POPULATION KINETICS OF RAT PERIPHERAL B CELLS

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Studies of B lymphopoiesis in the bone marrow of adult mice (1, 2) and rats (3) suggest that the rate of production is sufficient to totally replace the peripheral B cell pool within 4–5 d. This assumes first that there is no significant loss of cells between the stages of μ chain gene rearrangement and light chain gene rearrangement, and second that most of the newly produced surface membrane IgM^+ B cells are exported from the marrow. While the scale of export is difficult to assess because of the diffuse nature of the bone marrow, in situ labeling has shown that significant numbers do emerge into peripheral lymphoid organs (4, 5). More convincingly, the rate of repopulation of the B cell compartment after depletion by long-term treatment with anti-μ and anti-δ antibodies is very rapid and correlates well with this estimate of 4–5 d (6). Is the turnover of B cell populations in normal adult animals as rapid as this?

Methods for estimating the rate of turnover of peripheral, recirculating B cell populations rely on the assumption that one can identify the entry of B cells, newly emerged from the marrow, into the peripheral, recirculating pool because they are derived from proliferating precursors. By infusing rodents continuously with a label such as [^{3}H]thymidine that is incorporated into DNA during cell division, one can estimate the proportion of the peripheral B cell pool that divides during the period of infusion and thereby obtain an idea of the number of new B cells that have entered the pool. Long-term infusion experiments show that over a 5-d period only ~20% of the peripheral pool is labeled (7) and that the average life span of mature, recirculating B cells is in the order of 6–7 wk (8, 9). If most recirculating B cells live >5 d, then not all virgin B cells can gain entry into this pool and a form of selection must occur.

However, infusion techniques have been criticized for two reasons. First, [^{3}H]thymidine may have cytotoxic or cytostatic effects that lead to a selective underestimate of short-lived cells. Second, the stress inherent in the surgical intervention required for infusion and analysis of recirculating cells also depletes short-lived populations (10). Freitas et al. (11, 12) using hydroxyurea to kill cells as they enter S phase show that in mice given two injections of this drug, separated by 7–8 h, the majority (80–90%) of B cells in peripheral lymphoid organs are killed. These workers favor the hypothesis that the peripheral B cell compartment consists largely of cells newly derived from the marrow that turn over in a matter of days.

It is not clear which of these methods gives us the more accurate estimate of

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B cell life span. We have used a method for labeling dividing cells that is designed to minimize stress. Rats have been given bromodeoxyuridine (BUdR)[1] in their drinking water and then tissue sections of peripheral lymphoid tissues have been stained with an mAb to BUdR. B cells within the recirculating pool can be reliably identified in follicles of the spleen and lymph nodes (13, 14). The results suggest that the turnover of the recirculating B cell pool is in the order of weeks. An estimate of the rate of incorporation of new B cells into the recirculating pool is important when considering the expression of B cell repertoire and the induction and maintenance of antibody responses (12, 15). We have attempted to assess this rate of incorporation by distinguishing between virgin B cells derived from dividing precursors in the marrow and cycling cells that are part of antigen driven clones.

Materials and Methods

Animals. PVG/Ciu rats and PVG-RI-a[a] (DA) congenic strains (16) were supplied by the Institute for Biomedical Research, Füllinsdorf, Switzerland, after which they were maintained under standard laboratory conditions in the animal facility of the Basel Institute. All animals were used at 10–16 wk of age and within experiments were sex matched.

Drug Treatment. [^{3}H]TdR (TRA-61 5 Ci/mMol = 185 GBq/mMol; Amersham International, Amersham, United Kingdom) was injected intraperitoneally twice daily at a dose of 0.5 μCi/body weight/day; Cold thymidine (Sigma Chemical Co., St. Louis, MO) was administered at 50 μg/g body weight.

