AGE-DEPENDENT DEFICIENCY OF B LYMPHOCYTE LINEAGE PRECURSORS IN NZB MICE*

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Animals with genetically determined defects in the development of lymphoid and hemopoietic systems have proven useful for understanding how these processes are normally regulated (1–4). There is reason to believe that New Zealand strain mice, which have long been used as a model for autoimmune disease, will similarly be important for studies of B lymphocyte differentiation. These animals are said to develop large numbers of polyclonally activated and secreting cells together with B cells that are atypical in terms of cell surface Ig and resistance to anti-μ antibodies, tolerance, and serum inhibitors (5–10). Many of these features, which presumably relate to autoantibody formation, have been conferred on normal irradiated recipients by grafting with New Zealand Black mouse (NZB) hemopoietic cells (11–15). Such findings suggest that it might be possible to localize defects of their humoral immune system to the B lineage or microenvironmental elements that affect B cell formation.

It has recently become apparent that precursor cells capable of giving rise to functional B cells display distinctive surface antigens and can mature under appropriate culture conditions (16, 17). These and other characteristics allowed us to compare such pre-B cell populations in bone marrow of normal and NZB mice of different ages. Dramatic temporal changes were observed in the B cells and their precursors in NZB bone marrow, and this indicates that B cell formation might virtually cease in these animals by adult life.

Materials and Methods

Animals. Inbred DBA/2 and BALB/c mice were purchased from Cumberland View Farms, Clinton, TN. CBA/H-T6T6, partially immunodeficient CBA/N, and NZB.*xid* mice were from our own colonies. NZB/BinJ mice, obtained from The Jackson Laboratory, Bar Harbor, ME, were used interchangeably with NZB/umc mice from our own colony. Sex-related changes were not exhaustively examined in this study, and although it did not obviously influence our results, the sex of the animals is indicated in each table and figure.

Cell Suspensions. Cell suspensions were prepared, freed of small and large debris, depleted of surface immunoglobulin-positive (sIg^+)[1] B cells, and depleted of Sephadex G-10 adherent cells exactly as described in our previous reports (17, 18). Thy-1.2-bearing cells were depleted by first incubating cells with a 1:200 dilution of monoclonal HO-13-4 antibody (19), followed by

* Aided by grants AI-11843, AI-12741, AG-00541, NS-11457, CA-17404, and CA-20816 from the U. S. Public Health Service, Research Career Development Awards AI-00193 and AI-00265, and the March of Dimes Birth Defects Foundation.

[1] *Abbreviations used in this paper:* cμ, cytoplasmic μ chains of IgM; FCS, fetal calf serum; LPS, lipopolysaccharide; sIg, surface immunoglobulin; SRBC, sheep erythrocytes.

a wash and exposure to a 1:10 dilution of selected rabbit complement for 45 min at 37°C. This procedure removed >95% of the Thy-1^+ cells in spleen cells, as determined by immunofluorescence.

Cell Cultures. Semisolid agar cultures containing 25 μg/ml of lipopolysaccharide (LPS), 2-mercaptoethanol, fetal calf serum (Flow Laboratories, Rockville, MD; lot 29101296), amino acids, and vitamins were prepared as described in detail elsewhere (20). Cells lacking surface Ig but capable of giving rise to sIg^+, clonable B cells were detected by 48-h liquid preculture in 96-well microtiter plates (3040; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) at a density of 10^6 cells/ml of RPMI 1640 medium with appropriate additives (17). These were then harvested, washed, counted, and either examined by immunofluorescence for sIg^+ cells or plated in semisolid agar cultures. Numbers of proliferating foci were determined after 6–7 d of incubation, and all results were expressed in terms of initial cell numbers, to reflect both specific cell losses and total numbers of functional B cells that were produced (17).

Immunofluorescence. Viable cells bearing sIgM were detected with $F(ab')_2$ fragments of rhodamine-labeled goat anti-mouse μ antibodies, and cells containing μ chains were detected in acid ethanol-fixed, cytocentrifuge preparations with the same antibody as previously described (21). A high molecular weight B-lineage-associated antigen was detected on cells with monoclonal rat 14.8 antibody using purified, fluorescein-labeled mouse anti-rat Ig antibodies (22).

