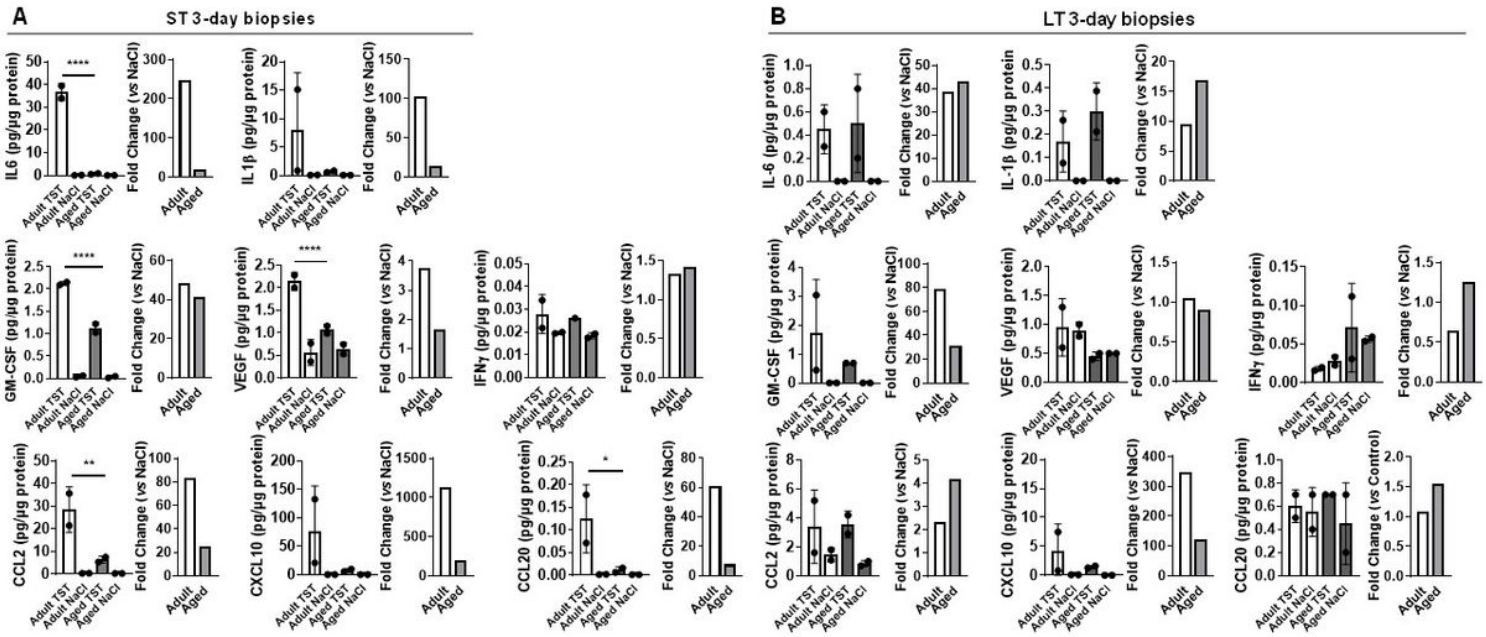

Figure 3

Decreased immune mediator production in aged baboon skin challenged with TST. Levels of immune proteins in (A) ST 3-day biopsies and (B) LT 3-day biopsies from adult (A) and aged (O) vaccinated baboons were detected by Luminex assay. Shown for each analyte: protein concentration normalized per µg of protein in skin tissue homogenates (left) and the fold change of TST protein levels vs saline (right). One-way ANOVA post-Tukey analyses Adult TST vs Aged TST, *p<0.05, **p<0.01, ****p<0.0001.

