PRECURSORS OF MURINE B LYMPHOCYTES

Physical and Functional Characterization, and Distinctions from Myeloid Stem Cells*

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The precursors of B lymphocytes have previously been distinguished and characterized by various parameters including their ability to generate B cells after adoptive transfer, expression of surface markers and/or B cell function after short-term induction in vitro, and their synthesis but not surface display of immunoglobulin (Ig) heavy chains (1–19). The extent to which these cells are heterogenous in fetal and adult life is not known, and it is also not clear to what extent their numbers depend on differentiation from multipotential hemopoietic stem cells. The ability to form foci of proliferating hemopoietic cells in spleens of irradiated recipients is usually ascribed to uncommitted colony-forming stem cells (CFU-s)[1] but recent studies suggest that differentiation options for some CFU-s may be restricted (20). Our previous studies identified one or more cell types which were capable of restoring colony-forming B cells (CFU-B) to partially immunodeficient CBA/N mice (6, 19). These cells were neither surface Ig^+ (B cells) nor closely related to CFU-s. That is, the restorative capacity of various cell supensions did not correlate with numbers of either CFU-s or lymphocytes, and furthermore newly formed B cells in irradiated, reconstituted mice were not spatially related to the clonal progeny of CFU-s. Our present observations further define these pre-B cell populations and demonstrate by means of a monoclonal antibody that they may be distinguished from CFU-s from an early stage of embryonic life. These findings presage studies of factors that regulate lymphoid system development and raise interesting questions about initial events in the differentiation of this lineage. Among these are the following: (*a*) whether precommitted cells arise at a very early age and have sufficient self-renewal and differentiation potential to obscure the subsequent contribution of less mature, uncommitted stem cells, and (*b*) whether truly multipotential stem cells comprise a very small subpopulation of cells detected by the in vivo splenic focus (CFU-s) assay.

Materials and Methods

Mice and Irradiation. The mice used in these studies were all raised in our own laboratory and kept in a laminar flow facility under specific pathogen free conditions. CBA/H-T6T6 mice

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[1] *Abbreviations used in this paper:* CFU-B, colony-forming unit B cell; CFU-c, colony-forming unit-culture assay; CFU-mega, colony-forming unit-megakaryocyte; CFU-s, colony-forming unit spleen cell; LPS, lipopolysaccharide; SRBC, sheep erythrocyte.

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were originally obtained from the breeding colony of Dr. O. Stutman (Sloan-Kettering Institute, New York) whereas the CBA/N mice were obtained from the National Institutes of Health (Bethesda, Md.). For both the CFU-s assay and reconstitution experiments, mice were exposed to 1,050 rad emitted from a ^{137}Cesium irradiator delivered at a dose rate of 113.5 rad/min (J. L. Shepherd & Associates, Glendale, Calif.). Timed pregnancy was determined by examining mice each morning for the presence of a vaginal plug, and the day of appearance of a plug was considered day 0.

Cloning Assays. Single-cell suspensions of fetal and adult tissues were obtained as previously described (19). Colony-forming unit spleen (CFU-s) assays were performed according to the methods of Till and McCullough (21). Usually 5×10^4 adult bone marrow or 10^5 fetal liver cells were injected into CBA/H-T6T6 recipients, which had been exposed to 1,050 rad. Spleen foci were enumerated on day 8 or 9 after injection.

For both the clonable B cell assay (CFU-B) and the granulocyte/macrophage colony-forming unit-culture assay (CFU-c) we utilized McCoy's 5A medium (Grand Island Biological Co., Grand Island, N. Y.) containing 15% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), 0.3% agar (Difco Laboratories, Detroit, Mich.) and supplemented as previously described (19). CFU-c were stimulated with 0.1 ml of 10 times concentrated WEHI-3 supernates added to 1-ml culture plates. Clonable B cells were stimulated with lipopolysaccharide (LPS) (*Salmonella typhosa* W0901; Difco Laboratories) and/or with 0.1 ml of 10% sheep red blood cells (SRBC) (Flow Laboratories, Inc.), which had been previously washed five times with 5 vol of phosphate-buffered saline. After the cultures had gelled, they were incubated for 6 d in a humid atmosphere containing 7% CO_2 and maintained at 37°C. A dissecting microscope was used to enumerate the colonies. Cultures that contained SRBC were fixed in 0.3% acetic acid, which lysed the erythrocytes and thereby allowed the visualization of B cell colonies. Colony-forming unit-megakaryocyte (CFU-mega) assays were performed as previously described (22).

