

# Mature Murine B and T Cells Transferred to SCID Mice Can Survive Indefinitely and Many Maintain a Virgin Phenotype

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## Summary

To seek information on the potential lifespan of normal B and T lymphocytes, lymph node (LN) cells from unprimed mice were transferred to H-2-identical severe combined immunodeficiency (SCID) hosts. At a population level, the donor B and T cells survived for at least 10 mo post-transfer with no reduction in their numbers. In terms of antibody production, LN-injected SCID mice remained responsive to several different antigens and contained unprimed precursors of memory cells for  $\geq 6$  mo post-transfer. Most of the B and T cells recovered from LN-injected SCID mice expressed the typical virgin phenotype of mature lymphocytes from young mice. These findings suggest that many of the transferred lymphocytes might have remained in interphase as virgin cells from the time of injection. This did not apply to all of the transferred cells, however, because 20–40% of CD4<sup>+</sup> cells from long-term SCID hosts displayed a memory phenotype, 7% incorporated 2-bromodeoxyuridine over 5 d, and total numbers of B and T cells increased gradually (twofold) over a 10-mo period. Collectively, the data favor the view that the pool of mature B and T cells in adult mice is largely self sufficient: some of the cells proliferate, presumably in response to environmental antigens, but many mature cells can remain quiescent for prolonged periods. Input of new cells from the primary lymphoid organs continues, but at a much reduced rate relative to young life.

Most mature B and T cells in the secondary lymphoid organs of mammals reside in the recirculating lymphocyte pool (RLP)<sup>1</sup> (1–3). Recirculating lymphocytes originate in the primary lymphoid organs: T cells arise in the thymus whereas B cells are formed in the fetal liver, bone marrow, and Peyer's patches. The release of newly formed lymphocytes from the primary lymphoid organs is substantial in young animals and leads to a rapid entry of lymphocytes into the RLP. The contribution of the primary lymphoid organs to the RLP of adults, however, is less clear.

For T cells, four lines of evidence suggest that the RLP of adults is relatively independent of the thymus. First, labeling experiments with rodents given repeated injections of tritiated thymidine have shown that many of the mature T cells of adults can remain in interphase for a period of several months, implying that the input of new T cells into the pool is not extensive (4–6). Second, thymectomy of adult mice causes

only a very slow decline in the size of the RLP (7). Third, by 6 mo of age, the production of new T cells by the thymus is very limited, i.e.,  $\approx 10^5$  cells/d compared with  $2 \times 10^6$  cells/d at 3 wk of age (8). Fourth, mature T cells fail to disappear after transfer to athymic (nude) hosts; in fact, when transferred in small numbers, the T cells undergo considerable expansion (9, 10). Collectively, these data indicate that, once formed in young life, the pool of mature T cells in adults is relatively autonomous and requires little input of new T cells from the thymus.

The situation with B cells is more complex. Based on the rate at which small B cells divide after repeated administration of tritiated thymidine (4–6) or 2-bromodeoxyuridine (BRDU) (11, 12), some groups have concluded that many mature B cells can survive in interphase for a period of several weeks. There is also evidence that B cells responsive to TNP coupled to Ficoll can survive for prolonged periods after transfer to TNP-Ficoll-unresponsive hosts (mice with X-linked immunodeficiency) (13). However, other workers assert that B cells have a very short lifespan. Based on (a) the extent of lymphocyte depletion seen in mice given hydroxyurea (which kills dividing cells); and (b) examining the rate at which LPS-reactive B cells disappear after adoptive transfer, these

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<sup>1</sup> Abbreviations used in this paper: BRDU, 2-bromodeoxyuridine; CFU, colony-forming unit; CGG, chicken gamma globulin; HRBC, horse red blood cells; HU, hydroxyurea; LN, lymph node; PFC, plaque-forming cells; RLP, recirculating lymphocyte pool.

workers maintain that most mature B cells have a lifespan of <48 h (14–17). This notion is widely held (18–20) and has led to the view that, unlike T cells, the pool of mature B cells in adults needs constant replenishment from the primary lymphoid organs.

To seek direct evidence on the lifespan of B and T cells, we have investigated the fate of mature lymph node (LN) lymphocytes transferred to mice with severe combined immunodeficiency (SCID); these mice have an autosomal recessive mutation that prevents the formation of functional B and T cells (21, 22). The notion that mature B cells are short-lived cells predicts that the B cells in the LN suspensions would survive only a few days after adoptive transfer. Conversely, based on studies with athymic hosts (9, 10), the transferred T cells would be expected to undergo extensive proliferation. In practice, the transferred B and T cells survived in SCID hosts for at least 10 mo in relatively constant numbers. The cells remained fully immunocompetent and most retained the “virgin” phenotype of typical recirculating lymphocytes. The data favor the view that, once formed, the pool of mature lymphocytes (including both B and T cells) remains relatively independent of the primary lymphoid organs.

## Materials and Methods

**Mice.** Male BALB/c and C.B-17 SCID mice were obtained from the breeding colony of the Research Institute of Scripps Clinic. The origin of the SCID mice has been considered elsewhere (23).

**Monoclonal Antibodies.** The following mAbs were used: anti-Thy-1.2 (Jlj) (24), anti-CD4 (GK1.5) (25), anti-CD8 (3.168.8) (26), anti-B220 (14.8) (27), anti-heat-stable-antigen (Jlld) (24), anti- $\mu^s$ ,  $\mu^b$ ,  $\delta^s$  and  $\delta^b$  (Pharmingen, San Diego, CA), anti-CD45RB (23G2) (TexStar Monoclonals, Dallas, TX), SM3G11 (3G11) (28), and MEL-14 (29).

**Preparation of LN Cells.** Sterile suspensions of pooled cervical, axillary, mesenteric, and inguinal LN collected free of blood were prepared with a tissue homogenizer. For injection, LN cells were injected intravenously via the tail vein.

**Mitogen Responses.** Using RPMI supplemented with 10% FCS, glutamine, and 2-ME (30), doses of  $2 \times 10^5$  spleen or LN cells were cultured with Con A (2–5  $\mu\text{g}/\text{ml}$ ) or LPS (50  $\mu\text{g}/\text{ml}$ ) in 200- $\mu\text{l}$  volumes under standard tissue culture conditions. [ $^3\text{H}$ ]TdR incorporation was measured on day 2 or 3 at 8 h after pulsing cultures with 1  $\mu\text{Ci}$  [ $^3\text{H}$ ]TdR.

**Specific Antibody Responses.** As described elsewhere (31), aliquots of spleen cell suspensions were assayed for plaque-forming cells (PFC) to SRBC and horse red blood cells (HRBC) using the Cunningham modification of the Jerne method. A polyvalent rabbit anti-mouse Ig antiserum was used to detect enhanced PFC. PFC to chicken gamma globulin (CGG) (Cappel Laboratories, Malvern, PA) were detected using SRBC coated with chicken anti-SRC antibody (32). PFC to TNP were detected using TNP-coupled SRBC (13).

**Immunization for Specific Antibody Responses.** Primary responses to SRBC and HRBC were induced by injecting doses of 0.1 ml of 25% suspensions of red blood cells intravenously. Primary responses to TNP-Ficoll were induced with 20  $\mu\text{g}$  TNP-Ficoll injected intravenously. To measure anti-CGG responses, mice were primed intraperitoneally i.p. with 500  $\mu\text{g}$  alum-precipitated CGG and then boosted with 100  $\mu\text{g}$  soluble CGG intraperitoneally 6

wk later. For all antigens, spleen PFC responses were measured on day 7.

