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Altered Memory B-Cell Homeostasis in Human Aging

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Previous studies of age-associated immune system changes revealed alterations in expressed immunoglobulin heavy chain variable domain repertoires, and variability in the fraction of expressed heavy chain variable domain genes with mutations. To test whether the latter finding reflected a variation in memory B-cell numbers, we measured circulating memory B cells of 11 healthy elderly subjects, 173 nursing-home residents, and 34 healthy young adults. A large fraction of old adults have low values for memory cells both as a percentage of all B cells and as an absolute memory B-cell concentration. The range of both values is much wider in old adults than in young adults, and it is much wider than the range of T-cell concentrations. Memory B-cell concentration, which was positively correlated with memory T-cell concentrations but inversely related to in vitro T-cell responses to mitogens, may reflect highly individual rates of immune senescence, and it may serve as an amplified marker of underlying T-cell function.

THE immune system undergoes many changes in the aging process, as reflected in diminished T-cell responses to recall antigens, decreased antibody formation to foreign antigens, and increased autoantibody formation (summarized in several articles in Vol. 160 of *Immunological Reviews*, 1997). Diminished immune responsiveness is a predictor of morbidity and mortality (1,2). It may underlie the fact that infectious disease is one of the most frequent causes of morbidity and mortality in the elderly population (3), particularly in nursing-home patients (3,4). Over many years of study, one of the most consistently demonstrated manifestations of age-related immune system decline is reduced responsiveness to immunization (5–13). There is a pressing need to clarify mechanisms underlying poor responses and to predict which people may need more than simple vaccination for protection against infection.

We previously compared immunoglobulin (Ig) VH cDNA libraries (where VH is the heavy chain variable domain, comprising V_H, and D_H, and J_H segments) from circulating B cells of old (over the age of 65) and young (mean age 35) humans (14). In the young adults, approximately two thirds of the IgM-encoding VH cDNA clones had zero to three V_H segment mutations. There was much more variation among the elderly group in the size of this low mutation fraction of clones, ranging from 22% to 85%. In addition, V_H4 family expression was increased in older subjects, whereas V_H3 family expression dominated in young adults (14).

The variability in mutation frequency in VH cDNA clones from the elderly group led us to test more widely whether that finding reflected a similarly high variability in memory B-cell populations among senior individuals. Memory cells, in both IgM and IgG subclasses, have in fact been partially defined by the presence of numerous V region mutations (15), but prohibitively extensive sequencing would be required to study a large number of individuals by use of that criterion. In recent years, a simpler measurement has become possible, as a cell-surface glycoprotein, CD27, a member of the tumor necrosis factor (TNF) family, has been identified as a memory B-cell marker (16). CD27 is expressed on the majority of T cells and on some B cells. $CD27^+$ B cells have mutations in their Ig V genes (17), and they are also classified as memory B cells on the basis of function and morphology (18,19). Therefore, we examined circulating B cells from a number of elderly and young subjects by staining the cells with anti-CD19 (a pan-B cell marker) and anti-CD27. We confirmed that, in young adults, the memory B cells made up 20–60% of the B-cell compartment. Among a sample of healthy elderly subjects, however, we found that a large fraction had lower levels and the group had a lower mean value. Among 173 nursing-home residents, we also found a large fraction with low numbers of memory B cells, measured as either a percentage of total B cells or as the concentration of cells per cubic millimeter of blood; however, there was an exceptionally wide range of memory B-cell numbers in the nursing-home population. Because aging-associated alterations, in both mice and humans, are generally more pronounced in T-cell than in B-cell function (20,21), and these T-cell changes may indeed be the underlying factors in altered B-cell responses (22) and poor responses to vaccines, we also tested whether the memory B-cell numbers were correlated with changes in T-cell subset concentrations and in vitro T-cell responses to mitogenic stimulation.