Sedimentation Velocity Separation. Single-cell suspensions of adult bone marrow or 14-d fetal liver cells were allowed to sediment through a continuous bovine serum albumin gradient (0.2–2%) according to the method of Miller and Phillips (23). After 3.5 h, fractions were collected, the number of viable nucleated cells were enumerated and then appropriately pooled. A specific volume of these pooled fractions were then analyzed either by direct cloning or by injection into either irradiated or unirradiated CBA/N mice. The number of clonable B cells that emerged in the spleen of recipient mice were assesed 2–6 wk later. To assay for CFU-s, a specific number of cells from each fraction (5×10^4 for adult bone marrow and 10^5 for fetal liver) were injected into irradiated CBA/H-T6T6 recipients. In the figure presented, the data are normalized as percentage of peak fraction.

Antibody and Complement-mediated Cytotoxicity. The preparation of rat anti-mouse brain monoclonal antibody, fusion procedure, and maintenance of the 19B5-producing hybridoma has been previously described (24).

Single cell suspensions of fetal liver or adult bone marrow were prepared and washed in RPMI-1640 medium containing 10 mM Hepes. 5×10^6 cells were centrifuged and resuspended in 50 μl of undiluted 19B5 supernate or control medium and allowed to stand at room temperature for 30 min. The cells were then washed two times with 5 ml of RPMI-1640 and Hepes, resuspended in 200 μl of rabbit complement previously absorbed on spleen cells, and diluted 1:8. After incubation for 45 min at 37°C, the cells were washed one more time and resuspended in 5 ml of medium. Based on the initial cell number appropriate portions were then utilized for in vivo or in vitro assay.

Quantitative adsorbtion was performed as previously described using both the pre-B cell line 70Z/3 and the sIg^+ B cell line WEHI-231 (24–26). The latter cells were obtained from Dr. N. Warner (Alberquerque, N. Mex.).

Results

Sedimentation Velocity Characterization. Sedimentation at unit gravity resolves cell populations primarily on the basis of size differences (23). Using this method, it is obvious that functional B cells in adult marrow, which are capable of immediate colony formation sediment at approximately 3 mm/h and are distinct from precursors

that restore clonable B cells to irradiated CBA/N mice (Fig. 1 A). The latter sediment on average at around 5 mm/h and overlap CFU-s in this respect. We consistently found that pre-B cells in fetal liver were more rapidly sedimenting than their counterparts in adult marrow (Fig. 1 B). B lymphocyte precursors detected by transferring cells to unirradiated CBA/N recipients had sedimentation characteristics (not shown) similar to those illustrated here using irradiated recipients. These observations are compatible with earlier characterizations of functional B cells and their immediate precursors using this method of cell separation (1, 27, 28). Particularly noteworthy here are the suggestions of differences in pre-B cells in fetal and adult tissues and nonidentical but overlapping profiles of CFU-s and B cell precursors.

Kinetics and Dose-Response Analyses. The rate of emergence of functional B cells in irradiated mice after grafting could depend on the number of committed progeniter cells transferred and/or their stage of differentiation. These variables were examined first by monitoring B cell regeneration with time after injection of single doses of fetal liver and also by studying the effect of graded cell doses at a single time interval. Clonable B cells were detectable within 1 wk after irradiation and grafting with either 13- or 16-d fetal liver, however the number of CFU-B was higher and appeared sooner after injection with cells from 16-d donors (Fig. 2). In other experiments even 5 d were sufficient for the emergence of clonable B cells from 16-d fetal liver grafts. Also not

