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A role for mature B cells in bone marrow transplantation

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SUMMARY

The proliferative potential of membrane Ig (mIg)-bearing B lymphocytes was assessed in an adoptive transfer system based on the use of non-inbred rabbits matched for major histocompatibility (MHC) antigens and mismatched for immunoglobulin (Ig) allotypes. Cell suspensions made from spleens (SP), mesenteric lymph nodes (LN), or bone marrow (BM) of allotype b^4b^5 rabbits were deprived of B cells with mIg of the b4 type by adherence to plastic dishes coated with affinity-purified anti-b4. When such b4-depleted cell populations were injected into newborn hosts of allotype b^6b^6, stable and lasting chimerism promptly resulted, in which donor-derived products were almost entirely of the b5 allotype. Chimeras formed by transfer of unfractionated cells from b^4b^5 donors, on the other hand, exhibited a predominance of the b4 allotype, as seen in the living donors. BM but not SP or LN contained precursors capable of differentiating into mIg^+ lymphocytes in culture, but no evidence was obtained for engraftment and differentiation by such B-cell precursors or more primitive stem cells *in vivo*. These studies suggest a potentially significant role for mature B cells in reconstituting the immune system of human transplant recipients.

INTRODUCTION

In earlier studies, we developed a rabbit model for human bone marrow transplantation (BMT) in which we showed that life-long B-cell chimerism was readily induced in recipients of bone marrow (BM), spleen (SP) or lymph node (LN) cells from donors matched with recipients for antigens of the major histocompatibility complex (MHC) (RLA types). Engraftment was readily observed by measuring Ig products of donor and recipient origin on cells and in serum of the chimeras (Adler *et al.*, 1981). This experimental model proved useful for the characterization of cells found in donors undergoing chronic allotype suppression (Adler *et al.*, 1981, 1984) and also for assessing the colonizing efficiency and functions of memory cells from primed donors (Adler & Adler, 1984, 1985; Claassen *et al.*, 1987; Seferian, Rodkey & Adler, 1987).

The studies cited yielded considerable evidence in support of an important role for mature B cells in the colonization of recipients, a role that has received only scant notice in the history of studies in human BMT. In these studies, stem cells have traditionally received major attention because they are essential for reconstituting the haematopoietic system of patients whose diseased or ablated marrows require replacement. In addition, T cells in the marrow transplants have been the subject of intensive investigation, originally because alloreactive T cells were found to mediate graft-versus-host disease (GVHD) in rodents and humans (Korngold & Sprent, 1978; Deeg & Storb, 1984; Reisner *et al.*, 1981; Vallera *et al.*, 1981; Prentice *et al.*, 1984; Waldmann *et al.*, 1984) and, more recently, because either the same or other T cells appear to facilitate engraftment of the donor's cells (Martin *et al.*, 1985; Champlin, 1987; Patterson *et al.*, 1986). Although it is well known that patients receiving BMT experience lengthy delays in their attainment of a fully functioning immune system (Witherspoon *et al.*, 1981; Atkinson, 1983; Buckley *et al.*, 1986), the idea that mature lymphocytes in the donor inoculum could be a self-renewing source of specifically immunocompetent cells has received little consideration.

In our studies we have demonstrated that SP and LN cells were equally effective and decidedly superior to BM in establish-

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Abbreviations: Ab, antibody; ACT, ammonium chloride-TRIS; BM, bone marrow; BMT, bone marrow transplantation; EBSS, Earles's Balanced Salt Solution; EMEM, Eagle's Minimal Essential Medium; FBS, fetal bovine serum; GARFab, goat anti-rabbit Fab; GVHD graft-versus-host disease; HA, haemagglutination; HI, haemagglutination inhibition; Ig, immunoglobulin; ISC, Ig-secreting cells; LN, lymph node; MHC, major histocompatibility complex; mIg, membrane Ig; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; RFC, rosette-forming cells; RLA, rabbit lymphocyte antigen; SP, spleen; SRBC, sheep red blood cells.