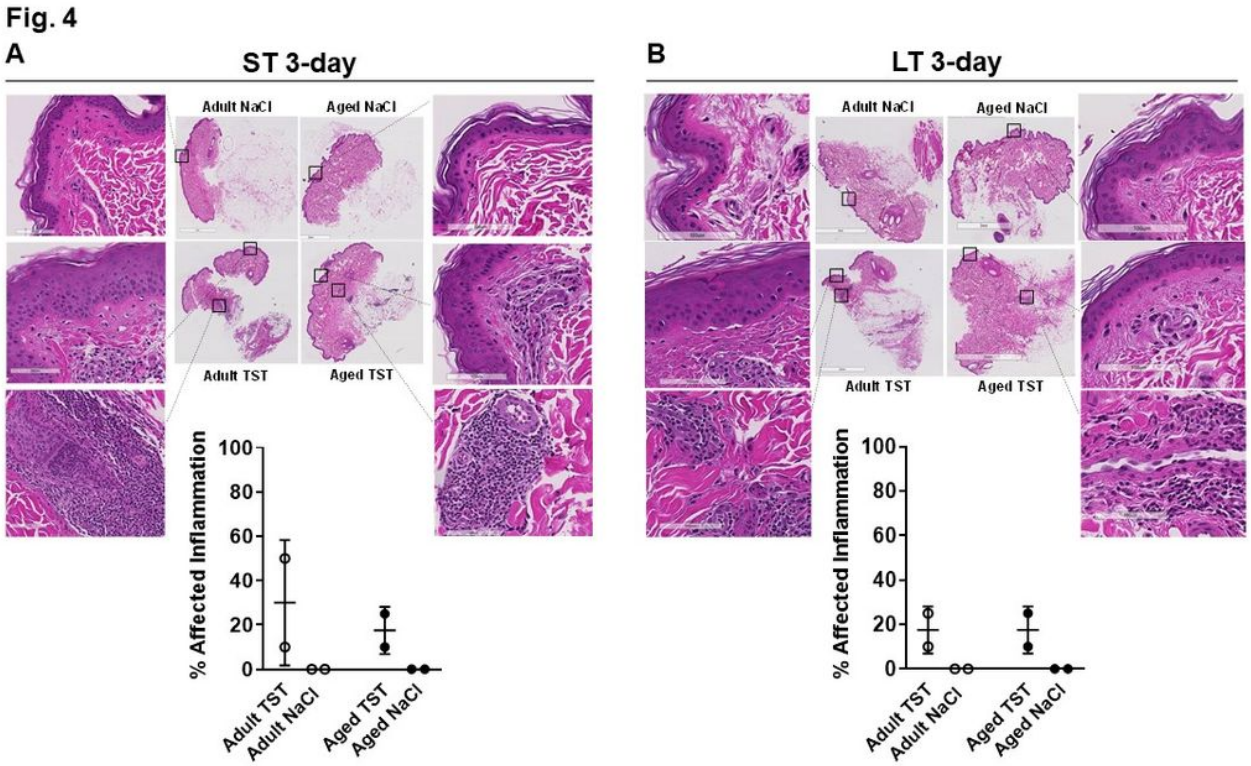

Figure 4

Similar cell infiltration and skin histological analysis in adult and aged baboons. H&E stained skin tissue from adult and aged baboons was evaluated for cell infiltration, inflammation, and skin structural changes in ST 3-day biopsies (A) and LT 3-day biopsies (B). Percent affected inflammation is quantified on the left. Representative images (right) are shown of adult (left) and aged (right) skin tissue in response to NaCl (top) and TST (bottom) for each time point.