BUdR (Sigma Chemical Co.) was dissolved in distilled water at a concentration of 0.8 mg/ml. Water bottles were replenished with fresh water + BUdR every day. Groups of rats receiving BUdR intraperitoneally were given two daily injections of 50 μg/g body weight. These dose rates are as described by Wynford-Thomas and Williams (17).

Hydroxyurea (HU) (Sigma Chemical Co.) was dissolved in PBS, pH 7.2, at 0.25 g/ml and administered at a dose of 1 g/kg body weight. Two such doses were given by intraperitoneal injections separated by 7 h as described (11). All injections were done without anesthesia.

Detection of BUdR in Tissue Sections. Tissues were frozen in liquid nitrogen and stored dry at −70°C before cutting. Cryostat sections were cut (5 μm) and dried for at least 1 h at room temperature before fixing in acetone for 20 min at 4°C. Slides were dried before storage or staining. Dissociation of histones and partial denaturation of DNA were carried out as described (17). Briefly, this involved treatment of the sections with 1 M hydrochloric acid at 60°C for 8 min followed by extensive washing in staining/washing buffer (0.05 M Tris-HCl saline, pH 7.5). This method gave better staining results than the method described by Becton Dickinson & Co., Mountain View, CA (0.07 M sodium hydroxide 5–10 min at room temperature followed by neutralization with 0.1 M sodium borate, pH 8.5). BUdR was detected by staining with a mouse mAb purchased from Becton Dickinson & Co. This antibody was applied to sections at a dilution of 1:50 and after washing was, in turn, detected with rat anti–mouse IgG (H+L) conjugated to peroxidase (Jackson Immunoresearch Laboratories, Hamburg, Federal Republic of Germany). The reaction was developed with diaminobenzidine. Slides were counterstained in Mayer's hematoxylin.

Nonspecific crossreactivity of anti-BUdR antibody with nondividing cells in BUdR-treated rats was checked by staining brain tissue. No staining was observed.

Detection of Surface IgM in Cell Suspensions. Spleen and lymph node suspensions were prepared by standard methods using a steel gauze. A rosetting technique described previously (13) was used to detect surface membrane IgM or IgD. Rabbit anti–rat IgM and anti-IgD were the generous gifts of Professor H. Bazin, Experimental Immunology

[1] *Abbreviations used in this paper:* BUdR, bromodeoxyuridine; HU, hydroxyurea; PALS, periarteriolar lymphocytic sheath.

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Unit, University of Louvrain, Brussels. These were coated onto sheep red blood cells with chromic chloride (13).

Autoradiography. Slides for dipping in autoradiographic emulsion, after staining with immunoperoxidase, were dehydrated and dried. Slides were dipped in Kodak NTB-2 nuclear track emulsion (Eastman Kodak Co., Rochester, NY) diluted 50% in distilled water + 1% glycerol. The emulsion was melted and kept in a 42°C water bath during the whole procedure. After dipping, slides were placed on a cold plate to solidify for 5 min and then put into racks and boxed, together with silica gel. All dark room procedures were carried out under Kodak Safelight filter GBX-2. Exposure times varied, but good results were normally obtained within 2–3 weeks at 4°C. Autoradiographs were developed in a 1:10 dilution of Kodak Dektol developer (5 min) quickly washed, fixed (standard rapid fixative, 5 min), and then washed again before counterstaining in Mayer's hematoxylin, dehydration, clearing (in histosol), and mounting.

Quantitation of Labeled Cells in Tissue Sections. Labeled cells were counted in tissue sections rather than in cell suspensions because this facilitated the rapid differentiation of lymphocyte subpopulations by well-defined histological compartments. For instance, the follicles within the spleen and lymph node consist almost entirely of recirculating B lymphocytes (14), while the B cells in the splenic marginal zone are sessile (13, 18). Paracortical areas of lymph nodes and periarteriolar lymphocytic sheath (PALS) in the spleen are predominantly T cell areas. As a control, identification was aided by double staining for surface membrane IgM and BUdR; equivalent results were obtained. Counting of labeled cells (either [^{3}H]TdR or BUdR) was done by a systematic random sampling procedure described by Weibel (19). This involves counting the positive and negative cells in several different randomly chosen areas within defined lymphoid compartments. For instance, upwards of 1,000 cells were counted in at least five follicular structures within one lymph node; this often involved two sections taken at different levels of the node. All histological compartments were counted in this way and the proportion of positive cells is expressed as a percentage of the B or T lymphocytes within that compartment. For all compartments this involved counting of at least 1,000 cells.

