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*J Immunol* 2003; 170:1267-1273; doi: 10.4049/jimmunol.170.3.1267
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Enhanced Differentiation of Splenic Plasma Cells but Diminished Long-Lived High-Affinity Bone Marrow Plasma Cells in Aged Mice

Shuhua Han,* Kaiyong Yang,† Zeynep Ozen,* Weiyi Peng,* Ekaterina Marinova,* Garnett Kelsoe,† and Biao Zheng2*

In the present work, we have dissected the mechanisms responsible for the impaired humoral responses in aging. We found that there was a substantially higher level of Ab-forming cells in the spleens of aged mice than that of young controls. However, the number of high-affinity, class-switched Ab-forming cells was severely decreased in the spleen of aged mice. The accumulation of low-affinity IgM Ab-forming cells in the spleens of aged animals was not due to a deficiency in isotype switching because the number of total IgG1 splenic plasma cells was not significantly reduced. Remarkably, plasma cells of both low and high affinity were significantly diminished in the bone marrow of aged mice compared with that of young mice. The results from reconstitution experiments showed that aged bone marrow was less supportive for plasma cells derived from young splenic B cells. These findings suggest that humoral immune deficiency in aging results from at least two mechanisms: the inability to generate sufficient numbers of high-affinity Ab-forming cells, which is a result of diminished germinal center reaction, and the defective bone marrow environment that has diminished ability to support the selection and survival of long-term Ab-forming cells. The Journal of Immunology, 2003, 170: 1267–1273.

In the BM, Ag-specific AFCs can be detected for at least 1 year after immunization (6). The long-term presence of AFCs may be attributable to two possible mechanisms: BM plasmacytes that are intrinsically long-lived or AFCs that are continuously generated by the activation of memory B cells with persisting Ags. Recent experiments have shown that most murine BM AFCs in a secondary immune response are long-lived and not derived from the differentiation of proliferating, activated memory B cells (6, 7, 11). Thus, any failure to support the generation of this long-lived effector population would result in short-term and low-affinity Ab responses, precisely what is observed in the elderly.

Recent work has clearly established that the presence of long-lived AFCs in the BM is dependent on the GC reaction. It has been shown in our earlier work that the successive administration of the anti-CD40 ligand Ab MR1 disrupts GCs without inhibiting early AFC foci associated with the periarteriolar lymphoid sheath (12). Thus, MR1 treatment enables us to block specifically the generation and differentiation of cells that pass through GC reaction. The effects of MR1 treatment on the generation of AFCs in BM have been clearly demonstrated in that mice treated with MR1 showed significant reduction of high-affinity AFCs in BM during the late primary response, whereas the frequency of low-affinity AFCs was unaffected, suggesting that GCs are critically involved in the generation of BM AFC precursors of high affinity, but not of precursors of low affinity (13). The serum titer of high-affinity, but not of low-affinity, Abs was also reduced and resulted in the impaired affinity maturation of serum Abs. Therefore, these data strongly support that GCs are the principal sites where BM AFC precursors of high affinity are generated and are indispensable for affinity maturation of serum Abs.

Reduced GC responses are correlated with declining humoral immunity in aging, in that there is a severe reduction in GC reaction in elderly people and aged animals (14). Although the diminished GC response is likely the result of age-associated impairment in lymphocyte function, the reduction of GCs may itself make an
important contribution to further immunological dysfunction in aging. To understand the mechanisms responsible for the short-term and low-affinity Ab responses in the aged, we have used the clonally restricted Ab response of C57BL/6 mice to the (4-hydroxy-3-nitrophenyl)acetyl (NP) hapten (15, 16) to study the generation and selection of long-lived, high-affinity AFCs in aged mice and young adult controls.

Materials and Methods

Mice, Ags, and immunization

Aged C57BL/6 mice (24 mo old) of both sexes were obtained from the National Institute on Aging, National Institutes of Health. Young C57BL/6 mice (2 mo old) of both sexes were purchased from The Jackson Laboratory (Bar Harbor, ME). Congenic C57BL6/Igha mice were also from The Jackson Laboratory. Animals were housed in microisolator cages, provided with sterile food, water, and bedding, and maintained on a 12-h day/night cycle. Sucinic anhydride esters of NP were reacted with chicken γ-globulin (GGG; Sigma-Aldrich, St. Louis, MO) or BSA (Biochemical, Cleveland, OH). Mice were immunized i.p. with 50 μg of NP-CGG conjugate precipitated in alum.