Fig. 5

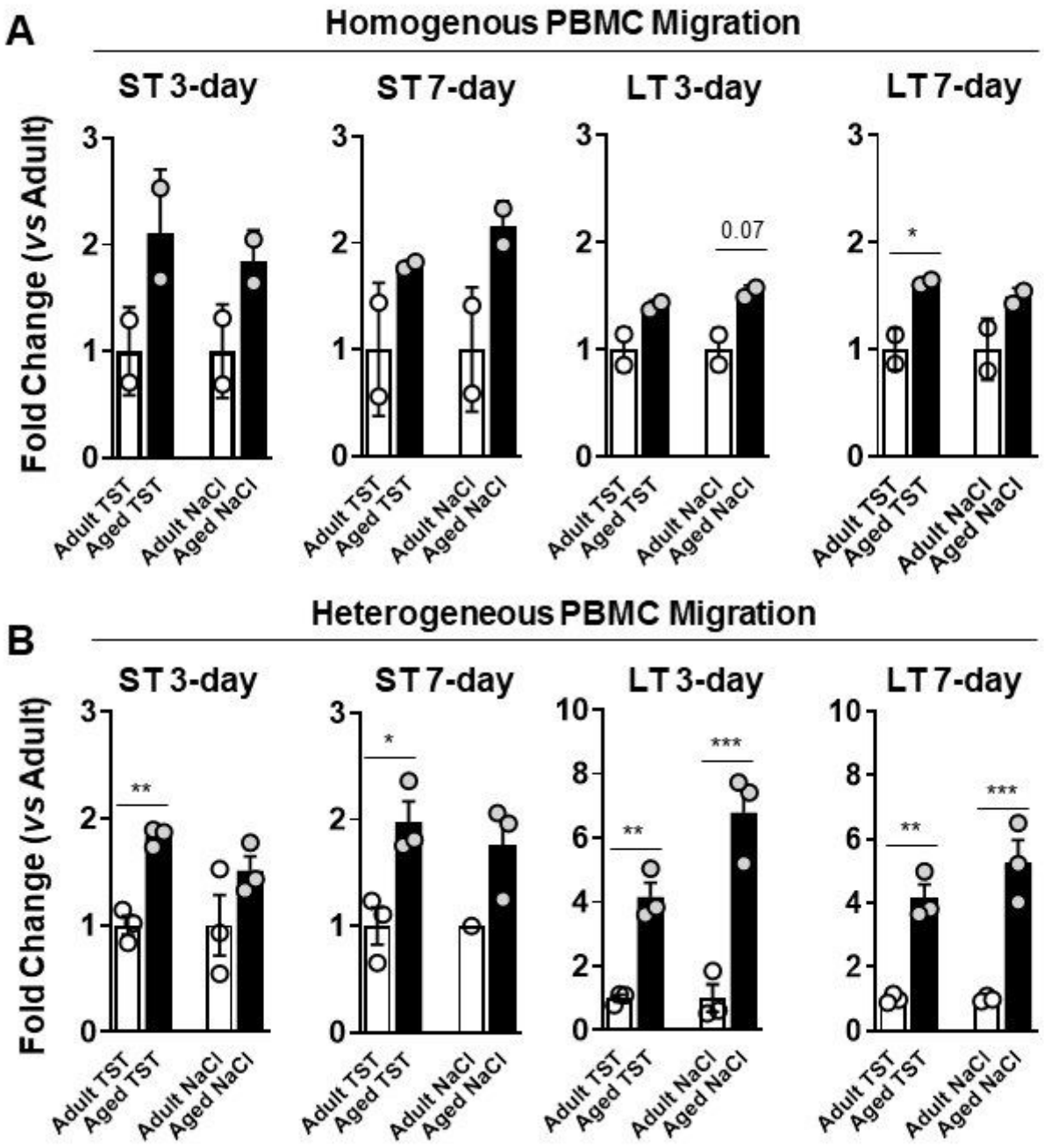

Figure 5

Increased cell migration of aged PBMCs in response to skin tissue homogenates. (A) Homogeneous migration of adult and aged PBMCs in response to skin tissue homogenates from TST and NaCl ST 3-day and 7-day biopsies and LT 3-day and 7-day biopsies. Each dot represents one animal. (B) Heterogeneous migration of adult and aged PBMCs in response to skin tissue homogenates from TST and NaCl ST 3-day and 7-day biopsies and LT 3-day and 7-day biopsies. Each dot represents a replicate, performed in triplicate, from adult or aged PBMCs by age group. Shown is fold change vs adult for

homogeneous (A) or heterogenous (B) experimental setup. Student's t test Adult TST vs Aged TST and Adult NaCl vs Aged NaCl, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Fig. 6