**Thoracic Duct Cannulation.** Thoracic duct cannulation was performed as described elsewhere (33, 34).

**Flow Cytometry.** To detect expression of CD4, CD8, and B220, cells were incubated with unconjugated mAb (rat origin), washed, and then incubated with FITC-labeled mouse anti-rat IgG (Pel-Freez Biologicals, Rogers, AR) followed by further washing.  $\mu^+$  cells were detected by incubating cell suspensions with biotinylated rabbit anti-mouse  $\mu$  antibody (affinity-purified rabbit anti-MOPC 104E antiserum) followed, after washing, with PE-conjugated streptavidin (Biomed, Foster City, CA). For  $\mu$  vs.  $\delta^s$  staining, cells were incubated with biotinylated rabbit anti- $\mu$  antibody followed (after further washing) by FITC-conjugated anti- $\delta^s$  mAb and then PE-conjugated streptavidin; under the conditions used the anti-IgD $^s$  antibody failed to stain B cells expressing the SCID (IgD $^b$ ) allotype (tested on C.B 20 spleen). For detecting expression of CD4 vs. CD45RB, MEL-14, or 3G11, cells were incubated first with unconjugated anti-CD45RB, MEL-14, or 3G11 mAb and then, after washing, with FITC mouse anti-rat IgG or FITC rat anti-mouse IgG (H+L chain specific) (Jackson ImmunoResearch, West Grove, PA); after incubation with normal rat serum, the cells were then incubated with PE-conjugated anti-CD4 mAb (Becton Dickinson & Co., Mountain View, CA). In all staining procedures, the final incubation step was performed in the presence of propidium iodide (2  $\mu\text{g}/\text{ml}$ ). To detect stained cells,  $1\text{--}2 \times 10^4$  fresh viable stained cells were analyzed on FACS IV $^{\text{®}}$  or FACScan $^{\text{®}}$  flow cytometers (Becton Dickinson & Co.).

**Lymphocyte Turnover.** Mice were given sterile drinking water containing BRDU (0.8 mg/ml) (Sigma Chemical Co., St. Louis, MO) (12); to prevent decay of BRDU, the drinking water was changed daily using freshly prepared BRDU. Using a modification of the approach of MacKay et al. (35), cells from BRDU-treated mice were incubated with biotinylated anti-CD4, anti-CD8, or anti- $\mu$  antibody followed by Texas red-conjugated streptavidin. After washing, the cells were fixed in 70% EtOH and the DNA was denatured using 3N HCl containing 0.5% Tween 20. The cells were then washed and resuspended in 0.1 M di-sodium tetraborate, pH 8.5, for 2–3 min, washed again, and stained with FITC-conjugated anti-BRDU antibody (Becton Dickinson & Co.). Stained cells were analyzed with a FACStar $^{\text{®}}$  Plus flow cytometer (Becton Dickinson & Co.).

## Results

The experiments discussed below involved the transfer of normal adult BALB/c LN B and T cells to young (2 mo old) unirradiated C.B-17 SCID mice; BALB/c and C.B-17 are identical except for an Ig allotype difference. LN cells were used as donor cells because, in contrast to spleen, bone marrow, and blood, LN cells (collected free of blood) are virtually devoid of stem cells.

**Survival of B and T Cells in SCID Mice Injected with  $5 \times 10^7$  BALB/c LN Cells.** Although a small proportion of SCID mice show leakiness in terms of oligoclonal production of IgG-producing plasma cells, typical B and T cells are quite rare even in Ig $^+$  SCID mice (22; and see below). In our colony of pathogen-free SCID mice, the cellularity of the spleen is unusually low ( $\sim 3 \times 10^6$  viable cells) (Table 1) and LN are almost acellular ( $< 0.5 \times 10^6$  cells for pooled LN). Most of the cells in SCID spleen are positive for Jlld

and/or Thy-1 (not shown), but cells expressing markers of mature B ( $\mu$ , B220) or T (CD4, CD8) cells are very rare (Table 1). To examine the survival of mature B and T cells in SCID hosts, doses of  $5 \times 10^7$  normal BALB/c LN cells were transferred to SCID mice intravenously under sterile conditions. The injected LN cells comprised a mixture of mature B cells (18%  $\mu^+$ , 20% B220<sup>+</sup>) and T cells (55% CD4<sup>+</sup>, 19% CD8<sup>+</sup>).

As shown in Table 1, pooled spleen and LN from SCID mice given  $5 \times 10^7$  BALB/c LN cells contained a mean of  $2.4 \times 10^7$  viable cells at 2 mo post-transfer and  $3.1 \times 10^7$  at 6 mo. These numbers were substantially higher than for uninjected SCID mice ( $\sim 3 \times 10^6$  cells) but were approximately eightfold lower than for normal BALB/c mice ( $\sim 2 \times 10^8$  cells in pooled spleen and LN) (not shown). B ( $\mu^+$ , B220<sup>+</sup>) cells were found predominantly in the spleen (20–25%) but were also found in small numbers in LN (6–8%); these findings are in accordance with the evidence that mature B cells localize preferentially in spleen rather than LN after intravenous injection (36). The proportion of T cells was higher in LN ( $\sim 85\%$ ) than spleen ( $\sim 40\%$ ), and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio ( $\sim 3:1$ ) was unchanged relative to the LN cells originally injected. In terms of total yields, the recovery of viable T and B cells relative to the numbers initially injected ranged from 30–55%. These percentages probably represent a twofold underestimate of the donor cells present in all of the lymphoid organs. This projection stems from the finding that, even at 4–24 h post-injection, only 40–60% of intravenously injected <sup>51</sup>Cr-labeled B and T cells can be recovered from spleen plus (easily accessible) LN (36). It should also be mentioned that preparation of cell suspensions from solid lymphoid organs inevitably leads to appreciable

loss of cells (20–40%) as the result of trauma. For these reasons, the total numbers of donor cells surviving in the SCID hosts were probably slightly in excess of the numbers originally injected.

A priori, one could argue that the mature lymphocytes recovered from long-term LN-injected SCID mice arose from small numbers of stem cells contaminating the injected LN cells. Alternatively, the recovered cells might be of SCID origin. The experiments described below address these possibilities.

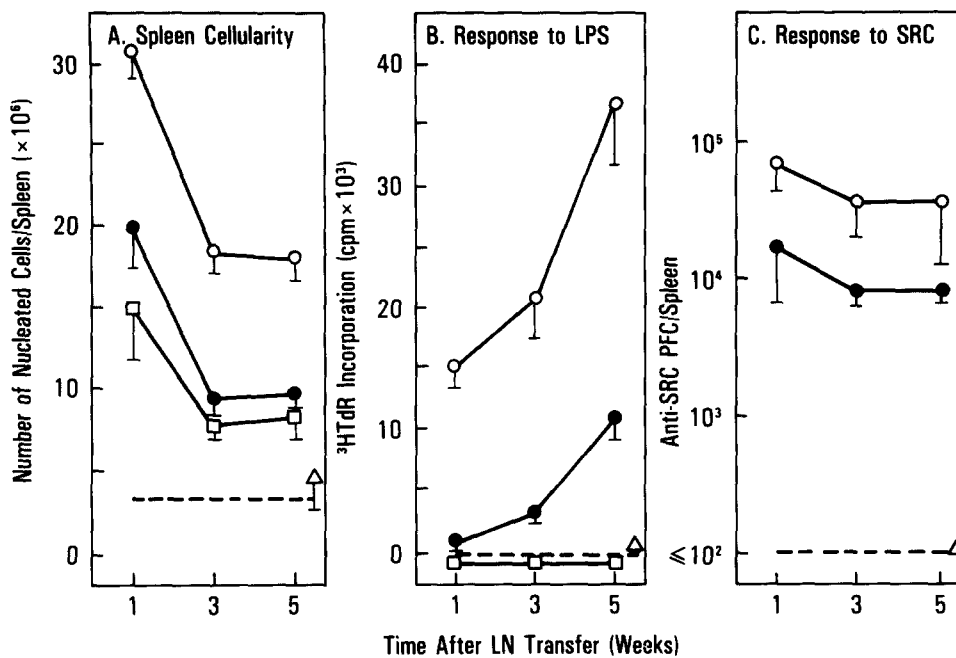
*Origin and Function of B and T Cells in LN-injected SCID Mice.* With regard to the possibility of stem cell contamination, colony-forming units (CFUs) were almost undetectable in the injected LN cells. In three experiments with a total of 24 mice, CFUs measured at 7–10 d after transfer of  $5 \times 10^7$  BALB/c LN cells to irradiated (1,000 rad) BALB/c or B10.D2 mice were found in only four mice (two to three colonies/spleen), and then only at early stages (day 7–8) post-transfer (data not shown); irradiated recipients of  $5 \times 10^7$  LN cells all died within 3 wk with signs of hematopoietic failure (e.g., small spleens), implying that the LN suspensions lacked pluripotential stem cells.

