EVIDENCE OF FUNCTIONAL LYMPHOCYTES IN SOME
(LEAKY) scid MICE

By GAYLE C. BOSMA, MICHAL FRIED, R. PHILIP CUSTER, ANN CARROLL, DAVID M. GIBSON* AND MELVIN J. BOSMA

From the Institute for Cancer Research, Fox Chase Cancer Center,
Philadelphia, Pennsylvania 19111; and the *Department de Biochimie, Université de
Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

Lymphoid and myeloid cells represent distinct lineages of a common hematopoietic stem cell (1–3). This distinction is dramatically illustrated in the autosomal recessive mouse mutant, scid.[1] Mice homozygous for the scid mutation (scid mice) are severely deficient in B and T lymphocytes whereas other hematopoietic cell types such as erythrocytes, monocytes, granulocytes, and megakaryocytes (all members of the myeloid series) are present in normal number (4, 5). Although the scid mutation appears to affect only lymphocyte development (4–10), it is not yet clear what stage of lymphoid differentiation is impaired or arrested.

Recent results suggest that the effects of the scid mutation become manifest after the commitment of lymphoid cells to the B and T cell pathways. First, early transcription of unrearranged H chain and TCR loci, which presumably signals the opening of these loci to factors responsible for gene recombination (11–16), is detectable in scid fetal liver and thymus, respectively (Schuler, W., A. Schuler, and M. J. Bosma, unpublished results). Second, although cells with H chain (or TCR) gene rearrangements cannot be directly demonstrated in freshly harvested lymphoid tissues of adult scid mice (17), early B cell lines with H chain gene rearrangements can be recovered from Abelson murine leukemia virus–transformed scid bone marrow cells (17) and from long-term cultures of scid bone marrow cells (18). There is also indication of early T cell development as thymic lymphomas with rearranged TCR-γ and TCR-β alleles spontaneously appear in ~15% of scid mice (5, 17, 19). It is striking, however, that the majority of rearranged H chain and TCR alleles in transformed scid lymphocytes show abnormal J region deletions. The deletions remove all J-coding exons of a given J region and appear to result from attempted D to J or V to J joining; they vary in size and extend both 5' and 3' of the deleted J regions (17, 19). Evidence of abnormal J-associated deletions has also been reported for rearranged H chain alleles of long-term B cell lines derived from scid bone marrow cells (18).

To explain the abnormal J-associated deletions and how they might account

[1] Abbreviations used in this paper: scid, severe combined immune deficiency; SPF, specific pathogen–free.

for the *scid* phenotype, we recently proposed the following (17). The *scid* mutation causes highly error-prone Ig and TCR gene rearrangements; consequently, most developing *scid* lymphocytes lack an antigen receptor due to nonproductive rearrangements at both alleles of a critical antigen receptor locus (e.g., H chain). The apparent absence of these nonfunctional cells in *scid* lymphoid tissues may result from their rapid turnover (elimination). On the other hand, developing *scid* lymphocytes that by chance make two productive rearrangements at the appropriate Ig or TCR loci would survive and express an antigen receptor. Antigen-dependent clonal expansion of one or more of these relatively rare cells could account for our earlier observation (4) that some *scid* mice (~15%) appear leaky in that they produce detectable serum Ig.

Leaky *scid* mice are important to understanding the nature of the *scid* defect and are the subject of this report. They will be referred to as *scid*(Ig⁺) mice. As shown here, most *scid*(Ig⁺) mice contain only a few clones of Ig-producing B cells; they also appear to contain a limited number of functional T cells. The regulation of these relatively few lymphocytes may be minimal as many *scid*(Ig⁺) mice have abnormally high concentrations of serum Ig and/or develop T cell lymphomas. Clearly, *scid*(Ig⁺) mice are not normal, and possible explanations for their appearance are discussed.

Materials and Methods

Mice. The *scid* mutation occurred in an H chain congenic partner strain of BALB/cAnIcr known as C.B-17 (4). C.B-17 mice that are homozygous for the *scid* mutation are here designated as *scid* mice; heterozygotes are designated as *scid*/+ mice.

All *scid* and *scid*/+ mice as well as the normal mice (C.B-17 [H-2ᵈ], C3H/HeJ [H-2ᵏ], and [C.B-17 × C3H/HeJ]F₁) used in these studies were derived from specific pathogen–free (SPF) breeder stocks of the ICR animal facility (SPF mice have a defined flora and are free of all known mouse pathogens). The SPF breeder stocks were maintained behind a barrier in rooms with HEPA-filtered air and were housed in microisolator cages (Lab Products Inc., Maywood, NJ) containing sterilized food and water. Progeny of the breeder stocks were transferred into a conventional animal room at 3–4 wk old and were maintained thereafter as "non-SPF" mice in microisolator cages containing sterilized food and water; mice were transferred to clean sterile cages within a class II type safety cabinet (Bellco Glass, Inc., Vineland, NJ). Non-SPF mice were used exclusively in all experiments except where noted.

Ig Quantitation. An ELISA (20) was used to quantitate serum Ig-κ concentrations. This was done in the manner previously described for a competitive RIA (21, 22). Briefly, microtiter wells (Dynatek Laboratories, Inc., Alexandria, VA) were first coated with purified myeloma κ chains of MOPC-46B (23) and then with rabbit antisera to IgG2a Fab fragments of the MOPC-173 myeloma protein (23). The reference antigen was the IgG1-κ myeloma protein of MOPC-31c (23) which was conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) by glutaraldehyde cross-linking (24). The relative amount of bound enzyme conjugate in each well was ascertained by addition of paranitrophenyl phosphate (Sigma Chemical Co.). The extent of paranitrophenyl phosphate hydrolysis was measured at 405 nm in an MR 580 Micro-Elisa Auto-Reader (Dynatek Laboratories, Inc.). Serum Ig-κ concentrations were calculated from the standard curve.

Ig Isotypes. Serum samples were assayed for the presence of the major Ig isotypes (IgM, IgG3, IgG1, IgG2b, IgG2a, and IgA) by double diffusion analysis in micro-Ouchterlony plates. Affinity-purified goat (and rabbit) antisera specific for the above mouse Ig isotypes were obtained from Litton Bionetics (Charleston, SC).

IEF of L Chains. Ig from normal BALB/c and C.B-17 sera, *scid*(Ig⁺) sera or from myeloma ascites was isolated by absorption onto protein A–Sepharose (Pharmacia Canada

Ltd. Dorval, Québec) followed by elution in 8 M urea, 0.05 M Tris-HCl, pH 8.0. The eluted material was precipitated with methanol and redissolved in 8 M urea, 0.05 M Tris-HCl buffer for reduction and alkylation. Reduction and alkylation with [^{14}C]iodoacetamide (Amersham Corp., Arlington Heights, IL) was carried out in a two-step procedure as described (25). H and L chains were separated by PAGE at pH 3.0 in the presence of 8 M urea. After electrophoresis, the light chain zone was cut from the gel and transferred to the surface of an IEF gel containing 2% carrier ampholines (LKB Instruments, Inc., Gaithersburg, MD, pH 3–10) and 6.6 M urea. IEF, fixing, and drying of the gel have been described (25). The autoradiograms represent 3–6-d exposures.

