Bone Marrow Precursor Cells from Aged Mice Generate CD4 T Cells That Function Well in Primary and Memory Responses

Sheri M. Eaton, Alexander C. Maue, Susan L. Swain and Laura Haynes

*J Immunol* 2008; 181:4825-4831; doi: 10.4049/jimmunol.181.7.4825

http://www.jimmunol.org/content/181/7/4825

---

**References**

This article cites 33 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/181/7/4825.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Bone Marrow Precursor Cells from Aged Mice Generate CD4 T Cells That Function Well in Primary and Memory Responses

Sheri M. Eaton, Alexander C. Maue, Susan L. Swain, and Laura Haynes

Understanding how aging impacts the function of memory CD4 T cells is critical for designing effective vaccines. Our studies show that immunological memory generated during youth functions well into old age, whereas that generated later in life functions poorly. This is the result of declines in the function of naive CD4 T cells from aged individuals and contributes to reduced efficacy of vaccines in the elderly. To begin to identify the cause of this defect, we examined the function of memory T cells generated from bone marrow precursor cells (BMPC) from young or aged mice in young hosts. In two different models, memory cells derived from young and aged BMPC exhibit good ex vivo and in vivo function. Importantly, memory CD4 T cells generated from aged BMPC exhibit potent cognate helper function for humoral responses, which are critical for effective immunization. These results indicate that there are no apparent age-related intrinsic defects in BMPC with regards to generation of functional memory T cells. The Journal of Immunology, 2008, 181: 4825–4831.

Aging has dramatic effects on the immune system and especially on the function of naive CD4 T cells, contributing to significant declines in the efficacy of vaccines in elderly populations (1–3). With increasing age, naive CD4 T cell numbers decline and their response to Ag is diminished (4–6), leading to reduced expansion and differentiation of these aged cells both ex vivo and in vivo. Importantly, the cognate helper activity of naive CD4 T cells from aged individuals is also significantly reduced, contributing to dramatic declines in humoral responses following vaccination (7–9).

These defects seen in naive CD4 T cell responses could be a result of changes induced at any stage in T cell development, from the pleuripotent hematopoietic stem cell to the peripheral naive T cell. Our hypothesis is that age-related defects in naive CD4 T cell function may be related, in large part, to the chronological age of the peripheral T cells. New T cell production declines dramatically with age, with an ∼100-fold decrease by 1 year of age, mainly due to thymic involution (10). While thymic output declines, the total number of T cells in aged individuals remains constant (11). Thus, in aged individuals, naive T cells are on the average older, due to decreased thymic output, and this increased chronological age may be involved in the development of defects associated with aging.

The studies presented in this report analyze the origins of the multiple defects in aged naive CD4 T cell function by further examining the functional capacity of new T cells produced by bone marrow precursor cells (BMPC) from aged mice, focusing on their ability to generate functional immune memory. Immunological memory is one of the central features of the immune system and is defined as the ability of the immune system to respond more efficiently to a second encounter with the same pathogen (12), which is the basis for protection from infection following vaccination. Long-lived memory T cells persist after initial priming of young individuals and can function well into old age. In contrast, it has been shown that for CD4 and CD8 T cells, new immune memory generated in older individuals exhibits reduced function compared with that generated in the young (13–15). These results suggest that it is the age of the naive T cell when it is initially primed that is the important factor in determining how well subsequent memory T cells will function and that naive T cells with aging defects give rise to memory cells also expressing those defects.

Importantly, age-related defects in the ability to generate new memory T cell function contribute to the reduced efficacy of vaccinations found in older individuals. Since the elderly are increasingly targeted for vaccinations for infectious diseases such as influenza (1–3), understanding the defects in the generation of immune memory in the aged is critical. One of the first steps in defining this defect is to determine whether BMPC from aged animals already possess intrinsic defects that preclude the ability to generate naive T cells that have the capacity to become fully functional memory T cells. In this study, we have used an adoptive transfer model to generate memory cells using naive CD4 T cells from young and aged TCR transgenic (Tg) mice and from BM chimeras generated with BMPC from young or aged donors. This model is unique and very useful since it allows us to determine the effects of age on naive and memory CD4 T cells independent from other aging effects on the immune system. In addition, we have examined, for the first time, the ability of newly generated CD4 T cells derived from aged BMPC to become functional memory CD4 T cells in the context of young hosts. Importantly, our results show that new CD4 T cells generated from aged BMPC, under these conditions in young hosts, can generate highly functional memory cells that respond well to Ag both ex vivo and in vivo.