To probe the issue of stem cell contamination of LN in more depth, we studied the kinetics of lymphoid reconstitution in SCID mice given LN cells vs. bone marrow (BM) cells, i.e., a rich source of stem cells. When (unirradiated) SCID mice were injected with a dose of  $10^5$  T-depleted BALB/c BM cells (containing  $\sim 30$  CFUs), lymphoid reconstitution was minimal even at 5.5 wk post-transfer. At this stage, cell counts in the spleen remained low (Fig. 1 A) and responses of spleen cells to Con A (not shown) or LPS (Fig. 1 B) were almost undetectable. The thymus was small (not

**Table 1.** Recovery of T and B cells from SCID Mice Given  $5 \times 10^7$  Normal LN Cells 2–6 mo Earlier

Marker	Uninjected SCID		$5 \times 10^7$ BALB/c LN → SCID assayed at 2 mo				$5 \times 10^7$ cells → SCID assayed at 6 mo			
	Cells in spleen	Total cells in spleen	Cells in spleen	Cells in LN	Total cells in spleen + LN	Total recovered cells vs. number injected	Cells in spleen	Cells in LN	Total cells in spleen + LN	Total recovered cells vs. number injected
	%	$\times 10^{-6}$	%	%	$\times 10^{-6}$	%	%	%	$\times 10^{-6}$	%
–	–	3.3	–	–	24.3	–	–	–	31.3	–
$\mu$	0.3	<0.1	19.9	6.3	3.9	29.8	25.0	7.9	5.3	55.8
B220	2.4	<0.1	27.3	8.0	5.4	40.4	24.8	8.1	5.4	56.8
CD4	2.0	<0.1	32.9	64.7	10.5	36.8	26.7	63.2	8.7	28.9
CD8	1.8	<0.1	10.6	20.1	3.2	33.6	12.1	22.5	3.6	33.7

Cell suspensions from pooled cervical, axillary, inguinal, and mesenteric LN were prepared under sterile conditions and injected intravenously into unirradiated SCID mice aged 6–8 wk. The lymphocyte composition of the injected cells was 20% B220<sup>+</sup> (18%  $\mu^+$ ), 55% CD4<sup>+</sup>, and 19% CD8<sup>+</sup>. The recipients (four mice/group) were killed 2 or 6 mo later to prepare suspensions of spleen cells and pooled LN cells. Cells suspensions from each individual mouse were counted and then phenotyped by standard techniques using flow cytometry (see Materials and Methods).  $\mu^+$  cells were detected with biotinylated rabbit anti-mouse  $\mu$  antibody (affinity-purified rabbit anti-MOPC 104E antiserum) followed, after washing, with PE-conjugated streptavidin. Expression of other markers was detected with rat anti-mouse mAbs using FITC-labeled mouse anti-rat Ig mAb as a second reagent (see Materials and Methods).



**Figure 1.** Early reconstitution of B and T cell function in LN-injected SCID mice but not in BM-injected SCID mice. C.B-17 SCID mice were injected intravenously with doses of  $3 \times 10^7$  (O),  $10^7$  (●), or  $3.3 \times 10^6$  (□) normal BALB/c LN cells or with  $10^5$  T-depleted (anti-Thy-1 mAb + C-treated) BALB/c BM cells (Δ); uninjected SCID mice (---) were used as a control. Groups of three mice/group were killed at the times indicated and assayed individually. (A) mean (SD) of total numbers of viable nucleated cells/spleen; (B) mean (SD) of [<sup>3</sup>H]TdR incorporation in cultures containing  $2 \times 10^5$  spleen cells plus LPS measured on day 2, 8 h after addition of 1 μCi [<sup>3</sup>H]TdR (Table 2); (C) geometric mean (SD) of total numbers of enhanced anti-SRC PFC/spleen (Table 2). The recipients of BM cells were assayed at only one time point, 5.5 wk post-transfer.

shown) and there was no PFC response to the T-dependent antigen, SRC (Fig. 1 C). By 7–8 wk post-transfer, however, BM-injected SCID mice showed clear evidence of B and T cell reconstitution. Responses to B and T mitogens and SRC were easily detectable at this stage and the thymus showed increased cellularity (Table 2). Lymphoid reconstitution at

7–8 wk post-transfer was substantial with injection of  $3 \times 10^5$  BM cells, moderate with  $10^5$  cells, and undetectable with  $3 \times 10^4$  cells (Table 2).

The above findings indicate that functional B and T cells do develop in BM-injected SCID mice, but not before 5 wk post-transfer. With injection of LN cells, by contrast, B and

**Table 2.** Mitogen and Anti-SRC Responses by SCID Mice Given Graded Doses of LN Cells or BM Cells 2 mo Earlier

Mice tested (no. of mice/group)	Response to:			No. of viable thymocytes /mouse
	Con A*	LPS*	SRBC†	
C.B-17 SCID (13)	-1.3	-0.2	<0.1	1.4
$3 \times 10^7$ BALB/c LN → C.B-17 SCID (3)	240.2	37.3	47.0	2.2
$10^7$ BALB/c LN → C.B-17 SCID (3)	211.5	11.1	16.4	1.1
$3 \times 10^6$ BALB/c LN → C.B-17 SCID (3)	121.1	-1.2	6.4	1.5
$3 \times 10^7$ Ig <sup>-</sup> BALB/c LN → C.B-17 SCID (3)	209.3	-2.9	0.4	NE <sup>§</sup>
$3 \times 10^5$ BALB/c BM → C.B-17 SCID (4)	173.6	16.4	62.1	40.0
$10^5$ BALB/c BM → C.B-17 SCID (4)	123.6	8.0	30.8	8.9
$3 \times 10^4$ BALB/c BM → C.B-17 SCID (4)	0.9	1.1	<0.1	0.6

Young adult C.B-17 SCID mice (Table 1) were injected intravenously with normal LN cells, B cell-depleted (Ig<sup>-</sup>) LN cells, or BM cells. LN cells were depleted of B cells by two sequences of negative panning on anti-Ig-coated plastic dishes; the eluted cells consisted of 69% CD4<sup>+</sup>, 27% CD8<sup>+</sup>, 0.7% B220<sup>+</sup>, and <0.3 μ<sup>+</sup> cells. BM cells were flushed from the marrow cavity by standard techniques and treated with anti-Thy-1 mAb plus complement before injection to remove mature T cells. Mice were killed at 7–8 wk post-injection, 1 wk after intravenous injection of 0.1 ml of a 25% solution of SRC. Aliquots of spleen cell suspensions were assayed for PFC to SRBC using a polyvalent rabbit anti-mouse Ig antiserum to detect enhanced PFC. Responses to mitogens were measured by culturing doses of  $2 \times 10^5$  spleen cells with Con A (2–5 μg/ml) or LPS (50 μg/ml) in 200-μl volumes under standard tissue culture conditions.