Calculation and Expression of Results. After each cell separation/manipulation step, numbers of nucleated cells were counted and values adjusted to reflect the incidence of the assayed cell type in the original suspension. For example, results shown were obtained by depleting sIg^+ cells and then determining the percentages of cells in the resulting suspensions that stained with 14.8 antibody. If 10^6 initial cells were placed in anti-μ-coated dishes and 9×10^5 B-depleted cells were recovered before immunofluorescence analysis, then the results would have been transformed by multiplication by 0.9. This procedure was used with all of the data that were generated at the different time points. It is therefore possible to compare directly any of the findings that result from examination of the same bone marrow suspensions before manipulation, after B cell depletion, and after liquid preculture. Relationships between independent variables were tested by Pearson correlation analysis, and a two-tailed Student's *t* test was used to test significance of differences (23).

Results

Precursors in Adult NZB Mice. A majority of the B cells from marrow of normal mice are removed by incubation in cold, anti-IgM-coated petri dishes, and very few cells in the nonadherent fraction can respond to mitogens in semisolid agar cultures. However, if the B cell-depleted suspensions are incubated in suitable liquid medium before plating in agar, a substantial number of sIg^+ clonable B cells are detectable (17, 18). Experiments of this kind have yielded similar results when young adult A/J, AKR/J, BALB/cJ, CBA/H-T6T6, CBA/J, C57BL/6J, C58/J, DBA/2Cum, and RF/J mice, or 1-yr-old BALB/c, CBA/H, and DBA/2 mice were studied (17, 18, and unpublished observations). In contrast, when marrow from NZB mice of at least 16 wk of age was depleted of B cells and held in liquid cultures, there was no evidence of emergence of functional cells (Table I). This finding corresponds to a low incidence of cells in NZB marrow with distinctive characteristics of B cell precursors. That is, cells with detectable cytoplasmic but not surface μ chains of IgM (21) and sIg^- marrow cells bearing a B-lineage antigen detected by monoclonal 14.8 antibody (22) were rare in marrow of these mice. On some occasions, as in this experiment (Table I), numbers of 14.8^+ cells were reduced by more than the expected amount by incubation in anti-μ-coated plates. Other observations suggest that this might reflect depletion of very low density sIg^+ cells (unpublished observations).

Studies of marrow-ablated ^{89}Sr-treated mice suggest that B cells could be formed in

TABLE I

Deficiency of Immediate B Cell Precursors in Adult NZB Mice

	In primary cultures				Recovered after 48-h preculture	
	sIg^+ cells $\times 10^{-4}$	14.8^+ cells $\times 10^{-4}$	$c\mu^+$ cells $\times 10^{-4}$	Clonable B cells	sIg^+ cells $\times 10^{-4}$	Clonable B cells
NZB bone marrow*						
Unseparated	6.0‡	12.0		8,475§		10,880
Depleted of sIg^+ cells	0.1	1.0	0.16	606	0.28	484
CBA/H bone marrow						
Unseparated	7.1	18.7		4,400		4,329
Depleted of sIg^+ cells	0.1	6.8	4.8	226	3.6	2,152

* Bone marrow cells were taken from 16-wk-old female mice.

‡ sIg^+, 14.8^+, and $c\mu^+$ cells are expressed as per 10^6 initial nucleated cells.

§ B lymphocyte colonies per 10^6 initially cultured cells. These data are from a single experiment and are representative of many examinations of mice of this age.

TABLE II

Immediate B Cell Precursors in Bone Marrow, Spleen, and Lymph Nodes of NZB and Control Mice

	In primary cultures			Recovered after 48-h preculture	
	sIg^+ cells $\times 10^{-4}$	$c\mu^+$ cells $\times 10^{-4}$	Clonable B cells	sIg^+ cells $\times 10^{-4}$	Clonable B cells
NZB bone marrow*					
Unseparated	6.0‡		10,037§		7,056
Depleted of sIg^+ cells	0.2	0.09	354	0.2	762
NZB spleen					
Unseparated	17.0		43,650		22,660
Depleted of sIg^+ cells	0.25	0.06	168	0.02	62
NZB lymph nodes					
Unseparated	12.0		24,990		15,854
Depleted of sIg^+ cells	0.2	<0.05	92	0.01	84
BALB/c bone marrow					
Unseparated	8.0		21,700		6,552
Depleted of sIg^+ cells	0.7	2.5	152	7.76	2,867
BALB/c spleen					
Unseparated	39.0		29,400		20,921
Depleted of sIg^+ cells	0.7		254		39
BALB/c lymph nodes					
Unseparated	15.0		5,100		7,235
Depleted of sIg^+ cells	0.6		419		31

* Cell donors were 16-wk-old female mice.