Cell Transfers. Recipients (PVG/Ciu rats carrying Kla light chain allotype) were given 5.5 Gy γ irradiation (Gamma Cell 40 ^{137}Cs source; Atomic Energy of Canada Ltd., Kanata, Ontario) 1 d before cell transfer. Single cell suspensions were made from lymph nodes (several sites) from congenic PVG-RI-a^a rats that bear the Klb light chain allotype. 4.5×10^8 lymph node cells ($\sim 1.5 \times 10^8$ Klb-bearing B cells) were injected intravenously into groups of irradiated or nonirradiated recipients. Donor cells were enumerated within spleen and cervical lymph node suspensions. Surface membrane Klb expression was detected with FH8, a rat mAb described previously (20). Biotinylated FH8 was detected with rhodamine-labeled streptavidin (Inotech AG, Wohlen, Switzerland). Total B cells were enumerated by double staining of suspensions with sheep anti-rat IgGAM+L–fluorescein conjugate (Inotech AG).

Results

Labeling of Dividing Cells with BUdR. To avoid the possibility of selective depletion of short-lived populations that may result from the stress produced by repeated injections, rats were fed on BUdR in their drinking water. Groups of rats were maintained for 1, 3, and 5 d on BUdR; the proportion of cells that incorporated BUdR in their DNA is shown in Table I. Although not indicated, the rapidly proliferating cells of germinal centers all become labeled even after one injection of BUdR (animals killed 8 h later). Within the recirculating B cell population of the follicles, ~20% become labeled over a 5-d period. A similar proportion of T cells in the spleen and lymph nodes contain BUdR after 5 d of labeling. It is striking that the percentage of labeled cells in the marginal zone (sessile B cells) is consistently higher than in the recirculating, follicular population at 1, 3, and 5 d (28% as opposed to 20% at 5 d). The results demonstrate

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TABLE I

Labeling of Dividing Cells in Spleen and Lymph Node Compartment after Infusion with BUdR

Days of BUdR infusion	Percentage of labeled cells				
	Spleen			Lymph node (cervical)	
	Follicle (B cells)	Marginal zone (B cells)	PALS (T cells)	Follicle (B cells)	Paracortex (T cells)
Single injection	3.0 ± 1.1*	2.9 ± 0.4	3.5 ± 2.1	2.4 ± 0.5	2.5 ± 1.4
1	5.3 ± 1.8*	7.1 ± 0.6	5.9 ± 1.6	5.0 ± 0.1	5.8 ± 0.6
3	12.0 ± 1.2*	14.7 ± 1.4	12.3 ± 1.4	13.7 ± 1.8	11.3 ± 0.6
5 (oral)	20.0 ± 2.0‡	28.0 ± 5.4	19.5 ± 2.2	21.3 ± 1.1	17.7 ± 1.2
5 (i.p. injection)	18.5 ± 1.8‡	22.3 ± 4.8	17.5 ± 2.2	19.9 ± 4.2	18.4 ± 3.0

* Mean ± SD of three animals is shown for top three rows.
‡ Mean ± SD of four animals is shown for bottom two rows.