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ing B-cell chimerism and that the colonizing efficiency of cells from these tissues paralleled their content of membrane Ig-bearing (mIg^+) B cells (Adler & Adler, 1987). Further, in the experimental model under discussion, where B-cell chimerism was attained in >95% of the recipients, chimerism with regard to T cells or erythrocytes was demonstrable only in exceptional recipients, namely, rabbits that had received RLA-incompatible cells and had survived a GVH reaction (Adler, Le Beau & Adler, 1983; Adler *et al.*, 1984). In addition, when grafts contained unequal proportions of cells with distinctive allotypic markers, donor-derived cells in the chimeras continued to produce Ig in these same proportions indefinitely (Adler *et al.*, 1981; Saito & Adler, 1986), suggesting that seeding of recipients was by clones of allotype-committed donor-derived B cells. Finally, it was shown that specific B-cell memory could be transmitted through three successive serial transfers over a period of 1·5 years and that such donor-derived cells underwent enormous clonal proliferation in response to antigenic challenge in the recipient (Adler & Adler, 1984, 1985). These clones were essentially the sole functional survivors of B cells derived from the original donor that could be identified in the secondary and tertiary recipients (Adler & Adler, 1985).

The main aim of this study was to establish formal proof of the hypothesis that even when deliberate antigenic stimulation is not used, donor-derived B lymphocytes in cell suspensions prepared from transplanted SP, LN, or BM are important for colonization of recipients with B lymphocytes of donor origin. Investigation of this question was facilitated by using donor rabbits that were heterozygous with regard to the kappa light chain allotypes of the *b* group, i.e. b^4b^5, and noting the effect of removing mIg^+ cells of one allelic type, b4, from the cells to be transplanted.

MATERIALS AND METHODS

Rabbits

The rabbits used in this investigation were bred and raised in the Animal Resources Center at St Jude Children's Research Hospital. They were from a colony of outbred rabbits that are typed for class I (RLA-A) and class II (RLA-D) antigens of the MHC, as well as for Ig allotypes on kappa light chains (*b* locus) and on the variable portion of heavy chains (*a* locus). Cell donors and transplant recipients used in the current series of experiments were all of RLA type C/C with regard to both the RLA-A and RLA-D loci (Adler *et al.*, 1984).

Cell preparation and transfers

Rabbits used as cell donors were killed by an i.v. injection of sodium pentobarbital (Abbott Laboratories, N. Chicago, IL). Spleen (SP) and mesenteric lymph node (LN) cells from a single adult donor were prepared, and in some cases pooled, as described earlier (Adler *et al.*, 1981; Saito & Adler, 1986). Spleen cells were treated with ammonium chloride-TRIS (ACT; Sigma) buffer to lyse red cells. Bone marrow was flushed from the femurs and tibiae using Earle's Balanced Salt Solution (EBSS; M. A. Bioproducts, Walkersville, MD) containing 0·1% preservative-free heparin. Clumps were dispersed by repeated aspiration and fat removed by flotation at 4°. The cells were then pelleted by centrifugation at 250 *g* for 10 min at 10°, resuspended in EBSS containing 100 μg deoxyribonuclease (DNAase I, Sigma, St Louis, MO) per ml for 5 min at 37°, pelleted again, and washed once with EBSS. Red blood cells were lysed by treatment with ACT buffer, followed by washing, and finally the cells were resuspended in EBSS for counting and viability determinations. All cell suspensions were adjusted to 10^8 viable cells per millilitre in EBSS for i.p. injection into 1–2-day-old recipients.

Antisera and purified antibodies

Anti-allotype sera were prepared in rabbits by methods described elsewhere (Inoue & Adler, 1982). In order to avoid cross reactions, most antisera were made in allotype-heterozygous rabbits; e.g. anti-b4 in b^5b^6 rabbits. All antisera were tested for possible cross-reactions by passive haemagglutination (HA; Adler & Adler, 1980), and when necessary were absorbed on columns of cyanogen-bromide-activated Sepharose 4B (Pharmacia, Piscataway, NJ) to which appropriate allotypic IgG had been conjugated. Purified anti-allotype antibody (Ab) was separated from whole antiserum by absorption to and elution from similar columns (Inoue & Adler, 1982). An antiserum prepared in a goat immunized with Fab fragments of rabbit IgG was used as a source of purified goat anti-rabbit Fab (GARFAb; Ohama & Adler, 1984).

Measurement of allotypic Ig in serum

A quantitative haemagglutination-inhibition (HI) assay was used to measure relevant allotypes in serum. This method has been described in detail elsewhere (Adler & Adler, 1980).