‡ sIg^+ and $c\mu^+$ cells are expressed as per 10^6 initial nucleated cells.

§ B lymphocyte colonies per 10^6 initially plated cells. These data are from a single experiment that is representative of three analyses that included control BALB/c and CBA/H mice of up to 1 yr of age.

extramedullary sites in adult animals (24). A survey of spleen and lymph nodes from 16-wk-old (Table II) and 1-yr-old NZB and BALB/cJ mice (not shown) did not reveal the presence of B cell precursors in these tissues. This indicates that formation of B cells in extramedullary sites is normally minimal in either strain of animals.

The possibility that B cell precursors from NZB mice mature more slowly in culture

than those of other strains of mice was also tested. Numbers of sIg-bearing and functional B cells were maximal in liquid cultures of B cell-depleted DBA/2 marrow after 2–3 d of incubation, and then recovery of viable cells declined rapidly (Fig. 1). Over this interval there was no evidence of substantial maturation of B cell precursors in cultures of 4-mo-old (or 10-mo-old, not shown) NZB marrow. The initial background of clonable B cells in sIg^- suspensions of younger NZB mice was higher than normal (see below), but these did not become more numerous with liquid culture.

Suppressor and Accessory Cells in NZB Marrow. Two types of experiments suggest that neither abnormalities in thymic-derived cells nor generation of suppressor cells account for the failure of adult NZB marrow to give rise to functional B cells in vitro. First, depletion of Thy-1-bearing marrow cells had little influence on the cloning efficiency of marrow B cells or formation of B cells from their immediate precursors in culture (Table III). Also, the addition of NZB (or NZB.*xid*) marrow to cultures of B cell-depleted DBA/2 marrow did not prevent the emergence of newly formed B cells (Table IV). Our previous studies (17) revealed that optimum maturation of B cell precursors in culture depends on interaction with accessory cells and that adherent, macrophage-like cells can replace at least one of these. Adherent peritoneal exudate cells from NZB mice restored the ability of adherent cell-depleted sIg^- cultures of DBA/2 mice to give rise to clonable B cells (Table V). Adequate accessory function was also provided by allogeneic CBA/N bone marrow (which lacks clonable B cells) or congenic NZB mice that carry the *xid* mutation of CBA/N mice (25). All of these experiments indicate that adult NZB marrow is deficient in B cell precursors rather than cells that influence their maturation in vitro. However, the studies performed to date do not reveal what types of regulatory abnormalities might result in this condition, and it remains to be determined whether defects in nonlymphoid "microenvironmental" elements are responsible.

Age-related Changes in Bone Marrow Populations. The composition of NZB and normal

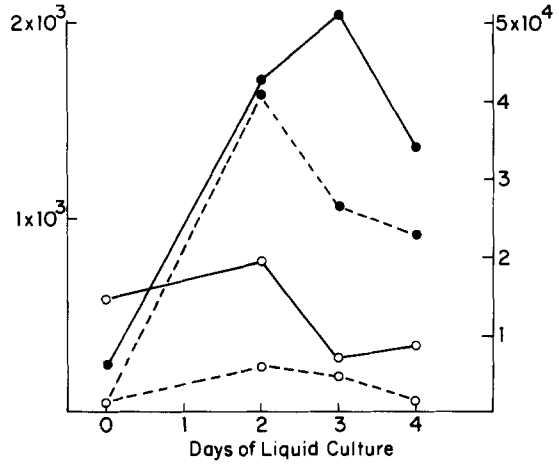

FIG. 1. Kinetics of maturation of sIg^- precursors in liquid cultures of B cell-depleted bone marrow. B cell emergence was detected as $sIgM^+$ cells using immunofluorescence or cells that can clone in response to mitogens in semisolid agar cultures. Suspensions were prepared from male 18–wk NZB (○) or male 16–wk DBA/2 (●) mice, and results are expressed as per 10^6 initial cells. ——, clonable B cells; -----, sIg^+ cells.