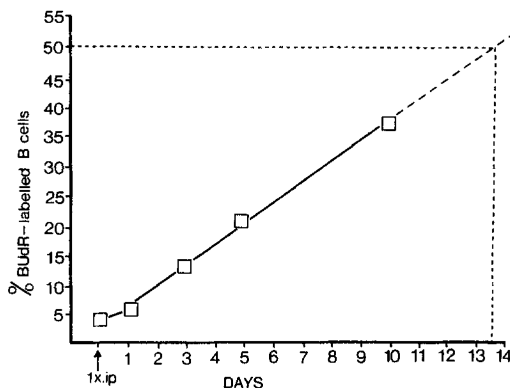

FIGURE 1. The increase in the proportion of BUdR-labeled cells in the spleen and lymph node follicles (recirculating B cells) during BUdR infusion for 1, 3, 5, and 10 d. The 10-d value is derived from sequential [^{3}H]TdR and BUdR infusion (see Table II). Extrapolation of this linear graph shows that 50% of B cells are labeled in 13.5 d.

that only 20% of the B cells in the recirculating pool are derived from a cell division within 5 d.

The results in Table I illustrate that repeated injections of BUdR may cause an underestimate of short-lived populations; however, the differences are small and are not the explanation for the large discrepancies suggested by alternative methods (10). However, oral administration of BUdR was convenient and was used in further experiments.

Estimate of B Cell Life Span. If the labeling values within the recirculating B cell pool (follicles) are plotted as in Fig. 1, one can see that the rate of increase of labeled cells over 5 d is linear. In fact, labeling with [^{3}H]thymidine and BUdR over two consecutive 5-d periods shows that after 10 d of labeling the proportion of cells carrying either or both labels is ~37% (Table II). The curve is linear over a 10-d period and so it seems valid to extrapolate to estimate that 50% of B cells within the recirculating pool will be labeled by 13.5 d.

It is not clear if the rate of labeling will remain linear between 50 and 100%; certainly, previous work has suggested that there is a small proportion (~5%) of cells that remain unlabeled even after several months of [^{3}H]thymidine infusion (21), although it is not clear if these were B cells or T cells. However, I think that it is reasonable to suppose that the vast majority of the recirculating B cell pool will have turned over within ~4 wk (extrapolation to 100% = 27 d). Thus,

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TABLE II
Incorporation of [^{3}H]TdR and BUdR after Sequential 5-d Labeling Periods

Days 1 2 3 4 5 6* 7 8 9 10 11

[^{3}H]TdR ⟵⟶ BUdR ⟵

	Percent of labeled cells‡		
	[^{3}H]TdR only	[^{3}H]TdR + BUdR	BUdR only
Lymph node follicle	12.9 ± 1.3	3.15 ± 0.4	17.55 ± 0.9
Splenic follicle	14.9 ± 1.3	3.25 ± 0.5	17.65 ± 1.6
Splenic marginal zone	9.88 ± 1.4	8.59 ± 1.9	23.68 ± 1.2

* On day 6, rats were given two injections of cold thymidine.
‡ Mean ± SD of four animals; the proportion of the total B cells carrying a single or both labels.

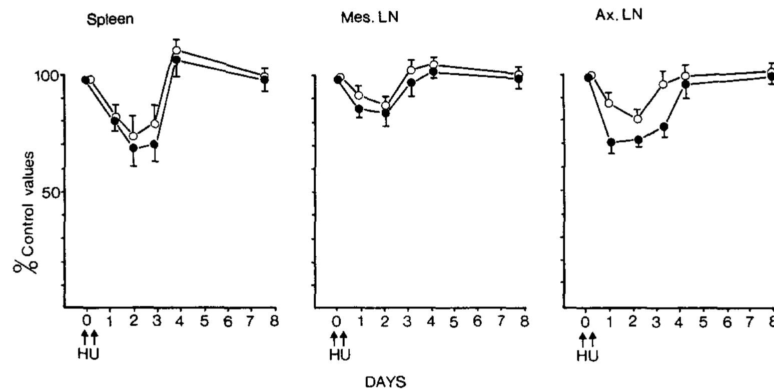

FIGURE 2. Depletion of B cell numbers in spleen, and mesenteric and axillary lymph nodes after two injections of HU. Results are calculated by expressing the values from experimental animals as a percentage of the mean control group value (rats given two injections of saline). Means and standard deviations are indicated. Each group contained four animals. (●) IgM$^+$ populations, (○) IgD$^+$ populations.

the life expectancy of a new B cell entering the recirculating pool of the rat is ~4 wk.