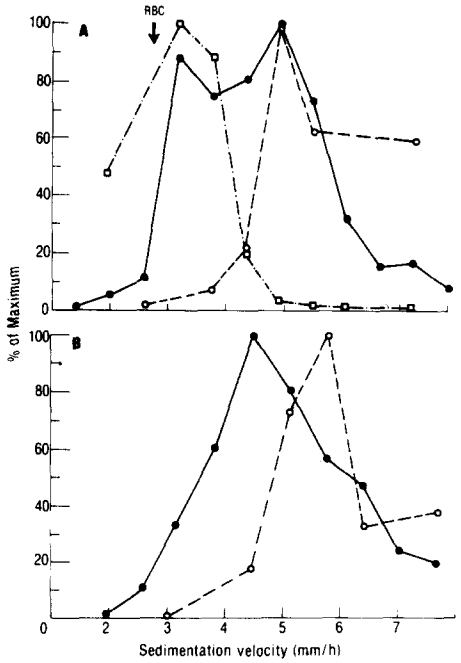

FIG. 1. Sedimentation velocity analysis of CBA/H-T6T6 cells showing profiles of: total nucleated cells (●); CFU-B cells present immediately after sedimentation (□); and CFU-B cells present in the spleens of irradiated/reconstituted CBA/N mice 2 wk after grafting (○) CBA/N recipients that received 1,050 rad before the transfer of a known volume of CBA/HT6T6 cells from each fraction. Also shown are the peak of the sedimentation profiles of erythrocytes (RBC) (arrow). The majority of CFU-S in adult marrow sedimented at 4.2–4.8 mm/h, whereas the majority of fetal liver CFU-S sedimented at 4.5–5.5 mm/h. In each case the data are expressed as the percent maximum response. (A) Adult bone marrow; (B) 14-d fetal liver.

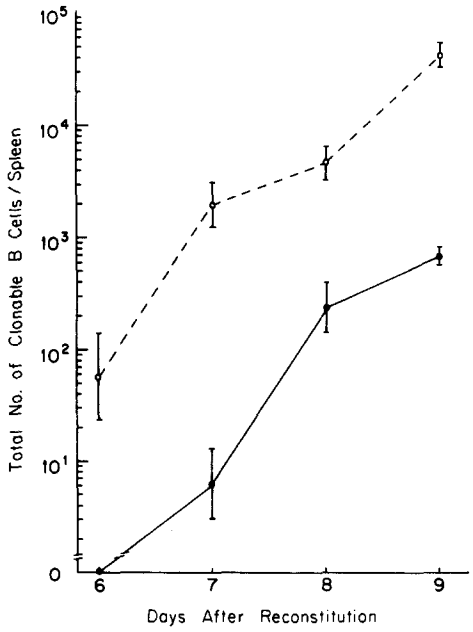

FIG. 2. Emergence of clonable B cells in irradiated (1,050 rad) CBA/N recipients of 5×10^6 13 (●)- or 16(○)-d fetal liver. Each point represents the geometric mean from 7–16 mice individually assayed.

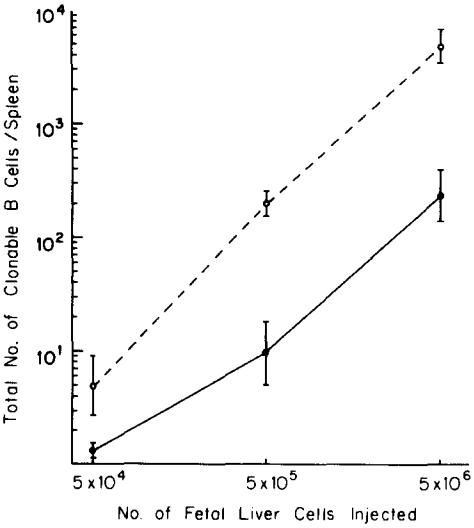

FIG. 3. Number of clonable B cells in irradiated (1,050 rad) CBA/N recipients 8 d after reconstitution with various doses of either 13(●)- or 16(○)-d fetal liver. Each point represents the geometric mean from 7–16 mice individually assayed.

illustrated is the fact that with time after grafting, i.e., after 14 d, differences in numbers of functional cells resulting from these two ages of fetal liver are insignificant. It should be noted that the incidences of CFU-s in these two-cell suspensions were similar ($3.04 \overset{\times}{\div} 1.1/10^5$ for 16-d and $3.86 \overset{\times}{\div} 1.1/10^5$ for 13-d fetal liver). Fig. 3 shows