TABLE III

Thy-1$^+$ Cells Do Not Influence Functional B Lymphocyte Maturation in Liquid Cultures

	Clonable B cells	
	Present initially	Recovered after 48-h preculture
NZB bone marrow*		
Unseparated	10,067‡	7,214
Depleted of Thy-1$^+$ cells	7,350	4,606
Depleted of sIg$^+$ cells	155	472
Depleted of Thy-1$^+$ and sIg$^+$ cells	115	592
BALB/c bone marrow		
Unseparated	6,000	9,108
Depleted of Thy-1$^+$ cells	4,648	4,600
Depleted of sIg$^+$ cells	123	2,231
Depleted of Thy-1$^+$ and sIg$^+$ cells	349	2,190

* Bone marrow donors were 15-wk-old female mice.

‡ B cell colonies per 10^6 initially plated cells. Essentially similar results were obtained in two additional experiments.

TABLE IV

NZB Bone Marrow Does Not Contain Active Suppressor Cells That Prevent B Cell Formation

sIg$^+$ cell-depleted cell suspension	Present initially		Recovered after 48-h preculture	
	sIg$^+$ cells $\times 10^{-4}$	Clonable B cells	sIg$^+$ cells $\times 10^{-4}$	Clonable B cells
NZB bone marrow*	0.57	606‡	0.29	444
DBA/2 bone marrow	0.25	280	3.40	1,754
NZB + DBA/2 bone marrow			3.25	2,315

* Bone marrow donors were male 15-wk-old (NZB) and 14-wk-old (DBA/2) mice.

‡ B cell colonies per 10^6 initially plated cells. These data are representative of those obtained in five separate experiments of this kind.

TABLE V

Influence of Accessory Cells on B Cell Formation in Liquid Precultures

	Present initially	Clonable B cells recovered after 48-h preculture with				
		—	DBA/2 PEC	NZB PEC	CBA/N marrow	NZB.*xid* marrow
NZB bone marrow*						
Depleted of sIg$^+$ cells	460	464				
Depleted of sIg$^+$ and adherent cells	193	34	39	35	41	82
DBA/2 bone marrow						
Depleted of sIg$^+$ cells	61	1,617				
Depleted of sIg$^+$ and adherent cells	28	53	818	1,231	1,022	1,206

* Marrow donors were 15-wk-old females, and results are expressed as B lymphocyte colonies detected per 10^6 initial cells.

‡ Liquid cultures were set up with adherent peritoneal exudate cells (PEC) or whole marrow cells as sources of accessory cells. Variations of this experimental design were used in six separate experiments and yielded similar results.

CBA/H bone marrow was investigated as a function of age. No significant differences in total cellularity were noted between the two strains of mice from birth to 5 mo of age (not shown). In unseparated bone marrow, the cloning efficiency of NZB cells in semisolid cultures was elevated at all time points (Fig. 2). However, an age-related decline was obvious for numbers of sIg^+ B cells detectable by immunofluorescence and B-lineage cells revealed by staining with monoclonal 14.8 antibody.

The same specimens were also depleted of Ig-bearing cells with anti-μ-coated dishes before analysis (Fig. 3). After this separation, residual B cells (detectable by immunofluorescence) always comprise <1% of the nucleated cells, and nonspecific cell loss is minimal (26). B cell precursors detected by their content of μ chains of Ig and absence of sIgM were elevated in 4–5-wk-old NZB mice, and these declined rapidly after 7 wk of age. This was closely correlated ($r = 0.8646$, $P < 0.005$) with changes in cells detected by the 14.8 antibody in NZB marrow, and, in fact, numbers of $sIg^-,c\mu^+$ cells were nearly equivalent to sIg^-, 14.8^+ cells at all times after 3 wk of age (compare Fig. 3a and 3b). All identifiable cells of the B lineage in these mice might therefore synthesize and/or display Ig chains. In contrast, in normal CBA/H marrow, numbers