Depletion of Peripheral B Cells with Hydroxyurea. A protocol involving two injections of HU separated by 7–8 h is designed to kill all dividing cells within an animal (11, 12). To see if rat B cells were depleted to the same extent as those of mice (11, 12) we treated rats with HU at the dose of 1 g/kg body weight. The results are shown in Fig. 2. The depletion in the spleen was 32% for the μ^+ populations and 28% for the δ^+ B cells. In mesenteric lymph nodes μ^+ and δ^+ populations were depleted by 16% and 13%, respectively, while in the axillary lymph node these figures were 29% and 20%. The nadir of B cell depletion in the spleen was 2 d after treatment, while in the lymph nodes it appeared to be 1 d earlier.

Immunohistological examination of the lymph nodes from HU-treated rats

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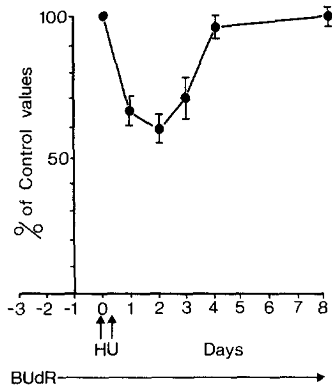

FIGURE 3. HU-induced depletion in spleen IgM+ B cell numbers in rats during infusion with BUdR. The results are calculated by expressing the values from experimental animals as a percentage of mean control group values. Means and standard deviations are indicated. Each group contained four animals. The control group too was maintained on BUdR throughout the experiment.

revealed two striking observations: (*a*) the lack of germinal centers and (*b*) large accumulations of B cells in cortico-medullary and perilymphatic areas that are particularly apparent in the axillary lymph nodes. This pattern is usually seen in activated lymph nodes.

Is BUdR Cytostatic? Although we have sought to minimize stress effects in these experiments, methods that involve the incorporation of a nucleotide analog into cellular DNA are potentially cytostatic or even cytotoxic (10). To check whether BUdR at the dose used in these experiments was cytostatic, rats were given BUdR in their water for 3 d before receiving two injections of HU. If BUdR was cytostatic then cells would not be synthesizing DNA and HU would not have its normal depletion effect on splenic B cells. The result of this experiment is depicted in Fig. 3. Depletion of splenic surface IgM+ B cells to ~60% of control values observed in rats treated with BUdR is similar to that seen in normal rats given HU (Fig. 2) and indicates that BUdR is not cytostatic. Spleen B cell numbers in rats given BUdR for 5 d were 91% of control values (± 1.5% SD, four rats) indicating minimal direct toxic effect of BUdR.

The Proportions of Newly Derived B Cells and Antigen Activated B Cells in the Recirculating Pool. The population of cells that incorporates BUdR during period of continuous labeling is made up of two sorts of cells: First, virgin B cells newly emerged from the marrow, which although they may not be dividing themselves have within the previous 24 h been derived from a proliferating precursor (1, 2); and second, mature B cells within the recirculating pool that divide in response to a stimulus of some kind, most commonly antigen, and appear to undergo several rounds of division. To assess the proportions of these populations, rats have been injected with [^{3}H]thymidine for 5 d then given one injection of an excess of cold thymidine followed 18 h later with BUdR in their drinking water and they were maintained on this for a further 5 d. In this way using immunoperoxidase staining for BUdR and autoradiography to detect [^{3}H] thymidine it is possible to estimate the proportion of B cells that divide only once or continue to divide during separate consecutive 5-d periods.

