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Loss of Naive T Cells and Repertoire Constriction Predict Poor Response to Vaccination in Old Primates

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Aging is usually accompanied by diminished immune protection upon infection or vaccination. Although aging results in well-characterized changes in the T cell compartment of long-lived, outbred, and pathogen-exposed organisms, their relevance for primary Ag responses remain unclear. Therefore, it remains unclear whether and to what extent the loss of naive T cells, their partial replacement by oligoclonal memory populations, and the consequent constriction of TCR repertoire limit the Ag responses in aging primates. We show in this study that aging rhesus monkeys (Macaca mulatta) exhibit poor CD8 T cell and B cell responses in the blood and poor CD8 responses in the lungs upon vaccination with the modified vaccinia strain Ankara. The function of APCs appeared to be maintained in aging monkeys, suggesting that the poor response was likely intrinsic to lymphocytes. We found that the loss of naive CD4 and CD8 T cells, and the appearance of persisting T cell clonal expansions predicted poor CD8 responses in individual monkeys. There was strong correlation between early CD8 responses in the transitory CD28+ CD62L− CD8+ T cell compartment and the peak Ab titers upon boost in individual animals, as well as a correlation of both parameters of immune response to the frequency of naive CD8+ T cells in old but not in adult monkeys. Therefore, our results argue that T cell repertoire constriction and naive cell loss have prognostic value for global immune function in aging primates.

Protean immunity declines with age, exposing the elderly to an increased risk from severe infections. The underlying causes of immunosenescence are multiple and not completely understood, yet the T cell compartment exhibits consistent and pronounced age-related changes (1). These include thymic involution and decreased production of naive T cells, diminished responsiveness to TCR signaling that culminates in blunted T cell proliferation, reduced IL-2 production and effector T cell differentiation (2), lowering or inversion of CD4/CD8 T cell ratios (3), reduction in naive and an increase in memory cells (4), telomere shortening (5), and TCR repertoire constriction (6, 7). It was shown that the occurrence of T cell clonal expansions (TCEs) in mice (8) and humans (9) is an age-dependent phenomenon associated with dramatic losses of TCR repertoire (10). Consistent with all of the above changes, there is some evidence that in mice, impaired CD8 response to virus infections (11, 12) may partially correlate to age-related repertoire losses (10, 13).

We and others have shown previously that old primates harbor diminished naive T cell pools (14, 15) and that the percentage of naive cells correlates inversely to the occurrence of TCE in rhesus monkeys (16). Associations between age-related TCR repertoire constriction and diminished immune function were experimentally demonstrated in rodents (13, 17). However, experiments in short-lived inbred animals, maintained in specific pathogen-free conditions throughout their lifetime, often cannot predict the relevance of these homeostatic changes for the T cell function in elderly humans.

To address these issues, we investigated CD8 T cell and Ab responses to primary vaccination with modified vaccinia Ankara (MVA) in long-lived, outbred, and pathogen-exposed rhesus macaques (Macaca mulatta). We observed age-related losses in primary responses to vaccine, despite largely unaltered dendritic cell (DC) function. Old monkeys exhibited a loss of T cell repertoire diversity as well as an overall loss of naive cells, which both correlated to poor CD8 response to Ag. Initial CD8 responses, in turn, correlated to maximum Ab responses to MVA. Therefore, our data argue that loss of naive T cell populations and repertoire predicts poor responses to vaccination and might be a major contributor to immune senescence in aging primates.

Materials and Methods

Animals and virus

Colony-bred male and female RM s of Indian origin were maintained according to the federal, state, and local guidelines. All experiments were approved by the Animal Care and Use Committee at the Oregon National...
Primates were immunized by two s.c. injections of 5 × 10^7 PFU of MV A into the left and right pectoral area respectively. Animals were anesthetized by ketamine injection for bleeding. Bronchoalveolar lavage (BAL) collection and biopsies at indicated time points. BAL cells were harvested by a 40 ml saline wash of one lung side. Two skin biopsies were sampled from painted and unpainted sites, respectively, by punch biopsy. Draining lymph nodes were surgically resected and analyzed by flow cytometry (FCM) as described below.