Generation and analysis of IghaIghb chimeric mice

Recipient young (2-mo-old) and aged (24-mo-old) C57BL/6 (Igha) mice were irradiated with 400 rad using a 157Cs irradiator. Eighteen hours later, the mice were injected i.v. with 5 × 10^5 spleenocytes from congenic C57BL/6 (Ighb) mice (2 mo old) that had been immunized with 50 μg of NP-CGG conjugate 12 days earlier. The recipient mice were then given an i.p. injection of 50 μg of NP-CGG conjugate precipitated in alum 6 h later. The reconstituted mice were analyzed for NP-specific AFC and Ab production 6 wk later with anti-IgG1 and anti-IgM′ Abs (BD PharMingen, San Diego, CA).

ELISPOT

The frequencies of NP-specific AFCs from both splenocytes and BM cells were estimated by ELISPOT assay using two different coupling ratios of NP-BSA, NP2-BSA, and NP25-BSA. Nitrocellulose filters were coated with 50 μg/ml NP2-BSA, NP25-BSA, or BSA in PBS at 4°C overnight and then blocked with 1% BSA in PBS. Splenocytes (10^5 cells/well) or BM cells (5 × 10^5 cells/well) were incubated on the nitrocellulose filters in 96-well plates at 37°C in 5% CO2. After a 2-h incubation, nitrocellulose filters were washed with distilled water once, PBS containing 50 mM EDTA once, followed by PBS containing 0.1% Tween 20 twice and PBS once. Filters were double stained with AP-conjugated anti-mouse IgM and HRP-conjugated anti-mouse IgG1 Abs. AP and HRP activities were visualized using 3-amino-9-ethylcarbazole and naphthol AS-MX phosphate/Fast Blue BB, respectively. The frequencies of high affinity and total AFCs was determined from NP2-BSA- and NP25-BSA-coated filters after background on BSA-coated filters was subtracted.

Several J558L myeloma lines (H−; A1) transfected with an IgG1 expression vector carrying different VDJ rearrangements derived from NP-binding B cells were incubated at 100 cells/well on nitrocellulose filters coated with these two NP-BSA conjugates to determine the threshold of Ab affinity which can be detected by each NP-BSA conjugate. Transfectomas secreting NP-binding Ab with an association constant (Ka) = 2.0 × 10^11 μM−1 (H33Ly/1A1) could be detected by both NP2-BSA and NP25-BSA. However, transfectomas with a Ka = 10^10 M−1 could be detected by NP25-BSA, but not by NP2-BSA. Transfectomas with a Ka = 2.3 × 10^9 M−1 could not be detected by either NP-BSA. Thus, AFCs secreting Abs with a Ka ≥ 2.0 × 10^11 M−1 can be detected with NP-BSA, while those with a Ka ≤ 10^11 M−1 but <2.0 × 10^9 M−1 can only be detected by NP25-BSA.

Measurement of serum Abs

Similar to ELISPOT assays, Abs specific for NP were detected by ELISA using the two different coupling ratios of NP-BSA as the coating Ags. Briefly, 96-well flat-bottom plates (Falcon; BD Biosciences, Oxford, CA) were coated with 50 μg/ml NP2-BSA or NP25-BSA in 0.1 M carbonate buffer (pH 9.0) at 4°C overnight. On each plate, H33Ly/1A1 or B1-8, two mAbs recognizing the NP hapten (Ka = 2.0 × 10^11 M−1) were included as a control. After washing with PBS containing 0.1% Tween 20, HRP-conjugated goat anti-mouse IgG1 or IgM Ab was added and incubated at room temperature for 1 h. HRP activity was visualized using a tetramethylbenzidine peroxidase substrate kit (Bio-Rad, Hercules, CA) and OD were determined at 450 nm. The concentrations of anti-NP Abs were estimated by comparison to standard curves created from the H33Ly/1A1 (for IgG1) or B1-8 (for IgM) control on each plate. To estimate the affinity of NP-binding Abs in the sera, the ratios of NP2-binding to NP25-binding Abs were calculated.