Detection of mIg^+ cells and Ig-secreting cells

A rosette assay based on the use of sheep red blood cells (SRBC) to which affinity-purified anti-allotype Ab or GARFab had been linked was used to detect viable lymphocytes with membrane-bound Ig (mIg). A detailed description of this assay, its specificity and significance, has been provided earlier (Inoue & Adler, 1982). Peripheral blood lymphocytes (PBL) secreting Ig of relevant allotypes were enumerated by means of an allotype-specific reverse haemolytic plaque assay (Ohama & Adler, 1984). Lymphocytes for this assay were isolated from heparinized rabbit blood by density gradient centrifugation on Lymphoprep (Nyegaard and Co., Oslo, Norway), as described earlier (Adler *et al.*, 1977).

Depletion of mIg^+ lymphocytes by specific adherence

Cell suspensions containing mIg^+ lymphocytes of the b4 and b5 types from allotype b^4b^5 rabbits were depleted of $b4^+$ cells by adherence to anti-b4 on plastic dishes (Falcon Labware, Oxnard, CA, 100 × 15 mm), using an adaptation of methods described earlier (Mage, McHugh & Rothstein, 1977; Wysocki & Sato, 1978). In brief, affinity-purified anti-b4 made in b^5b^6 rabbits was diluted to 50 μg per ml in 0·15 M phosphate-buffered saline (PBS), and 5 ml of diluted Ab were added to each plate, which was maintained overnight at 4°. The Ab was decanted and the plates washed four times with PBS and once with PBS containing 1% fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY), to saturate unbound sites. The plates were then over-layered with PBS until the moment of use. Suspended cells at 5×10^7 in 5 ml of EBSS were added to each plate and allowed to settle at 4° for 1 hr. Halfway through the incubation period, the non-adherent cells were resuspended by gentle swirling. The non-adherent cells were then swirled again and

Table 1. Allotype-specific depletion of lymphocytes from b^4b^5 rabbits

Rabbit no.	Cell source	Group	mIg$^+$ cells (% RFC) Fab	b4	b5	b6	Viable cell recovery (% of input)
3431	SP	C*	30·5	24·2	11·8	0	
		E*	7·3	1·0	7·2	0·8	61
	LN	C	38·8	25·0	15·7	0·2	
		E	6·3	0·8	6·5	0·2	40
	BM	C	15·7	12·3	5·5	0	
		E	8·2	1·2	6·2	0·3	44
6147	SP	C	28·0	19·7	9·2	0·3	
		E	7·2	1·0	7·2	0	54
	LN	C	31·3	21·2	10·8	0·2	
		E	7·5	0·8	7·2	0	55
	BM	C	15·3	9·7	4·7	0·2	
		E	6·0	1·0	4·7	0·5	48
5134	SP-LN Pool	C	36·3	23·7	12·7	0·3	
		E	12·9	1·2	12·2	0	46
6032	SP-LN Pool	C	38·3	28·3	15·5	0·3	
		E	12·5	1·0	13·8	0·3	38

*C, control (unfractionated cells); E, experimental (b4-depleted cells).

The percentage of mIg$^+$ cells was determined by the formation of rosettes (RFC) in the presence of SRBC coated with anti-rabbit Fab, anti-b4 or anti-b5. Anti-b6 SRBC were used as a control for specificity of rosette formation.

collected by pipetting, taking care not to disturb the adherent layer. The plates were washed three times with PBS to collect residual non-adherent cells, and the combined non-adherent cells were pelleted at 250 *g* for 10 min at 10° and resuspended in EBSS for counting.

Cell cultures

Conditions developed earlier (Inoue & Adler, 1982) were used for the culture of SP, LN or BM cells. Cell suspensions prepared from SP or BM were first treated with ACT buffer to lyse RBC. Each culture consisted of 4×10^6 cells in 0·2 ml of Eagle's Minimal Essential Medium (EMEM) supplemented with 10% FBS in the well of a 24-well Linbro plate (Flow Labs, McLean, VA). Incubation was from 1 to 7 days at 39° in a humidified atmosphere of 5% CO_2 in air, after which cells were removed from duplicate wells with a rubber policeman, washed twice with EMEM, and evaluated for viability and the presence of mIg.