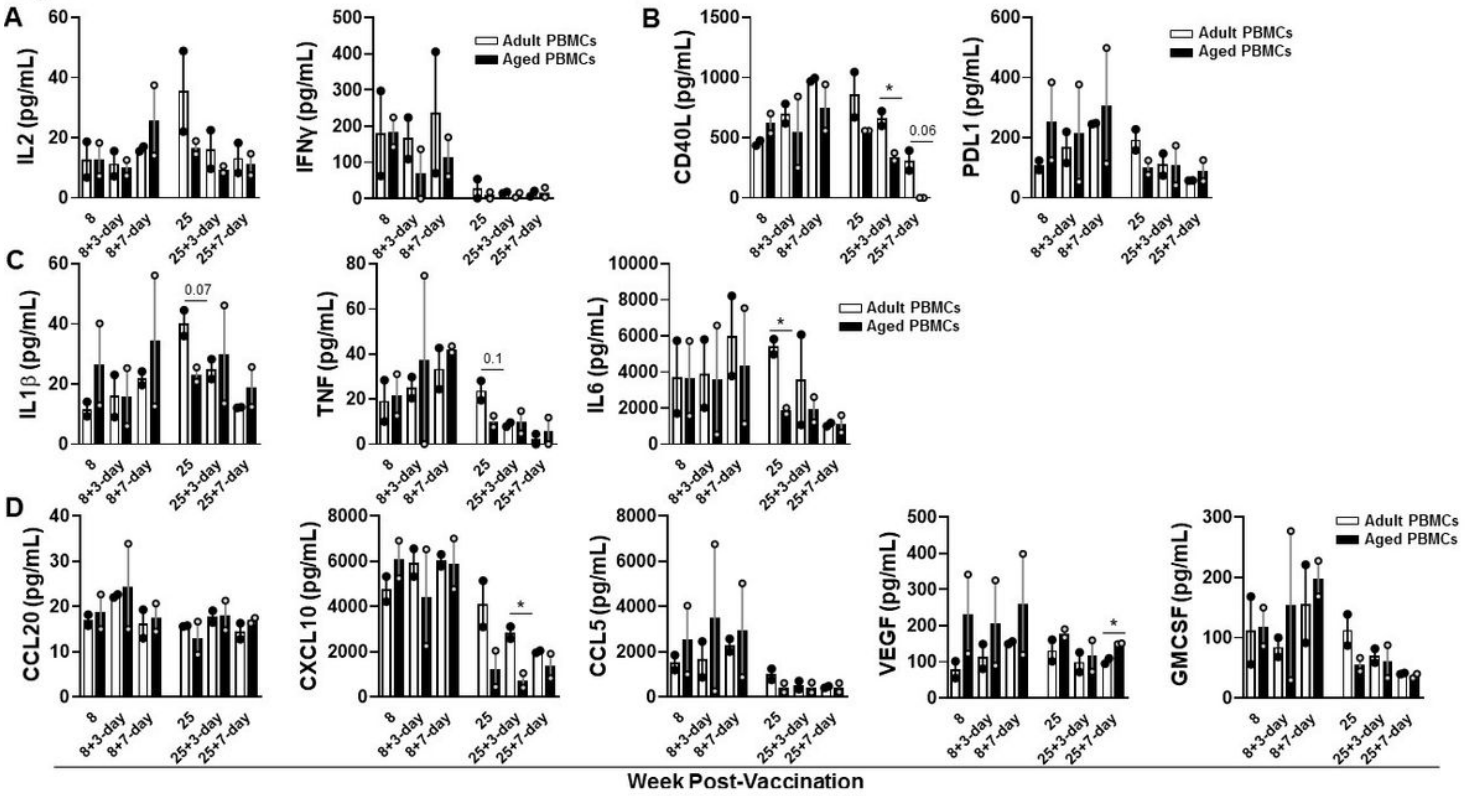

Figure 6

Antigen-stimulated PBMCs from aged baboons are functionally similar to adult PBMC responses. PBMCs from adult and aged vaccinated baboons from the time points indicated were stimulated with CFP for 5 days. Supernatants were collected and antigen-specific responses were detected by Luminex for production of (A,C) cytokines, (B) ligands, and (D) chemokines and growth factors. Student's t test Adult vs Aged PBMCs, *p<0.05.