The affinity threshold of Ab binding to each NP-BSA conjugate was determined by using several mAbs with different affinities for NP. H33Ly/1A1 bound equally well to both NP-BSA conjugates, while a mAb with a Ka = 10^10 M−1 showed a 20-fold lower binding to NP2-BSA than to NP25-BSA. Another mAb with a Ka = 2.3 × 10^9 M−1 had a 10-fold lower binding to NP25-BSA than the one with a Ka = 10^10 M−1. Thus, Abs with a Ka ≥ 2.0 × 10^9 M−1 can be detected with NP2-BSA and those with a Ka ≤ 2.0 × 10^9 M−1 but <2.0 × 10^8 M−1 can be detected with NP25-BSA.

Flow cytometry

Single-cell suspensions were prepared and RBCs were depleted by incubation in 0.83% NH4Cl; cells were then washed with PBS (pH 7.4) containing 2% FCS and 0.08% sodium azide at 4°C. To estimate the prevalence of GC B cells, cells were stained with FITC-labeled GL-7, PE-conjugated anti-B220 (BD PharMingen), and 7-aminoacridinomycin D (Molecular Probes, Eugene, OR) after incubation with anti-FcγRI/III to block FcγR-mediated binding. Events were collected on a FACSort machine (BD Biosciences) and the percentage of GL-7+B220+ cells were calculated from live lymphocytes selected by forward side scatter pattern and exclusion of 7-aminoacridinomycin D using CellQuest software (version 3.01; BD Biosciences).

Results

Augmented splenic AFC response but decreased GC reaction in aged mice

To determine the ability of aged mice to mount an AFC response after immunization with T-dependent Ags, we measured the frequencies of splenic NP-specific AFCs at both early and late stages of the primary response. Remarkably, at day 16 after immunization, the number of NP-specific AFCs in aged mice was ~3-fold higher than that in young control animals (Fig. 1a). More surprisingly, at day 70 of the response, there was still a substantial level of NP-binding AFCs in the spleens of aged mice, whereas only a minimum level of splenic AFCs was present in young animals (Fig. 1a). This enhanced and long-lasting splenic AFC response in aged mice was confirmed by in situ immunohistology. Significantly higher number of plasma cells was present in the spleens of aged mice than that in young control mice (Fig. 1b).

To exclude the possibility that this high level of NP-binding AFCs in aged animals may be the result of chronic exposure to certain environmental Ags that activate NP-specific or cross-reactive B cell clones, we examined the spleens of aged mice that did not receive NP immunization (day 0). The result clearly showed that the frequencies of NP-binding AFCs in unimmunized spleens were minimal (Fig. 1a), confirming that these splenic NP-binding AFCs in old mice were indeed specifically induced by immunization of NP-CGG.

Since the majority of splenic AFCs in aged mice are IgM Ab producers (Fig. 2a), it is possible that the increased level of IgM-secreting AFCs in aged spleens is the result of a deficiency in isotype switching that causes the accumulation of IgM-secreting plasma cells. However, this seems not the case since the number of total (NP25-binding) splenic IgG1 AFCs in aged mice was similar to that in young control mice (63 ± 20 vs 55 ± 8 cells/10^6 splenic cells; Fig. 2a). Thus, our work demonstrates that, contrary to conventional wisdom, the plasma cell differentiation pathway in the spleens of old animals is enhanced following T-dependent Ag stimulation.