METHODS

Subjects

The study population consisted of two elderly groups, one composed of free-living healthy subjects and the second composed of nursing-home residents. We measured memory B cells in blood from 11 elderly free-living volunteers, aged 65–82 years (average 74.5 ± 5), recruited at the Metabolic Research Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. They were screened by interview to ensure they did not have autoimmune diseases, allergies, lymphomas, or recent infection (within the previous 3 weeks), and were not being treated with steroids, immune suppressants, antihistamines, or chemotherapeutic agents. Smokers and drinkers of more than three alcoholic beverages per day were also excluded. The 173 nursing-home subjects, 68 men and 106 women, were 75 years of age or older (average 83.1 ± 7.8). At the time, they were being enrolled in a study of the effect of Vitamin E supplementation on their response to immunization with influenza vaccine and resistance to spontaneous infections. Blood samples were obtained before treatment. To minimize effects of concurrent disease or malnutrition, we excluded individuals who had less than a 6-month life expectancy, were bed bound or room bound continuously for 3 months, required nasogastric or other tube feeding, had long-term intravenous or urethral catheters, had a tracheostomy or were ventilator dependent, or had protein energy malnutrition, defined as albumin < 3.0 g/dl and body mass index < 18 kg/m^2. Also excluded were people being treated with cancer chemotherapy agents, immunosuppressive drugs, systemic steroids, antibiotics (chronic), or uncommon dietary supplements. The 173 study subjects were selected from a screened population of 210 residents. For comparison, we studied a young adult group consisting of 9 men and 25 women, 23–40 years old (average 28.1 ± 3.7). They were screened by the same criteria as the healthy elderly group. Protocols were approved by the Tufts/New England Medical Center Human Investigation Review Committee.

Cell Preparation and Flow Cytometry

Peripheral blood mononuclear cells (PBMC) were isolated, as described previously (23), from buffy coat cells of 20-ml blood samples provided by 8 of the older and 12 of the younger healthy free-living donors. PBMC were isolated by Ficoll–Paque medium density centrifugation (Pharmacia, Uppsala, Sweden), and approximately 10^6 PBMC (a 50-μl sample) were stained with 20 μl of undiluted antihuman CD19-fluorescein isothiocyanate (FITC) and 20 μl of CD27-phycoerythrin (PE) mAb (Pharmingen, San Diego, CA). The sample was incubated with antibodies for 30 minutes at 4°C in the dark, after which cells were washed twice with 4 ml of phosphate-buffered saline (PBS), with 5 minutes of centrifugation at 120 × *g*. Cells were suspended with 0.5 ml of PBS and measured by flow cytometry with a FACSCalibur (where FACS stands for fluorescence-activated cell sorting; Becton Dickinson, San Jose, CA). 25,000 cells were counted and gated on the PBMC population. Gates were then set up for CD19 positive, or CD19$^+$, cells (B cells) and CD27 positive, or CD27$^+$, cells (memory B cells).

Because it was not possible to collect large blood samples from nursing-home donors, we used the FACS Lysing Solution procedure (Becton Dickinson) to analyze a small fraction of a 5-ml blood sample obtained from each of 173 residents. We also tested 34 young and 3 healthy elderly adults with this method. Cells in 50 μl of whole blood were stained with 10 μl of undiluted antihuman CD19-FITC and 10 μl of antihuman CD27-PE mAb. With other 50-μl samples, T-lymphocyte subsets were measured by three-color staining with the following: 10 μl of antihuman CD3-PerCP mAb (Becton Dickinson), as a marker for T cells; 10 μl of anti-CD4-FITC or anti-CD8-FITC for CD4/CD8 subsets; and 10 μl of anti-CD45RO-PE as a marker for memory T cells or 10 μl of anti-CD45RA-PE (Pharmingen) as a marker for naïve T cells. After incubation of whole blood with appropriate antibodies for 30 minutes at room temperature in the dark, cells were incubated with 1 ml of FACS Lysing Solution for 10 minutes at room temperature in the dark. Cells were washed with 1 ml of PBS, with 5 minutes of centrifugation at 300 × *g*, suspended with 0.2 ml of PBS, and analyzed in the FACSCalibur instrument. There were 25,000 cells counted, and gates were set on PBMC and B cells as just described, or on PBMC, CD3, CD4/CD8, and memory and naïve T-cell populations.