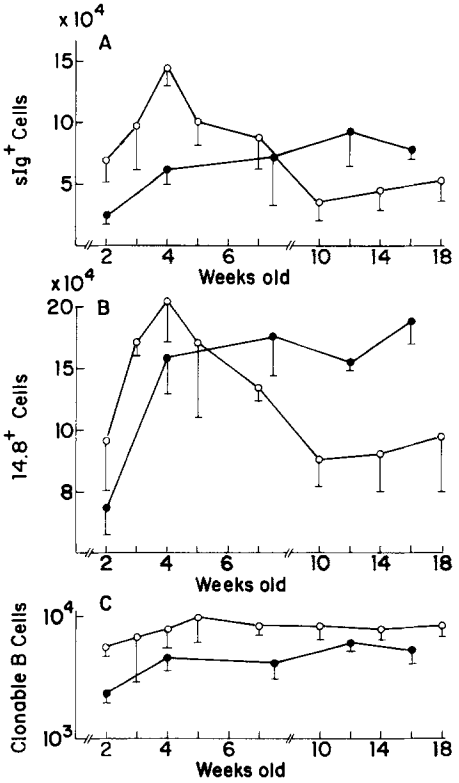

FIG. 2. Age-dependent changes in bone marrow B cells detectable by immunofluorescence (A), cells bearing a B-lineage antigen detected with monoclonal 14.8 antibody (B), and clonable B cells (C). Each point represents the mean ± SD of results from 3–4 NZB (○) or CBA/H-T6T6 (●) mice. These data and those presented in Figs. 3 and 4 were derived from the same samples and are expressed such that they can be directly compared (see Materials and Methods).

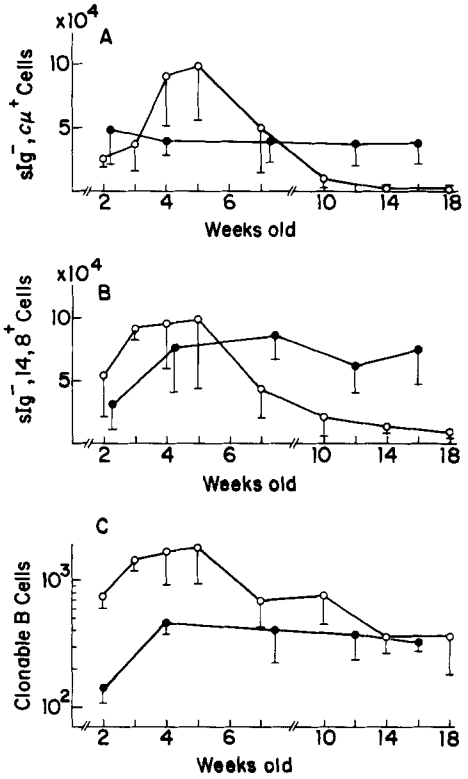

FIG. 3. The specimens described in Fig. 3 were depleted of sIg^+ cells by incubation in cold, antibody-coated dishes and then assayed for cells containing μ heavy chains of IgM (A), bearing a B-lineage antigen (B), and capable of clonal proliferation (C). ○, NZB mice; ●, CBA/H mice. Note that different scales were used for Fig. 2C and Fig. 3C.

of $sIg^-,c\mu^+$ cells were relatively constant and were usually exceeded by sIg^-, 14.8^+ cells at all ages.

It is noteworthy that although >90% of the functional B cells in CBA/H marrow were depleted along with Ig-bearing cells at all ages, a substantial number of "background" clonable cells were seen in NZB marrow suspensions. That these were lymphoid colonies was proven by their complete sensitivity to anti-μ antibodies added to the cultures (not shown).

The influence of age on the incidence of cells in NZB marrow that could give rise to sIg^+ and clonable B cells in vitro was striking (Fig. 4). That is, they increased in very young animals to exceed normal values at 4–5 wk of age and then rapidly declined. It is interesting that the incidence of $sIg^-,c\mu^+$ cells in NZB marrow closely correlated with numbers of clonable B cells ($r = 0.9259$; $P < 0.001$) and numbers of sIg^+ cells ($r = 0.8293$; $P < 0.005$) arising after liquid culture. Similar concordance was seen between sIg^- cells detected with the 14.8 antibody and numbers of B cells arising in liquid cultures. However, these were not significantly correlated in normal CBA/H mice ($P > 0.05$).