It has been well established that the GCs in the secondary lymphoid tissues are the site for somatic hypermutation of V(D)J rearrangements of Ag receptors, Ag-driven clonal selection for high-affinity Abs, and generation of the B cell memory compartment (17). We have examined the GC responses in the spleens of the
same animals used for analysis of AFC and Ab responses to NP-CGG. As expected, in situ immunohistologic staining shows that GC reaction in old mice was dramatically decreased compared with that in young controls. Consistent with previous reports (14, 18), GCs formed in the spleens of aged animals were significantly smaller and fewer in number as determined by the GC marker peanut agglutinin (Fig. 1b). Using the GC B cell-specific Ab GL-7 (19), we further determined the GC response by flow cytometric analysis. We found that, in aged mice, the numbers of B220^+GL-7^+ GC B cells in the spleens were less than or equal to one-third of those in young mice at 16 days after immunization, when the GC reaction reaches its peak in a primary immune response (Fig. 1c). These observations demonstrated that, compared with young control animals, aged mice are profoundly defective in GC reaction and the Ag-activated B cells may mostly undergo the pre-GC AFC differentiation pathway.

Aged mice are deficient in generating high-affinity AFCs
To further analyze the Ab responses in aged mice, we examined the NP-specific AFCs of different isotypes and affinities in the spleen and bone marrow. The results showed that, at day 16 of the response, the majority of the elevated splenic AFCs in old mice were low-affinity (NP_{25}-binding only) IgM producers, whereas the level of high-affinity (NP_{5} binders) AFCs in aged mice was significantly decreased compared with young animals (mean ± SE, 18 ± 6 vs 36 ± 4/10^6 splenic cells; Fig. 2a). More strikingly, in the BM of aged mice, only a small number of low-affinity IgM AFCs was observed, whereas NP_{5}-binding IgG1 AFCs were virtually undetectable (~1 cell/10^6 BM cells; Fig. 2b). In marked contrast, the vast majority of NP-specific BM AFCs in young animals were high-affinity NP_{5}-binding IgG1 producers (Fig. 2b).

The diminished levels of high-affinity AFCs in aged mice are well mirrored in the serum NP-specific Abs (Fig. 2c). NP_{5}-binding IgG1 serum Abs were almost absent in aged mice. Only small amounts of low-affinity (NP_{25}-binding only) Abs were detected in the sera of aged mice 16 days after primary immunization.

These observations demonstrate that, in aged mice, although B cells are able to respond to immunizing T-dependent Ags and produce high levels of Ag-specific IgM-secreting AFCs in the spleen, the ability to generate high-affinity AFCs is severely diminished.

BM AFCs are significantly decreased in aged mice
The total number of splenic AFCs at day 16 of the response in aged mice is significantly higher than that in young mice, with the frequency of 288/10^6 splenic cells or 80/10^6 splenic cells, respectively (Fig. 2a). In marked contrast, the frequency of BM AFCs in

FIGURE 1. Enhanced splenic AFC response and diminished GC reaction in aged mice. a, At 0, 16, and 70 days after immunization with 50 μg of NP-CGG, numbers of NP-binding AFCs from spleens of young (□) or aged (■) mice were determined by ELISPOT assays using NP_{25}-BSA-coated membrane. Both IgG1- and IgM-secreting AFCs were counted. b, Splenic sections of young and old mice at 16 days after immunization were stained with peanut agglutinin for GCs (red) and anti-IgM Ab (blue). Arrows indicate foci of plasma cells. Original magnification, ×100. c, The percentages of GC B cells out of splenic live lymphocytes were assessed by costaining with anti-B220 and GL-7 Abs. Percentages of cells in each quadrant are indicated.
Aged mice is only about half of that in young controls (17 vs 36 in 10^6 BM cells; Fig. 2b). We further analyzed the NP-specific AFCs at a late stage of the response to determine whether the capability of generating and maintaining long-lived plasma cells has altered in aging. It has been shown that during the late stage of a primary immune response in normal animals, most of the long-lived plasma cells reside in the BM (6, 7, 11). Indeed, by 70 days after immunization, increased numbers of BM AFCs (42/10^6 BM cells) were found in young animals compared with that of day 16 and the vast majority of NP-specific AFCs were NP5-binding IgG1 producers (Fig. 3b). However, the frequency of BM AFCs in aged mice was decreased compared with that of the early stage of the anti-NP response (8/10^6 cells vs 17/10^6 cells). Most of the AFCs present in old mice at 70 days after immunization were low-affinity IgM Ab producers and these low-affinity AFCs were largely retained in the spleen (Fig. 3a).