Lymphocyte Proliferation Measurement in Whole Blood Culture

Five milliliters of blood was collected in heparin-containing Vacutainers (Becton Dickinson) after a 10-hour (overnight) fast. The whole blood was diluted at 1:5 with complete RPMI 1640, that is, RPMI 1640 supplemented with 100,000 U/l penicillin, 100 mg/l streptomycin (Gibco Laboratories, Grand Island, NY), 2 mmol/l L-glutamine (Gibco), and 25 mmol/l Hepes (Sigma, St. Louis, MO). The diluted blood was incubated in 96-well round-bottom plates (Nunc, Denmark), in the presence or absence of the T-cell mitogens concanavalin A (Con A; Sigma) at 5, 25, 50, and 100 mg/L or phytohemagglutinin (PHA; Difco Laboratories, Detroit, MI) at 1, 5, and 50 mg/L for 72 hours at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Cultures were pulsed with 18.5 μBq of [^{3}H]-thymidine (specific radioactivity 247.9 GBq/mol; PerkinElmer Life Sciences, Boston, MA) during the final 4 hours of incubation. The cells were harvested onto glass fiber filter mats (Wallac, Gaithersburg, MD) by a Tomtec harvester (Wallac), and cell proliferation was quantified as the amount of [^{3}H]-thymidine incorporation into DNA as determined by liquid scintillation counting in a 1205 Betaplate counter (Wallac). The counter had an efficiency of more than 50% for ^{3}H. Data are expressed as counts per minute (cpm) and adjusted for the percentage of lymphocytes in each whole blood sample as determined by manual microscopic readings. Sufficient cells for these tests were obtained from 162 of the 173 blood samples.

Statistical Analyses

Differences between the study populations and genders were evaluated by use of a two-way analysis of variance with the age group and gender as study factors. Pearson correlation coefficients were used to study the association between lymphocyte subsets and the correlation of cell con-

centrations to in vitro T-cell responses. Multiple linear regression was used to test whether CD4 cell concentration and in vitro T-cell response were together predictive of memory B-cell concentration.

Results

CD27 Measurement Is a Reproducible Assay

The findings of variability in mutation frequency in VH cDNA clones from elderly people led us to test whether there was similar variability in memory B-cell numbers. We first analyzed 20-ml blood samples donated by free-living aged volunteers, using Ficoll–Paque density centrifugation to separate PBMC. We then used a FACS Lysing Solution assay to analyze 50-μl samples of whole blood from nursing-home residents. Dual staining allowed clear identification of $CD19^+$ $CD27^+$ B cells (Figure 1). Because we used two methods, some blood samples from healthy subjects were tested with both assays. Results for the two assays were very similar. For five subjects, the percentage of memory B-cell values from the Ficoll–Paque and FACS Lysing methods, respectively, were 42.5 and 41; 36 and 36.5; 42 and 42.7; 52.4 and 53; and 22.5 and 20. We also tested reproducibility of the FACS Lysing method itself by comparing two blood samples drawn at different times, several weeks apart, from given individuals. Examples of repeated assays for percentage of memory B cells for five subjects were 3.1 and 4; 13.9 and 12.8; 18.1 and 18.7; 25.2 and 24.3; 36 and 39; and 93.1 and 92.6. Thus, the assay was reproducible and values for an individual were stable over a period of weeks.

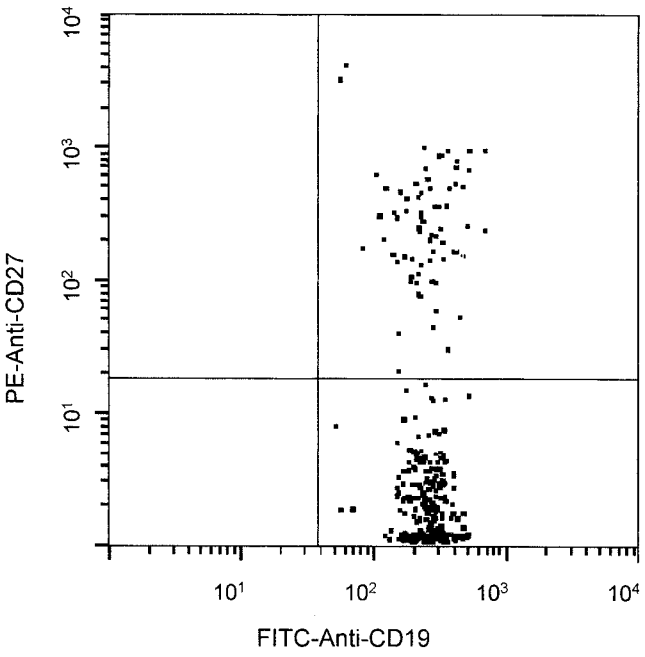

Figure 1. Fluorescence-activated cell sorting (FACS) of $CD19^+$ cells into $CD27^+$ (upper right) and $CD27^-$ (lower right) populations. Peripheral blood mononuclear cells were isolated by using either the Ficoll–Paque or FACS Lysing methods as described in the Methods section. There were 25,000 total cells counted, and gates were set to separate PBMC while memory B cells were evaluated by dual staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD19 mAb as a marker for B cells and phycoerythrin (PE)-conjugated anti-CD27 mAb as a marker for memory B cells.