All of these findings indicate that non-Ig synthesizing B-lineage cells diminish in very young NZB mice, and this is followed by parallel declines in immediate precursors

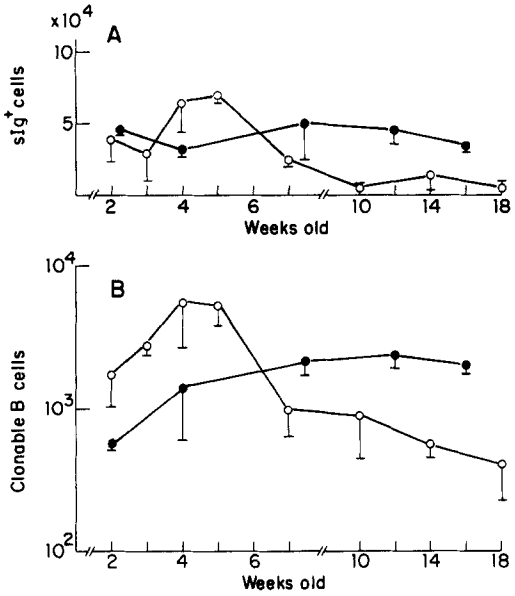

FIG. 4. The same B cell-depleted bone marrow samples described in Fig. 3 were placed into liquid cultures for 48 h. Recovered cells were tested for sIg expression (A) or ability to clone in semisolid agar cultures (B). ○, NZB mice; ●, CBA/H mice.

of B cells detected by $c\mu$ expression or ability to mature in culture. In preliminary studies (with Dr. D. Harrison of The Jackson Laboratories), marrow from 2-yr-old CBA/J mice was found phenotypically and functionally to tend to resemble that of adult NZB mice, and this suggests that the deficiency of B cell precursors in these animals might be explained in terms of an accelerated aging process.

Discussion

There have been many reports describing abnormalities in development and function of the humoral immune system of New Zealand strain mice, but this is the first investigation of the immediate precursors of B cells in animals of this strain. Three independent assessments indicated that such cells decline to almost undetectable numbers in bone marrow by the time the animals are mature. At that time it was not obvious that suppressor cells were directed to that compartment or that accessory cells were defective. These observations call attention to age-related changes that occur in this model of autoimmune disease and suggest that further investigation will provide insight into processes that normally regulate B lymphocyte formation.

The functional assay for B cell precursors that was used in this study has been described and discussed in detail elsewhere (16–18). It is based on the finding that such sIg^- cells have little ability to clone in semisolid agar cultures but acquire this after preincubation in conventional liquid tissue culture medium. Efficient formation of sIg-bearing and clonable B cells in this interval depends on close interaction with accessory cells, one type of which can be replaced by adherent peritoneal macrophages (17, and Table V). Adult (>15-wk-old) NZB marrow contained few sIg^- cells that gave rise to B cells in liquid culture, and these were not rescued by addition of adherent peritoneal exudate cells from normal DBA/2 mice. Furthermore, if defects

in regulatory cells were present in NZB marrow, one might expect also to find them in congenic NZB.*xid* mice (25). That is, the defective *xid* gene that has been backcrossed onto these animals should selectively render their B cells incapable of responding in semisolid cultures, but all other cells, including microenvironmental elements, should be of NZB type. Marrow from these animals was as effective as CBA/N marrow in supporting the terminal differentiation of precursors from adherent cell-depleted DBA/2 marrow (Table V). All of this indicates that adherent accessory cells in NZB marrow and peritoneal exudate are probably normal. In an earlier study, we obtained indirect evidence for a type of nonadherent accessory cells that are present in adult marrow but limiting in fetal liver (17). The present studies do not directly test the competence of such cells in NZB mice. One approach to this question is to enrich putative B cell precursors from normal and NZB marrow (using monoclonal 14.8 antibody) and place them in culture with various sources of accessory cells. In several experiments of this kind, positively selected B cell precursors from CBA/H marrow matured normally when cultured with NZB.*xid* marrow (H. Jyonouchi, unpublished observations).

Mice injected with *C. parvum* develop prostaglandin-secreting suppressor cells that selectively inhibit marrow B-lineage cells (18). It seemed possible that adherent cells of this kind or suppressor cells of the T lineage might be active in adult NZB mice. However, depletion of Thy-1 antigen-bearing cells or appropriate mixing experiments did not provide evidence for this. Therefore, our failure to detect B cell precursors with the liquid culture assay is not attributable to suppressor cells. On the other hand, these experiments do not exclude the possibility that immunoregulatory abnormalities occurring at an early age resulted in the deficiency detected in adult animals.