Materials and Methods

Mice

All mice used in these studies were bred and housed in the Trudeau Institute animal facility. AND TCR Tg mice, which express a Vβ3/Vα11 TCR...
specific for a peptide of pigeon cytochrome c (PCC) (16), were used as a source of young (2–4 mo) and aged (16–18 mo) CD4 T cells. AND TCR Tg mice crossed with green fluorescence protein (GFP) expressing mice (17) (AND.GFP) and B6.SJL-Ptprca Pep3/BoyJ (CD45.1) mice were used as BM donors. For BM studies, young mice were 2–4 mo and aged were 26–27 mo. Hosts for BM transfers and memory cell generation were either young B10.BR or C57BL/6 mice. All animal studies were approved by the Trudeau Institute Animal Care and Use Committee.

Preparation of BM chimeras

Each experiment involving the generation of BM chimeras and analysis of CD4 T cell function from these chimeras was conducted at least twice. BM was obtained from the bones of the hind limbs of three to four young or aged AND.GFP Tg mice or CD45.1 mice. Mature T cells were depleted by positive selection with anti-CD4 and anti-CD8 MACS magnetic beads (Miltenyi Biotec). No CD4 or CD8 positive cells could be detected by flow cytometry following this depletion. BMPCs cells (10^7) were transferred i.v. into hosts that had been lethally irradiated (950 Gy). At specified time points after BM transfer, lymphocytes from spleens and peripheral lymph node of young and aged chimeric mice were assayed.

Efferent and memory T cell generation

Naive CD4 T cells were enriched from spleens and pooled peripheral lymph nodes by negative selection with magnetic beads (Miltenyi Biotec). For ex vivo studies, memory cells were pooled from three to four individual memory mice and cultured with Ag/APC to determine cytokine production and expansion potential. Memory cells generated from young naive T cells underwent similar expansion (Fig. 1A) and anti-IFN-γ (XMG1.2, 10 µg/ml), and anti-IL-4 (clone RM4–5), PE anti-CD4 (clone IM7), PE anti-CD44 (clone IM7), PE anti-CD62L (clone MEL-14), and PE anti-CD25 (IL-2Rα) were used (all purchased from BD Pharmingen): PerCP-Cy5.5 anti-CD3 (clone 145–2C11), allophycocyanin (APC) (clone 7D4), biotin anti-CD8 (clone 53–6.7), and PE anti-CD38 (clone 90). FITC-PNA was purchased from BD Biosciences. Flow cytometry was conducted using a FACSCalibur flow cytometer (BD Biosciences) and the data were analyzed with FlowJo software.

Cytokine detection

Supernatants from stimulated CD4 T cells were collected after 24 h and assayed for the presence of IL-2, IL-4, and IFN-γ by ELISA.

Statistical analysis

Statistical significance was determined by Student’s t test or ANOVA analysis. Values of p < 0.05 were considered significant.

Results

Memory responses of naive CD4 T cells from aged mice are defective

To assess the extent of age-related defects in memory CD4 T cell function, an adoptive transfer approach was used (13, 20). Naive CD4 T cells (CD44^lowCD62L<sup>high</sup>CD25<sup>+</sup>) from young (2–4 mo) and aged (16–18 mo) AND TCR Tg mice were used to generate Th2 effectors. We have shown previously that the presence of exogenous IL-2 and IL-4 during effector generation overcomes defects in expansion and differentiation of aged T cells and allows for comparable effector differentiation of the young and aged CD4 T cells (5). Memory cells were generated by transferring Th2 effector populations into T cell-deficient hosts and allowing them to return to rest. Memory cells generated from young naive cells were assayed after either 1 or 12 mo, and those generated from aged naive cells were assayed after 1 mo. This approach allowed for the determination of whether aging of naive CD4 T cells influences the response of memory CD4 T cells derived from them.

Following CD4 T cell enrichment, equal numbers of memory cells were restimulated with peptide/APC to determine cytokine production and expansion potential. Memory cells generated from young naive T cells underwent similar expansion (Fig. 1A) and IL-4 production (Fig. 1B) whether they were assayed at 1 or 12 mo. In contrast, memory T cells recovered 1 mo after transfer of effectors generated from aged naive CD4 T cells exhibited significantly less expansion and IL-4 production upon restimulation.