Western Blot Analysis. Cell lysates of lymph node, spleen, and bone marrow were prepared, reduced, and subjected to electrophoresis in 10% polyacrylamide gels as previously described (26). Prestained molecular weight standards (Bethesda Research Laboratories, Gaithersburg, MD) were applied to all gels. The contents of the gel were electroblotted onto nitrocellulose after which the nitrocellulose blot was overlaid sequentially with goat anti-IgM (Litton Bionetics) and ^{125}I-IgM of MOPC-104E. After autoradiographs of the blot were made, the process was repeated with goat anti-IgG-κ (Litton Bionetics) and ^{125}I-IgG1-κ of MOPC-31c. Proteins were radiolabeled with ^{125}I (Amersham Corp.) using the chloramine T method of Hunter (27). Details of the above Western blot procedure are described elsewhere (26).

FACS Analysis. Aliquots of spleen cells (10^6 cells) from *scid*, *scid*/+, and C.B-17 normal mice were incubated with antibodies specific for various lymphocyte surface antigens. The antibodies included FITC-conjugated rabbit anti–mouse IgM (Litton Bionetics), FITC-conjugated (Fab')2 fragment of goat anti–mouse IgG (Fab')2 specific antibody (Cappel Laboratories, West Chester, PA), rat monoclonal anti-Ly5(B220) (28), FITC-conjugated rat monoclonal (30-H12) anti–Thy-1 (29), rat monoclonal anti–mouse Ly-1 (29), and FITC-conjugated rabbit anti–rat Ig (from R. L. Coffman, DNAX, CA) as a second-stage reagent. The incubations, washings, and counterstaining with ethidium bromide to exlude dead cells were carried out as previously described (4, 5). Cells were analyzed on a FACS II (Becton Dickinson & Co., Mountain View, CA).

Mitogen Stimulation. *scid*, *scid*/+, and CB-17 normal spleen cells were depleted of erythrocytes by Tris-ammonium chloride lysis and plated at 5 × 10^5 cells/well (96-well plates, Costar, Data Packaging Corp., Cambridge, MA) in RPMI-1640 medium supplemented with 10% FCS, 2 × 10^{-5} M 2-ME, 10 mM Hepes, and 2 mM L-glutamine. Medium was also supplemented with Con A (5 µg/ml; Sigma Chemical Co.) or with bacterial LPS (5 µg/ml; Difco Laboratories, Inc., Detroit, MI). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 48 h after which 1 µCi [^{3}H]thymidine (New England Nuclear, Boston, MA) was added to each well. After an additional 18-h incubation, cells were harvested using a MASH filter harvester and assayed for [^{3}H]thymidine incorporation by β-scintillation counting.

Skin Grafts. The grafting of full-thickness skin grafts was done according to the procedure of Billingham and Silvers (30). *scid* mice were grafted on one flank with allogenic skin of (C.B-17 × C3H/HeJ)F$_1$ or C3H/HeJ mice and with a control graft (from *scid* or C.B-17 normal mice) on the other flank. Grafts were covered with a small telfa sterile pad (Curity; Colgate-Palmolive Co., New York, NY) and held in place with 1-in clear First Aid Tape (Johnson and Johnson, New Brunswick, NJ) wrapped around the thorax. The bandage was removed 10 d later and grafts were checked closely for possible rejection over the next several weeks.

Histologic Preparations. Tissues were fixed in Carson's 10% neutral formol (31), embedded in methacrylate glycol (JB-4; Polysciences, Inc., Warrington, PA), and sectioned at 2 µm on a Sorvall JB-4 microtome (DuPont Instruments, Newtown, CT). They were stained routinely with hematoxylin and eosin.
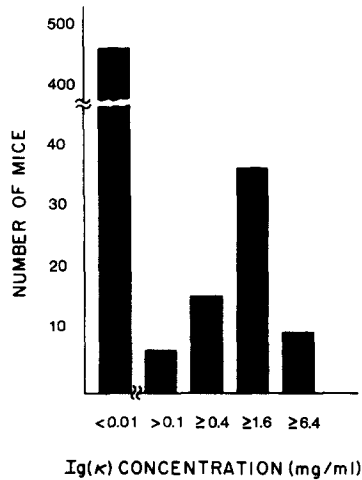
FIGURE 1. Histogram of serum Ig-κ concentrations in non-SPF *scid* mice. All mice were 3–4 mo old.

TABLE I

Frequency of scid(Ig⁺) Mice as a Function of Age and Environment

Group	Environment	Age	Number of mice		Ig⁺
			Tested	Ig⁺	
		mo			%
A	SPF	3–4	222	12	5.4
B*		3–4	40	1	2.5
		8–9	40	2	5.0
		14–15	40	13	32.5
C	Non-SPF	3	486	66	13.6
D		4	263	48	18.2
E		5	217	50	23.0
F*		3–4	76	9	11.8
		7–8	76	14	18.4
		12–14	76	36	47.4

* Select groups of very old mice (>12 mo old) that were also tested at 3–4 and 7–9 mo old.

Results

Serologic Analysis of scid(Ig⁺) Mice

Frequency. Routine serological testing of 3–4-mo-old *scid* mice revealed circulating Ig in ~15% of the mice. As illustrated in Fig. 1, 78 of 545 mice analyzed had serum Ig-κ concentrations ranging from 0.10 to >6.4 mg/ml; the remaining 467 mice (*scid*[Ig⁻] mice) lacked detectable Ig-κ (<0.01 mg/ml). Mice with >0.05 mg/ml of serum Ig-κ are designated as *scid*(Ig⁺) mice.

The chance of a given *scid* mouse becoming Ig⁺ appeared to be influenced by both its environment and age. As shown in Table I, ≤5% of 3–9-mo-old SPF mice typed Ig⁺. However, when SPF mice were removed from their barrier environment at 3–4 wk old and subsequently maintained as non-SPF mice in a conventional animal facility for 2–8 mo, the percentage of Ig⁺ mice went up to
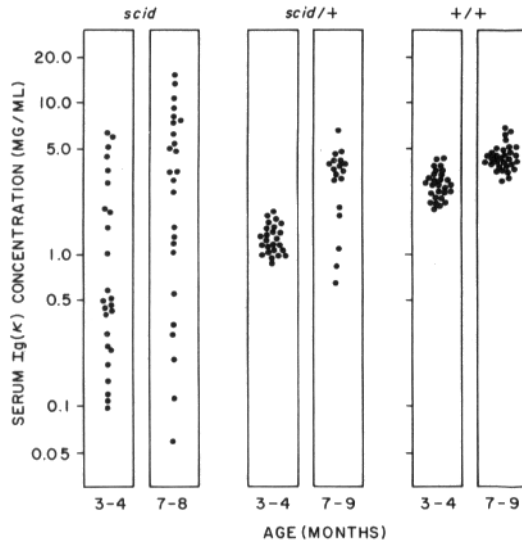
FIGURE 2. Serum Ig-κ concentrations in individual *scid*(Ig⁺), *scid*/+, and C.B-17 normal mice (+/+). Each point represents an individual mouse at the respective ages. The same *scid*(Ig⁺) mice were tested at 3–4 and 7–8 mo old.