RESULTS

Depletion of allotype-specific B lymphocytes

In order to assess the role of mature B lymphocytes in haematopoietic cell engraftment, we used lymphoid cell populations (spleen, mesenteric lymph node, or bone marrow) from adult rabbits of allotype b^4b^5 and depleted lymphocytes with membrane Ig of the b4 allotype by adherence to purified anti-b4 on plastic dishes. The efficacy and specificity of this procedure are illustrated by results shown in Table 1, taken from a total of 20 depletion procedures. The proportion of mIg$^+$ cells in SP and

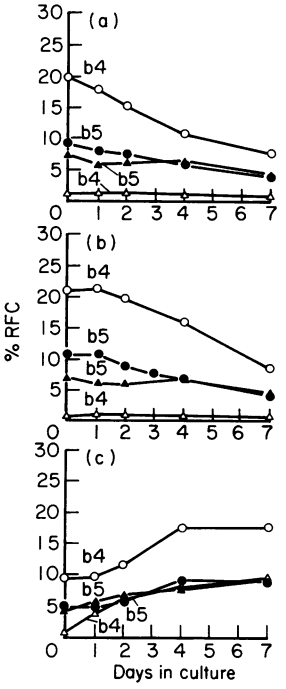

Figure 1. Allotype-specific mIg$^+$ cells in cultures of unfractionated or b4-depleted cell suspensions from b^4b^5 rabbits. (a) SP; (b) LN; (c) BM. Open and closed circles represent unfractionated cells, and triangles symbolize b4-depleted cell cultures.

LN suspensions was very similar and was in the range of 28–38% before fractionation. There were approximately twice as many b4$^+$ as b5$^+$ cells; this ratio of b4 to b5 expression is commonly observed in b^4b^5 rabbits (Kindt, 1975; Ohama, Adler & Saito, 1985). After one cycle of adherence on anti-b4 plates, the b4$^+$ population of cells was reduced from a mean of 24% to 1% or less. The proportion of b5$^+$ cells, which might have been expected to increase as a result of b4 cell depletion, remained essentially unchanged or decreased slightly, reflecting the fact that some B cells adhere non-specifically to plastic surfaces. BM populations had a lower representation of mIg$^+$ cells than did SP or LN (mean in six experiments of 11%, of which about 7% were b4$^+$ cells), and less than 0·5% of b4$^+$ cells remained in the non-adherent fraction. Total viable cells recovered following the separation procedure varied from 38% to 61% of input in the data shown.

Membrane Ig expression by cultured lymphoid cells

The effects of allotype-specific B-lymphocyte depletion were first investigated in cell culture experiments. Unfractionated and b4-depleted cells from SP, LN or BM were compared by the enumeration of mIg$^+$ cells of the b4 and b5 types after 1–7 days of culture. Typical results illustrating three such experiments are presented in Fig. 1. In cultures of unfractionated SP or LN cells, a gradual decline in the proportion of mIg$^+$ lymphocytes of both the b4 and b5 types took place with time and in cultures of b4-depleted cells, no evidence for regeneration of lymphocytes with mIg of the b4 type was obtained. In contrast, BM cells showed a definite capacity for generating mIg$^+$ cells during the first 4 days of culture. This occurred in cultures of unfractionated as well as b4-depleted cells. Although membrane Igb4

Table 2. Allotype-specific Ig secretion by donor-derived lymphocytes in chimeric rabbits

Rabbit	Transferred cells	ISC/10^6 blood lymphocytes b4 (D)*	b5 (D)	b6 (R)*	% b4†
T80	Unfractionated	740	469	1344	61
T81	Unfractionated	1064	578	1179	65
T87	Unfractionated	164	103	3272	61
T79	b4-depleted	72	439	3715	14
T86	b4-depleted	54	405	3170	12
T88	b4-depleted	51	457	3076	10

* D, donor-derived; R, recipient type.

† Calculated as:

$$\frac{b4}{b4+b5} \times 100.$$

Allotype-specific Ig-secreting cells (ISC) were determined using purified lymphocytes obtained from peripheral blood of chimeric rabbits at age 3 months.

expression in cultures of b4-depleted cells never reached that of unfractionated cells, the results demonstrate that precursors of lymphocytes committed to b4 or b5 mIg expression are present in the BM of rabbits but not in SP or LN.