The results of this analysis, shown in Table II, indicate that 3% of the B cells within the recirculating pool (splenic and lymph node follicles) divide during separate 5-d periods in that they have incorporated both labels. This figure may be an underestimate due to dilution of [^{3}H]thymidine label caused by continued

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TABLE III
Survival of Donor B Lymph Node B Cells in Recipient Rats 8 wk after Transfer

Recipients	Percent donor B cells (number × 10^6)	
	Spleen	Lymph node
Irradiated (n = 4)	30.5 ± 2.1 (27.6 ± 6.0)	28.3 ± 3.6
Nonirradiated (n = 4)	8.0 ± 0.82 (7.4 ± 2.64)	9.0 ± 1.83

proliferation during the second 5-d period. However, the maximum figure for activated B cells must be 6% for the following reasons: the percentage of B cells carrying [^{3}H]thymidine at day 11 is 18.1% (14.9 + 3.2%, spleen follicle values, Table II) and the percentage of [^{3}H]thymidine-labeled cells after 5 d is 21.5% (± 1.1 SD, four rats; data not shown). Thus, between 5 and 11 d there has been a loss of only 3%. This loss is likely to be due to cell death as well as dilution of label. If these values are recalculated in terms of the proportion of labeled B cells rather than total B cells then one can see that between 15 and 30% of labeled cells are part of activated clones, while 70–85% are newly formed virgin B cells. The fact that 70% of the B cells labeled with [^{3}H]thymidine during the first 5 d remain within secondary lymphoid tissues for a further 6 d tells us, first, that new B cells that are incorporated into the recirculating pool have a life span in excess of 6 d and, second, that most new B cells do not continue to divide in the periphery.

The labeling pattern within the sessile B cell population of the marginal zone is in marked contrast to the recirculating B cell pool. Here there is a loss of 40% from the [^{3}H]thymidine-labeled population over the subsequent 6 d: 31.1% (8.50 + 23.68) down to 18.47% (8.59 + 9.88). Also, the proportion of double-labeled cells is higher; 8.59% of marginal zone B cells (28% of labeled cells).

Long-term Survival of Transferred Cells in Irradiated and Nonirradiated Recipients. Survival of transferred cells over long periods of time in chimeric animals demonstrates the potentially long life span of clones of primed (20) and unprimed B cells (22). The transfer of nonprimed B cells into adoptive hosts should allow an estimate of the half-life of peripheral B cell populations to be made. 4.5 × 10^8 lymph node cells (~1.5 × 10^8 Klb B cells) were transferred (intravenously) into irradiated and nonirradiated Kla congenic rats. 8 wk later spleen and lymph nodes were taken and the proportion of donor cells was enumerated. Table III shows that transferred B cells have the potential to survive in both irradiated and intact hosts for at least 2 mo. If we assume that the B cells in the spleen represent ~25% of the peripheral B cell pool then in irradiated hosts 1.1 × 10^8 of the initial 1.5 × 10^8 inoculum have survived while in intact (nonirradiated) hosts the decay is from 1.5 × 10^8 to 0.3 × 10^8. The rate of decay in nonirradiated hosts is consistent with a population half life of about 4 wk. The slower decay of transferred cells in irradiated recipients illustrates that B cell life span is not an inherent property of the cells but may be determined by available space within lymphoid microenvironments.

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Discussion

B Cell Life Span. The results of BUdR incorporation clearly show that only 4% of the recirculating B cell pool becomes labeled each day. Extrapolation of this rate leads us to conclude that the entire recirculating B cell pool turns over in ~4 wk. This estimate of the average B cell life span is not very different from the 6 wk suggested by Sprent (8, 9, 23). However, this estimate does appear to contradict the work of Freitas et al. (11, 12), which indicates that the B cell pool in mice turns over in a matter of days. These authors have stated that the methods used by Sprent (8, 9, 23) and others (24) cause stress that leads to underestimates of short-lived populations (10). For this reason we used oral administration of BUdR but found that the degree of labeling differed little if the drug was injected intraperitoneally twice daily. We have also demonstrated that this nucleotide analog has no cytostatic or direct toxic effects at the dose used. We do not have any information about the amount of BUdR absorbed via the oral route but are confident that this does not lead to errors because of the similarity to labeling indices obtained with intraperitoneal injection of either BUdR or [^{3}H]thymidine.