13–23%. In the non-SPF environment, the proportion of Ig⁺ mice appeared to increase slightly from 3 to 9 mo old. At 12 mo and older, the percentage of individuals with detectable Ig increased dramatically to 32% in SPF mice and to 46% in non-SPF mice. However, the levels of Ig in many of these very old Ig⁺ mice were low (<0.2 mg/ml), unlike those of 3–8-mo-old *scid*(Ig⁺) mice (see Fig. 2). All subsequent analyses pertain to mice 3–9 mo old.

It is important to note here that the *scid*(Ig⁺) phenotype is not inherited. This is clear from pedigree analyses of *scid*(Ig⁺) and *scid*(Ig⁻) mice. Also, we have not been able to increase the frequency of *scid*(Ig⁺) mice by selective breeding.

Variable Ig Levels. Fig. 2 emphasizes the enormous variation in Ig-κ concentrations between individual *scid*(Ig⁺) mice. The values for 3–4-mo-old *scid*(Ig⁺) mice were widely scattered (from 0.1 to >10.0 mg/ml), in striking contrast to the tightly clustered values of age-matched *scid*/+ and C.B-17 normal homozygotes (+/+). When the same *scid*(Ig⁺) mice were again tested at 7–8 mo old, all but one mouse showed higher levels of Ig-κ. The mean Ig-κ concentration ± the standard deviation at 7–8 mo old was 4.49 ± 4.53 mg/ml *vs.* 1.59 ± 2.04 mg/ml at 3–4 mo old.

It is of interest to note that the mean Ig-κ concentration in 3–4-mo-old *scid*/+ mice (1.3 ± 0.3 mg/ml) was significantly less than that of age-matched C.B-17 normal mice (3.0 ± 0.72 mg/ml). Also, at 7–9 mo old, ~25% of tested *scid*/+ mice (5/18) retained relatively low levels of Ig-κ; the remaining mice showed Ig-κ levels (~4.0 mg/ml) comparable to those of age-matched C.B-17 normal mice. These differences may reflect a slower development of functional lymphocytes in *scid*/+ heterozygotes than in C.B-17 normal mice.

Representation of Ig Isotypes. Table II indicates the different distribution patterns of H chain isotypes that were observed in 48 *scid*(Ig⁺) mice. 8% of the mice (group A) were positive for all H chain isotypes tested (μ, γ3, γ1, γ2b, γ2a, and α), 40% lacked only α (group B), 27% lacked α and γ2a (group C), 15% lacked α and one or more γ isotypes (γ1, γ2b, γ2a) (group D), and 10% lacked

Table II
Representation of H Chain Isotypes in scid(Ig⁺) Mice

Group	Mice	Distribution patterns of H chain isotypes*					
		μ	$\gamma3$	$\gamma1$	$\gamma2b$	$\gamma2a$	α
	n %						
A	4 (8)	+	+	+	+	+	+
B	19 (40)	+	+	+	+	+	−
C	13 (27)	+	+	+	+	−	−
D	7 (15)	+	+	[		]	−
E	5 (10)	−	+	[		]	−

* The presence or absence of a given isotype is scored with + or −; mice in groups D and E lacked one or two of the γ isotypes enclosed by the brackets.
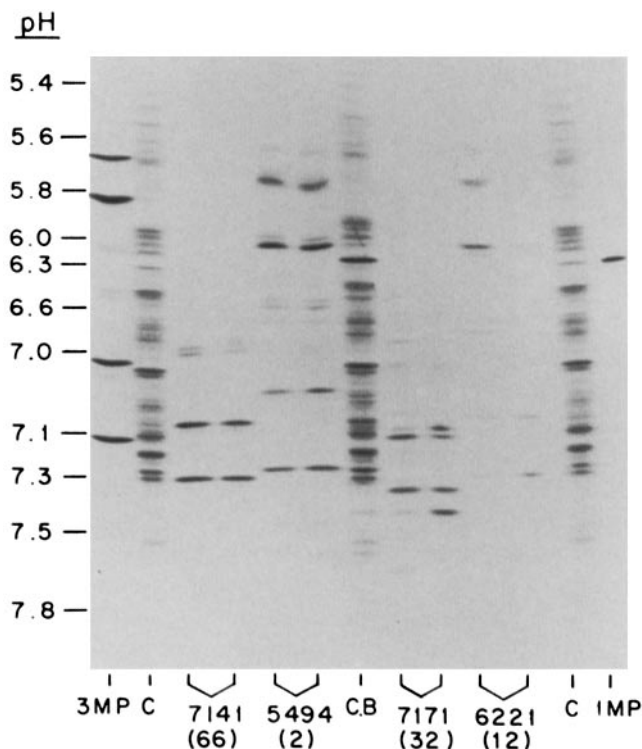

Figure 3. Illustration of very restricted (pauciclonal) IEF patterns of serum L chains in scid(Ig⁺) mice. Each mouse (7141, 5494, 7171, 6221) was sampled twice; the interval (in weeks) between each bleed is indicated in parentheses. L chains of normal BALB/c (C) and C.B-17 (C.B) serum and of purified myeloma proteins (MP) were included as controls. The first lane contained L chains from three myeloma proteins: PC-4050 (IgG2b-Vκ-21B); PC-7644 (IgG3-κ); and PC-8643 (IgG1-κ). The last lane contained the L chains of HOPC-1 (IgG2a-λ1). The pH across the gel was determined at 10°C using a surface electrode (Ingold, Urdorf, Switzerland).

both μ and α and one or more γ isotypes (γ1, γ2b, γ2a) (group E). Most scid(Ig⁺) mice (≥85%) lacked detectable λ light chains (data not shown).

