B-less: a Strain of Profoundly B Cell-deficient Mice Expressing a Human λ Transgene

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Summary

We have created several transgenic mouse strains that bear the human λ light chain gene driven by its own promoter and a mouse immunoglobulin heavy chain enhancer. The transgene is expressed in many tissues, with particularly high levels of expression in the bone marrow, thymus, spleen, and lymph nodes. One of these transgenic lines, *B-less*, displays a dramatic phenotype characterized by an acute susceptibility to bacterial and viral infections. Analysis of this strain shows it to be profoundly deficient in both immature (pre-B) and mature B cells, as well as in circulating immunoglobulin. The pre-B and B cell defects are cell autonomous, as judged by cell culture and bone marrow graft chimeras. Despite this B cell deficiency, the T cell lineage appears grossly normal as assessed by flow cytometric analysis and by its response to mitogen stimulation. Since an independently derived transgenic strain bearing the same human λ construct displays a partial *B-less* phenotype, it is likely that the B lineage deficiency is due to a dominant effect of transgene expression rather than to the insertional perturbation of an endogenous mouse gene. It is interesting that the deficiency phenotype is fully expressed in the FVB/N genetic background, but is suppressed in F_1 hybrids formed between the FVB/N and C57BL/6 inbred strains. Evidently, there are one or more dominant genetic suppressors of *B-less* in the C57BL/6 genome.

Ig genes undergo rearrangement in a precisely ordered fashion in which recombination of H chain gene segments precedes that of L chains, and recombination of κ chain gene segments precedes that of λ (1). The mechanism responsible for this ordered rearrangement is not known, but is thought to be related to transcriptional controls in which cryptic promoters close to the separate germ line gene segments become activated in an ordered fashion (2). This activation is thought to be necessary for Ig gene recombination and to be governed by the rules of allelic exclusion, a process by which only one H chain gene can functionally recombine in each B cell, and by the related rules of isotype exclusion, by which only one L chain gene can rearrange functionally in each B cell. The available data from Ig transgenic mice suggest that the presence of functional Ig transgenes in developing B cells results in allelic and isotypic exclusion of the corresponding endogenous Ig alleles. H chain transgenes inhibit rearrangement of endogenous H chain genes, and L chain transgenes inhibit rearrangement of endogenous L chain genes (3). Further, it is the membrane-bound form of the Ig H chain that is required to exclude the expression of endogenous alleles, and not the secreted version of the H chain (4, 5).

As might be expected, mice bearing Ig H or L chain transgenes have significant perturbations of B cell populations (6, 7). For example, L chain transgene expression appears to result in the reduction of early pre-B cells, suggesting that L chain transgenes may interfere with very early stages of B cell development (8). Although a slight impairment of the B cell response to bacterial antigens has been observed (9), no dramatic immunodeficiency has been reported for any of these mice (3). It is therefore likely that these exclusionary rules are sufficiently "leaky" to permit the escape of sufficient numbers of B cells, so as to preserve a functional immune response in such animals.

Our experiments were designed to test further features of these exclusionary rules, and to explore the transcriptional controls that govern the expression of λ L chain genes. Accordingly, we prepared a fusion transgene consisting of a rearranged human λ L chain gene artificially activated by an Ig H chain enhancer. Several transgenic animals bearing this construct were derived and one of these displayed a profound immunodeficiency and sensitivity to infectious disease. Since immunodeficient mice have proven to be valuable experimental models for elucidating the roles of specific cell types in the immune response, we have characterized this line and speculated regarding the molecular pathogenesis of this defect. Several B cell deficiencies have been described in mutant mice. However, unlike our example, none provides a complete,

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specific elimination of all stages of B cell lymphopoeisis (10, 11). For example, an interesting transgenic mouse expressing the interferon response factor 1 (IRF-1)[1] transgene is also severely deficient in mature B cells, but retains significant numbers of bone marrow B cell precursors (12). A second immunodeficient transgenic mouse has been derived by targeted disruption of the membrane exon of the Ig μ chain gene (13). These animals, though clearly B cell deficient, appear to have normal numbers of bone marrow pre-B cells. By contrast, the *B-less* strain reported herein is profoundly deficient in B cells of the earliest identifiable stages.