The discrepancy between the data presented here (as well as that of Sprent) and that of Freitas et al. (11, 12) can be explained both on technical grounds and on differences in interpretation. Data derived from transfer between LPS responder and nonresponder congenic mice cannot be easily interpreted when one considers that LPS stimulates 20–35% of splenic B cells and only 20% of lymph node B cells (25–27). Treatment of rats with HU results in a depletion of peripheral B cells that is in general similar to that seen in mice (11, 12). The lag of 2 d before full depletion is reached in the spleen suggests that HU may be killing a precursor population; for instance, bone marrow B cell precursors. In lymph nodes the depletion is more rapid (within 1 d), and histological examination indicates that this is due to the loss of rapidly dividing germinal center B cells. Indeed these structures may contain 10–20% of lymph node B cells (28). Neither virgin B cells entering the spleen nor germinal center B cells are part of the recirculating B cell pool. Thus, while the recirculating B cell pool of the rat (consisting of $\sim 10^9$ cells) contains relatively long-lived cells there are at least two other pools of short-lived cells: (*a*) 5×10^8 virgin B cells produced per day from the marrow (3) appear to survive only a few days; and (*b*) cells proliferating in response to antigen in germinal centers. These short-lived B cells over a period of 1 wk certainly outnumber the cells in the recirculating pool; however, they are compartmentalized (29) and require selection for entry into this longer-lived pool.

Incorporation of New B Cells into the Recirculating Pool. To differentiate between labeling due to incorporation of new B cells into the recirculating pool from that due to antigen-driven proliferation we assume that B cells newly produced from a dividing precursor population within primary lymphoid organs are quiescent as they emerge into the periphery (1, 2), while mature lymphocytes that respond to an external stimulus undergo a clonal expansion that involves several rounds of cell division. The proportion of labeled cells in these two categories and hence an estimate of the rate of incorporation of new B cells into the recirculating pool was obtained by infusion with [^{3}H]TdR and then BUdR

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over sequential 5-d periods. It seems reasonable to assume in the double-label experiment that the vast majority of [^{3}H]TdR single-labeled cells surviving to the end of the 11-d period represent virgin B cells that were incorporated into the recirculating pool during the first 5 d of [^{3}H]TdR infusion.

The proportion of cells within the recirculating pool that are derived from antigen-activated clones has an upper limit of 6% over 11 d or 0.3–0.6% per day. The number of new B cells that are incorporated into the recirculating, peripheral pool is 17% over 5 d or 3.4% per day. 70% of these new B cells remain in the recirculating pool without dividing for at least a further 6 d. The remaining 30% of new B cells either die or become part of activated clones. The total size of the peripheral B cell pool of the rat is $\sim 10^9$ cells (3), and so this means that $\sim 3.4 \times 10^7$ new B cells enter the recirculating pool per day. Deenen et al. (3) have recently calculated that the bone marrow of a rat produces $\sim 5 \times 10^8$ new B cells per day. While these figures obviously carry some degree of error it is probably true to say that only 5–10% of new B cells become stably incorporated into the recirculating pool. The rest ($>4.5 \times 10^8$/day) have a very short lifespan of 1–2 d, most of which is probably spent in the spleen. There is a delay of ~2 d between entry of transferred bone marrow B cells into the spleen and their appearance in lymph nodes (5, 30).

There are two possible sources of error in this analysis: (*a*) Virgin B cell precursor divisions that overlap the two labeling periods. If these were substantial many more double-labeled cells would be apparent; (*b*) antigen-activated B cells that proliferate only during one labeling period. The low proportion of double-labeled cells, however, suggests that the number of cells undergoing several rounds of division is small. We may underestimate the number of B cells derived from germinal centers that carry only one label because their rapid proliferation dilutes [^{3}H]thymidine to an undetectable level. The maximum for this underestimate can only be ~3% of the peripheral pool (loss of [^{3}H]thymidine-labeled cells between days 5 and 11 is 21–18%).