Although ex vivo cytokine production and expansion after restimulation of memory CD4 T cells to Ag indicates that the memory cells share defects with the naive cells from which they were derived, earlier studies indicated profound defects in the ability of naive CD4 T cells from aged mice to provide cognate help to B cells and to generate a robust humoral response (7). Thus, we examined the in vivo cognate helper function of these young and aged Th2 memory CD4 T cells. Th2 effectors were generated and transferred into T cell-deficient hosts, which were immunized 1 or 12 mo later with NP-PCC in alum. Two weeks after immunization, the expansion and differentiation of NP-specific B cells was examined. NP-specific B cells were identified by flow cytometry following staining with NP-allophycocyanin. Memory cells generated from young naive CD4 T cells exhibited good cognate helper activity at both 1 and 12 mo time points. Both the expansion and germinal center (GC) differentiation (PNA<sup>high</sup>CD38<sup>low</sup>) of the NP-specific B cells and the serum NP-specific IgG1 titers were significantly higher when memory cells generated from young naive cells provided help (Fig. 1, C and D) compared with memory cells generated from aged naive CD4 T cells. Importantly, numbers of donor memory cells were similar whether they were from aged or young naive cells 2 wk following immunization, so it does not seem that the dramatic functional differences were due to reduced survival of memory CD4 T cells from aged naive cells (Fig. 1E). Thus, aging led to marked reductions in the functional capacity of memory cells derived from aged naive CD4 T cells including poor recall expansion, reduced cytokine production, and severe impairment of B cell helper activity in vivo.

T cells derived using BMPC from young and aged TCR Tg mice

The ability of aged BMPC to give rise to naive T cells that generate functional memory was examined to determine...
whether these aged stem cells exhibited intrinsic defects. BMPC from young and aged AND.GFP mice were transferred into young lethally irradiated hosts. After 3-mo reconstitution, the phenotype and function of the newly generated naive TCR Tg CD4 T cells was assessed. Fig. 2A shows that re-population of both GFPTg and TCR Tg (Vβ3+3) CD4 T cells from young and aged precursor cells was similar and that cells derived from donor BM expressed a naive phenotype Tg+GFP+ CD4 T cells was assessed. Fig. 2A shows that repopulation of both GFP+ and TCR Tg+ (Vβ3+) CD4 T cells from young and aged precursor cells was similar and that cells derived from donor BM expressed a naive phenotype Tg+GFP+ CD4 T cells was assessed. Fig. 2A shows that repopulation of both GFP+ and TCR Tg+ (Vβ3+) CD4 T cells from young and aged precursor cells was similar and that cells derived from donor BM expressed a naive phenotype.

**FIGURE 1.** Memory cells generated from aged naive T cells function poorly. Th2 effector populations generated from naive AND TCR Tg CD4 T cells harvested from young or aged mice were transferred to T cell-deficient hosts (107 per host) and allowed to return to rest and become memory cells. Memory populations generated from young naive T cells were assayed at 1 (shaded bars) or 12 mo (striped bars) post transfer; those generated from aged naive T cells were assayed at 1 mo (open bars). A, Memory cells were pooled from three to four memory mice and enriched for CD4 T cells. Equivalent numbers (106) of memory cells were restimulated with peptide Ag and APC. Fold expansion was determined after 4 days of culture. B, Production of IL-4 was measured by ELISA in supernatants after 24 h. C, Memory mice were immunized with NP-PCC/alum. On day 14, the number of NP-specific cells in the spleen of individual mice was determined by flow cytometry following staining with NP-allophycocyanin; the number of NP+GC+ cells was determined by gating on PNAhighCD38low cells. D, The endpoint titers of NP-specific IgG1 in the serum of each memory mouse were determined by ELISA. E, The number of Tg+ CD4 memory T cells on day 14 following immunization was determined by flow cytometry by gating on Vβ3/CD4 cells. Data shown for A and B is mean ± SE for four experiments starting with young naive cells (both 1 and 12 mo time points) or two experiments starting with aged naive cells; all used pooled memory cells from three to four memory mice for ex vivo study; data shown for C and E is mean ± SE of four individual mice. *, p < 0.05 by one-way ANOVA.

**FIGURE 2.** TCR Tg CD4 T cells generated from young and aged BMPC can differentiate into functional Th2 effectors. BMPC cells (105) from young or aged AND.GFP mice were transferred i.v. into young B10.BR hosts that had been lethally irradiated (950cGy). After 3 mo, the generation of new T cells was assessed. Naive CD4 T cells were pooled from eight to nine individual mice for analysis and effector generation. A, The percentage of peripheral lymphocytes expressing GFP and the TCR transgene (Vβ3) as well as cell surface phenotype was examined by flow cytometry. CD4/Vβ3 dot plot is gated on GFP+ cells. CD44, CD62L, and CD25 histograms are gated on CD4/Vβ3+ cells; dotted gray lines represent isotype controls; data shown is from a representative experiment. Naive CD4 T cells generated from young or aged BMPC were cultured to generate Th2 effector populations. After 4 days, (B) the fold expansion and (C) IL-4 production upon restimulation by these effectors was analyzed; each point represents the results of a single experiment.
These naive, newly generated CD4 T cells were used to generate Th2 effector populations in the presence of IL-2, IL-4, and blocking Ab to IFN-γ. The expansion (Fig. 2B) and IL-4 production (Fig. 2C) of the resulting effector populations were very similar whether they were derived from young or aged precursors. Thus, T cells generated from both young and aged BMPC gave rise to functional Th2 effectors.