Fig. 7

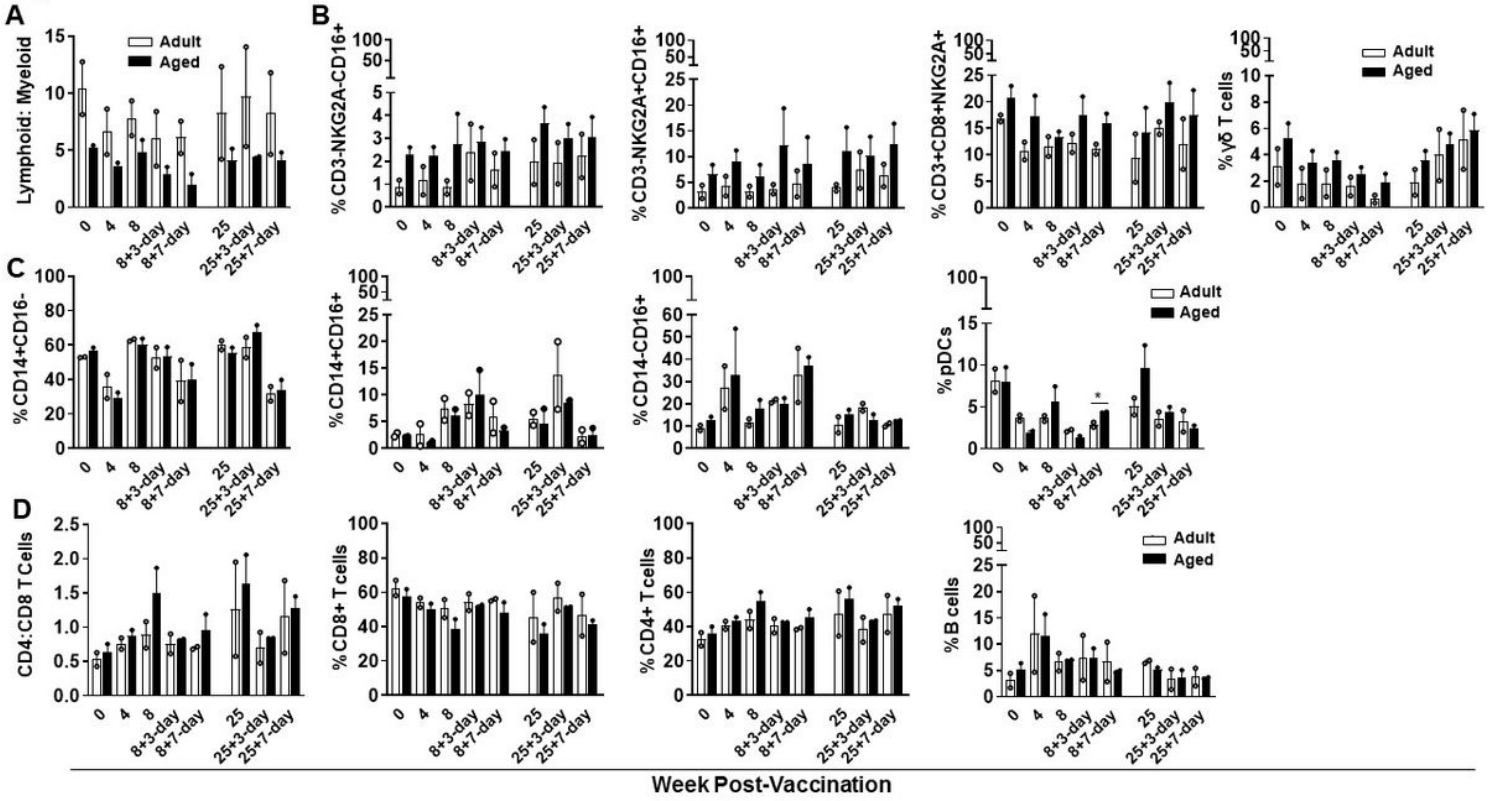

Figure 7

Age-related changes in peripheral blood phenotypes, independent of BCG-vaccination, are observed in aged baboons. PBMCs from adult and aged vaccinated baboons from the time points indicated were stained for surface markers and analyzed by flow cytometry. (A) Lymphoid to myeloid ratio, defined as CD3+CD20+NKG2A+ cells to CD3-CD20-NKG2A- cells. (B) Percentage of CD3-NKG2A-CD16+ and CD3-NKG2A+CD16+ NK cells, CD3+CD8+NKG2A+ NKT cells, and □□TCR+ T cells. (C) Percentage of CD14+CD16- classical, CD14+CD16+ intermediate, and CD14-CD16+ non-classical monocyte populations and CD123+ plasmacytoid DCs. (D) Percentage of lymphoid populations:CD4 to CD8 ratio, total CD8 T cells, total CD4 T cells, and B cells.