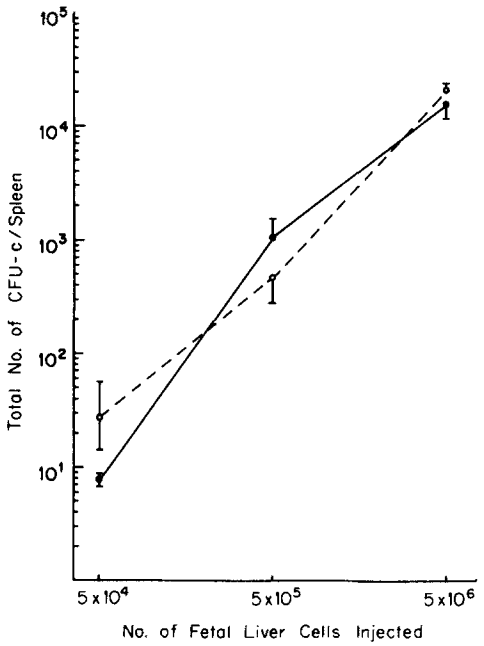

FIG. 4. Number of granulocyte-macrophage progenitor cells (CFU-c) in irradiated (1,050 rad) CBA/N recipients 9 d after reconstitution with various doses of either 13(●)- or 16(○)-d fetal liver. Each point represents the geometric mean of four to five mice individually assayed.

TABLE I

*Lack of Correlation between Clonable Myeloid and Lymphoid Cells in Fetal Liver-grafted, Irradiated Mice**

Age of fetal liver	No. of cells injected	No. of CFU-s contained among injected cells‡	No. of colony-forming units/spleen in recipient mice 1 wk after reconstitution§	
			CFU-c	CFU-B
d				
13	5×10^6	$233 \times\!\div 1.3$	$3{,}142 \times\!\div 3.2$	$53 \times\!\div 2.7$
16	5×10^5	$17 \times\!\div 1.3$	$358 \times\!\div 1.2$	$52 \times\!\div 2.0$

* Summary of data from four separate experiments in which CBA/H-T6T6 fetal liver cells were injected into irradiated (1,050 rad) CBA/N recipients.

‡ Geometric mean of the number of CFU-s injected based on separate determinations in which the splenic focus-forming ability of 10^5 cells was assessed in 10–12 irradiated (1,050 rad) CBA/H recipients 8–9 d after reconstitution. Incidence of endogenous foci was <1 in 10 spleens.

§ Geometric mean of the number of CFU-c or CFU-B detected in the spleens of irradiated CBA/N recipients 1 wk after reconstitution. In each of four experiments, three to five mice were individually assayed. CFU-c were stimulated with WEHI-3 supernates, and CFU-B were potentiated with LPS plus SRBC as described in Materials and Methods.

that at 8 d after transplantation, approximately 10 times the number of 13-d cells had to be injected to achieve the level of B cell restoration obtained with 16-d fetal liver.

TABLE II

*Effect of Pretreatment with 19B5 plus C on Colony-forming Units in CBA/H-T6T6 Bone Marrow**

Cell type	Stimulus	No. of colony-forming units, 10^5 bone marrow cells		
		Medium plus C	19B5 plus C	Percent reduction
CFU-s	—	29.6 ± 4.8	28.2 ± 5.8	5
CFU-c	CSA‡	219 ± 5	216 ± 21	1
CFU-mega	CSA‡	5 ± 6	15 ± 19	0
CFU-B	LPS§	363 ± 43	114 ± 29	69
CFU-B	SRBC§	429 ± 59	237 ± 37	45

* 5×10^6 bone marrow cells from 10- to 12-wk-old CBA/H-T6T6 mice were exposed to 19B5 antibody and complement (C) as described in Materials and Methods. A volume equivalent to that which contained 10^5 cells before antibody plus C treatment was utilized in each experiment.

‡ 0.1 ml of 10 times concentrated supernate of media conditioned by WEHI-3 cells was added to each 1-ml agar culture containing 10^5 cells. Mean numbers of granulocyte/macrophage progenitors (CFU-c) or megakaryocyte progenitors (CFU-mega) in three to five replicate plates. Because of the low incidence of CFU-mega's the number shown is based on 10^6 cells plated.

§ Mean number of B cell colonies determined from three to five replicate cultures stimulated with LPS (10 μg/ml) or SRBC (1%).