Synthesis of b4 allotypic Ig by adoptively transferred SP and LN cells

In order to test the idea that allotypically committed B lymphocytes play a significant role in permanent colonization of transplant recipients, we injected equivalent numbers of viable nucleated cells from unfractionated or b4-depleted populations of b^4b^5 rabbits into newborn RLA-matched recipients of the b^6b^6 allotype. Typically, equal numbers of SP and LN cells (about 5×10^8 each) were obtained from each donor, and this served as a cell inoculum for five to six recipients, each of which received 10^8 b4-depleted or unfractionated cells. The transplanted animals were bled at regular intervals, beginning at age 3 weeks and continuing up to age 6 months or more, and their blood lymphocytes as well as serum Ig were analysed for allotypes of donor (b4 and b5) and recipient (b6) origin.

All of the successfully engrafted animals became mixed lymphoid cell chimeras, as manifested by their continued production of both donor-derived and recipient allotypes. Data presented in Table 2 show the allotype distribution among Ig-secreting cells in chimeric rabbits that were injected neonatally with b4-depleted or unfractionated cell suspensions from b^4b^5 donors. Both groups of chimeras had considerable numbers of donor-derived B lymphocytes, and the normal preponderance of b4 over b5 production was seen in the control group. In contrast, recipients of b4-depleted cells had very few lymphocytes secreting b4 Ig in comparison to their component of b5-secreting lymphocytes.

Results of serum Ig measurements in two representative rabbits that received unfractionated or b4-depleted SP+LN cells from the same donor are shown in Fig. 2. The dominance of b4 over b5 production and the magnitude of total donor-derived Ig synthesis were notably stable over a 6-month period in the

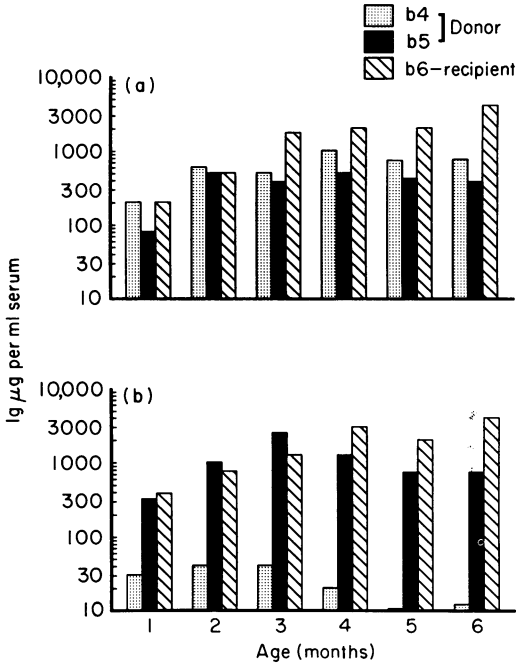

Figure 2. Serum concentrations of allotypic Ig in allotype b^6b^6 recipients of pooled SP+LN cells from b^4b^5 donors. (a) Recipient of unfractionated cells; (b) recipient of b4-depleted cells.

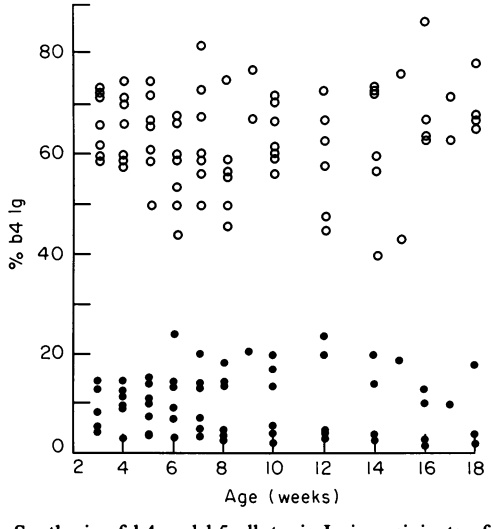

Figure 3. Synthesis of b4 and b5 allotypic Ig in recipients of pooled SP+LN cells from b^5b^5 donors. Serum concentrations of b4 and b5 allotypes are expressed as the percentage of Igb4 in total donor-derived Ig,

$$\frac{b4}{b4+b5} 100.$$

(○) Recipients of unfractionated b^4b^5 cells; (●) recipients of b4-depleted b^4b^5 cells.