We recently described a group of monoclonal antibodies (including clone 14.8) that detect an antigen that is displayed on cells of the B lineage from an early stage (22). It appears that virtually all Ig-bearing cells as well as $c\mu^+$ cells and precursors that can quickly give rise to functional B cells are recognized by this antibody (27). The incidence of 14.8^+ cells that did not bear Ig steadily declined in NZB mice after 5 wk of age and closely corresponded with changes in $sIg^-,c\mu^+$ cells. In fact, it appears likely that from 4 wk of age these two categories of cells were almost completely overlapping in NZB mice. This follows from the fact that all $c\mu^+$ cells in marrow are 14.8^+ (27) and from comparison of the incidences of these among sIg^- populations (Fig. 3a and 3b). In normal CBA/H marrow, on the other hand, numbers of sIg^-, 14.8^+ cells exceeded $c\mu^+$ cells, and the sizes of these two populations did not closely correspond during ontogeny.

One interpretation of these results is that a category of early B cell precursors with the phenotype $sIg^-,c\mu^-,14.8^+$ rapidly disappear after 3 wk of life in NZB mice, and this leads to a premature shutdown of myeloid B cell production. This might indicate that regulation of B cell formation normally is controlled at the level of such cells. In this context, it is noteworthy that anti-μ suppression experiments indicate that the rate of small lymphocyte formation in marrow is not influenced by the size of the mature B cell pool (28). However, in addition to the B lineage, 14.8 antibody at least weakly binds to subpopulations of peripheral T cells, and the contribution of these to non-Ig synthesizing, 14.8^+ populations in normal marrow remains to be determined. The close correlation of precursors detected by cytoplasmic μ chains with 14.8 antibody or by means of the liquid culture assay in NZB mice might also minimize

doubts about the relevance of $c\mu^+$,sIg^- cells to normal B-lineage differentiation (29, 30).

When sIg^+ cells were removed from suspensions of lymph node cells with anti-Ig-coated petri dishes, clonable B cells were diminished by >95%. This provides an indication of the efficiency of B cell depletion with this separation technique because lymph nodes are not thought to contain precursors of B cells (16). Usually no more than 10% of the functional B cells in normal adult bone marrow suspensions remain after this procedure, and we concluded that the immediate precursors of B cells must have little ability to proliferate in these mitogen-containing, semisolid agar cultures (26). In the present study, we noted that young NZB marrow gave a significant number of "background" colonies when plated after B cell depletion (Fig. 3c). That these were lymphoid colonies was determined by the fact that inclusion of anti-μ in the semisolid cultures completely prevented their proliferation. We also found some cells of this kind in liver and spleen of normal newborn CBA/H mice (H. Jyonouchi, unpublished observations). It is possible that these represent precursors that are just hours away from acquiring sIg and functional capability. They might also be newly formed B cells that display Ig in insufficient quantities to adhere to the antibody-coated dishes or to be detected by immunofluorescence microscopy. Another possibility to be considered is that the B cell precursors of young NZB mice might be able to mature even in semisolid cultures. A possibly related finding was that B cell precursors as well as functional B cells were consistently elevated in very young NZB mice. This could reflect a period of hyperactivity in which B cells are being rapidly formed. Further studies of this critical age might reveal whether this is intrinsic to the B cell precursors or attributable to accessory cells that influence B cell maturation (17).

The age-related changes that occur in B lymphocyte precursors of NZB mice are particularly interesting. Our assay procedures make it possible to estimate the size of the compartment that immediately precedes sIg^+ functional B cells but do not give direct information about the rate of the pre-B to B cell transition. However, it seems unlikely that substantial numbers of B cells emerge from precursors in NZB mice after 15 wk of age. Radioactive thymidine incorporation studies indicate that the rate of total small lymphocyte formation might gradually decline in marrow of normal C3H mice after 4 wk of age (28, 31). On the other hand, transplantation experiments suggest that during an entire normal lifespan, multipotential stem cells do not lose their potential for forming lymphoid and myeloid cells (32–33). We have initiated studies with very old normal CBA strain mice and found that B cell precursors were reduced at 2 yr but not at 8–10 mo of age (Landreth, Jyonouchi, Kincade, and Harrison, unpublished observations). Furthermore, numbers of clonable B cells decline prematurely in NZB mice, and this is first obvious with bone marrow populations (34, and unpublished observations). New Zealand mice might therefore provide a model for genetically determined, premature senescence of the humoral immune system, and this is consistent with the well-known early involution of the thymus in these animals (7). One prediction of these findings is that the repertoire of antibody specificities could become restricted with time in NZB mice. That is, if maintenance of diverse antibody responsiveness depends on constant input of newly formed B cells, this might not occur in these animals.