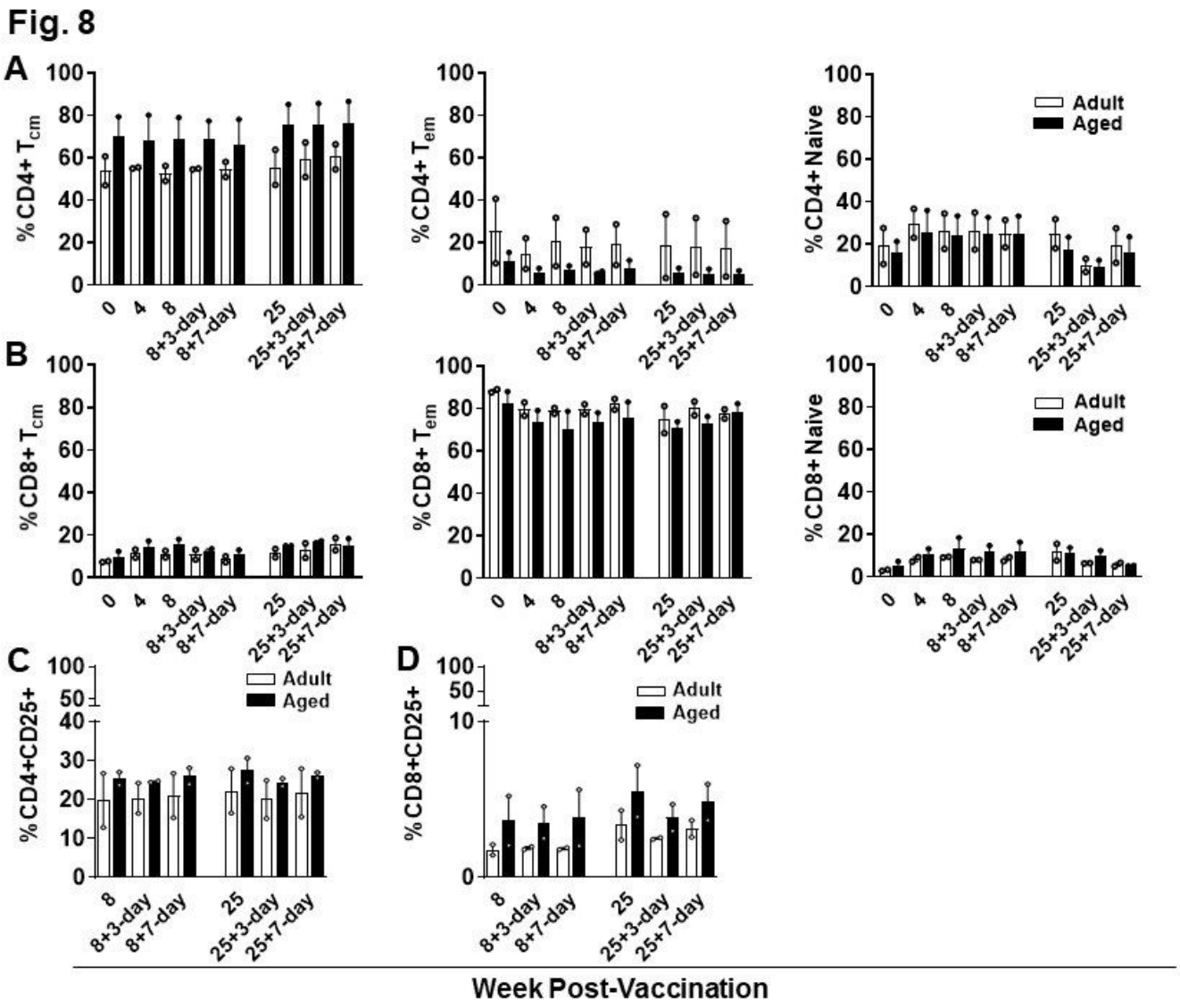

Figure 8

Peripheral blood memory T cell populations are similar between vaccinated adult and aged baboons. PBMCs from adult and aged vaccinated baboons from the time points indicated were stained for surface markers and analyzed by flow cytometry for memory T cell populations: Tcm defined as CD95+CD28+, Tem defined as CD95+CD28-, naïve T cell defined as CD95-CD28+. (A) Percentage of CD4 Tcm, CD4 Tem, and naïve CD4 T cells. (B) Percentage of CD8 Tcm, CD8 Tem, and naïve CD8 T cells. (C) Percentage of CD4 and CD8 T cells expressing CD25.