ELISA

Costar ELA/RA plates (Corning, Corning, NY) were coated with VACV-WR lysates as a source of Ag. Duplicates of serially diluted plasma were incubated on the plates for 1.5 h followed by three washes. The plates were then incubated for 1 h with goat anti-monkey IgG(γ)-HRP (Nordic Immunological Laboratories, Tilburg, The Netherlands). The plates were again washed three times before o-phenylenediamine (Sigma-Aldrich, St. Louis, MO) in citrate buffer was added. The reaction was stopped with 1 M HCl. The 490 nM absorbance was assessed in a Molecular Devices optical reader (Molecular Devices, Sunnyvale, CA) using the Soft Max program. The titers were calculated from the linear phase, as 0.1 intersecting points, of IFN-γ responses. Ag-specific responses were defined by subtracting the background IFN-γ responses in uninfected controls from IFN-γ values observed in vaccinia-stimulated samples.

Ab staining and flow cytometry

Surface staining was performed as shown earlier (16), with following modifications: in baseline phenotype stainings, we used anti-CD4–APC-Cy7 (clone OX-4; Biolegend, San Diego, CA) Abs instead of anti-CD4 (clone L200); we added anti-CD45RA–PE-Cy5.5 (clone MEM-56; Invitrogen, Carlsbad, CA) and anti-CCR7–PE-Cy7 (clone 3D12; BD Biosciences, San Jose, CA) to the phenotypization Ab panel. For intracellular cytokine staining, cells were first surface stained with anti-CD4–PerCP-Cy5.5, anti-CD8–ECD, anti-CD95–APC, anti-CD62L–PE (clone SK11; BD Biosciences), and anti-CD28–biotin Abs, followed by incubation with streptavidin-Qdot525. Cells were subsequently fixed for 5 min with 100 μl IC Fixation buffer (eBioscience, San Diego, CA), followed by 5 min permeabilization with 100 μl Permeabilization Buffer (eBioscience) and 30-min incubation with anti–IFN-γ–FITC (clone 48–B3; BD Biosciences) and anti–TNF-α–PE-Cy7 (clone Mab11; BD Biosciences). Cells were washed and reconstituted with a murine three-laser LSR-II cytometer (BD Biosciences). Cytometric results were analyzed by FlowJo 8.2 software (Tree Star, Ashland, OR). To define the percentage of naive CD8 cells, we multiplied the percentage of CD8 cells in the CD45RA+CD11a+ gate by the percentage of CD31 “CD95” in the CD45RA+CD11a+ subset and divided their product by 100. Frequency of IFN-γ–responsive cells was defined by adding values from IFN-γ– and TNF-α– and IFN-γ–TNF-α– quadrants. Responses in CD62L+CD28+ or CD62L–CD28– subsets were defined by progressive gating.

IL-12 release from stimulated DCs

DC stimulation and IL-12 detection performed were essentially as described (20). In brief, DCs were enriched by a 5-d incubation of CD14+ blood monocytes with 1000 U/ml GM-CSF and 100 U/ml IL-4, followed by a 2-d stimulation with 1 μg/ml CD40L. IL-12 in supernatants was quantified by ELISA in a SpectraMax Plus reader (Molecular Devices).

MHC class Iβ-restricted Ag processing and presentation

Primates were divided into two groups as described above. Human DCs were isolated by flasks adherence and then incubated with IL-4/ GM-CSF for 5 d (21). To test the ability of DCs to process and present Ag to CD8+ T cells, DCs were used as APCs in limiting quantities and responding T cells in excess in an IFN-γ ELISPOT assay (22). We used readily available human MHC class Iβ (MHC-Iβ)-restricted CD8+ T cell clones recognizing an Ag contained in Mycobacterium tuberculosis cell wall, because the MHC-Iβ molecule is conserved sufficiently between rhesus monkeys and humans such that rhesus DCs can present to the human CD8+ T cells (D.A. Lewinsohn, G. Swarbrick, and D.M. Lewinsohn, unpublished observations). Specifically, DCs (10,000 cells/well) were incubated with M. tuberculosis cell wall (30 μg/ml) for 1 h. DCs and then T cell clones (10,000 cells/well) were added and supplemented with IL-2 (0.5 μg/ml) and tested in the IFN-γ ELISPOT assay.

DC migration assay

Animal hair was clipped, but not shaved, and skin was painted with a solution of 100 mg/ml fluorescein in a solution of 10% DMSO, 45% acetone, and 45% dibutyryl cAMP.

A day later, skin and draining lymph node biopsies samples were collected from painted and control (unpainted contralateral) sites to each monkey. The epidermis was removed from the dermis, fixed with paraformaldehyde, and stained for HLA-DR or CD3. In addition, stained for HLA-DR or CD3. Langerhans cells were enumerated by fluorescent microscopy.