Low Values of $CD27^+$ Cells in Free-Living Older Population

The mean of the values for percentage of $CD27^+$ B cells for the older individuals (22.5% ± 9.7) was significantly lower than that for young adults (35% ± 12.5; $p = .002$, by Student's t test). Five (45%) of 11 healthy free-living older subjects (average age 76.75 ± 3.4) had 20% or fewer memory B-cells, and 1 of those had fewer than 10%, whereas only 3 (9%) of 34 young adults had 20% or fewer memory B cells (Figure 2). Thirty (88%) of the 34 young adult values were in the previously reported normal range of 20–60%, and 1 (3%) was higher.

Percentage of Memory B Cells in Nursing-Home Residents

We measured memory B cells in blood samples from 173 nursing-home residents (average age 83.1 ± 7.8 years) at the time of their enrollment into a study of the effect of Vitamin E on immune responses to influenza vaccine. Blood samples were taken before dietary treatment or immunization. We measured two values: first, percentage of memory B cells, that is, memory B cells ($CD19^+$ $CD27^+$) as a percentage of all $CD19^+$ B cells; and second, memory cell concentrations, that is, memory B cells per cubic millimeter.

As in the healthy elderly group, a large fraction of subjects had low values for percentage of memory B cells: 61 of 173 (35%) of the senior donors, compared with 3 of 34 (9%) of the young adults, had fewer than 20% memory B cells (Figure 3). Fifteen of the senior adults (9%), but none of the young adults, were more than 2 *SD* below the mean for the young adults (i.e., <10% memory B cells). In addition, however, unusually high levels were also measured for some nursing-home residents, 21 of whom (12%) were

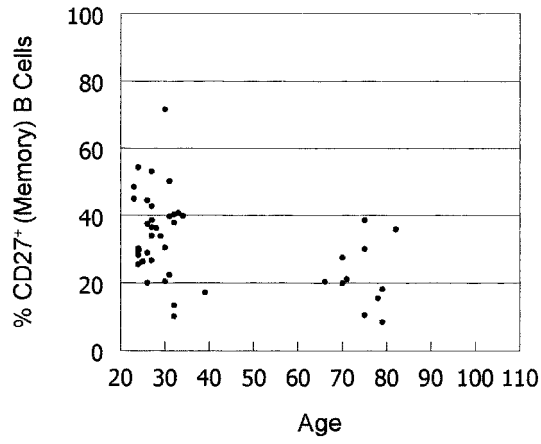

Figure 2. Percentage of memory B cells in young adults and healthy free-living elderly subjects. The percentage of memory B-cell values were determined by dual staining with fluorescein isothiocyanate-conjugated anti-CD19 mAb and phycoerythrin-conjugated anti-CD27 mAb after gating on peripheral blood mononuclear cells, identified by forward and side scattering.

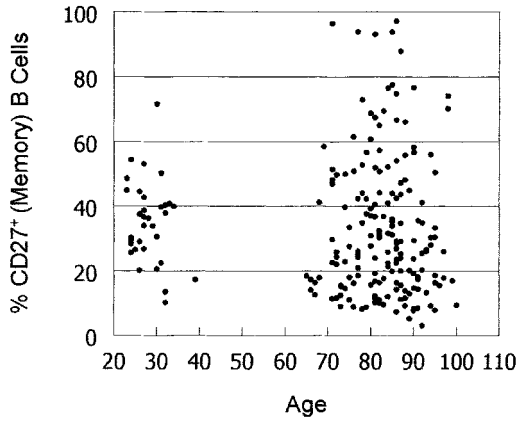

Figure 3. Percentage of memory B-cell values in young adults and nursing-home residents. The percentage of memory B-cell values were determined by dual staining with fluorescein isothiocyanate-conjugated anti-CD19 mAb and phycoerythrin-conjugated anti-CD27 mAb after gating on peripheral blood mononuclear cells, identified by forward and side scattering.

more than 2 *SD* above the mean for the young adults; only 1 of the 34 younger people was in that range. As already noted, very low or very high values were reproducible for a given individual when samples were tested at intervals of up to several weeks. The wide range of values in the senior group, from 3% to 97% memory B-cells, reveals altered regulation of circulating B-cell populations in the elderly group in comparison with younger people. That result is consistent with our previous observation of greater variability in the frequency of mutated IgM clones in cDNA libraries from peripheral B cells of healthy elderly subjects in comparison with young adults (14).