Materials and Methods

Transgenic Mice. The complete DNA sequence of the rearranged human λ gene from the U266 cell line is available in the EMBL Data Library under the accession number X51754. Detailed structural information on this gene was presented previously (14). The human λ clone consists of an 8.5-kb EcoRI to HindIII fragment, containing the Vλ pseudogene, Vλ1, as well as the active U266λ gene rearranged with the λ_2 constant region gene. To prepare the λ plasmid, we inserted the 976-bp XbaI fragment containing the mouse Ig H chain enhancer into the HindIII site downstream of the λ gene by blunt end ligation as shown in Fig. 1. The 9.5-kb fragment containing the U266λ gene, and the fragment containing the Eμ (the murine μ H chain enhancer) were isolated after EcoRI digestion. DNA fragments for microinjection were excised from the plasmids and purified by electroelution after agarose gel electrophoresis. The fragments were injected into fertilized oocytes derived from the FVB/N × CD-1 mice (EG) or FVB/N (EH, EO, ET, and EZ) as described by Muller et al. (15). FVB/N mice are albino (c), agouti (A), H-2^q, and have the b alleles of Lyt-1–3. All transgenics are maintained by back-crossing male hemizygotes with +/+ FVB/N females. The EG strain (referred to as *B-less* in this text) has currently been back-crossed with FVB/N for over ten generations. This strain has been formally designated as Tg(O; HSA, IGL)1LED and is registered at the Jackson Laboratory, Bar Harbor, ME. Unless otherwise specified, all experiments with the *B-less* animals involve EG/+ hemizygous mice and +/+ wild-type littermates between four and ten wk of age. EG/EG homozygotes seldom live beyond weaning, and offspring raised by *B-less* mothers are generally unhealthy.

The Eμ-c-*myc* transgenic strain, EB, contains the human c-*myc* gene with the mouse Ig H chain enhancer inserted into the first c-*myc* intron (16).

Cell Lines and Culture. The λ/Eμ-c-*myc* cell line, EG/EB10298, is one of four such cell lines isolated from B cell lymphomas that arose in mice bearing both transgenes. This cell line was originally cultured from the tumor on bone marrow feeder layers (17), and later was weaned from the feeder to grow in RPMI supplemented with 10% FCS. Ableson murine leukemia virus–(A-MuLV)-transformed lines were derived from 14–17 d postcoitum (dpc) fetal liver cells, as described for bone marrow cells by Rosenberg and Baltimore (18).

DNA and RNA Analysis. DNA extraction and blot analyses were carried out essentially as described by Maniatis et al. (19) using the 1.0-kb BamHI fragment containing the U266λ constant region as a probe (see Fig. 1 *A*). RNA was extracted by disrupting cells and tissues in a solution of 4 M guanidine isothiocyanate plus 0.1 M 2-ME and centrifugation through CsCl (20). RNase protection analyses were performed essentially as in Melton et al. (21). The probe used for RNA analyses is a uniformly labeled RNA complementary to the U266λ V region, and it protects a 344-nucleotide fragment (14). Solution hybridizations were performed in the presence of 80% formamide at 50°C for 4–12 h and the protected fragments were analyzed on 5% acrylamide/urea sequencing gels.

The rearrangement status of the Ig genes in the A-MuLV transformed cell lines was determined by Southern blotting genomic DNA from the cells after restriction digestion with BamHI and EcoRI. The blots were sequentially probed with random hexamer-labeled DNA fragments containing J_H, C_κ, and U266 V_λ sequences.