Cells from the inguinal (draining) and axillary (control) lymph nodes were harvested 24 h after hindquarter FITC painting. Cells were surface stained using CD83–PE (ImmunoTech, Westbrook, ME), CD3–PerCP (BD Pharmingen, San Diego, CA), CD86–allophycocyanin (BD Pharmingen), and stained for HLA-DR–Cy3, Langerhans cells were enumerated by fluorescent microscopy.

CDR3 length polymorphism assay

T-cell receptor CDR3 length polymorphism was assayed essentially as described previously (16). In brief, cDNA from 5 to 10×10^6 PBMC was subjected to 24 separate PCR Vβ-specific PCR reactions. PCR products were separated by PAGE and quantified by densitometry.

Statistical analysis

All statistical analysis was calculated with SAS software, version 9.1.3 (SAS Institute, Cary, NC) using repeated-measure ANOVA or nonparametric two-tailed statistical analysis. Single time point comparisons of CD8 subsets from young and old monkeys were done by t distribution-based Wilcoxon-Mann-Whitney U test. Kinetics of CD8 responses in young and old monkeys were compared by repeated measures ANOVA. Due to skewed data distribution, nonparametric correlations were calculated throughout unless indicated otherwise.

Results

Poor response to vaccination in old monkeys

To define the immune response to vaccination in aging primates, and exclude possible influences associated with the maturation of the immune system, we compared cohorts of old (18–25 y old, average 21.8 y) and adult, nonjuvenile RM (7–10 y old, average 8.1 y). Monkeys were primed, and boosted 8 wk later, with MVA as a representative replication-defective live vaccine that would be considered a safe candidate for vaccination of older adults. We analyzed a large group of 21 adult and 29 old rhesus monkeys, divided in eight independent cohorts, consisting of at least two adult and three old monkeys/cohort. Because Ab is responsible for long-term antipox immunity postvaccination, we tested Ab responses by indirect ELISA to MVA at baseline, on days 28 and 42 following priming, and days 14, 28, and 49 postbooster. Responses were quantified as logarithmic fold increases of Ab titer over baseline, and all experimental results were pooled and compared between age cohorts. Average Ab responses to MVA
were weaker in old than in young monkeys at all times, and this difference became significant upon boost (Fig. 1A), strongly arguing that humoral immune response to MVA vaccination may be weaker in aging primates than in young ones.

MVA vaccination is known to induce CD8 responses believed to play a role in clearing primary poxvirus infection. To analyze broad CD8 responses to MVA, we developed an in vitro stimulation assay with infectious vaccinia as source of Ag (see Materials and Methods). Preliminary results showed suboptimal in vitro restimulation of frozen samples (data not shown). Therefore, we performed all stimulation assays on fresh samples. We measured by flow cytometry the CD8 IFN-γ response to antigenic restimulation by a 16-h in vitro infection of blood and BAL cells with VACV. Due to the high technical demands of this protocol, this procedure was performed on three randomly selected cohorts consisting of 11 adult and 9 old monkeys. We compared the kinetics of CD8 responses in blood on days 7, 14, 28, and 42 post-vaccination. The response peaked at day 14 in both age groups and decreased thereafter, yet was overall significantly lower in old monkeys (Fig. 1B). Therefore, CD8 T cells from old monkeys exhibited significantly weaker IFN-γ responses upon brief in vitro Ag restimulation than their adult counterparts. We next tested whether this result represented an overall reduction in Ag-specific response or merely a redistribution of Ag-specific CD8 T cells from the bloodstream into tissues. To that effect, we collected BAL cells on days 14, 28, and 42 postinfection and used them in Ag-stimulation assays to monitor for the development of Ag-specific CD8 T cell response in tertiary tissues. In the adult monkeys, the BAL response peaked at day 28 rather than 14 and was even more intense than observed in the blood (Fig. 1C). By contrast, the response was undetectable in the majority of old monkeys, and the age-related difference was more pronounced than in blood. Therefore, the age-related loss of CD8 function manifested itself in multiple compartments.