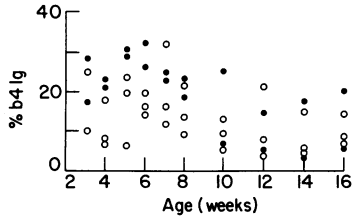

Figure 4. Synthesis of b4 and b5 allotypic Ig in recipients of b4-depleted LN or BM cells from b^4b^5 rabbits. Results are expressed in terms of the percentage of b4 Ig in total donor-derived Ig (b4 + b5). (○) Recipients of b4-depleted BM; (●) recipients of b4-depleted LN cells.

animal injected with unfractionated cells from a b^4b^5 donor. In contrast, Fig. 2b shows that in an animal injected with b4-depleted cells from the same b^4b^5 donor, a considerable amount of donor-derived Ig was produced, but it was almost entirely of the b5 allotype.

Additional data establishing the importance of allotypically committed B cells in engraftment are presented in Fig. 3. These results represent an additional group of b^6b^6 rabbits injected at birth with pooled SP + LN cells (unfractionated or b4-depleted) from b^4b^5 donors. Serum samples obtained at ages 3 to 18 weeks were analysed for allotypes of the b4 and b5 (donor) as well as b6 (recipient) types. The results, expressed as the percentage of b4 in total donor-derived Ig, illustrate the dramatic and lasting effects of removing mIg^+ cells of the b4 type from the cell inoculum. As expected, most of the Ig produced by donor cells was of the b4 type in recipients of unfractionated cells, while in recipients of b4-depleted cells the proportions of donor-derived Ig products were reversed, with b4 accounting in many instances for less than 5% of the total donor-derived Ig. When data from the same two groups of animals were analysed for the degree of chimerism, i.e. the proportion of donor type Ig (b4 + b5) relevant to total Ig (b4 + b5 + b6) in serum, no pattern of differences were seen between the recipients of b4-depleted or unfractionated cells (data not shown). This suggests that in the absence of b4 production, cells of the b5 type compensate to maintain similar donor:recipient Ig levels.

Effect of B-lymphocyte depletion on transplanted BM

Since BM is the tissue most often used for human haematopoietic reconstitution, it was of particular interest to determine what part could be played by committed B lymphocytes in the marrow in colonizing the host's lymphoid system. Therefore, allotype b^6b^6 rabbits were transplanted with BM from b^4b^5 donors after depleting mIg^+ lymphocytes of the b4 type by adherence (Table 1). Synthesis of b4 and b5 Ig by the resulting chimeras was compared with that of rabbits that had been injected with b4-depleted LN cells, a source generally believed to be devoid of pluripotent stem cells (Gallagher, 1983). Results depicted in Fig. 4 show that over a 16-week period the proportion of b4 Ig in recipients of b4-depleted BM was similar to that of rabbits that received b4-depleted LN cells. The deficiency of b4 Ig production caused by depleting mIg^+ cells of the b4 type from BM or LN was also comparable to that in recipients of b4-depleted pooled SP + LN cells previously shown in Fig. 3. Recipients of unfractionated BM, on the other hand, synthesized more b4 than b5 Ig (data not shown), as had recipients of unfractionated SP or LN cells (Figs 2 and 3). These findings demonstrate immature precursor cells in BM to be much less effective in colonization under these transplant conditions than mature mIg^+ B cells.

DISCUSSION

Results presented here clearly demonstrate that mIg^+ B cells in spleen, lymph nodes or bone marrow of adult donors are the major source of engrafted B cells in chimeric rabbits. We have shown that removing a subpopulation of B cells committed to Ig synthesis of one allelic type severely and indefinitely curtailed production or that allotype in chimeric recipients injected with cells from allotype-heterozygous donors. Rabbits engrafted with unfractionated cells continued to produce donor-derived Ig in similar proportions of b4:b5 as observed in the living donor namely, 1·5–2:1 (Kindt, 1975; Ohama *et al.*, 1985). The deficiency in b4 Ig production was equally pronounced whether b4-depleted BM or LN cells were used as the inoculum. Since BM and LN are, respectively, the richest and the poorest sources of stem cells, this outcome supports the notion that in our model (i) stem cells are not a major source of donor-derived B-cell progeny and (ii) mature B cells are capable of engraftment and prolonged self-renewal. It was interesting to note that BM depleted of cells bearing mIg of the b4 type contained cellular precursors capable of differentiating *in vitro* into $b4^+$ B cells, but that no such regeneration of B cells occurred in cultures of SP or LN cells. The most likely immediate precursors of $b4^+$ B cells arising in cultured marrow are mIg^- pre-B cells containing cytoplasmic Ig only, and known to be present in BM but not in SP or LN of adult rabbits (Gathings *et al.*, 1982). Surprisingly, such cells, as well as other more primitive stem cells presumably present in the marrow, did not function as a major source of $b4^+$ B-cell progenitors in recipients of RLA-compatible cells. It is not possible to determine from the results presented here whether stem cells actually did not engraft or were merely prevented from differentiating to $b4^+$ lymphocytes by cellular control mechanisms such as clonal dominance effects. Since the transplanted rabbits in these experiments were not irradiated and were conditioned only by virtue of their neonatal state, the failure of b4 precursor cells in the marrow to engraft could be caused by limitations in 'biological space'. On the other hand, differentiation from stem cells could account for the small amounts of b4 Ig produced by recipients of b4-depleted cell transplants, or one could attribute this activity to the small number of $b4^+$ lymphocytes (1% or less) remaining in the non-adherent population after the depletion procedure.