Thus, our analysis of the splenic and BM AFC pools at the early and late stage of the Ab response clearly showed a shift of anatomic localization for AFCs between old mice and young controls. In young animals, the principal source of Ab production has shifted from the spleen at the early phase to the BM at the late phase of the response, whereas in old animals, the spleen has remained as the primary source of Ab production during the entire course of the response (Fig. 4a).

Earlier work has shown that long-term, class-switched, and high-affinity AFCs are selectively enriched in the BM (6, 7, 11). However, our present study demonstrated that, in addition to AFCs that produce high-affinity Abs, there was a significant number of AFCs that produce low-affinity Abs (32% of IgG1-producing AFCs) present in the BM of young control animals 70 days after immunization (Fig. 3), suggesting that normal BM can support plasma cells of various affinities. In contrast, IgG1-producing AFCs were almost absent in the BM of aged mice (Figs. 2b and 3b), although the levels of total IgG1-producing AFCs in the spleens at both early and late stages of the response were comparable between old and young animals (Figs. 2a and 3a). Therefore, our work strongly suggests that low-affinity AFCs generated in aged spleens failed to emigrate to or were unable to survive in the BM environment, resulting in accumulation of these low-affinity AFCs in the spleen.

Affinity maturation of Ab responses in aged mice can be partially achieved in the spleen

In young mice, the accumulation and persistence of high-affinity isotype-switched NP-specific AFCs in the BM correlated well with
the persistently high amount of NP-binding IgG1 Abs in the sera (Fig. 3c). At day 70 of the response, although the total amount of NP-specific serum Abs was decreased compared with the early stage of the response (Fig. 2c), the majority of the serum Abs were high-affinity (NP$_5$-binding) IgG1 Abs (Fig. 3c). In contrast, in aged animals, the levels of NP-specific serum Abs, particularly NP$_5$-binding IgG1 Abs, were severely reduced, consistent with the severely diminished BM AFCs (Fig. 3c).

In addition, our results also demonstrate that affinity maturation was achieved effectively in both BM and splenic AFC populations of young animals early during the primary immune response. By day 16 of the primary response, the proportions of high-affinity AFCs out of the total NP-specific IgG1 AFCs in the spleen and BM of young animals were 65 and 77%, respectively (Fig. 4b). However, the average affinity of NP-binding IgG1 Ab in the serum at this time was still low and only <10% of the total serum NP-binding Abs were NP$_5$ binders (Fig. 4b). This lag between affinity maturation of AFCs and affinity maturation of serum Abs presumably is the time needed for Ab production by high-affinity AFCs to replenish the serum Ab pool. By day 70 of the response, the vast majority of high-affinity IgG1 AFCs generated in the spleens of young animals were exported to the BM (Figs. 3b and 4a). There was no significant change in the affinity of BM AFCs in young animals between 16 and 70 days after immunization (Fig. 4b). These results are consistent with the observation that high-affinity AFCs are selectively enriched early in the primary immune response (7).

On the other hand, the splenic AFC pool of aged mice has remained as the principal source of Ab production throughout the entire course of the response since BM AFCs were still largely absent at 70 days after immunization (Fig. 4a). However, the number of high-affinity IgG1-producing splenic AFCs has increased from 25% at day 16 to 38% at day 70 (Fig. 4b). Additionally,
although the total amount of NP-specific Abs in aged mice is ∼5- to 20-fold lower than that in young animals during the course of the primary response (Figs. 2 and 3), the average affinity of the IgG1 Abs produced by aged mice was progressively increased. By day 70, the ratio of NP25- to NP5-binding IgG1 Abs produced in aged mice reached a level similar to that in young control animals. Fifty-four percent of the IgG1 anti-NP Abs present in aged animals were NP5 binders as measured by ELISA (Fig. 4b). These results suggest that affinity maturation of splenic AFCs as well as serum Abs could be partially achieved during an immune response in aged animals, indicating that the mechanisms of clonal competition and selection are still largely intact. However, unlike in young animals, splenic AFCs become the major source of long-term Ab production in aged animals.