No drastic loss of DC function in old monkeys
Ag processing and presentation by DCs is essential to T cell and, indirectly, most B cell responses. Thus, age-related decline in uptake, migration, processing, and/or presentation by DCs would all have the potential to decrease T cell (and, indirectly, via poor CD4 stimulation, B cell) responses to an Ag. For example, a reduction in numbers (23, 24) and migration (25) was seen in young monkeys infected with SIV, and reduced DC numbers were reported (26), but not confirmed (27), in aging mice. Therefore, we examined DC phenotype and function in adult and old monkeys. We quantified DCs in situ by morphometric immunohistology of bioptic

**FIGURE 1.** Age-related decline in RM primary CD8 response to vaccination. Eight experimental cohorts of old (n = 28) and adult (n = 21) RMs were vaccinated with MVA as described in Materials and Methods. MVA-specific Ab responses were calculated and displayed as fold increase of ELISA titers over baseline (A). cohorts of young and old animals were compared at indicated time points post prime (upper panel) or boost (lower panel) by repeated measures ANOVA and assessed for significance ("p > 0.05; *p < 0.05). Histograms indicate group means; error bars show SEM. B, Kinetic of IFN-γ CD8+ cell responses in blood on days 7, 14, 28, and 42. Cells were in vitro stimulated with VACV-WR at multiplicity of infection of 1 for 15 h, followed by additional 2 h incubation with brefeldin A. Upon surface staining with anti-CD4 and anti-CD8, cells were fixed, permeabilized, and stained for cytokine expression. Frequency of IFN-γ+ cells in uninfected controls were subtracted from vaccinia-infected samples to eliminate background IFN-γ expression. Results are shown as means ± SEM from three pooled experiments. The p value from repeated measures ANOVA is indicated. C, Kinetic of IFN-γ responding CD8+ cells in BAL on days 14, 28, and 42 of adult and old monkeys. Note that age-related differences were more pronounced in BAL than in blood (see B). Pooled means ± SEM from three independent experiments and p value from repeated measures ANOVA are shown. D, Three representative contour plots of IFN-γ (y-axes) and TNF-α (x-axes) expression in control CD8 T cells (upper panels) or vaccinia-stimulated cells (lower panels). ns, not significant.
skin specimens and observed no age-related difference in the number of HLA-DR⁺ cells (Fig. 2A). Painting the skin of our monkeys with fluorescein and enumerating the fluorescently labeled HLA-DR⁺ cells in the skin or CD11c⁺ cells in draining LN showed no aging-related decrease in the number of DCs leaving the skin or reaching the draining LNs. Likewise, fluorescein-labeled CD80⁺ or CD86⁺ cells reaching draining LNs did not show age-related differences (data not shown). Therefore, DC numbers and in vivo migration were intact in old monkeys.

We next addressed the possibility that DCs from old primates provide inferior costimulatory response. For instance, infant monkeys exhibit a DC defect in IL-12 responses to in vitro stimulation, resulting in strongly decreased response to MVA vaccination despite relatively intact T cell function (L. Byrd, T. Robinson, G. Swarbrick, D.M. Lewinshon, and D.A. Lewinsohn, manuscript in preparation). However, in our cohorts of adult and old monkeys, we observed no differences in IL-12 DC response to moderate CD40L stimulation (Fig. 2B) or to strong stimulation with a combination (20) of TNF-α, PGE₂, the β form of pro-IL-1, and IL-6 (not shown).

Finally, the functional ability of DCs to present class I Ag was directly measured by their ability to present mycobacterial Ags in a class Ib-restricted manner to human CD8⁺ T cell clones in which sufficient conservation between MHC-Ib alleles allows recognition of Ag-expressing rhesus monkey DCs by these human T cells without the need for Mamu matching (D.M. Lewinshon and D.A. Lewinsohn, unpublished observations). There was no difference in response to adult and old DCs as measured by ELISPOT (Fig. 2C). In conclusion, within the constraints of our experimental models, we failed to uncover age-related differences in DC numbers, phenotype, migration, or Ag presentation function between adult and old rhesus monkeys, suggesting that other elements of the aging immune system, including lymphocytes themselves, likely cause suboptimal Ag-specific responses.

Loss of naive CD8 cells and repertoire diversity in old monkeys

We have previously shown that age-dependent loss of naive T cells in aging monkeys correlates to increases in naive cell proliferation and turnover (16). Because naive lymphocytes, which carry broadly diverse TCR repertoires, are required for the recognition of novel Ags, it was conceivable that the poor CD8 response to MVA in old monkeys reflected the age-related loss of naive CD8 cell numbers and TCR repertoire diversity.