In contrast to the recirculating pool the marginal zone B cell population contains larger numbers of double-labeled cells and the decay of [^{3}H]TdR single-labeled cells is much more rapid (40 vs. 15% loss after 6 d). This indicates that at least a proportion of marginal zone B cells are part of antigen-driven clones as has been proposed previously (31).

Selection of New B Cells. As <10% of new B cells become part of the mature recirculating pool some form of selection process must operate. Upon what criteria are new B cells selected for entry? We have previously shown (20) in K allotype–marked, bone marrow–recirculating cell chimeras, that thymus-dependent antigen can activate virgin B cells in the period immediately after immunization. Thus, the production of large numbers of B cells per day facilitates the screening of the potential repertoire in a very short time. New B cells with appropriate specificities are selected for entry into the recirculating pool. Whether antigen specificity is the only criterion upon which new B cells are selected is not clear. The number of antigenic epitopes required seems too large. Measurements of the frequency of B cells specific for a particular antigen vary from 1 in 10^3 (32) to 1 in 10^6 (33); however, if we take a value of 1 in 10^5 (34), then 10^5 antigenic epitopes are required to select $3–4 \times 10^7$ new B cells. If the

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interactions involved are low affinity, then numerically such a selection process may become feasible. The role of autoantigen in selecting "housekeeping" specificities may also be important.

The size of the peripheral B cell pool appears to be very tightly controlled; despite an input of very large numbers of new B cells from the marrow, only ≤10% become incorporated into the pool. It is not clear how B cells are counted but it does seem that their life span is not something that is programmed within the cells themselves. The decay of donor lymph node B cells is much more rapid in nonirradiated recipients than in irradiated hosts. Similar phenomena have been observed in other models (35, 36). Space within secondary lymphoid microenvironments appears to be an important factor.

It is clear from transferring B cell populations that some B cells may live much longer than 4 wk. However, we must be careful not to confuse B cell life span and clonal half-life, which may be very long (22). Analysis of established responses in long-term bone marrow–recirculating cell chimeras (20) and also the survival of primed B cells in adoptive hosts in the absence of antigen (Gray, D., and H. Skarvall, manuscript in preparation) suggest that long-term memory may be a consequence of continual restimulation of cells within the memory clone rather than long-lived, resting memory cells.

Summary

Currently available estimates of B cell life span vary from 4 d to 6 wk. The discrepancy may have arisen out of the selective effects of stress and drug cytotoxicity on short-lived populations. In this report, bromodeoxyuridine (BUdR), a drug that incorporates into the DNA of dividing cells, has been fed to rats in their drinking water, eliminating stressful injection procedures. Labeled cells in the recirculating B cell pool are identified in tissue sections using an mAb to BUdR. BUdR is shown to have no cytostatic effects at the dose used. Over a 5-d period of infusion, only 20% of the peripheral recirculating pool incorporate label (~4% per day); labeling over various periods indicates that the peripheral B cell pool turns over in ~4 wk. To distinguish between turnover due to incorporation of new B cells into the peripheral pool and division of antigen-activated B cells rats underwent two consecutive periods of labeling, first with [^{3}H]thymidine for 5 d and then with BUdR for a further 5 d. Virgin B cells newly derived from dividing precursors in the bone marrow do not continue to proliferate in the periphery, while activated cells undergo several rounds of division during both labeling periods. The results indicate that 3–4% of the peripheral pool is replaced by new B cells each day, while 0.3–0.6% become part of activated clones every day. Assuming that the peripheral pool of the rat contains 10^9 B cells, then $3–4 \times 10^7$ new B cells become stably incorporated per day. This represents ~10% of the putative output of the bone marrow.

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