The Th2 effector populations were then transferred to T cell-deficient hosts and allowed to return to rest for at least 4 wk. Fig. 3A shows that the resulting memory T cells that were recovered from the hosts expressed a typical central memory phenotype (CD44highCD62LhighCD25−) (22). If naive cells were defective, we would expect the memory cells to also exhibit functional defects as in Fig. 1. Thus, equal numbers of memory cells were stimulated ex vivo with peptide Ag and APC to determine their ability to become effector memory cells. The ability of these memory effectors to both expand (Fig. 3B) and secrete IL-4 (Fig. 3C) was very similar. These results suggest that the naive CD4 T cells generated from aged BMPC did not express heritable aging defects that precluded generation of naive CD4 T cells that could give rise to functional memory cells under these conditions.

The in vivo cognate helper activity of these memory cells derived from young and aged BMPC was also examined. Hosts containing memory T cells were immunized with NP-PCC in alum, and, on day 14, the response of NP-specific B cells was assessed. Fig. 3D shows that the expansion and GC differentiation of NP-specific B cells was very similar when CD4 T cells generated from young or aged BMPC were used to provide help. In addition, the serum titers of NP-specific IgG1 were similar (Fig. 3E). Thus, in contrast to memory derived from conventional naive cells that are recovered from aged hosts, both ex vivo and in vivo memory CD4 T cells generated from naive cells newly generated from aged BMPC function much like those generated from young BMPC.
CD4 populations from normal aged mice that contain an increased proportion of memory phenotype cells (4). The new CD4 T cells derived from young and old BMPC were stimulated with anti-CD3/anti-CD28 to generate Th2 effector populations. The fold expansion of the effectors over the 4 days of culture was similar for both populations (Fig. 4B) and both exhibited a typical Th2 cytokine profile, with high levels of IL-4 and little IL-2 or IFN-γ (Fig. 4C and data not shown).

These Th2 effector populations were then transferred into T cell-depleted CD45.2 hosts and allowed to return to rest. After 4 wk, equal numbers of these memory cells were stimulated ex vivo with anti-CD3/anti-CD28 to examine function. The fold expansion and IL-4 production of these memory cells was very comparable (Fig. 5A and B). Thus, as with the TCR Tg model, the new polyclonal CD4 T cells generated from young and aged BMPC from CD45.1 mice generated memory cells that responded similarly ex vivo.

To examine the in vivo cognate helper function of these memory CD4 T cells, memory mice were immunized with NP-OVA in alum and the NP-specific B cell response was assessed on day 14. Memory CD4 T cells generated from both young and aged BMPC exhibited robust helper activity resulting in expansion and GC differentiation of responding B cells (Fig. 5C) and high titers of NP-specific IgG (Fig. 5D) in NP-OVA-immunized mice compared with PBS immunized controls. Since these studies examined a polyclonal response, we also included a control group of hosts that did not receive transferred Th2 effectors (no T cell group) to demonstrate that the observed helper activity was provided by the transferred memory cells and not residual host cells.

**Discussion**

Elderly populations are often targeted for vaccinations due to their increased susceptibility to infectious diseases such as influenza. However, the efficacy of these vaccinations in the elderly is significantly reduced compared with younger populations (1–3). This is mainly due to age-related changes in naive T cell responses, which contribute to declines in the generation of functional memory T cells in older individuals.

Our earlier studies in mouse models indicated that immunological memory generated during youth functioned well into old age, but that naive CD4 T cells in aged mice were functionally defective and gave rise to functionally defective memory cells even when the generation of effectors was enabled by addition of IL-2 and IL-4 that overcome aging defects in naive cell response (13). These memory cells generated from aged naive CD4 T cells exhibited dramatic functional defects including significantly reduced expansion and cytokine production upon restimulation with Ag ex vivo and reduced cognate helper function for NP-specific B cell responses. The novel work presented in this current study indicate that BMPC harvested from aged mice retain the capacity to give rise to newly generated naive cells that do not display these aging defects and are able to themselves become highly functional memory cells. In two different models, memory cells generated from...
aged BMPC respond well to restimulation with Ag ex vivo and, importantly, exhibit robust cognate helper activity.