Three weeks before immunization, we defined the percentage of naive CD4 and CD8 T cells in blood by FCM. Naive CD4 T cells were defined as CD45RA⁺CD11a⁻CD28⁺CD95⁻CD31⁻CCR7⁻ and naive CD8 as CD45RA⁺CD11a⁻CD31⁻CD95⁻, which allowed stringent calculation of naive T cell fractions. Significantly lower frequency of naive CD4 (p = 0.0089) and CD8 T cells (p = 0.0075) was observed in old monkeys (Fig. 3A). This relative loss was a reflection of the loss of naive cells in absolute terms, counted as the number of naive CD4 and CD8 T cells per volume unit of blood, because the aged cohort showed significantly lower absolute counts of naive CD4 (p = 0.022), and CD8 cells (p = 0.0088) (Fig. 3B).

To assess the loss of TCR repertoire diversity in the aging monkey cohort, we defined the CD3 length polymorphism of their TCR Vβ-chains by a set of 24 PCR reactions specific for individual Vβ families (28). Vβ families exhibiting a single PCR peak were defined as likely containing a TCE. TCEs indicate severe repertoire constriction of the affected Vβ family (8), and their frequency correlates inversely to the frequency of naive CD8 cells in RMs (16). In mice, TCEs wax and wane over time, with only some of them becoming stable (29), and can be classified into CD49d⁺CD122⁺ cells, which are unstable upon adoptive transfer.
transfer and could be responding to Ag, and CD49d^hiCD122^hi, which are Ag independent, stable upon adoptive transfer, and arise preferentially in old rodents (30). Thus, we analyzed the TCE in our monkey cohorts over four consecutive yearly time points prior to immunization, and Vβ families showing a TCE pattern in at least two most recent time points were defined as Vβ families with a stable TCE. Old animals exhibited a significant (p = 0.0247) increase in the frequency of stable TCE (Fig. 3C), arguing for an age-related decrease in TCR repertoire. Moreover, naive CD8 T cell frequency correlated inversely (Spearman r = −0.7003; p = 0.0008) to the frequency of stable TCEs (Fig. 3D), and a similar correlation (Spearman r = −0.7253; p = 0.0004) was observed between stable TCE and naive CD4 frequency (data not shown), arguing for a link between the size of the naive cell populations and repertoire diversity. In conclusion, aging monkeys showed an increase in stable TCE, and a decrease in naïve T cell frequency and absolute counts, arguing for an aging-related loss of TCR repertoire.

**Stable TCE and loss of naïve CD8 is linked to poor immune response**

It was conceivable that the loss of naïve T cells and repertoire diversity (Fig. 3) may be responsible for the failure of CD8 cells to respond to vaccination with a novel Ag (Fig. 1). In that case, there should be direct correlation between these phenomena. The frequency of naïve blood cells correlated to peak CD8 responses (Fig. 4A). Spearman’s correlation test (r = 0.8636) revealed a significant (p < 0.0001) correlation when old and adult values were pooled, but also for adult RM values in isolation (Spearman r = 0.6909; p = 0.01). Because the majority of old monkeys displayed almost no CD8 response in lung tissue (Fig. 1C), a correlation between naïve cell counts and tissue CD8 responses could not be established in this age group (data not shown). Therefore, naïve cell frequency predicted peak CD8 responses to MVA in adult but not in old monkeys. To examine whether stable TCE affected CD8 responses, we compared CD8 responses in monkeys with at least one stable TCE-positive population (TCE^+) to those without clonally expanded Vβ populations (TCE^-). TCE^+ animals showed significantly (p = 0.0019) lower Ag responses than TCE^- animals in BAL on day 28 (Fig. 4B). When the two age groups were considered separately, we could observe similar trends: The only two old animals showing any response in lung tissue were the two TCE^- animals, whereas the three adult animals that exhibited TCE were among the four lowest-ranked Ag responders in their age cohort (Fig. 4B).