The wide range in percentage of memory B-cell values could not be explained by concurrent clinical conditions, which did not differ in subjects with unusually low, intermediate, or high values. On the basis of exclusion criteria, no people in the study were receiving immune suppressants, chemotherapy, or systemic steroids, which could influence lymphocyte populations, and there was no evidence of gross malnutrition in any subject. Medications were virtually identical in both groups, and included mostly agents to treat hypertension, cardiac arrhythmias, atherosclerosis, blood coagulability, depression or psychosis, bowel and urinary bladder dysfunction, and osteoarthritis (the latter was treated mainly with nonsteroidal anti-inflammatory agents). Many subjects in all groups were taking vitamin and mineral supplements. Subjects with low (average 8 ± 1.5; n = 14), medium (average 43 ± 14; n = 15, randomly chosen), and high (average 82 ± 11; n = 15) values for percentage of memory B cells were taking an average of six medications in each group. The low-value group for percentage of memory B cells comprised 3 men and 11 women; the medium- and high-value groups each had 6 men and 9 women.

Memory B-Cell Concentrations in Nursing-Home Subjects

Total leukocyte counts and the percentage that were both $CD19^+$ and $CD27^+$ were combined to yield the number of memory B cells per cubic millimeter. The means of logarithmic values for the old group were significantly lower than those for the young group (p = .001) (Table 1). Ninety-one of the nursing-home subjects (53%) had fewer than 35 $CD27^+$ B cells/mm^3; 51 of them (29%) had fewer than 20 and 12 of them (7%) had fewer than 10 (Figure 4). In contrast, only 1 of 34 young adults (3%) had fewer than 35 memory B cells/mm^3.

Although the dominant pattern in the elderly group was a reduction in memory B-cell concentration as well as in percentage of B cells that are memory cells, and 50% of senior adults had lower memory B-cell concentrations than all but one young adult, some senior adults had unusually high concentrations. With all samples considered, there was a nearly 900-fold range in $CD27^+$ $CD19^+$ B-cell concentration among the elderly group, from 3 to 2670 cells/mm^3 of blood (Figure 4). Three of the values were particularly high outliers, but even when they were excluded, the range was 170-fold, from 3 to 510 cells/mm^3. In 34 young adults, in contrast, all memory B-cell concentrations were within just an 8-fold range (35 to 285 cells/mm^3). Within the nursing-home population, the variations in memory B-cell concentrations were much greater than the 8.5-fold variation in the lymphocyte or T-cell concentrations, indicating a selective alteration in regulation of B-cell dynamics in some subjects.

Among the nursing-home residents, we did not find a significant gender-based difference in $CD27^+$ B cell numbers. There were no significant differences between old and young subjects in total T-cell numbers, or in $CD4^+$ or $CD8^+$ T cells per cubic millimeter (Table 1). To further test our assays, we measured markers of other lymphocyte subpopulations as well. Consistent with previous analyses, we found a

Table 1. Peripheral Blood Lymphocytes in Nursing-Home and Young Populations

					Memory		Naïve	
Parameter	B Memory	B Cells	CD4	CD8	CD4	CD8	CD4	CD8
Old (n = 173)	1.67 ± 0.033	2.24 ± 0.026	3.05 ± 0.012	2.63 ± 0.02	2.88 ± 0.013	2.44 ± 0.022	2.47 ± 0.026	2.01 ± 0.029
Young (n = 34)	1.97 ± 0.082	2.46 ± 0.066	3.00 ± 0.030	2.62 ± 0.051	2.70 ± 0.033	2.28 ± 0.055	2.67 ± 0.065	2.32 ± 0.072
p Value	.001	.002	.133	.873	<.0005	.007	.005	.005

Notes: All values are cells/mm,^{3} mean ± *SD*. Lymphocytes were gated to separate peripheral blood mononuclear cells and evaluated by dual staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD19 mAb as a marker for B cells, phycoerythrin (PE)-conjugated anti-CD27 mAb as a marker for memory B cells, anti-human CD3-PerCP mAb as a marker for T cells, CD4-FITC/CD8-FITC for CD4/CD8 subsets, CD45RO-PE as a marker for memory T cells, and CD45RA-PE as a marker for naïve T cells. The data were analyzed by using a two-way analysis of variance with gender and age group as study factors. There were no gender effects or age-by-gender interactions for any of the lymphocyte measurements; therefore, we report the mean for each age group for both sexes combined.