Restricted Heterogeneity of L Chains. IEF of L chains in serum of 3–9-mo-old scid(Ig⁺) mice gave very restricted IEF patterns. Forty-one scid(Ig⁺) mice were examined. All of these mice had distinct and restricted IEF patterns unlike the complex and indistinguishable patterns of individual C.B-17 normal mice. Representative results are shown in Figs. 3 and 4. Most scid(Ig⁺) mice, as illustrated in Fig. 3, showed only 4–10 major bands corresponding to 1–3 clones of Ig-
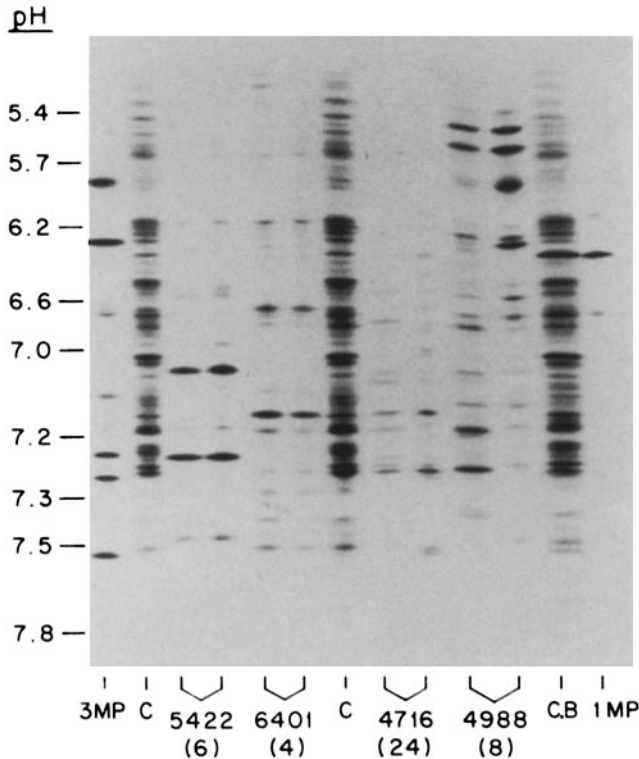
FIGURE 4. Illustration of restricted IEF patterns of serum L chains in *scid*(Ig⁺) mice. Each mouse (5422, 6401, 4716, 4988) was sampled twice; the interval (in weeks) between each bleed is indicated in parentheses. L chains of normal BALB/c (*C*) and C.B-17 (*C.B*) serum and of purified myeloma proteins (*MP*) were included as controls. The first lane contained L chains from three myeloma proteins: FLOPC-1 (IgG1-Vκ-1A); PC-7477 (IgG3-Vκ-21E); and PC-1229 (IgG2b-Vκ-21A). The last lane contained the L chains of HOPC-1 (IgG2a-λ1). The pH across the gel was determined at 10°C using a surface electrode (Ingold).

producing cells as judged from the number of L chain bands obtained with one (*last lane*) or a mixture of three myeloma proteins (*first lane*). Other mice showed more complex IEF patterns consisting of >10 bands (*e.g.*, 6401, 4988 in Fig. 4). Some *scid*(Ig⁺) mice displayed the same IEF pattern for many weeks (*e.g.*, 7141 in Fig. 3 and 5422 in Fig. 4). In other mice the pattern changed with time; i.e., new bands appeared along with retention of old bands (*e.g.*, 4716 in Fig. 4). In some cases, there was a clear loss of certain bands (*e.g.*, 4988 in Fig. 4).

Western Blot Analysis of Spleen and Bone Marrow Cell Lysates.   Spleen and bone marrow cell lysates of *scid*(Ig⁺) mice were subjected to electrophoresis in 10% polyacrylamide containing 0.1% SDS; the contents of each gel were electroblotted onto nitrocellulose and the blots were sequentially coated with 0.5% casein, anti-μ, ^{125}I-IgM, and exposed to X-ray film for 3 and 16 h. The same was done for spleen, lymph node, and bone marrow cells of C.B-17 normal mice. The blots were later sequentially coated with anti-γ/κ–specific sera and ^{125}I-IgG-κ as described below. Using this procedure, the spleen lysates of 12 *scid*(Ig⁺) mice were analyzed and found to contain γ and κ chains; 9 of these lysates also contained detectable μ chains. Representative results are shown in Fig. 5. No μ chains were detected in the bone marrow lysates of *scid*(Ig⁺) mice and neither μ, γ, nor κ chains were found in spleen lysates of *scid*(Ig⁻) mice (data not shown).

As illustrated in Fig. 5a, the apparent molecular mass of the μ chains in the spleen lysate of *scid*(Ig⁺) mice (lane *4*) was equivalent to the smaller of two distinct molecular masses of μ chain (80 kD and 76 kD) found in the spleen lysate
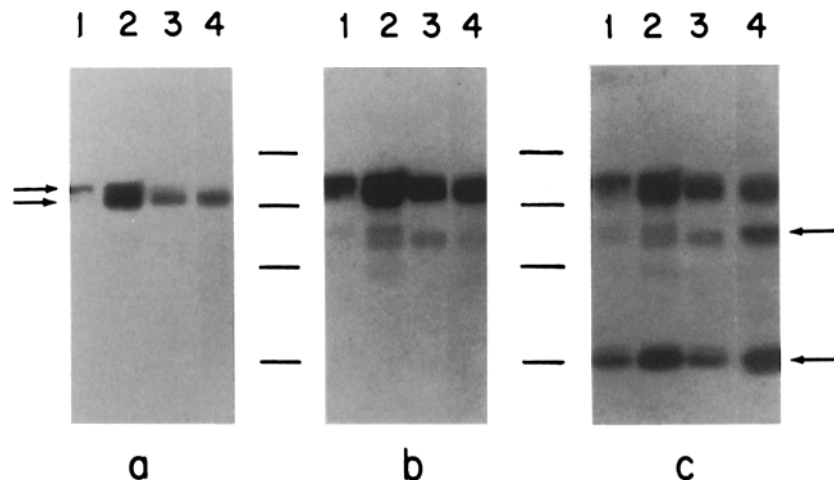
FIGURE 5. Western blot of reduced cell lysates (subjected to electrophoresis in 10% poly-acrylamide gels containing 0.1% SDS) of lymph node (*1*), spleen (*2*), and bone marrow (*3*), from a C.B-17 normal mouse vs. spleen (*4*) of a *scid*(Ig$^+$) mouse. The blot was first sequentially overlaid with affinity-purified anti–mouse IgM and ^{125}I-IgM to reveal μ chains as shown in autoradiograph; (*a* and *b*) 3- and 16-h exposure. A second sequential overlay of affinity-purified anti–mouse IgG-κ and ^{125}I-IgG-κ revealed κ-specific bands of ~25 kD (lanes 1–4) and a 56-kD band in lane *4* that presumably corresponds to γ chains. This is shown in (*c*) 16-h exposure. The positions of the molecular mass standards (92, 68, 43, and 25 kD) are indicated between each autoradiograph.

of C.B-17 normal mice (lane *2*). Interestingly, the 80-kD μ chains predominated in C.B-17 lymph node cells (lane *1*) while the 76-kD μ chains predominated in C.B-17 bone marrow cells (lane *3*). This striking disproportion in the distribution of the 80-kD and 76-kD μ chains was regularly observed and whether it reflects tissue-specific differences in the proportion (or glycosylation) of membrane and/or secreted forms of μ chains is not clear. What is clear, however, is that the μ-containing cells in the spleen of *scid*(Ig$^+$) mice appeared to resemble those found in normal bone marrow.