**Loss of naïve T cells predicts immediate CD8 responses to MVA in old monkeys**

Our results indicated that the loss of TCR repertoire and naïve T cells might compromise CD8 responses in old primates. Because such a mechanism would affect immediate CD8 responses, we explored the initial and early responses. Naïve cells are CD28^+ whereas activated effector T cells are characterized by the CD62L^-CCR7^- phenotype (31) and are predominantly CD28^- in rhesus monkeys (15). We showed previously that polyclonal in vitro stimulation of CD8 cells results in a progressive loss of CD28 from cell surface upon subsequent rounds of cell division (14), but the kinetics of this loss upon Ag stimulation in vivo had not been studied in old primates. IFN-γ responses to Ag restimulation were significantly stronger in the CD28^- than in the CD28^+ subset of CD62L^- CD8 T cells on day 7 postimmunization (Fig. 5A), but not at later time points (not shown). Moreover, the kinetic of CD28 expression on IFN-γ^- CD8 cells showed that MVA-specific cells had a significant subset of CD62L^- CD28^- on day 7 and changed toward the CD62L^- CD28^- phenotype by day 14, which was maintained thereafter, in both adult (Fig. 5B) and old monkeys (not shown). Ag responses to restimulation in the transitional CD28^-CD62L^- subset of activated T cells were significantly reduced in old monkeys compared with adult counterparts at day 7 postvaccination (Fig. 5C), arguing strongly that old animals show poor immediate CD8 responses to a neo-Ag. We correlated the frequency of naïve T cells prior to immunization to the immediate CD8 response. Spearman’s correlation between naïve CD8 cell frequency and immediate Ag responses (CD28^- cells, blood, day 7) was significant (r = 0.55; p = 0.015) when adult and old animals were pooled, but also in animals within the old cohort (r = 0.74; p = 0.037). This correlation was highly significant (r = 0.82; p = 0.001) in monkeys that had <20% of naïve cells (Fig. 5D) but not in those with large naïve populations (not shown). Monkeys with low naïve cell frequencies included all old animals, and 4 out of 11 of the adult ones, which was in line with the significant loss of naïve cells in aging (Fig. 3). To define if the absolute count of naïve CD8 cells defined the frequency of immediate CD8 responses to MVA vaccination, we correlated the absolute count of CD8 cells in old monkeys, as shown in Fig. 3B, to the frequency of IFN-γ responders within the CD28^-CD62L^- pool (Fig. 5E). These parameters showed a remarkably tight correlation by both nonparametric (Spearman r = 0.93; p = 0.0009) or parametric statistical analysis (Pearson r = 0.98; p < 0.0001), arguing that the naïve cell count in blood of old primates defines the initial CD8 response to vaccination. In contrast, the absolute count of naïve CD4 cells correlated less tightly (Spearman r = 0.80; p = 0.047) with CD8 response (not shown). Therefore, the aging-related loss of naïve T cells, especially CD8 T cells, predicted the poor initial CD8 response to MVA in old primates.

Our results provided an opportunity to test whether the early CD8 responses also correlate with the ability to mount humoral responses in aging. To that effect, we examined the intensity of the immediate Ag response in the CD28^- subset of CD8 cells and correlated it to Ab responses in individual monkeys. Most interestingly, the CD28^-CD62L^- CD8 response correlated to the peak Ab-specific responses, both in younger adults and old animals (Fig. 5F).

In conclusion, our results argued that the age-related losses of naïve T cells and TCR repertoire have consequences for the immune function and that frequencies of naïve T cell populations in blood have potential value as predictors of immune responses to vaccination.

**Discussion**

In this study, we longitudinally followed immune responses in adult and old monkeys before and during prime-boost vaccination with MVA. We have shown in this paper that CD8 T cell and Ab
Early CD8 responses to MVA are attenuated in old monkeys. A, The frequency of IFN-γ responders in CD28\(^+\) and CD28\(^-\) subsets of individual adult monkeys at day 7 postvaccination was pairwise compared by Wilcoxon signed rank test, and \( p \) value is shown. Similar results \(( p = 0.0039)\) were observed in old animals (not shown). B, In vitro VACV stimulation was followed by FCM as in Fig. 1B. The frequency of CD28\(^+\) and CD28\(^-\) cells in CD62L\(^-\) IFN-γ responders was calculated for each individual adult monkey and time point (see supportive Fig. 3 for gating). Means and SEM for each time point are shown. C, The frequency of IFN-γ responders in CD28\(^+\) subsets of adult or old animals was compared by Mann-Whitney \( U \) test, and \( p \) value is shown. Symbols indicate values relative to individual monkeys; horizontal bars indicate means. D, Frequency of naive CD8 lymphocytes prior to MVA immunization (x-axis) was correlated to IFN-γ responses in CD62L\(^-\) CD28\(^+\) cells at day 7 following infection (y-axis) in animals with <20% of naive CD8 cells prior to vaccination. Symbols show values in individual adult (●) or old monkeys (△); the line shows the linear regression curve. E, Naive CD8 counts per milliliter blood prior to MVA immunization (x-axis) was correlated to IFN-γ responses in CD62L\(^-\) CD28\(^+\) cells at day 7 following infection (y-axis) in old animals. Symbols show values in individual adult monkeys; the line shows the linear regression curve. F, Immediate CD28\(^+\)CD62L\(^-\) CD8+ T cell IFN-γ responses from Fig. 2C (x-axis) were correlated to Ab titers at day 14 postboost (y-axis). Values for individual adult (●) or old (△) animals and the linear regression curve for combined datasets are shown.