\* [<sup>3</sup>H]TdR incorporation (cpm × 10<sup>3</sup>) of triplicate cultures measured at 8 h after pulsing cultures with 1 μCi [<sup>3</sup>H]TdR; cultures were harvested on day 2. Background responses (cpm with cells cultured without mitogens) have been subtracted from the data.

† Geometric mean of total numbers of PFC/spleen/mouse (× 10<sup>3</sup>).

§ Not examined.

T cell function was evident from the earliest time point examined (1 wk) and then persisted indefinitely (see below). Spleen counts (total nucleated cells) and anti-SRC responses decreased approximately twofold after 1 wk and then reached a plateau at 3–5 wk (Fig. 1). Interestingly, reciprocal kinetics were observed for LPS responsiveness: responses to LPS were low at 1 wk and then rose progressively to reach plateau levels at 5–8 wk (Fig. 1, Table 2) (see Discussion). By all parameters tested, the lymphoid reconstitution seen in LN-injected SCID mice was directly proportional to the number of LN cells injected. This was particularly clear for responses to SRC (Table 2).

The marked difference in the kinetics of lymphoid reconstitution in SCID mice given LN vs. BM cells makes it most unlikely that the mature B and T cells found in long-term LN → SCID mice arose from contaminating donor stem cells. In the case of T cells, it should be noted that injection of even large doses of LN cells failed to cause enlargement of the host thymus (Table 2), implying that the thymus remained nonfunctional. As a more stringent test for the origin of B cells in the LN-injected mice, SCID mice were injected with LN cells negatively panned on anti-Ig-coated plates; this procedure would be expected to selectively remove mature B cells but spare stem cells. As shown in Table 2, SCID mice injected 8 wk before with B-depleted LN cells showed strong T cell function (high Con A responses) but were almost totally devoid of B cell function (very low or undetectable responses to LPS and SRC). To check whether the B cells in LN-injected SCID mice were of donor or host origin, B cells from SCID mice given normal unseparated LN cells were stained for donor (BALB/c, allotype a) vs. host (C.B-17, allotype b)  $\delta$  and  $\mu$  allotype. Cells expressing host allotype were very rare and the vast majority of the recovered B cells expressed donor allotypic markers (see below).

**Long-term Function and the Generation of Memory Cells.** The data discussed above indicate that LN-injected SCID mice give strong primary responses to SRC for at least 8 wk post-transfer. In other studies, high anti-SRC responses were seen in LN-injected SCID mice even at 6 mo post-transfer (the latest time point examined) (not shown). Responses to three other antigens are shown in Table 3. The main aim in these experiments was to determine whether the B cells surviving in LN-injected mice could serve as unprimed precursors of memory cells. CGG was selected for these studies; in contrast to SRC, primary responses to CGG are very low and heavily dependent on adjuvant (32), implying that the responding cells are virgin cells (see Discussion). SCID mice injected with normal unprimed LN cells were left for various periods and then primed with CGG in adjuvant. 6 wk later, the mice were boosted with soluble CGG and also immunized with HRBC and TNP-Ficoll (a type II T-independent antigen). Secondary PFC responses to CGG and primary responses to HRBC and TNP-Ficoll were measured 1 wk later. Each mouse tested responded well to all three antigens (relative to normal donor strain mice), even as late as 7.5 mo post-transfer. Whereas responses to HRC and TNP-Ficoll consisted of a mixture of IgG (enhanced) and IgM (nonenhanced) PFC, the response to CGG was composed solely (>99%)

of IgG PFC. Since primary responses to soluble CGG are almost undetectable (32), the anti-CGG response of LN-injected SCID mice can be regarded as a true secondary response.

Collectively, these data indicate that LN-injected SCID mice remained responsive to four different antigens for at least 7 mo post-transfer. In the case of CGG responses, the data suggest that unprimed (virgin) precursors of memory cells survived in SCID hosts for at least 6 mo (see Discussion).

**Expression of Virgin vs. Memory Markers.** As discussed earlier, yields of B and T cells from pooled spleen and LN of SCID mice injected 4–6 mo before with a dose of  $5 \times 10^7$  BALB/c LN cells were quite similar to the numbers of cells originally injected (Table 1). The simplest explanation for this finding is that the bulk of the transferred cells remained in interphase from the time of injection. If so, most of the surviving lymphocytes would be expected to display a virgin phenotype, e.g.,  $\mu + \delta$  expression for B cells and CD45RB expression for T cells.

In the experiment shown in Fig. 2, spleen and LN cells from SCID mice injected with  $3 \times 10^7$  LN cells 3.5 mo before were stained for  $\mu$  vs.  $\delta$  expression using non-allele-specific anti- $\mu$  antibody and donor ( $\delta^a$ )-specific anti- $\delta$  antibody. It can be seen that, as in normal BALB/c mice, most of the B cells from LN-injected SCID mice showed double staining for  $\mu$  and  $\delta^a$ , both in spleen and LN. The ratio of  $\mu^+ \delta^+ / \mu^+ \delta^-$  cells in the LN-injected SCID mice was almost as high as in the control donor strain mice. In addition to  $\delta$  expression, nearly all of the B cells in LN-injected SCID mice expressed the Jlld antigen (not shown), a marker for unprimed but not memory B cells (24). CD45RB expression on CD4<sup>+</sup> cells from LN-injected SCID mice vs. normal donor strain mice is shown in Fig. 2. It is evident that most of the CD4<sup>+</sup> cells in both groups of mice were CD45RB<sup>+</sup>.

**Turnover.** To examine the turnover of B and T cells in LN-injected SCID mice, BRDU (a DNA precursor) was added to the drinking water. Cell suspensions from the mice were double stained for BRDU incorporation vs.  $\mu$ , CD4, or CD8 expression. In preliminary experiments, BM cells taken from normal 3-mo-old mice after 5 d on BRDU water showed 85–90% BRDU incorporation. This finding established that the concentration of BRDU in the drinking water was adequate for detecting dividing cells. BRDU incorporation by spleen and LN cells from normal adult (5-mo-old) BALB/c mice is shown in Fig. 3. The data show the mean percent BRDU labeling of B ( $\mu^+$ ) cells and T (CD4<sup>+</sup>, CD8<sup>+</sup>) cells pooled from two to three mice/group. For LN, BRDU labeling of B and T cells was quite low and reached only 10–12% by day 15. BRDU labeling was more rapid in spleen cells and rose from 8–18% on day 5 to 16–24% on day 15; early labeling was more prominent in CD4<sup>+</sup> cells (18% on day 5) than in  $\mu^+$  cells or CD8<sup>+</sup> cells (11% and 9%, respectively, on day 5). BRDU labeling of LN-injected SCID mice was tested on a batch of four SCID mice given a dose of  $5 \times 10^7$  BALB/c LN cells 5 mo before. These mice were placed on BRDU water for 5 d. As shown in Fig. 3, BRDU-labeling of B and T cells recovered from the spleen of LN-injected SCID mice was quite limited, i.e., 7% for CD4<sup>+</sup>