Expansion of nonlymphoid cells in the spleens of irradiated and reconstituted mice in these experiments was assessed by determining numbers of granulocyte-macrophage colony-forming cells (CFU-c) at the same intervals. In contrast with the temporal and numerical disparity evident in the generation of B cells (Figs. 2 and 3), the emergence of CFU-c from 16- or 13-d donors was indistinguishable (Fig. 4). This discrepancy between CFU-s numbers injected and numbers of expanding CFU-c, on the one hand, and numbers of clonable B cells on the other, is dramactically shown in Table I. The pooled data are given from four experiments in which the reconstitution potential of 5×10^5 16-d fetal liver cells was compared with a log greater number of 13-d cells. Both the input numbers of stem cells (CFU-s) and numbers of CFU-c recovered in the recipient spleens 1 wk after grafting reflected the log-fold difference in numbers of transferred cells, whereas the number of functional B cells recovered was approximately the same. This might suggest that a closer precursor-product relationship exists between CFU-s and CFU-c than between CFU-s and cells of the B lineage. It could also be argued that the incidence of CFU-s and CFU-c is similar at 13 and 16 d, whereas numbers of B cell precursors expand considerably over this interval.

Surface Antigen Characterization. Differentiating cells early in the B cell lineage may express unique surface receptors as milestones of their development. These could be useful in discriminating between stages of development as well as determining the size of various compartments in different tissues and periods of ontogeny. Of initial interest to us was an antigen known to be expressed on several established cell lines having pre-B cell characteristics but not demonstrable on CFU-s (24). This antigen has not been completely defined, but it is identified by monoclonal rat antibody, 19B5, which was prepared by fusing rat spleen cells immune to mouse brain with

TABLE III

Effect of Exposure to 19B5 plus C on CFU-s and B Cell Precursors

Experiment No.	Cell type injected and No. of weeks after reconstitution	No. of CFU-s contained among injected cells*			No. of clonable B cells/spleen recovered from recipient mice‡		
		Medium plus C	19B5 plus C	Percent reduction	Medium plus C	19B5 plus C	Percent reduction
1	Adult BM (2)	16.6 ×÷ 1.2	19.4 ×÷ 1.1	0	38,467 ×÷ 1.4	2,078 ×÷ 1.7	95
2	Adult BM (2)	29.4 ×÷ 1.1	27.8 ×÷ 1.1	5	22,321 ×÷ 1.5	1,051 ×÷ 1.8	96
3	Adult BM (2)	21.8 ×÷ 1.1	20.6 ×÷ 1.1	6	3,051 ×÷ 1.3	1,256 ×÷ 1.5	60
4	Adult BM (2)	19.6 ×÷ 1.1	18.6 ×÷ 1.1	5	12,796 ×÷ 1.1	4,263 ×÷ 1.6	67
5	Adult BM (2)	7.6 ×÷ 1.2	6.8 ×÷ 1.1	10	8,109 ×÷ 1.2	643 ×÷ 3.1	92
	B-depleted BM (2)§	6.4 ×÷ 1.3	6.4 ×÷ 1.3	0	8,955 ×÷ 1.3	795 ×÷ 1.4	91
6	Adult BM plus untreated CBA/N bone marrow (2)‖	ND¶	ND¶		10,926 ×÷ 1.2	1,857 ×÷ 1.4	83
7	13-d FL (2)	4.4 ×÷ 1.2	3.3 ×÷ 1.3	23	8,612 ×÷ 2.1	338 ×÷ 3.1	96
8	13-d FL (2)	8.0 ×÷ 1.1	8.6 ×÷ 1.1	0	5,179 ×÷ 1.3	1,905 ×÷ 1.3	63
	(6)	8.0 ×÷ 1.1	8.6 ×÷ 1.1	0	223,313 ×÷ 1.2	11,153 ×÷ 2.6	95

* Geometric mean of the number of CFU-s injected based on separate determinations in which the splenic focus-forming ability of adult bone marrow (BM) or fetal liver (FL) cells as assessed in 8–12 irradiated (1,050 rad) CBA/H recipients 8 d after reconstitution. A volume equivalent to that which contained 10^5 cells before antibody plus C treatment was injected into each recipient. Statistical analysis by Wilcoxon's rank test reveals no significant difference between the medium plus C and 19B5 plus C groups ($0.9 > P > 0.8$).