responses to vaccination with MVA are significantly diminished in old primates. Our data examining DC and CD8 phenotypes, function, and diversity all suggest that this defect may not lie with DC and that it may be cell autonomous for CD8 responses based upon lack of observable defects in DC function and strong correlation between diminished CD8 responses and the loss of naive CD8 T cell numbers and TCR repertoire diversity.

In our experiments, one striking finding was that humoral and CD8 responses strongly correlated in individual monkeys. This could mean that the two arms of the immune response aged in a coordinated, synchronous fashion, which would be somewhat surprising given the general heterogeneity of the aging process. Alternatively, it was possible that another cell/process, lying upstream of these effector responses, could be undergoing age-related decline in function or structure in these animals. One candidate would be DC, and our experiments all but excluded their age-related loss of function in the experiments described above. In contrast, Schwaiger et al. (32) have described a CD25\(^+\) CD8\(^+\) Th-like cell in aging humans, whose presence strongly correlated with successful outcome of Ab-response to influenza vaccination, and we cannot exclude at the present that a similar cell may exist in monkeys. Finally, another candidate for a common link could be the CD4 T cell, known to play a role in both CD8\(^+\) and B cell responses. Although our attempts to directly measure Ag-specific responses of these cells have failed, and the resolution of this issue will have to await further experimentation, our results argue that the loss of naive cells was seen not only in the CD8 but also in the CD4 cell pool, which could explain reduced Ab responses. Moreover, our analysis of TCE was not subset-specific and therefore showed reduced repertoire diversity that would be expected to affect CD4 cells as well. Additional experiments will be necessary to define more precisely the loss of repertoire diversity in CD4 subsets.

Lack of any measurable defects in DC function is in concert with some (27) but not other (26) studies published in mice. Our experiments failed to reveal discernible age-related differences in Ag uptake, emigration from skin, immigration into draining lymph nodes, IL-12 production, and Ag presentation. If confirmed, these results would raise a question as to whether and what extent short (er) lifespan of DC compared with lymphocytes may render them relatively more resistant to the manifestations of aging. Regardless, it would be naive to expect that any given cell type would show no effects of aging; it is more important to understand whether these effects have functional consequences, and our results suggest that DC aging may have fewer functional consequences than T cell aging.

What defect(s), then, matter the most in CD8\(^+\) (and CD4\(^+\)) T cell aging? Three distinct defects (or clusters of defects) that are important for the response to new microbial challenge have been described in greater or lesser detail: 1), cell-autonomous defects in T cell signaling (starting with synapse formation and culminating with transcriptional events leading to effector T cell differentiation), which were studied chiefly in CD4 T cells (33, 34); 2), numerical reduction in naive CD4 and CD8 T cells; and 3), loss of TCR repertoire diversity among total (13) and, more importantly, naive (17) CD8 T cells. It is highly likely that these three factors potentiate each other, although at the present we still lack quantitative parameters and qualitative studies that would tease their relative contributions apart. Our results do not address 1), but they speak in favor of the importance of 2) and 3) in functional responses of old monkeys to vaccination.

In conclusion, our data argue that old primates have poor responses to novel MHC-I restricted Ags and that the age-related loss of CD8 function is intrinsic to the shrinking T cell repertoire in old age, consistent with previous results in aging rodents (13). Because TCR repertoire and CD8 responses correlated to the size of the naive cell pool, we propose that phenotyping of blood lymphocytes may yield prognostic value for the evaluation of likely responses to vaccination with live replication-defective vaccines, in which low frequency of naive CD8 T cells might predict poor cellular responses to immunization in elderly or middle-aged vaccinees.
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Disclosures

The authors have no financial conflicts of interest.

References

Corrections


The second and sixteenth authors’ names were published incorrectly. The correct names are Susan Smyk-Pearson and Michael K. Axthelm.

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