In studies using naive TCR Tg CD4 T cells, we were able to eliminate variables that may change with aging, such as enhanced proportions of peripheral memory and regulatory T cells and changes in TCR repertoire (4, 23, 24). This model has allowed us to definitively show that there are intrinsic changes in naive CD4 T cells from aged TCR Tg mice that lead to reduced responses to Ag both ex vivo and in vivo (5, 7, 25). These aged naive CD4 T cells can be stimulated, under polarizing conditions with IL-2, to generate Th1 and Th2 effector populations that are very similar to young effectors with regards to expansion and cytokine production (5). Importantly, when these aged effectors are allowed to return to rest, either in vivo (13) or ex vivo (S. L. Swain and H. Tsukamoto, unpublished observations), age-related defects in expansion and cytokine production are re-expressed. Thus, there is a fundamental difference in the young and aged naive CD4 T cells that can be temporarily overcome but, we suggest, cannot be permanently reversed.

Since there is a dramatic reduction in the production of new naive T cells with increasing age (26), naive CD4 T cells in aged individuals must exhibit longer lifespans to maintain peripheral T cell numbers, as our recent studies demonstrate (S. L. Swain and H. Tsukamoto, manuscript in preparation). As these naive T cells age in the periphery, they can possibly undergo homeostatic turnover or have environmentally induced changes in their gene expression, resulting in functional declines in response to Ag. Another potential source of this observed age-related defect in the generation of functional memory T cells is a decline in the intrinsic ability of aged BMPC to generate new T cells. For instance, stem cells in the BM are potentially subjected to the same stresses of longevity that seem to impact peripheral naive cells and render them more defective. In addition, one must consider whether there is a decline in the ability of the aged BM microenvironment to support BMPC or a decline in the ability of an aged thymus to support the production of new T cells. Our previous studies suggested that aged bone marrow, even in an aged host, could give rise to a substantial population of new naive CD4 T cells and that the immediate function of these cells was equivalent to populations derived from young BMPC (27).

Our novel studies presented in this report have examined whether BMPC from aged mice have the capacity to give rise, in young lymphopenic hosts, to new naive CD4 T cells that can become functional memory cells. Several studies have examined the influence of aging on BMPC function and the production of new T cells. Weissman and colleagues (28) demonstrated that stem cells from young and aged mice exhibit indistinguishable progenitor activities in vivo. In a subsequent study, they also showed that aged stem cells exhibit up-regulation of myeloid lineage genes and genes involved in leukemic transformation, which might account for reduced lymphocyte and enhanced production of myeloid lineage cells with aging (29). With regard to T cell production, Gui et al. (30) reported that T cell precursors are recruited similarly to both young and aged thymuses, but that the aged thymus exhibited an altered architecture that did not support the expansion and differentiation of the precursors into mature T cells. Thus, aging may influence stem cell function and new T cell production at several different levels.

To determine the genesis of the observed defect in function of memory cells derived from aged naive CD4 T cells, we began by examining the intrinsic ability of BMPC from young and aged animals to generate new CD4 T cells in young lymphopenic hosts and determined whether these new T cells could give rise to functional memory populations. These experiments are a critical first step to determine whether there are intrinsic age-related changes in BMPC function, distinct from other components in the aging animal, such as BM microenvironment and thymic involution. In both TCR Tg and polyclonal models, our results demonstrate that new naive CD4 T cells generated in lethally irradiated young hosts from aged BMPC can differentiate into functional memory cells that exhibit good responses both ex vivo and in vivo. Importantly, we also show for the first time that memory T cells derived from the newly generated CD4 T cell exhibit potent helper activity leading to good GC formation and IgG production, both of which are important for proper responses to vaccination.

Now that we have demonstrated that there are no detectable age-related intrinsic defects in the ability of BMPC from aged mice to generate new naive T cells and, subsequently, memory T cells, in lymphopenic hosts, the next step in our studies will be to examine the impact of the aged host environment on new T cell production. A careful analysis of age-related changes in the BM microenvironment and on new T cell production by an aged involution thymus will further our understanding of the defects observed in naive T cell function in aged individuals (5, 6, 13). Thymic involution begins at sexual maturity in mice (31) and is thought to be a major obstacle with regards to new T cell production with aging and other lymphopenic conditions (32). Although there has been little success in reversing thymic involution in the past, we are very encouraged by a recent study showing that enhancement of new T cell production in aged animals by an IL–7 fusion protein improved T cell responses to influenza infection (33). Thus, it is quite encouraging that this approach could improve the immune response to infection or vaccination in the elderly.

Disclosures
The authors have no financial conflict of interest.

References