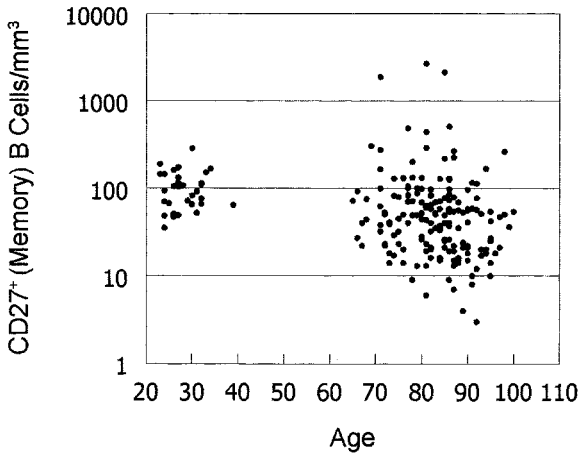

Figure 4. Memory B-cell concentrations in blood of young adults and nursing-home aged residents. Cell concentrations were evaluated by combination of total leukocyte counts with data from dual staining with fluorescein isothiocyanate-conjugated anti-CD19 mAb and phycoerythrin-conjugated anti-CD27 mAb after gating on peripheral blood mononuclear cells, identified by forward and side scattering.

significant increase in the concentration of memory components of the $CD4^+$ and $CD8^+$ ($CD45RO^+$) cells in the old group and a significant decrease in their naïve $CD4^+$ and $CD8^+$ ($CD45RA^+$) cells (Table 1).

Relationships Between Memory B Cells and Lymphocyte Subsets

Because memory B-cell variations may result from underlying T-cell changes, we tested whether the $CD27^+$ B-cell concentrations were correlated with other cell measurements (Table 2). Using Pearson correlation coefficients (r values), we found the strongest correlations between $CD27^+$ B-cell numbers and total concentrations of T cells, $CD4^+$ T cells, or $CD4^+$ memory T cells (Figure 5A and Table 2). Memory B-cell concentrations were only weakly correlated with total or memory $CD8^+$ T cells, and they were not significantly correlated with naïve $CD4^+$ or $CD8^+$ T cells (Table 2).

Relationship Between Memory B Cells and T-Cell Function

As aging is associated with profound changes in T-cell function, which may underlie B-cell homeostasis, we tested

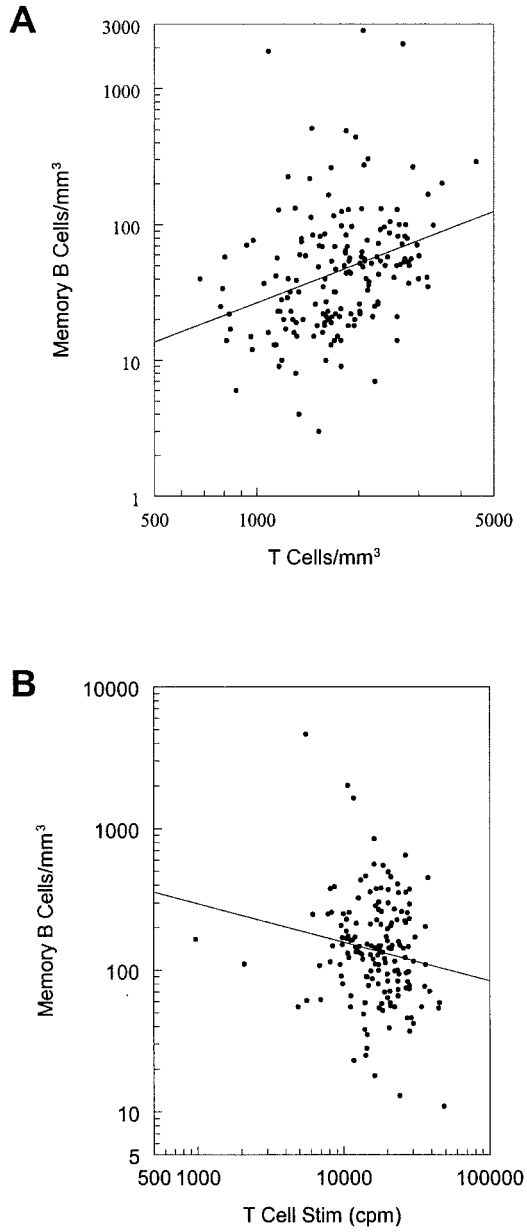

Figure 5. **A**, Positive correlation between $CD19^+$ $CD27^+$ (memory) B-cell concentrations and T-cell concentrations in blood samples from nursing-home residents. **B**, Inverse correlation between $CD19^+$ $CD27^+$ (memory) B-cell concentrations and magnitude of in vitro T-cell responses to stimulation by the mitogen concanavalin A.