‡ Geometric mean of the number of CFU-B detected in the spleens of three to five irradiated CBA/N recipients. Clonable B cells were potentiated by LPS (10 μg/ml) plus SRBC (1%). A volume equivalent to that which contained 10^5 cells before antibody plus C treatment was injected into each recipient. Statistical analysis by Wilcoxon's rank test reveals significant difference between the medium plus C and 19B5 plus C groups ($P < 0.001$).

§ B cell depletion was accomplished utilizing anti-μ-coated petri dishes as previously described (19). After depletion the marrow contained <0.5% sIg^+ cells, and clonable B cells were reduced by 95%.

‖ 10^5 CBA/N bone marrow cells were injected.

¶ ND, not done.

defective murine plasmacytoma cells (24). A partial analysis of the specificity of this cytotoxic antibody is shown in Table II. By quantitative adsorbtion we have also found that both the pre-B cell line 70Z/3, and the sIg^+ B cell line, WEHI-231, possess this antigen. The results of eight separate experiments in which adult or fetal hemopoietic cells from CBA/H-T6T6 mice were treated with complement alone or 19B5 and complement before being used to restore B cell function in CBA/N mice are shown in Table III. Experiments 1–5 demonstrate that immune elimination with this antibody substantially reduced the B cell regenerative capacity, whereas numbers of CFU-s in the transferred suspensions were unaffected. That self-renewing B cells do not contribute to numbers of newly formed B cells in this assay and thus are not the relevant targets of the antibody is demonstrated in experiment 5 where sIg^+ B cells were depleted before further manipulation. Because it is likely that the antigen detected by 19B5 is not restricted to lymphoid cells, untreated CBA/N marrow cells were added to control and antibody-treated cell suspensions before transfer in

experiment 6. CBA/N bone marrow thus provided accessory cells that may have been the target of antibody and complement lysis, whereas it does not contribute any clonable B cells. This had no effect on the emergence of B cells in the recipients, and we conclude that in our reconstitution assay the relevant targets of the 19B5 antibody must be cells other than CFU-s, mature B cells, or accessory cells in CBA/N bone marrow. Precursors of B lymphocytes in 13-d fetal liver were similarly susceptible to cytolysis with this antibody and complement (experiments 7 and 8), and numbers of clonable B cells were still extremely low in recipients of treated cells 6 wk after grafting (experiment 8). Treatment with 19B5 in the absence of complement did not influence B cell reconstitution (data not shown).

Discussion

In earlier studies we described B lymphocyte precursor cells in the mouse that appeared not to be closely related to cells detected by the in vivo spleen focus (CFU-s) assay (19). The observations reported here extend our understanding of these cells by showing that (*a*) they have sedimentation characteristics that distinguish them from B cells, may differ in fetal and adult life, and overlap but do not always coincide with CFU-s; (*b*) they become more efficient and/or more numerous with increasing fetal age; and (*c*) they bear at least one distinctive antigen from a very early stage of development.

For these investigations we utilized an adoptive transfer assay in which CBA/H-T6T6 cells are grafted into irradiated CBA/N recipients after which B cell generation is assessed by monitoring the emergence of colony-forming B cells. As before, we have taken advantage of findings that CBA/N mice lack colony-forming B cells, the cloning procedure is an efficient and easy method for detecting and enumerating functional B cells, and microenvironments of CBA/N mice adequately support the differentiation of B cells from the precursors of normal CBA/H-T6T6 mice (8, 19). It should be noted that whereas the absence of background makes CBA/N recipients convenient for these studies, this is not essential, and similar results have been obtained using irradiated CBA/H recipients (19).

Clonable murine B cells residing in various tissues and at different stages of development have now been characterized in terms of a number of cell surface and physical properties, including their sedimentation velocity at unit gravity (29–38). Our profiles for functional B cells in adult marrow are similar to those previously reported by others and are strikingly different from those of precursor cells measured in the transfer system (1, 27, 28). Indeed, the latter are similar in this respect to cells shown to restore immune responsiveness after an interval to SRBC, cells capable of maturing in liquid cultures containing thymus filler cells to respond to LPS and secrete immunoglobulin, and cells defined by the presence of cytoplasmic but not surface IgM (1, 27, 28). The sedimentation characteristics of CFU-s are overlapping but not identical with those of these pre-B cells. We have also found that the sedimentation rates of the precursor cells found in the fetal liver differed from those of the adult bone marrow. Whereas the significance of this observation is not yet clear, it is interesting to note that previously we have found differences in the ability of these two populations to restore B cell function in unirradiated CBA/N recipients (19). Further study could reveal hitherto unknown heterogeneity in this category of cells.