Longer exposure of the blot in Fig. 5*a* revealed additional bands corresponding to a molecular mass of ~53 kD and ~57 kD (see Fig. 5*b*). These bands represent truncated μ chains and were described in an earlier report (26).

A second sequential coating of the blot shown in Fig. 5*a* and *b*, with anti-γ/κ specific antisera and ^{125}I-IgG-κ revealed a prominent 56-kD band in the *scid*(Ig$^+$) spleen lysate (Fig. 5*c*, lane *4*). This putative γ-specific band was not apparent after the first sequential coating with anti-μ (see Fig. 5*b*, lane *4*) and it clearly distinguished *scid*(Ig$^+$) mice from C.B-17 normal mice as it was not detected in the control spleen lysate (compare lane *2* in Fig. 5*b* and *c*). Consistent with expectation, bands corresponding in size to L chains (25–27 kD) appeared in all lanes of Fig. 5*c*. These results suggest that an abnormally high quantity of IgG is being produced (or concentrated) in the spleen of *scid*(Ig$^+$) mice.

Attempted Clonal Expansion of Ig-producing Scid Cells and Apparent Absence of Functional B Cell Precursors in Bone Marrow. Attempts to expand Ig-producing cell clones of *scid*(Ig$^+$) mice involved the following kind of cell transfer experiments. Splenic cells of individual *scid*(Ig$^+$) mice were injected intravenously into

scid(Ig⁻) recipients; i.e., the equivalent of one donor spleen was equally divided and transferred into two recipients. Seven of nine such experiments failed to result in detectable Ig in the recipients. However, in two experiments, Ig production was seen in both pairs of recipients beginning at 3–4 wk after cell transfer, Ig-κ levels (1–2 mg/ml) remained relatively constant over the next 6–8 wk and then declined to <0.1 mg/ml at 16–18 wk after cell transfer.

In another series of experiments, 5–10 × 10⁶ bone marrow cells of *scid*(Ig⁺) mice were injected intravenously into x-irradiated BALB/c mice. We found that bone marrow cells of six individually tested mice were unable to generate detectable IgG-producing cells of donor allotype. Control recipients, which were injected with as few as 2 × 10⁵ bone marrow cells of C.B-17 normal mice, expressed donor IgG allotype within 3–4 wk after cell transfer.

Cellular Analysis of scid(Ig⁺) Mice

Histopathology.   *scid*(Ig⁺) mice retained the same fundamental histologic abnormalities described previously for *scid*(Ig⁻) mice (4, 5). The notable difference in *scid*(Ig⁺) mice was the finding of irregularly scattered foci of lymphocytes differentiating to plasmacytes. Plasmacytic foci were detected in 27 of 50 *scid*(Ig⁺) mice examined. The foci (illustrated in Fig. 6) were found solely in the spleen of 9 mice, in one or more lymph nodes of 6 mice, and in nodes and spleen of 10 mice. One mouse contained plasmacytic foci in thymus and spleen, and another in thymus, spleen, and lymph nodes. Most splenic and thymic areas of plasmacytosis were small, the major ones being nodal. In all foci, there was an intermingling of lymphocytes, intermediate plasmacytoid lymphocytes, and classic plasmacytes. In six cases, the plasmacytic cytoplasm contained Russell bodies (intracellular inclusions of Ig) (32–34).

Lympho-plasmacytic activity was not found in ~50% of the necropsied *scid*(Ig⁺) mice. This is not surprising, in that our search was limited to a few thin sections of assorted lymphatic tissues and many foci were small and unevenly distributed. It is important, however, to note that no plasmacytic focus was found in the many *scid*(Ig⁻) mice examined.

FACS Analysis.   Splenic cells of *scid*(Ig⁺) mice appeared markedly deficient in the expression of common lymphocyte antigens. For example, cells expressing surface μ chains were not detected and few (<5%), if any, Ly-1⁺ or Ly-5(B220)⁺ cells were found (see Table III). Seven *scid*(Ig⁺) mice were tested for the presence of Ly-5(B220), a pre-B and B cell marker present on 37–49% of the control splenic cells. All seven mice lacked detectable Ly-5(B220) even though three of the mice, numbered 5507, 4988, and 4976, had 11, 16, and 66% of their splenic cells stain positive for Ig, respectively. These Ig⁺ cells presumably corresponded to a population of Ly-5(B220)⁻ plasmacytes (28).