Table 2. Tests for Correlations Between Log Memory B-Cell Concentrations and Other Cell Populations in Blood of Nursing-Home Residents

	Total Cells				Memory		Naïve	
Parameter	B	T	CD4	CD8	CD4	CD8	CD4	CD8
r	.760	.322	.300	.171	.324	.188	.086	.074
p	<.0005	<.0005	<.0005	.025	<.0005	.013	.259	.331

Notes: Pearson correlation coefficient (r) and significance (p values) were calculated for log(cells/mm^3) in 173 blood samples. In each column, correlation is tested for numbers of $CD27^+$ B cells and numbers of the designated cell population.

the relationship between memory B-cell concentration and T-cell responsiveness to in vitro stimulation with two mitogens. The same pretreatment blood samples were used for B-cell and T-cell function measurements. Unexpectedly, the person with the lowest memory B-cell concentration had the highest value for in vitro T-cell stimulation, whereas the person with the highest memory B-cell concentration was among the lowest for T-cell responsiveness. With all samples considered, there was a significant inverse correlation between memory B-cell concentrations and in vitro T-cell responsiveness (Figure 5B; $r = -.25$, $p < .001$ for Con A

stimulation). Similar values were measured with PHA stimulation. In view of this result, we then examined the relationships between T-cell subsets and in vitro responses to mitogen stimulation. We found an inverse correlation between in vitro T-cell responses and $CD4^+$ or $CD8^+$ memory T-cell concentrations or $CD8^+$ naïve cells (Table 3); the person whose cells gave the highest in vitro T-cell stimulation response was the same person whose cells measured the lowest for both memory B-cell and memory $CD4^+$ T-cell concentration. With all samples considered in multiple linear regression analysis, the memory B-cell concentration was significantly correlated with a combination of high CD4 memory T-cell concentration and low in vitro T-cell response to mitogen ($p < .001$ for a positive correlation with CD4 memory T cells and $p = .023$ for an inverse correlation with Con A stimulation). A similar correlation was found for the combination of CD4 memory cells and response to PHA stimulation ($p < .001$ and .037). There was no correlation of T-cell response with naïve $CD4^+$ cell concentrations.

Discussion

Following up previous findings on V gene segment usage and frequency of mutations in expressed Ig genes of circulating B cells, our study provides the first documentation that low circulating memory B-cell numbers occur in a large fraction of both healthy free-living elderly subjects and nursing-home residents. In addition, however, there was a remarkably wide range in memory B-cell numbers in the nursing-home residents, with a few having exceptionally high values. The nursing-home residents were older, on average, and had more possibility of concurrent diseases that could affect B-cell numbers. We did not, however, find unusual disease patterns or medication among those with very low or very high levels of memory B cells, and we had excluded subjects with any of several conditions that might in themselves alter immune cell function.

In the variability of memory B-cell fractions, the nursing-home residents resembled young patients with common variable immunodeficiency (CVID) who also had values ranging from less than 5% to 95% (24). CVID involves defects in both T-cell function and B-cell responses to T cells and their cytokine products. Similar dysregulation in both B cells and T cells may be associated with immune senescence (25). Indeed, T cells are more severely affected than B cells as the functional capacity of the immune system gradually declines with age (26,27), and it may be that T-cell alterations underlie B-cell dysregulation. The much greater variation in total and memory B-cell concentrations than in concentrations of total lymphocytes or of any T-cell subsets in the nursing-home residents reveals selectively altered B-cell regulation in aging or marked amplification of underlying T-cell alterations.

Table 3. Tests for Correlations Between T-Cell Responses to In Vitro Stimulation by Mitogen and Cell Populations in Blood of Nursing-Home Residents

				Memory		Naïve	
Parameter	Memory B Cells	CD4	CD8	CD4	CD8	CD4	CD8
r	−.250	−.127	−.429	−.228	−.410	.083	−.274
p	.001	.109	<.001	.004	<.001	.294	<.001

Notes: Pearson correlation coefficient (*r*) and significance (*p* values) were calculated for log(cells/mm^3) and log counts per minute, [^{3}H]-thymidine incorporation, in response to 25 μg/ml of concanavalin A for 162 blood samples. In each column, correlation is tested for [^{3}H]-thymidine incorporation and numbers of the designated cell population.