**BM of aged mice is less supportive for accumulation or survival of AFCs**

To understand the mechanism responsible for the reduced AFCs in aged BM, we generated chimeric mice by reconstituting irradiated young and aged C57BL/6 (Ighc) mice with splenocytes from young congenic C57BL/6 (Ighc) mice. To increase the frequencies of NP-specific lymphocytes, NP-primed splenocytes were used in transfer. Six weeks after immunization, AFCs of the Ighc-C "B" haplotype in the spleen and BM of young and old recipient mice were determined. In the spleen, the numbers of NP25- and NP5-binding AFCs of both IgM and IgG1 isotypes were comparable between young and aged animals (Fig. 5a). However, the levels of BM AFCs in aged mice were significantly decreased compared with those in young mice receiving the same preparation of splenocytes from IghC congenic mice (Fig. 5b). The frequency of total AFCs in aged BM was less than half of that in young BM (22 vs 56/106 BM cells); in particular, IgG1-type AFCs were almost undetectable in aged BM. Thus, although splenocytes from young Ighc-C congenic mice were able to mount similar levels of splenic AFC responses in irradiated young and aged animals, aged BM contained significantly less AFCs than young BM, suggesting that the ability of aged BM to support the accumulation or survival of plasma cells is significantly compromised.

**Discussion**

After immunization, aged individuals often generate significantly fewer Abs (20), maintain protective titers of serum Abs for much shorter periods (21), and produce Abs with affinity and/or avidity typically below that of young adult controls (22–24). These deficits may be responsible for increased susceptibility to infection in aged populations. The present study reveals that humoral immune deficiency in aging results from at least two mechanisms: failure to generate sufficient numbers of high-affinity AFCs and a defective BM environment that has diminished ability to support selection and survival of AFCs.

It is generally believed that the Ab responses in the aged are overall diminished. Therefore, it was unexpected that a significantly higher number of Ag-specific plasma cells was present in the spleens of aged mice compared with that in young control animals (Fig. 1). The spleens of old mice retained a significant amount of plasma cells even at day 70 of the response, at a time when splenic AFCs were largely absent in young mice (Fig. 1a). However, the vast majority of plasma cells in aged spleens were IgM producers (78% at day 16 and 92% at day 70, respectively), as opposed to the AFCs in young animals in which IgG1 producers were predominant (69% at day 16 and 98% at day 70, respectively; Figs. 2 and 3). Furthermore, this small number of IgG1 plasma cells in aged spleens was mostly low-affinity Ab producers (Figs. 2a and 3a). In contrast, the majority of the IgG1 plasma cells in young spleens were high-affinity Ab producers (65% at day 16 and a minimal number of splenic AFCs at day 70; Fig. 4). Therefore, these results demonstrate that although aged splenic B cells are able to be activated and differentiate into plasma cells, the capability of generating class-switched, high-affinity AFCs was impaired.

Results from our earlier work and other groups have clearly established the relationship between GC response and high-affinity long-term AFCs in the BM. Smith et al. (7) have shown that high-affinity AFCs generated by somatic hypermutation selectively accumulate in the BM compartment, suggesting that the precursors for BM AFCs are generated within GCs. Our earlier work has demonstrated the extent to which GCs contribute to the generation of AFCs, particularly those in the BM (12, 13, 25). The present study is consistent with earlier reports (14, 18) that GC reaction is progressively diminished during the process of aging. The most remarkable difference between GC responses in aged and young control mice is the almost complete absence of mutated V(D)J rearrangements in the old animals (18). Thus, our present results are consistent with the notion that the diminished GC response is responsible for the diminished number of high-affinity AFCs.

An interesting observation from this study is the presence of large numbers of IgM-secreting plasma cells in the spleens of aged mice. One possible explanation could be that the switching machinery is defective in aged mice. However, the number of total (NP25-binding) splenic IgG1 AFCs in aged mice was similar to that in young controls (Fig. 2a), suggesting that B cells in aged mice are capable of isotype switching. It has been shown that although isotype switching takes place mainly in the GCs, the process is initiated in the T cell zone and junction between the T and B zones (17, 26, 27). Another possibility is that the balance between AFC and GC differentiation pathways is tilted in aged mice. It has been generally accepted that Ag-activated B cells undergo two separate pathways. One is the pre-GC pathway in that activated B cells become plasma cells early in a primary immune response, whereas other activated B cells enter the GC pathway in lymphoid follicles (2–5, 17, 26). In aged mice, the GC differentiation pathway is inhibited, which may render most of the activated B cells undergo the pre-GC pathway of plasma cell differentiation, resulting in the increased Ag-specific AFCs in the spleen (Fig. 1a). Therefore, diminished GC reaction may also be responsible for the accumulation of plasma cells in the spleen.