Mitogen Responsiveness.   Splenic cells of *scid* (Ig⁺) mice were as unresponsive to lymphocyte mitogens as splenic cells of *scid*(Ig⁻) mice. This is shown in Fig. 7 for the B and T cell mitogens, LPS and Con A, respectively. In 16 *scid*(Ig⁺) mice tested, the LPS stimulation index was ≤3.0 as opposed to 30–60 for normal C.B-17 control mice (data not shown). The same *scid*(Ig⁺) mice were also tested for Con A responsiveness. In 14 mice the Con A stimulation index was ≤3.0; the stimulation indices in the remaining 2 mice were 4.9 and 6.0. In contrast, control
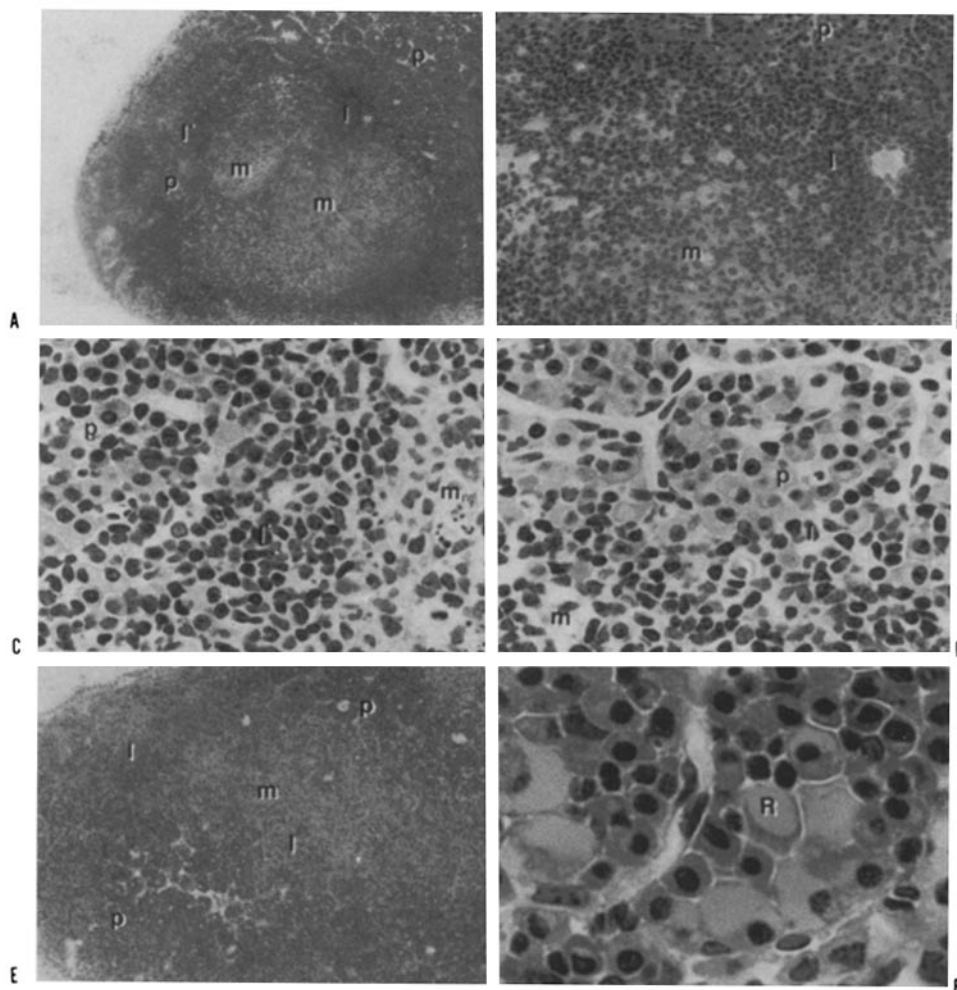
FIGURE 6. Cervical lymph nodes of scid(Ig$^+$) mouse showing multifocal sites of lymphocytes differentiating to plasmacytes with Russell bodies. (A) Two seemingly concentric or abutting areas (m), pallid by virtue of degenerating background cells and macrophage influx, each margined by a darker zone of lymphocytes (l) which blend with plasmacytes (p) that solidly occupy the remainder of the node (× 63). (B) Area of l in A distinguishes the three zones mentioned above. (× 252). (C) Area l' in A shows degenerated center on the right, with nuclear debris engulfed by macrophages (m), with lymphocytes (l) in the center, and plasma-cytoid lymphocytes (p) on the left (× 630). (D) Similar situation at the upper margin of B, with gradation from lymphocytes (l) to plasmacytoid lymphocytes (p) above. A macrophage (m) with engulfed cellular debris is seen in the lower left (× 630). (E) A second lymph node with changes similar to those noted in A. Nodes elsewhere were too small to harvest, and thymus and spleen were sparsely populated with lymphocytes, being similar to scid(Ig$^-$) mice (× 63). (F) Field from upper right of E, showing further maturation of plasmacytes with acquisition of Russell bodies (R) (× 1,250).

splenic cells of C.B-17 normal mice gave Con A stimulation indices ranging from 150 to 280 (data not shown). Splenic cells from six additional scid(Ig$^+$) mice (denoted with an x) gave comparable Con A stimulation indices expecting one mouse with an index of 21.0. LPS and Con A stimulation indices for scid(Ig$^-$)

TABLE III

Expression of Lymphocyte Surface Antigens on Splenic Cells of scid(Ig⁺) and Control Mice

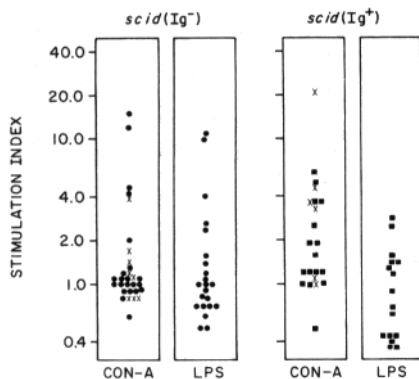| Mouse genotype	Mouse number	Serum κ	Cell surface markers (% positive cells)				
			μ	IgG(Fab)	B220	Thy-1	Ly1
		mg/ml					
C.B-17*scid*	3875	<0.01		4		11	
	3874	5.1		10		15	
	3879	4.7		9		13	
	4037	5.6			<1	10	
	4016	<0.01			<1	20	
	3427	<0.01		<1			
	4976	6.5		66	<1		
	4988	2.5		16	<1		
	5209	<0.01		<1	<1		
	5494	7.5	<1		2	2	2
	5407	5.0	3		3	4	5
	5498	6.5	<1	2	<1	3	6
	5507	10.6	<1	11	4	22	9
C.B-17*scid*/+	2973	1.8		44	37		
	2987	2.1		51	41		
	3011	1.6		45		31	
C.B-17(+/+)	2763	3.2	49	39	49	46	36
	2954	3.5	52	55	43	37	37

FIGURE 7. Proliferative response of spleen cells from individual *scid*(Ig⁻) and *scid*(Ig⁺) mice to Con A and LPS. The stimulation index is the ratio of [³H]-thymidine incorporation obtained with Con A or LPS to that obtained in medium alone. All values represent a single mouse. In most cases, each mouse was analyzed for both Con A and LPS responsiveness; some mice (x) were tested only for Con A responsiveness. The stimulation indices of control spleen cells (from C.B-17 normal mice) ranged from 150 to 280 in the case of Con A and from 30 to 60 in the case of LPS (data not shown).

mice were also generally negative (≤2.0). A few *scid*(Ig⁻) mice (<15%) showed indices ranging from 4.0 to 15.0.

Allograft Rejection. Although the preceding mitogen analyses failed to indicate the presence of functional T cells in the spleen of *scid*(Ig⁺) mice, such cells apparently did arise in *scid*(Ig⁺) mice. As shown in Table IV, many *scid*(Ig⁺) mice were able to reject allogeneic skin grafts. 14 *scid*(Ig⁺) and 34 *scid*(Ig⁻) mice were grafted on one flank with full-thickness allogeneic skin grafts from (C.B-17 × C3H/HeJ)F₁, or C3H/HeJ mice and on the other flank with syngeneic (control) grafts from *scid*(Ig⁻) or C.B-17 normal mice. 7 of the 14 *scid*(Ig⁺) mice completely rejected their allogeneic grafts in 12–33 d; the mouse that took 33 d rejected a second allogeneic graft in 12 d. 3 of the 14 mice appeared to reject part of their allogeneic graft; i.e., necrosis was observed in the middle of the graft and yet the

TABLE IV
Survival of Allogenic Skin Grafts on scid(Ig⁻) vs. scid(Ig⁺) Mice

Exp.	Recipients*	Number grafted	Number of mice with grafts			Elapsed time for complete rejection[‡]
			Accepted	Partially rejected	Completely rejected	
						d
	scid (Ig⁻)					
1		8	8			
2		7	7			
3		14	12	1	1	12
4		5	5			
Total		34	32	1	1	
	scid (Ig⁺)					
1		1			1	33[§]
2		3	1		2	13, 22
3		5	1	1	3	12, 14, 14
4		5	2	2	1	17
Total		14	4	3	7	