**Table 3.** Generation of Memory Cells to CGG in Long-term LN → SCID Mice

Exp.	Mice tested (no. of mice/group)	Time after transfer to SCID hosts	PFC/spleen		
			Secondary CGG*	Primary HRC*	Primary TNP*
		mo		$\times 10^{-3}$	
1	Normal C3H (3)	–	34.4 (1.09)	25.8 (1.12)	9.3 (1.17)
	C3H SCID (3)	–	<0.1	<0.1	<0.1
	$2.6 \times 10^7$ C3H LN → C3H SCID (3)	2.3	12.0 (1.56)	17.2 (1.14)	4.7 (1.29)
	Normal BALB/c (3)	–	12.1 (1.06)	21.5 (1.08)	8.8 (1.02)
	C.B-17 SCID (3)	–	<0.1	<0.1	<0.1
2	Normal BALB/c (3)	–	8.9 (1.15)	74.2 (1.03)	7.2 (1.23)
	$5 \times 10^7$ BALB/c LN → C.B-17 SCID (6)	2.5	17.7 (1.18)	21.5 (1.35)	4.5 (1.12)
3	Normal BALB/c (3)	–	10.3 (1.13)	74.9 (1.07)	9.9 (1.25)
	$5 \times 10^7$ BALB/c LN → C.B-17 SCID (3)	3.0	33.8 (1.48)	33.4 (1.50)	5.8 (1.51)
4	Normal BALB/c (3)	–	21.4 (1.12)	10.8 (1.04)	4.6 (1.07)
	$5 \times 10^7$ BALB/c LN → C.B-17 SCID (3)	7.5	10.8 (1.05)	4.4 (1.16)	2.5 (1.05)

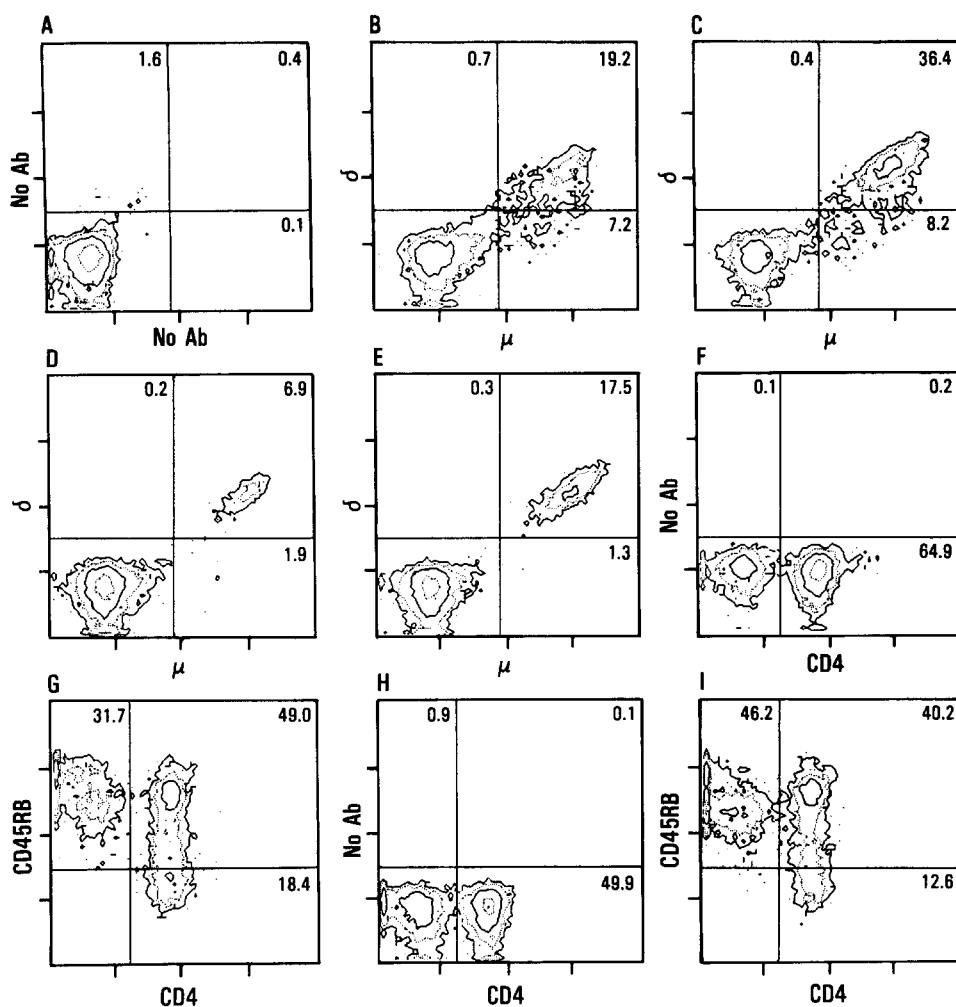
Young adult SCID mice were injected with sterile LN cells as for Tables 1 and 2; the C3H SCID mice used in Exp. 1 were bred and maintained at the Robert Wood Johnson Medical School, Piscataway, NJ. LN-injected SCID mice plus control mice were left for various periods and then primed intraperitoneally with 500  $\mu$ g alum precipitated CGG; 6 wk later, the mice received an intraperitoneal injection of a mixture of 100  $\mu$ g soluble CGG, 0.1 ml of 25% HRC, and 20  $\mu$ g TNP-Ficoll. The mice were then killed 1 wk later to measure splenic PFC specific for CGG, TNP, and HRC (see Materials and Methods). The data show the geometric mean ( $\times/\pm$ SE) of numbers of PFC/spleen for three mice/group measured on day 7.

\* Geometric mean ( $\times/\pm$ SE) of total numbers of PFC/spleen/mouse ( $\times 10^3$ ). Enhanced PFC are shown for responses to HRBC and CGG; nonenhanced PFC are shown for anti-TNP responses. Numbers of nonenhanced PFC were 70–90% lower than enhanced PFC for HRBC, and were undetectable for CGG PFC.

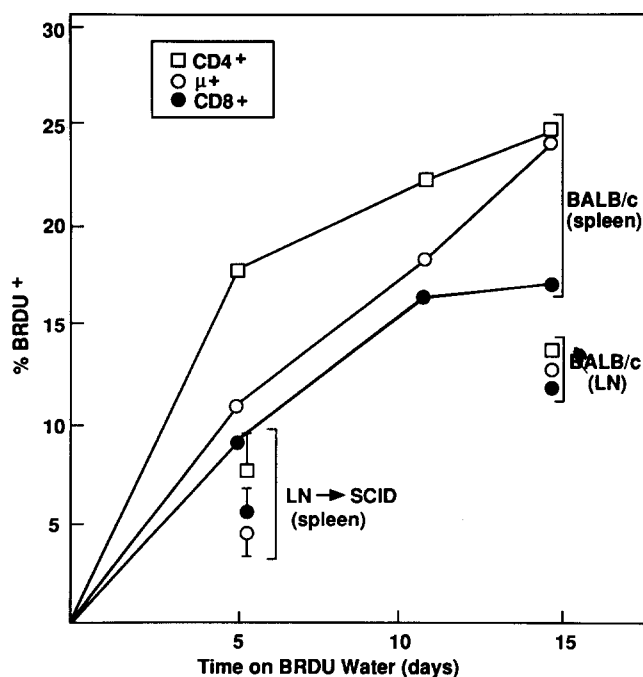
cells, 5% for CD8<sup>+</sup> cells, and 4% for  $\mu^+$  cells. At least for spleen, the labeling of B and T cells in LN-injected SCID mice was thus considerably less than in the control mice. It may be noted that, for B cells, the percent labeling of  $\mu^+$  spleen cells in the control mice is in agreement with recent data of Forster and Rajewsky (12).