Fig. 9

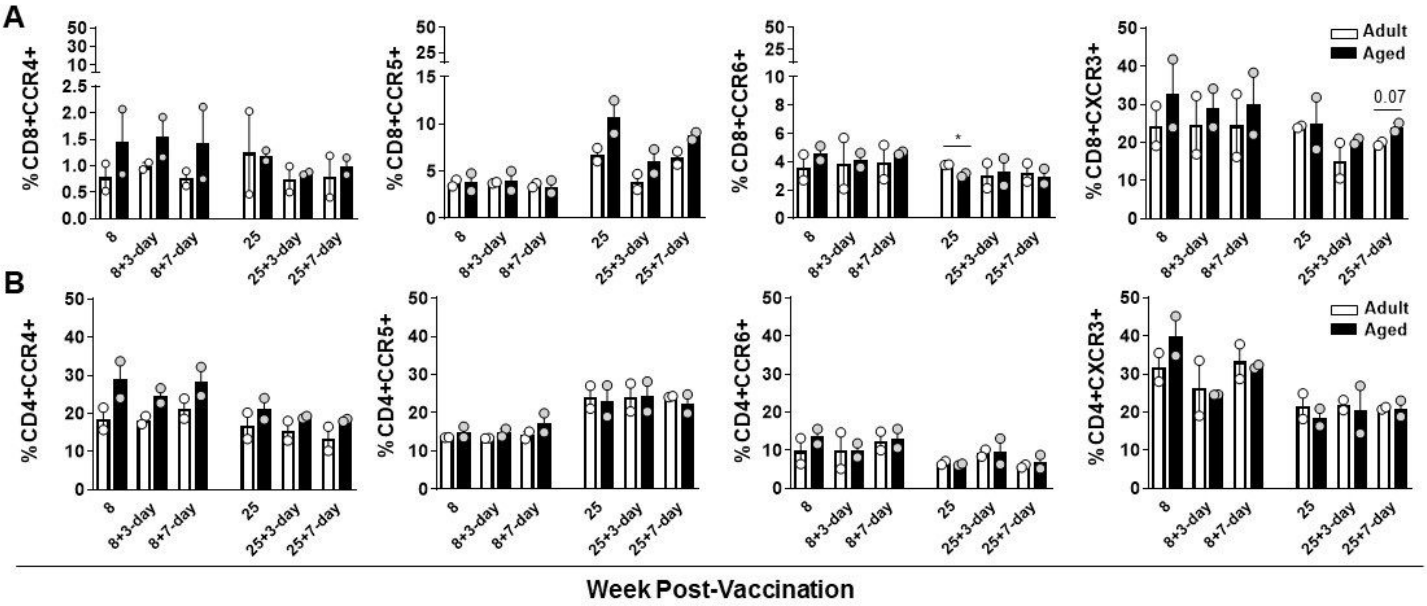

Figure 9

Aged T cells have slightly increased expression of skin homing receptors. PBMCs from adult and aged vaccinated baboons from the time points indicated were stained for surface markers and analyzed by flow cytometry for T cell expression the skin homing receptors CCR4, CCR5, CCR6, and CXCR3. (A) Percentage of CD8 T cells and (B) CD4 T cells expressing surface receptors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalFile1SCORDOTURNER11242020.docx
- AdditionalFile2SCORDOTURNER11242020.docx