The assay for memory cells was highly reproducible with blood samples drawn from one person at different times. A reproducible parameter characteristic of a particular elderly person is of interest because of the recognized variation in the rate of progression of immune senescence among different people and the correlation of immune senescence with morbidity and mortality. Studies of these subjects over time will test whether dysregulation in memory B cells, as measured with the CD27 marker, is a predictor of poor immunological responses and susceptibility to such problems as infectious diseases or cancer.

CD27 as a Marker

CD27 is a Type I transmembrane glycoprotein that belongs to the TNF receptor family (28). Originally identified as a T-cell activation antigen (TA1) (29), involved in regulation of T-cell responses (30), it is also expressed on some B cells and plays a role in T-cell-dependent B-cell activation and expansion (31). Several lines of evidence indicate that, among B cells, CD27 selectively marks the memory cell population. $CD27^+$ B cells express Ig genes with mutated V gene segments, whereas $CD27^-$ cells express unmutated Ig genes (17). Ligation of surface CD27 by CD70, a CD27 ligand present on the surface of T cells, can trigger differentiation of memory B cells into plasma cells (19,32) and, in the presence of IL-10 and IL-2, can enhance production of immunoglobulins (33,34). $CD27^+$ cells produce IgA, IgM, and IgG in response to stimulation with *Staphylococcus aureus* Cowan + IL-2; and they produce IgE in response to stimulation by IL-4 + CD40 (34). $CD27^+$ cells make up 20–60% of peripheral blood B cells in normal adults (17,35). Results with our group of 34 young adults are generally consistent with those reports, although 1 had a value above 60% and 3 had values between 10% and 20%. To our knowledge, the $CD27^+$ B-cell subset has not previously been quantified in elderly humans.

Factors Influencing Circulating B-Cell Populations

The numbers and proportions of naïve and memory circulating B cells depend on several factors, including the output of naïve cells from the bone marrow, homing and retention or recirculation of the cells in and from peripheral tissues such as spleen, lymph nodes, and mucosal lymphoid tissue, and the generation and release of memory B cells from germinal centers (reviewed in 36). These factors are not accessible to experimentation in this nursing-home population, but studies in experimental animals have revealed a marked decrease in the homing and retention of both B cells and T cells in the spleens of aging mice (37). Thus, decreased numbers of adhesion molecule receptors in the spleen matrix could account for the high numbers of circulating B cells in some of the nursing-home residents. The al-

tered expression of homing molecules, such as VCAM-1 (38), CD22 (39), CD21, and L-selectin (40), on the B cells themselves could be the subject of further investigation.

T-Cell Subsets and Function

Changes in subpopulations and functions of T cells in older people have been identified more consistently than changes in total T-cell numbers. For example, several investigators have reported an aging-related increase in activated–memory T cells and a decrease in naïve T cells (9,41,42). We also measured those two changes, but our findings emphasize the need to evaluate individual subjects. Considering group averages, for example, one measures increased memory T cells and decreased memory B cells; considering individuals, however, one finds that concentrations of memory T cells (particularly $CD4^+$ memory T cells) were positively correlated with memory B-cell numbers. That correlation is consistent with the role in which $CD4^+$ cells help to drive memory B-cell formation and suggests that low memory B-cell levels in some elderly people might reflect previously recognized deficiencies of T-cell activation (43). Although memory T cells may have been the most important determinants of memory B-cell concentrations, they do not appear to be primarily responsible for high in vitro T-cell responsiveness to mitogen, as revealed in the *inverse* relationship between $CD4^+$ and $CD8^+$ memory T-cell concentrations and mitogen-stimulated in vitro T-cell responses.

T-cell changes do not account for all aspects of the age-associated drop in B-cell numbers. The decrease in memory B cells correlates with a decrease in total B-cell concentrations (Tables 1 and 2), including naïve B cells ($p = .001$; not shown).

Potential Importance for Function

Effective disease prevention by vaccination depends on immunological memory involving both memory B cells and memory T cells (44), interacting in germinal follicles (45). Memory B cells produce antibodies rapidly in the secondary response and are a key component for secondary immune response. The high number of nursing-home residents with exceptional percentages of memory B cells may lead us toward understanding why elderly people have difficulty in mounting a protective response to protein, viral, or bacterial vaccines and why infectious diseases are the major causes of sickness and death in an aged population (9,10,46). We are currently testing whether an unusual level of memory B cells or a combination of unusual memory B-cell and memory T-cell numbers can be a predictor of immune responsiveness for a given individual.

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