There are a number of clearly documented abnormalities of the humoral immune system in NZB mice. These include their development of large numbers of polyclonal

Ig-secreting cells and clonable B cells that include an unusual $sIgM^+$ subset that proliferates in LPS-potentiated cultures in the presence of anti-μ antibodies (5, 34). In adult mice, the most unique cell surface distinction is a reduced ratio of μ:δ chains, and this is especially noteworthy on large splenic and lymph node cells (35–37). Studies of resistance of NZB B cells to tolerance have yielded conflicting and variable results, and it appears that the increased resistance of these mice to in vivo induced tolerance is largely attributable to T lineage and hormonal defects (9, 15, 37, 38). Reports of cell transfer experiments and studies of athymic NZB and NZB.*xid* mice seem to negate the importance of T cell abnormalities in production of autoantibodies and various B cell changes in NZB mice (9, 11, 38, 39). However, it has recently been shown that very small numbers of T cells can influence immune responses that were formerly thought to be T independent (40–42).

It now seems crucial to evaluate all findings relating to B cell defects in chronological terms, that is, whether abnormalities appear earlier than autoantibody production, earlier than other known defects, and consistently in all individuals. The development of significant numbers of polyclonal Ig-secreting cells was said to occur in T cell-depleted cultures of neonatal cells and reproducibly in all NZB mice from birth (43, 44). Our own experience with Ig-secreting cells measured by reverse plaque-forming cell assay (unpublished observations) is more like that reported by Izui et al. (45), where animal-to-animal variation was substantial, and significant differences were not consistent before several weeks of age. Also, in other reports, B cell abnormalities were not remarkable in very young NZB mice (36, 37).

We recently found (14) that it was possible to reciprocally transfer myeloid and B lymphoid abnormalities of NZB to DBA/2 mice by whole marrow transplantation. This followed reports that potential for autoimmune disease could be transferred with hemopoietic cells (11–13). Similarly, DBA/2 mice that were irradiated and grafted with NZB marrow later had a deficiency of B cell precursors, and NZB recipients of DBA/2 cells were found to have a normal incidence of these (46). Further dissection of this model might reveal whether intrinsic B-lineage defects or transferable, microenvironmental elements are responsible. It is also known that expression of autoimmunity and other NZB characteristics is under multigenic control (9, 47). Careful analysis of recently developed recombinant inbred lines (47, 48) might indicate whether accelerated aging of the humoral immune system is related to any of these. Another question under investigation is whether stem cells that precede 14.8-expressing cells prematurely decline in NZB mice. Such information could be helpful in identifying critical stages in B-lineage differentiation that are subject to regulation.

Summary

Adult NZB mice (>15 wk old) have very few bone marrow cells that can give rise to sIg^+ clonable B cells during liquid culture. This deficiency corresponds to extremely low numbers of cells with cytoplasmic but not surface μ chains of IgM and reduced numbers of cells bearing a high molecular weight B-lineage antigen. Depletion of Thy-1-bearing cells and appropriate mixing experiments did not provide evidence either that suppressor cells are responsible for this phenomenon or that accessory cells are defective in NZB mice. Nor did it seem that B cells were being produced in extramedullary sites. B cell precursors were detectable in very young NZB mice, exceeded control values at 4–5 wk of age, and then declined rapidly. In contrast, these

persisted for >1 yr in normal BALB/c, DBA/2, and CBA/H mice. It appears possible that intermediate stages in B-lineage differentiation become prematurely exhausted through an accelerated aging process in NZB mice. These chronological changes have implications for understanding the sequence of events that lead to B lymphocyte formation and the processes that normally regulate it.

Received for publication 17 February 1982.

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