*Size of the RLP in SCID Mice Given Small Doses of LN Cells.* The above findings complement the data on total cell recoveries (Table 1) and suggest that many of the B and T cells transferred to SCID hosts remain quiescent for prolonged periods. This finding contrasts with reports that T cells undergo marked expansion after transfer to athymic hosts (9, 10). Since T cell proliferation in athymic hosts is most prominent after injection of small doses of cells, it was considered important to study the effects of injecting SCID mice with small numbers of LN cells. The experiments discussed below involved SCID mice injected with  $10^7$  LN cells. Cell survival was quantitated by thoracic duct cannulation. This approach has two advantages over counting cells in spleen/LN suspensions. First, thoracic duct cannulation mobilizes cells from throughout the body (with the exception of the primary lymphoid organs) and thus gives a good indication of the total size of the RLP. Second, the high viability of TDL and the absence of nonlymphoid cells made TDL ideal for FACS<sup>®</sup> analysis. TDL from LN-injected SCID mice were collected over 48 h; cell outputs after 48 h were quite low.

TDL outputs from LN-injected SCID mice were examined at 1, 2, and 10 mo post-transfer from three to four mice/group. Total cell yields and the proportions of B and T cells in lymph samples collected from 0–24 and 24–48 h are shown in Table 4. Normal BALB/c (2–5 mo) and uninjected SCID mice (12 mo) were used as controls. TDL outputs from normal BALB/c mice were high and amounted to  $2.2 \times 10^8$  cells/mouse (comparable to total cell yields from pooled spleen + LN); CD4<sup>+</sup> cells were the major population in the first collection of lymph whereas B cells predominated in the second collection. Lymph from uninjected SCID mice was almost acellular ( $0.1 \times 10^6$  cells/mouse) and the few cells recovered from these mice resembled nonlymphoid cells. For LN-injected SCID mice, TDL yields at 1 mo post-transfer amounted to  $9.9 \times 10^6$  cells/mouse, i.e., very similar to the dose of  $10^7$  cells originally injected. TDL outputs increased slowly thereafter, i.e., to  $1.2 \times 10^7$  at 2 mo and  $2.0 \times 10^7$  at 10 mo. At each time point, the proportions of  $\mu^+$ , CD4<sup>+</sup>, and CD8<sup>+</sup> cells in the lymph showed little variation. CD4<sup>+</sup> cells were the predominant population, and CD4<sup>+</sup>/CD8<sup>+</sup> ratios were somewhat increased ( $\sim$ 5:1) relative to the cells originally injected (3:1).  $\mu^+$  cells (detected by rabbit anti- $\mu$  antibody) were rare in the first collection of lymph (3–5%) but reached 7–12% in the second collection (compared with 15% in the initial LN inoculum). To check the origin of the  $\mu^+$  cells, TDL from the



**Figure 2.** Surface phenotype of B and T cells in spleen and LN recovered from LN-injected SCID mice. The data are representative of several individual mice tested. (A-E)  $\mu$  vs.  $\delta$  ( $\delta^a$ ) expression on B cells recovered from C.B-17 ( $\delta^b$ ) SCID mice injected with  $3 \times 10^7$  BALB/c ( $\delta^a$ ) LN cells 3.5 mo before (A, B, and D); staining of B cells from normal (2-mo-old) BALB/c mice (C and E) is shown as a control. (F-I) CD4 vs. CD45RB expression on C.B-17 SCID mice injected with  $5 \times 10^7$  BALB/c LN cells 6 mo before (F and G); staining of aged-matched BALB/c mice (H and I) is shown as a control. (A) Spleen from LN-injected mouse (stained with secondary reagents only, see Methods). (B) Spleen from LN-injected SCID mouse. (C) Spleen from BALB/c control. (D) Pooled LN cells from LN-injected SCID mouse. (E) LN cells from BALB/c control. (F and G) pooled LN cells from LN-injected SCID mouse. (H and I) LN cells from BALB/c control. Comparison of B and C shows that spleens from LN-injected SCID and normal BALB/c mice both contain a major population of  $\mu^+ \delta^+$  cells together with smaller numbers of  $\mu^+ \delta^-$  cells;  $\mu^+ \delta^+$  cells form a near homogeneous population in the LN of these mice (D and E). Comparison of G and I indicates that CD4<sup>+</sup> cells from the LN of LN-injected SCID mice and age-matched BALB/c controls both consist predominantly of CD45RB<sup>+</sup> cells. For details of  $\mu$  vs.  $\delta^a$  staining and CD4 vs. CD45RB staining, see Materials and Methods.



second lymph collection of the mice tested at 10 mo post-transfer were also stained for donor ( $\mu^a$ ) expression. For each mouse, staining with the  $\mu^a$ -specific mAb was almost as high (10%) as with the non-allele-specific anti- $\mu$  antibody (12%) (Table 4).

The expression of putative virgin markers on B and T cells recovered from the LN-injected mice at 10 mo post-injection is shown in Fig. 4. For B cells, the majority of the  $\mu^+$  cells co-expressed the donor  $\delta^a$  allotype (Fig. 4, A-D). For CD4<sup>+</sup> cells, 56–70% of the cells showed high expression of the “virgin” markers, CD45RB, 3G11, and MEL-14 (E-H). This compared to 65–95% expression of these markers for TDL

**Figure 3.** Turnover of B and T cells in LN-injected SCID mice. C.B-17 SCID mice injected with  $5 \times 10^7$  BALB/c LN cells 5 mo before, together with 5-mo-old normal BALB/c mice as controls, were given drinking water containing BRDU for 5, 10, or 15 d (see Materials and Methods). LN and/or spleen cells from these mice were double stained for expression of BRDU vs.  $\mu$  (O), CD4 (□), or CD8 (●) (see Materials and Methods). The data show percent BRDU<sup>+</sup> cells for cell populations pooled from two to three mice/group for normal BALB/c mice and from four individual mice for the LN → SCID group. The source of the cells examined is shown in parentheses.

**Table 4.** Number and Phenotype of Thoracic Duct Cells Recovered from SCID Mice Injected with  $10^7$  BALB/c LN Cells 1–10 mo before

Mice cannulated (time after LN injection)	Surface markers of TDL						Total no. of TDL collected over 48 h (SD)
	0–24 h			24–48 h			
	$\mu^+$	CD4 <sup>+</sup>	CD8 <sup>+</sup>	$\mu^+$	CD4 <sup>+</sup>	CD8 <sup>+</sup>	
				%			$\times 10^{-6}$
Normal BALB/c	41	47	16	50	39	16	223.4 (16.9)
Uninjected C.B-17 SCID	–	–	–	–	–	–	0.1 (0.1)
LN → SCID (1 mo)	3	73	10	7	65	23	9.9 (2.3)
LN → SCID (2 mo)	3	77	13	10	68	17	11.8 (0.4)
LN → SCID (10 mo)	5	75	19	12	64	22	20.2 (0.9)
				(10.3)*			

Groups of SCID mice were left for 1–10 mo after intravenous injection of  $10^7$  BALB/c LN and then subjected to thoracic duct cannulation; normal BALB/c mice (3–5 mo) and SCID mice (12 mo) were used as controls. TDL collected over 24 h were stained for the markers shown (Table 1) using FACS<sup>®</sup> analysis. The data show mean values for 3–4 mice/group; each mouse was tested individually.

\* Staining with specific anti- $\mu^2$  mAb (instead of rabbit anti-mouse  $\mu$  antibody).

from young (2 mo) BALB/c mice (*I-L*) and 60–93% for TDL from older (5 mo) BALB/c mice (*M-P*). By these parameters, most of the CD4<sup>+</sup> cells from the LN-injected mice did not express a memory phenotype. Pgp-1 expression was not examined because most BALB/c T cells show high Pgp-1 expression even in young mice (37).

## Discussion

The main finding in this paper is that typical mature B and T cells in LN are very long lived, at least at a population level. Upon transfer to the pathogen-free environment of SCID mice, both cell types appeared to survive indefinitely with little change in their numbers, functions, or surface phenotypes.