The precise role of the CFU-s in hematopoiesis, and particularly in lymphopoiesis, is not clearly understood. Whereas these cells are generally thought to be pluripotent, a class of CFU-s with restricted differentiation options has also been identified (20).

Several studies have indicated that many of the cells capable of focal proliferation in the spleen of irradiated mice can potentially generate B lymphocytes (39–43). We have previously shown, however, that greater than 90% of spleen foci contain B cells, which are not derived from the CFU-s that gave rise to those foci, and we furthermore suggested that pre-B cells rather than stem cells were responsible for the majority of the B cells observed. A major prediction of this suggestion is that conditions can be found in which the numbers of CFU-s in a population would not correlate with the potential of that population to give rise to B lymphocytes. The dramatic difference between 10-d yolk sac and 12-d fetal liver in this regard is one example in which this prediction is fulfilled, although age- and organ-specific differences in CFU-s could be evoked to explain the discrepancy (19). In the present study a comparison between 13- and 16-d fetal liver again showed this lack of correlation between CFU-s numbers and B cells generation potential thus limiting the possible differences to age-related ones. This reservation is removed, however, by the further demonstration that the same population of either fetal liver or adult bone marrow could be manipulated, by immune selection, to demonstrate a lack of correlation between the presence of CFU-s and the ability to generate B lymphocytes. We are, therefore, left with the possibilities that the entry of stem cells into the B cell lineage is minimal compared with the expansive potential of precommitted cells and/or that the relevant stem cells for the humoral immune system are not well enumerated by the CFU-s assay. Of particular interest was the finding that committed progenitor cells of the granulocyte-macrophage lineage (CFU-c) recovered from the spleens of grafted mice did correspond with numbers of injected CFU-s. This finding is compatible with previous indications of the close relationship between these cells (44, 45).

These observations reinforce our thesis that committed progenitor cells for the humoral immune system (pre-B cells) are formed early in development and thereafter constitute the major precursor pool for the generation of B lymphocytes. Recent studies on the aging of CFU-s suggest that under normal conditions the contribution of these cells may indeed be negligible (46, 47). It is also of interest to note that the WW^v mouse, while severely deficient in stem cells, has normal number(s) of clonable B cells from birth (48). This may imply that most CFU-s are not relevant stem cells for the lineage to which clonable B cells belong or that the major expansion of the B cell precursor pool occurs at a poststem cell stage. Previously, it has been suggested that the CFU-s numbers do not correlate well with the long-term hematopoietic reconstitution capacity of various cell populations (49). Final resolution of these issues should provide useful information needed to understand the generation of tolerance, further insight into the nature of immunodeficiency states, and a better rationale for human transplantation techniques.

Summary

The emergence of functional B cells was monitored in irradiated or unirradiated CBA/N recipients of either adult bone marrow or fetal liver from CBA/HT6T6 donors. The cells that are primarily responsible for the generation of B lymphocytes, at least during the first 6 wk, are rapidly sedimenting (4.5–6 mm/h), lack surface

immunoglobulin, and are found in both the adult bone marrow and the fetal liver from day 12 onward. These pre-B cells are distinct from the colony-forming unit spleen (CFU-s) as demonstrated by the following criteria: (*a*) absence from yolk sac (19), (*b*) lack of correlation between CFU-s number and the ability to generate B cells in fetal liver populations of different ages of gestation, and (*c*) hybridoma antibodies that significantly inhibited B cell reconstitution but have no effect on CFU-s numbers. The antigen detected by this antiserum is present on both the fetal liver and bone marrow B cell progenitor, although its expression is not restricted to the B lineage. The pre-B cells that we monitor are not homogeneous, however, as both physical and functional differences are found. These observations reinforce our thesis that committed progenitor cells for the humoral immune system are formed early in development and thereafter constitute the major precursor pool for the generation of B lymphocytes.

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