* C.B-17scid mice were each grafted with skin of C.B-17 or C.B-17 scid mice (control grafts) on one flank and on the other flank with skin of (C.B-17 × C3H/HeJ) F1 (exp. 1 and 3) or C3H/HeJ mice (exp. 2 and 4). The mice in experiments 1 and 3 were grafted and maintained within a barrier facility as SPF mice.
[‡] The mean time for allograft rejection by normal C.B-17 mice was 14 ± 3 d.
[§] This mouse rejected a second graft in 12 d.

remaining portion of the graft persisted and grew hair. We denote this as incomplete rejection (see Table IV) rather than technical failure since this kind of result was not observed for any of the control syngeneic grafts. In contrast to scid(Ig⁺) mice, only 1 of 34 grafted scid(Ig⁻) mice completely rejected its allogeneic graft and only 1 showed evidence of incomplete rejection. The possibility that these two mice became Ig⁺ during the course of the experiment cannot be excluded because they unfortunately were not retested. Since alloreactive antibodies could not be demonstrated in the serum of scid(Ig⁺) mice (data not shown) and since the rejection of allogeneic skin grafts is a T cell–dependent process (35), the above data indicate that most scid(Ig⁺) mice contain functional T cells.

T Cell Lymphomas. Unlike their normal counterpart, scid mice are prone to develop lymphomas (4). Spontaneous lymphomas have been detected in ~15% of necropsied mice, they appear to arise in the thymus, and all examined to date type as T cell lymphomas (reference 5 and our unpublished results). As indicated in Table V, a disproportionate number of these lymphomas occur in scid(Ig⁺) mice. 13 of 41 necropsied scid(Ig⁺) mice (32%) were found to contain thymic lymphomas as opposed to 9 of 109 mice (8.3%). Both groups of mice ranged from 3 to 5 mo old. In an older group of scid(Ig⁺) mice, more than half of the mice (10/17) had thymic lymphomas.

Discussion

Evidence for Pauciclonal Ig-producing B Cells in scid(Ig⁺) Mice. As deduced from the preceeding results, most 3–9-mo-old scid(Ig⁺) mice appeared to contain ~1–3 clones of Ig-producing B cells. IEF of serum L chains from individual

TABLE V

Incidence of Thymic Lymphoma in scid(Ig⁻) and scid(Ig⁺) Mice

Mouse phenotype	Age range	Number of mice		Lymphoma incidence
		Necropsied	With lymphoma	
	mo			%
scid(Ig⁻)	3–5	105	9	8.2
scid(Ig⁺)	3–5	41	13	31.7
scid(Ig⁺)	5–9	17	10	58.8

scid(Ig⁺) mice showed very restricted IEF patterns which, in most cases, consisted of 4–10 bands. Equally restricted IEF patterns were obtained with L chains of 1–3 different myeloma proteins. Each *scid*(Ig⁺) mouse gave a distinct IEF pattern suggesting considerable diversity of B cell clones in the *scid*(Ig⁺) mouse population. In most individuals, a given IEF pattern persisted throughout the period of observation (2–5 mo) with little or no change. Thus, the appearance of new B cell clones was apparently minimal.

Recent analysis of splenic hybridomas from two *scid*(Ig⁺) mice has also indicated a paucity of Ig-producing B cell clones (36). 16 Ig-producing hybridomas were obtained from 1 mouse, 2 of which produced IgM and 14 of which produced IgG2b. Sequence analysis of the IgM-producing hybridomas along with 2 randomly picked IgG2b-producing hybridomas showed that all used Vh3609, Jh2, and a common D region. All 18 Ig-producing hybridomas obtained from another mouse produced IgG2b. Sequence analysis of two such hybridomas showed that both expressed the same Ig heavy chain variable region (Vh7183, Jh4, and DQ52) in addition to sharing common mutations. Apparently, the spleen in each of the above two mice contained only a single clone of Ig-producing plasmacytes. Interestingly, proliferating B cell clones were not evident in the spleens of some *scid*(Ig⁺) mice as no Ig-producing hybridomas were obtained (Solvason N., M. Fried, M. J. Bosma, and J. F. Kearney, unpublished results). This implies that the tissue distribution of Ig-producing plasmacytes is uneven and limited to a few lymphatic sites.

Our histologic findings support the above inference. Plasmacytic foci—consisting of lymphocytes, intermediate plasmacytoid lymphocytes, classic plasmacytes and, in some cases, plasmacytes with Russell bodies—were found in 27 of 50 *scid*(Ig⁺) mice examined. Usually, the foci were detected in only one or two lymphoid tissues of a given mouse. For example, in five mice where several lymph nodes were available for study, one or more nodes showed robust plasmacytosis while others were dissimilar, displaying lymphocytic depletion or hyperplasia without plasmacytic differentiation or early lymphomatous change. Even within a tissue, such as the spleen, foci were distributed irregularly; i.e., foci were detected in some but not all follicles or were sparsely scattered in the perifollicular red pulp. We interpret the restricted and patchy tissue distribution of plasmacytic foci as a reflection of multiple "hit and miss" cloning of a limited number of cell progeny from one or more lymphoid ancestor cells.

Ig class switching by individual B cell clones was not impaired as multiple

isotypes were detected in ≥90% of *scid*(Ig$^+$) mice. Most mice, however, lacked two or more of the six major serum Ig classes (IgM, IgG3, IgG1, IgG2b, IgG2a, and IgA); those classes most often absent corresponded to the most downstream H chain constant region genes (i.e., IgG2a and IgA) (37). Total Ig-κ concentrations in *scid*(Ig$^+$) mice ranged from 0.1 to >10.0 mg/ml. Ig-κ concentrations in a given mouse generally increased with time and often approached those found in plasmacytoma-bearing C.B-17 mice (our unpublished results). However, neither plasmacytomas nor any other kind of B cell malignancy have been detected in *scid*(Ig$^+$) mice. Therefore, we seem to be dealing with unregulated Ig production by a few "normal" B cell clones. Naturally occurring antigens may play an important role in the activation and clonal expansion of these B cell clones, because the appearance of *scid*(Ig$^+$) mice was environmentally dependent (see Table I). Preliminary results indicate that some B cell clones may be autoreactive. For example, hybridoma antibodies reactive to nuclear antigens were obtained from one of the two *scid*(Ig$^+$) mice cited earlier (36). Also, anti-DNA antibodies have been detected in the serum of many *scid*(Ig$^+$) mice (A. D. Steinberg and M. J. Bosma, unpublished results).