The capacity of mIg^+ lymphocytes to colonize and function as a self-renewing population in transplant recipients has received little direct attention to date. Earlier studies on adoptive transfer of antigen-primed lymphocytes in inbred rodents have involved short-term observations (Kincade, 1981) and have stressed the importance of continuous antigenic stimulation for the maintenance of antibody-forming clones (Nossal, 1963; Askonas, Williamson, & Wright, 1970). In the work reported here, deliberate stimulation of B cells was not required to achieve long-term engraftment; however, we cannot rule out stimulation due to environmental factors or to allogenic effects engendered by minor histocompatibility differences.

Studies by others (Craig & Cebra, 1971; Tseng, 1981; Leventon *et al.*, 1986) have suggested a role for mature isotype-

committed cells or immediate precursors of such cells found in Peyer's patches in seeding the gut-associated lymphoid tissue. However, these workers did not separate mIg$^+$ cells from mIg$^-$ precursors in their transplant inocula. Kurland, Ziegler, & Witte (1984) were able to reconstitute the B-lymphocyte lineage in immunodeficient mice by injecting long-term cultured BM cells demonstrably lacking in multi-potential stem cells, and their results also suggested, but did not identify clearly, a role for mature B lymphocytes in the cultures as engrafting cells. Very recent reports present evidence for engraftment and self-renewal by a small population of Ly-1$^+$ B cells found in mouse peritoneum but not in marrow (Hayakawa *et al.*, 1986; Förster & Rajewsky, 1987). Reconstitution of irradiated mice with this class of B cells appeared to depend entirely upon the transfer of mature Ly-1$^+$ B lymphocytes rather than on stem cells. Evidence for the existence of self-renewing Ig-bearing B cells in birds was reported by Pink, Ratcliffe & Vainio (1985), who concluded that such cells are the sole source of B cells in adult chickens. On the other hand, data reported earlier by Paige *et al.* (1979) led these authors to conclude that expansion of mIg$^+$ B cells in murine BM did not play a significant role in the generation of colony-forming B cells when transplanted to immunodeficient CBA/N mice. Clonable B cells arose at the same rate whether the marrow was obtained from normal or neonatally anti-μ-suppressed donors, and removal of BM-B cells by adherence to anti-Ig on plastic dishes yielded similar results. In contrast, results obtained with our experimental model clearly delineate an important role for B lymphocytes both in seeding the lymphoid system of transplant recipients and in providing a lasting source of B-cell proliferation. It is likely that the discrepancies noted arise from major differences in the animal models and methods used for assessing results.

The studies reported here confirm our hypothesis, deduced from earlier observations, that enduring B-lymphocyte chimerism in the RLA-defined rabbit results from engraftment by mature B cells rather than from stem cells transferred with the donor inoculum. This finding complements and extends our previously derived conclusions regarding the remarkable persistence and colonizing potential of donor-derived B-memory cells in chimeric rabbits (Adler & Adler, 1985).

Very recently, interest in the question of transferred adoptive immunity has revived because of the urgent need to accelerate the reconstitution of protective immunity in human BMT recipients and victims of immunodeficiency diseases (Wimperis *et al.*, 1986, 1987; Lum, Seigneuret & Storb, 1986; Shiobara *et al.*, 1986). Our data suggest the potential value of including B lymphocytes in human BM grafts, not only for the transfer of immunity to specific pathogens via memory cells, but also for seeding the recipient's lymphoid system with a portion of the donor's already expanded repertoire of immunocompetent lymphocytes.

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