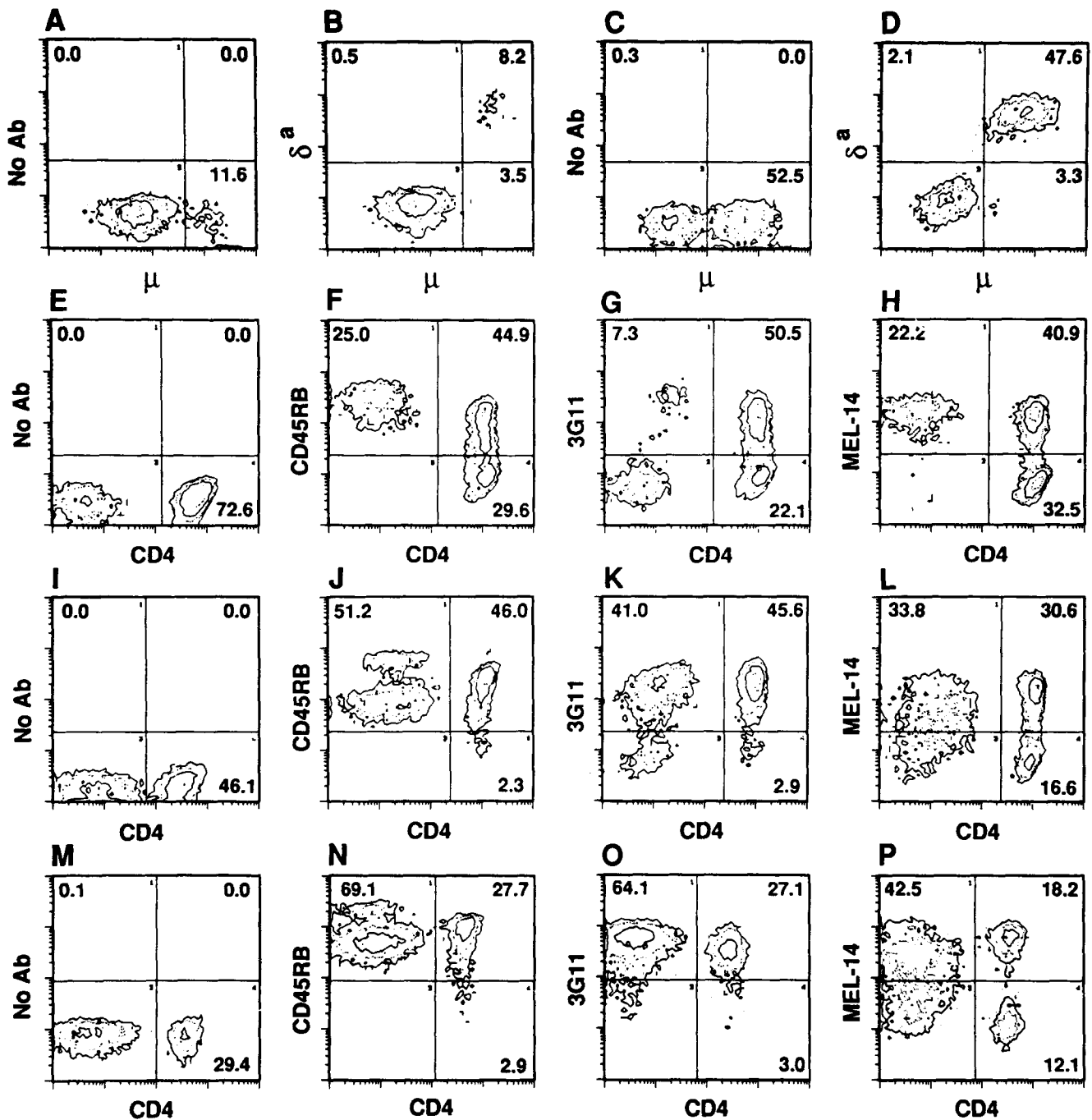
The possibility that the lymphocytes recovered from LN-injected SCID mice were derived from contaminating donor stem cells seems very unlikely. Most preparations of LN cells were devoid of stem cells and, unlike BM cells, injection of even large doses of LN cells failed to cause enlargement of the host thymus. In the case of B cells, strong evidence that these cells were derived from Ig<sup>+</sup> LN cells rather than stem cells was provided by the finding that B cell function was not seen in SCID mice given LN cells selectively depleted of Ig<sup>+</sup> cells. The possibility that the B cells in LN-injected mice were of host origin was ruled out by the finding that most of the B cells expressed donor ( $\mu^2$ ,  $\delta^2$ ) allotypic markers.

The finding that the numbers of B and T cells harvested from long-term LN-injected SCID mice were quite similar to the numbers originally injected implies that many of the injected cells remained relatively quiescent from the time of injection. Although we favor this possibility, it is clear that a proportion of the transferred cells became activated and/or entered cell cycle at some stage post-transfer. This was most

noticeable for CD4<sup>+</sup> cells. Thus, when these cells were recovered from SCID hosts at  $\geq 5$  mo post-transfer, 20–40% of CD4<sup>+</sup> cells displayed a memory phenotype, and 7% incorporated BRDU over a short (5-d) period. Some of these proliferating cells (or their progeny) appeared to survive for prolonged periods. Thus, after injection of a small dose of  $10^7$  LN cells, 48-h TDL outputs increased from  $10^7$  cells/mouse at 1 mo to  $2 \times 10^7$  cells at 10 mo, i.e., a two-fold increase. Substantially higher (eightfold) recoveries were seen in a recent experiment with  $2 \times 10^6$  input LN cells (our unpublished data). Despite these findings, it is striking that, even at 10 mo post-transfer, the majority (>50%) of CD4<sup>+</sup> cells from LN-injected SCID mice retained CD45RB, 3G11, and MEL-14 expression. Likewise, the B cells coexpressed  $\mu$  and  $\delta$  and, unlike typical memory cells (24), remained J11d<sup>+</sup>. By these parameters, the majority of the recovered B and T cells resembled virgin lymphocytes, i.e., the typical B and T cells found in normal young mice. One should caution, however, that retention of a virgin phenotype does not necessarily signify that the cells remained in interphase from the time of initial injection. In view of recent evidence that CD45RB<sup>-</sup> CD4<sup>+</sup> T cells can sometimes revert to CD45RB<sup>+</sup> cells on transfer (38), the expression of memory markers could simply be a sign of activation. After activation some of the cells might gradually lose their memory markers and thereby reacquire a virgin (resting) phenotype.

In view of reports that T cells undergo extensive proliferation and expansion after transfer to athymic (nude) hosts (9, 10), the relatively minor expansion of T cells seen in SCID recipients is surprising. Since T cell proliferation in nude hosts is most prominent with injection of small doses of T cells, the prevailing view is that proliferation is a reflection of homeostasis: the T cells expand in order to restore the pool size to normal. The receptor-ligand interactions involved in





**Figure 4.** Surface phenotype of B and T cells recovered from TDL of SCID mice injected with  $10^7$  BALB/c LN cells 10 mo before. (A–D)  $\mu$  vs.  $\delta$  ( $\delta^a$ ) expression on B cells recovered from TDL (24–48-h collection) of LN-injected SCID mice (A and B) vs. 5-mo-old normal BALB/c mice (C and D); in both groups of mice, the majority of the B cells were  $\mu^+\delta^+$ . (E–P) Expression of CD4 vs. CD45RB, 3G11, or MEL-14 on cells recovered from TDL (0–24-h collection) of SCID mice given  $10^7$  BALB/c LN cells 10 mo before (E–H), 2-mo-old normal BALB/c mice (I–L), and 5-mo-old normal BALB/c mice (M–P); the cells in E–H and I–L were stained at the same time, whereas the cells in M–P were stained in a separate experiment. For each group of mice, most of the CD4<sup>+</sup> cells showed coexpression of three putative markers for “virgin” (resting) T cells.

such homeostatic control are not discussed. In our view the simplest explanation is that T cell proliferation in nude hosts reflects a chronic response to endemic pathogens (which are common in immunoincompetent hosts); the pathogens are eventually destroyed and the pool size stabilizes. Since antigen-

presenting function for T cells *in vivo* is largely under the control of B cells (39, 40), the abundance of B cells in nude hosts could provide optimal conditions for T proliferative responses to pathogens. The relative paucity of B cells in LN-injected SCID mice could then be the key to why T cell

proliferation is much less in SCID than nude mice. This idea is being tested by injecting SCID mice with small numbers of T cells and large numbers of B cells.