Finally, the association of plasmacytosis and Russell body formation with hypergammaglobulinemia and autoreactive Ig deserves comment as this association also has been observed in various lymphoproliferative disorders and hyperimmune states. For example, plasmacytes with intracellular inclusions of Ig (Russell bodies) have been observed in myeloma patients (32), in germinal centers of patients with AIDS (38), in chronic inflammatory lesions (33), in hyperimmune animals (39), and in mice with lymphoproliferative and autoimmune diseases including the immune-deficient "viable motheaten" mouse (40, 41). In view of these observations and the results discussed above, we suggest that the development and persistence of the few Ig-producing B cell clones in *scid*(Ig$^+$) mice may be in response to chronic stimulation by self antigens and/or opportunistic microorganisms.

Indication of T Cells in scid(Ig$^+$) Mice. That *scid*(Ig$^+$) mice contained functional T cells in addition to Ig-producing B cells was evident from their ability to reject allogenic skin grafts, a T cell–dependent process (35). The presence of functional T cells has been recently confirmed by one of us (A. Carroll) by selectively growing clones of alloreactive T cells from the spleen of individual *scid*(Ig$^+$) mice. These clones are IL-2 dependent and show TCR-β gene rearrangements (unpublished results).

About half of the grafted *scid*(Ig$^+$) mice completely rejected their H-2^k allografts (see Table IV). This suggests that many *scid*(Ig$^+$) mice may contain ~20–40 functional T cell precursors, assuming that 1 in every 20 precursors can recognize a given foreign MHC haplotype (42, 43) and that the progeny of 1–2 precursors is sufficient to mediate allograft rejection. Nonetheless, the T cell repertoire must be very restricted since half of the grafted *scid*(Ig$^+$) mice failed to reject (or completely reject) their allografts. Experiments to address the issue of T cell clonality are currently underway.

Additional evidence for the development of T cells was the high incidence of T cell lymphomas in *scid*(Ig$^+$) mice. Approximately 40% of 3–9-mo-old *scid*(Ig$^+$) mice (23 of 58 necropsied mice) had thymic lymphomas. Whether these represent

transformed leaky T cells is not clear, however, because the majority of rearranged TCR-γ and TCR-β alleles in such lymphomas are aberrantly rearranged (17, 19) and most transformants would be expected to lack a functional T cell receptor. Possibly, most of the T cell lymphomas are secondary to the conversion of scid(Ig$^-$) into scid(Ig$^+$) mice; i.e., the growth and progression of incipient lymphomas may be promoted by the leaky T cells and their products.

Possible Implications of Scid(Ig$^+$) Mice. Despite the indicated presence of Ig-producing plasmacytes and alloreactive T cells, most scid(Ig$^+$) mice retained the same general histologic pattern of severe lymphocytic deficiency seen in scid(Ig$^-$) mice (5). Splenic cells lacked Ly-5(B220), Ly-1, and surface μ, and responded marginally, if at all, to LPS and Con A. Bone marrow cells lacked detectable intracellular μ chains and were unable to generate Ig-producing B cells after transfer into x-irradiated BALB/c mice. We conclude that the cellular event(s) responsible for the appearance of functional lymphocytes in scid(Ig$^+$) mice occurs infrequently and may result in uncontrolled terminal differentiation of the affected cells because resting lymphocytes, responsive to lymphocyte mitogens and/or expressing common lymphocyte surface antigens, were not detected.

Two explanations for the appearance of functional lymphocytes in scid(Ig$^+$) mice can be considered. Both assume that recombination of antigen receptor genes in scid mice is highly error prone resulting in the premature death of developing lymphocytes for lack of a functional antigen receptor. This does not preclude that the scid mutation could also result in a very low frequency of gene recombination such that the rate of lymphoid differentiation is severely reduced. The first explanation postulates that defective scid lymphocytes occasionally make (by chance) productive gene rearrangements at two critical antigen receptor loci (e.g., H and L chain or TCR-β and TCR-α). These rare cells with functional antigen receptors would presumably arise in all scid mice but remain latent until activated and clonally expanded as a result of chance encounter with the appropriate naturally occurring antigens. Most of the nonproductive gene rearrangements in these functional cells would be expected to consist of abnormal J-associated deletions similar to those previously described in transformed scid lymphocytes (17). The second explanation postulates a low rate of cell reversion such that developing scid lymphoid cells occasionally revert to normal in which case a fraction of the progeny cells go on to make two productive gene rearrangements at the appropriate Ig and TCR loci. Genetic reversion at either scid allele would presumably suffice to normalize a given cell. Lymphocyte progeny of these reverted cells would be expected to show conventional gene rearrangements as opposed to rearrangements with aberrant J-associated deletions.

Given either of the above explanations, how does one account for the joint presence of B and T cells in scid(Ig$^+$) mice? If the cellular events responsible for scid(Ig$^+$) mice were to occur infrequently and independently in early B and T cells, as required by the first explanation, then one must argue that the development of Ig-producing scid B cells is highly dependent on the presence of functional T cells and that screening for Ig$^+$ mice necessarily selects for mice with both cell types. Conversely, one would also have to argue that T cell development is dependent on the presence of B cells as there was no clear-cut

evidence of functional T cells in $scid(Ig^-)$ mice (see Table IV). The second explanation allows that developing *scid* lymphocytes may revert to normal at any stage of lymphoid ontogeny; e.g., before or after the commitment of lymphocytes to the B or T cell pathway. However, the detection of reversion events before commitment presumably would be favored as this would result in more functional progeny of both B and T cell types. This assumes that lymphoid stem cells have bipotentiality.

Clearly, the nature of the cellular event responsible for $scid(Ig^+)$ mice remains to be elucidated. The average number of such events per $scid(Ig^+)$ mouse and the time of their occurrence in lymphoid cell ontogeny will hopefully be resolved in future experiments with chimeric $scid(Ig^+)$ mice containing genotypically distinct and genetically marked populations of hematopoietic *scid* cells.

Summary

Although the majority of severe combined immune deficiency (*scid*) mice lack functional lymphocytes, some (2–23%) appear to develop a limited number of B and T cells between 3 and 9 mo old. Most of these leaky *scid* mice were shown to contain very few clones (≤ 3) of Ig-producing plasmacytes. Clonal progeny were distributed unevenly in the lymphatic tissues and appeared as discrete plasmacytic foci. In many cases, individual clones persisted for several months and produced abnormally high concentrations of Ig that included multiple isotypes. Functional T cells were inferred from the ability of leaky mice to reject allogeneic skin grafts, a T cell–dependent reaction. Interestingly, ~40% of leaky mice developed thymic lymphomas. In other respects, leaky mice resembled regular *scid* mice; e.g., their splenic cells failed to express common lymphocyte antigens (Ly-5[B220], Ly-1) and to proliferate in response to lymphocyte mitogens. Histologically, their lymphoid tissues retained the same general pattern of severe lymphocytic deficiency as *scid* mice.

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