In aged mice, although the levels of total serum Abs specific for the immunizing Ag is ∼5- to 20-fold lower than those in young control animals (Figs. 2c and 3c), remarkably, affinity maturation is still present. The proportion of serum NP5-binding IgG1 Abs increased from <10% on day 16 of the response to >50% on day 70 of the response (Fig. 4b). Consistently, the affinity of NP-binding IgG1 AFCs present in spleens of aged mice had increased significantly during the immune response (Fig. 4b). Since Ab affinity maturation is largely dependent on the selection and enrichment of high-affinity B cell mutants that are generated during the GC response, one obvious question, then, has emerged. How can affinity maturation be achieved in aged animals in which GC reaction and Ig somatic hypermutation are severely diminished? Earlier studies demonstrated that, like GC B cells in young mice, GC B cells in aged mice often contain V(D)J rearrangements with a single dominant CDR3 (18), implying active clonal selection within GCs. Furthermore, the unmutated V H 186.2 rearrangements recovered from the GCs of aged mice were associated with D elements typical of the primary NP response (18) and could be found in NP-specific hybridomas. Indeed, Abs from transfectomas generated by the expression of V(D)J fragments recovered from A17 GCs of aged mice avidly bind the NP and (4-hydroxy-5-iodo-
3-nitrophenyl)acetyl haptens (18, 28). Thus, although the mutational mechanism is impaired, aged mice still can achieve partial affinity maturation by selecting and enriching B cell clones that have higher affinity over other clones. The decreased extent of affinity maturation in aged mice is most likely due to the diminished capability of generating high-affinity B cell clones via somatic hypermutation.

Another interesting observation in the current work is that, unlike young mice in which the long-term Abs are mostly produced by long-lived AFCs in the BM, the majority of AFCs in old animals remain in the splenic environment and do not home to the BM (Figs. 3 and 4a). Two possible mechanisms may account for the lack of AFCs in the BM of aged mice: requirement of high affinity for plasma cells to be selectively accumulated in the BM and decreased capability of BM in supporting the survival of plasma cells. It is conceivable that an affinity threshold is enforced so that only high-affinity AFCs may be selectively exported to and enriched in the BM environment, whereas AFCs of low affinity either cannot make it to the BM or cannot survive in the BM environment. However, since low-affinity IgGl-producing AFCs were present in the BM of young mice (Fig. 3b), it is unlikely that AFCs in aged mice are excluded from the BM environment because of their low affinity. Instead, the absence of AFCs in aged BM is most likely due to the inability of the aged BM environment to support the survival of these cells. This notion is supported by our results from transfer experiments. Young donor splenic B cells were able to mount similar AFC responses in the spleens of young and aged recipients, but the numbers of BM AFCs in aged recipients were significantly diminished compared with that in young controls (Fig. 5), demonstrating that the BM environment in aged mice is defective for supporting plasma cells. This is consistent with earlier work showing that, with age, BM has become much less supportive due to impaired stromal cell function and decreased cytokine/growth factor production (29, 30). Therefore, apart from deficiency in generating high-affinity long-lived AFCs due to a comprised GC response, the diminished ability of aged BM to support the accumulation and survival of AFCs plays an important part in the humoral deficiency in aging.

The present study may have important clinical implications. It is important to realize that the aged populations not only have a decreased ability to generate sufficient numbers of effector cells during an immune response, but also have a defective microenvironment to support these effectors. Thus, the strategy for therapeutic immune potentiation to protect the elderly populations will be 2-fold, restoring the diminished GC response to produce sufficient high quality effectors on one hand and improving the microenvironment such as BM to sustain the survival and enrichment of effector cells on the other.

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