The finding that LN-injected SCID mice contained near-constant numbers of typical  $\mu^+ \delta^+$  cells for at least 10 mo post-transfer is difficult to reconcile with the dogma that most B cells are short lived. As discussed earlier (see introduction) the evidence that mature B cells have a short life span rests largely on two pieces of evidence: (a) the rapid destruction of B cells seen in mice given hydroxyurea (HU) (15); and (b) the disappearance of LPS-reactive B cells after adoptive transfer (14). With regard to HU, it is clear that injecting mice with HU destroys nearly all immature B cells in the marrow within a few days. The effects of HU on B cells in the secondary lymphoid organs, however, are quite variable. Some workers report that HU destroys >50% of B cells in spleen and TDL within 2 d (15, 16). By contrast, Rusthoven and Phillips (41) found that injection of HU caused no detectable decrease of  $Ig^+$  cells in the spleen, despite a 10-fold reduction in the marrow. This latter finding cannot be ignored. In our view the simplest explanation for the reports that HU can cause widescale destruction of B cells in spleen and TDL is that the mice studied were infected with pathogens: the rapid turnover of B cells in these mice reflected a proliferative response to antigens or mitogens. Whatever the explanation, demonstrating that HU kills a certain proportion of peripheral B cells merely indicates that these cells are dividing. The system provides no indication as to whether these cells are (a) short-lived cells of recent marrow origin, or (b) long-lived cells engaged in a proliferative response.

With regard to the lifespan of LPS-reactive B cells, the finding of Freitas and Coutinho (14) that responsiveness to LPS disappeared rapidly after B cell transfer is generally taken as evidence that LPS-reactive B cells (and B cells in general) are short lived. However, the alternative possibility is that LPS reactivity is restricted to a certain stage of differentiation: the transferred B cells do not die but simply differentiate to an LPS-unresponsive state. In this respect, it should be noted that LPS reactivity is not a property of all B cells. Most B cells in spleen (75%) and the vast majority (>90%) in LN and TDL are LPS unresponsive (15, 42). In the present study on LN-injected SCID mice, there was no evidence that LPS reactivity declined with time. Indeed, there was a considerable increase in LPS reactivity between 1 and 5 wk post-transfer. Whatever the explanation for this paradoxical increase, the data provide no support for the view that LPS-reactive B cells are short lived.

As argued above, the dogma that B cells are short lived (as against being in cell cycle) is based on specious reasoning. Why then is this notion so deeply ingrained? We suggest that it stems from the assumption that the known massive production of B cells in the marrow (18) has to be balanced by widescale migration of B cells from the marrow to the secondary lymphoid organs (in the same way as it used to be argued that release of T cells from the thymus must be extensive). The further assumption is that, to maintain homeostasis of the peripheral B cell pool, most of the B cells released from the marrow have to be short lived. This line

of reasoning rests on the assumption that the release of B cells from the marrow is indeed extensive. To our knowledge, however, there is no firm evidence that the release of B cells from the marrow is any more extensive than the release of T cells from the thymus (which is known to be quite limited, i.e.,  $\sim 10^6$  cells/d) (8). If release of B cells from the marrow were extensive, one would expect to find substantial numbers of early B cells in the bloodstream; yet, the proportion of  $Ig^+$  cells in blood is quite low, i.e.,  $\sim 30\%$  of PBL).

The present findings on the survival of B cells in SCID mice do not exclude the possibility that some peripheral B cells are short lived. However, the data do provide strong evidence that, at a population level, typical mature B cells from LN can have an almost indefinite lifespan. This finding is clearly difficult to reconcile with the view that most B cells represent recent emigrants from the marrow. Although one might cavil that the (pathogen-free) environment of SCID mice is unphysiological and not conducive to the homeostatic influences of antiidiotypic networks etc., the simplest conclusion from the data is that maintenance of the B cell pool in adults is relatively independent of the primary lymphoid organs. Although input of new cells from the primary lymphoid organs is clearly critical for the initial formation of the B cell pool, one can argue that, once formed, the B cell pool of adults becomes self sustaining: newly formed cells continue to enter the pool, but at a much reduced rate. There would then be a close similarity with the formation of the T cell pool. As discussed earlier (see Introduction), the considerable decline in thymic function after puberty and the long-term maintenance of T cell numbers after adult thymectomy implies that the T cell pool eventually attains self sufficiency.

With regard to memory, the "virgin" phenotype of most B and T cells recovered from LN-injected SCID mice deserves comment. The prolonged survival of these cells is difficult to reconcile with the notion that long-lived lymphocytes are memory cells (43). Although there is no question that memory itself is long lasting, the evidence that antigen can remain trapped in the lymphoid system for prolonged periods (44) raises the question whether memory is largely a reflection of persistence of antigen. In this respect, there is now convincing evidence that depriving primed B cells of antigen leads to a rapid disappearance of memory cells (45). Likewise, transient exposure of T cells to antigen *in vivo* can result in the rapid disappearance of many of the responding cells (46, 47). These data suggest that, at least in certain situations, memory (primed) cells are short lived. If so, what is the status of memory cell precursors? Are the virgin precursors of memory cells short lived or long lived? At face value the finding that LN-injected SCID mice retained the capacity to mount typical primary responses to three different antigens, SRC, HRC, and TNP-Ficoll, for many months post-transfer implies that virgin lymphocytes can be very long lived. However, because primary responses to these three antigens tend to be quite high, it can be objected that the precursor cells involved in these responses were primed through contact with environmental antigens. This objection is less applicable to responses to CGG because primary anti-CGG re-

sponses are quite low, and unlike secondary responses, are heavily dependent on priming with adjuvant (32). The cells participating in primary anti-CGG responses are thus good candidates for being true virgin cells. It is significant, therefore, that unprimed LN-injected SCID mice could be left for at least 6 mo before priming for secondary anti-CGG responses (elicited with soluble antigen), implying that the unprimed precursors of CGG memory cells have a prolonged lifespan.

Although these data favor the notion that the "primary" responses induced in LN-injected SCID mice are mediated by long-lived virgin cells, it is impossible to formally rule out the possibility that the cells had been previously primed through contact with environmental antigens. However, if memory cells are short lived (see above), one would expect priming with environmental antigens to shorten the lifespan

of the responding cells. Yet the cells surviving in SCID mice are clearly long lived.

In conclusion, the main finding in this paper is that transferring stem cell-depleted mature B and T cells to SCID mice does not cause the cells to disappear: the cells survive indefinitely with little or no change in their functions or surface phenotype. The data favor the notion that maintenance of the pool of mature lymphocytes in adult mice does not require constant replenishment from the primary lymphoid organs. Although some recruitment of new lymphocytes is known to continue into adult life (48, 49), the fact that the primary lymphoid organs atrophy progressively with age (50) implies that de novo formation of lymphocytes from stem cells is eventually quite limited. The pool of mature lymphocytes in adults might thus be largely self sufficient.

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