Protein Analyses. 200 μl of blood was removed via the retroorbital plexus of anesthetized mice for preparation of serum (and nucleated cells for DNA analysis). The concentrations of mouse Ig and human λ were determined by sandwich immunosorbant assay (22). Mouse sera or detergent extracts from tumor cell lines and A-MuLV-transformed cell lines were adsorbed to microtiter assay plates with either goat anti-mouse Ig or goat anti-human λ. The same antibodies, conjugated with alkaline phosphatase, were then bound to the adsorbed proteins for subsequent development and colorimetric determinations.

Flow Cytometry. Single cell suspensions prepared from lymphoid organs were either directly or indirectly stained with FITC-conjugated antibodies. The antibodies used were: FITC-conjugated mouse anti–human λ (free and bound, Nordic Laboratories, Capistrano Beach, CA); FITC-conjugated monoclonal anti–mouse κ (MRCox20; Bioproducts for Science, Inc., Indianapolis, IN); FITC-conjugated monospecific goat anti–mouse μ H chain (Southern Biotechnology Associates, Inc., Birmingham, AL); monoclonal rat anti–mouse B220 (Ly-5) (6B2, [23, 24]); monoclonal rat anti–mouse Sca-I (Ly-6A.2, a gift from Irving Weissman, Stanford University, Stanford, CA [25]); hamster anti–mouse T3 (clone 2C11, [26]); FITC-conjugated rat anti–mouse L3T4 (CD4) and Lyt2 (CD8) (Becton Dickinson & Co., Mountain View, CA). Unconjugated rat mAbs and hamster antibodies were labeled with FITC-conjugated goat anti–rat Ig or goat anti–hamster antisera (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD). The labeled cells were analyzed on a Cytofluorograf IIS (Ortho Diagnostic Systems Inc., Westwood, MA).

Immunohistochemical Analysis. Immunohistochemical analysis was performed with 24-μ sections cut from OCT-(Miles Laboratories, Inc., Elkhart, IN) embedded frozen spleens. Sections were fixed in acetone and blocked with normal goat serum. Hybridoma supernatants 6B2 (anti-B220 [Ly5]) or M5/49 (anti-Thy-1.1) were applied to the sections followed by a second biotinylated anti-rat antibody (Kirkegaard and Perry Laboratories, Inc.). The stained regions were visualized with the Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA), which uses avidin/biotin-conjugated horseradish peroxidase (HRP), and 3-amino-9-ethylcarbazole (AEC). After development, slides were quenched in 4% formaldehyde. Sections were counterstained with Lerner-1 hematoxylin (Lerner Laboratories, Pittsburgh, PA), and mounted with Glycergel (Dako Corp., Carpinteria, CA).

Bone Marrow Transplantation. For bone marrow transplantation, recipient mice were lethally irradiated in a Gammacell 40 ^{137}Cs source (Atomic Energy of Canada, Ltd.). Mice received a split dose

[1] *Abbreviations used in this paper:* AEC, 3-amino-9-ethylcarbazole; A-MuLV, Ableson murine leukemia virus; GAR-IG, goat anti–rat Ig; GAH-IG, goat anti–hamster Ig; HRP, horseradish peroxidase; IRF-1, interferon response factor 1; SPF, specific pathogen-free; XLA, X-linked agammaglobulinemia.

of 800 and 400 rad separated by 2–4 h. Within 24 h of irradiation, mice were injected via the tail vein with 0.2 ml of a suspension containing 10^7 bone marrow cells/ml (27). To facilitate detection of wild-type bone marrow, some experiments used marrow from the TG.XA transgenic mouse strain (referred to as wt*), an otherwise wild-type FVB/N mouse bearing an easily detected lacZ transgene (a gift of Radek Skoda).

Mitogen Stimulation Assays. Mitogen stimulation assays were performed as described in Mishell and Shiigi (28). Single-cell splenocyte suspensions were prepared and samples were transferred to wells of microtiter plates containing LPS, Con A, or control medium and incubated for 68 h. Cultures were pulsed with [^{3}H]thymidine (New England Nuclear, Boston, MA) for 6 h and ^{3}H uptake was determined.

Results and Discussion

Widespread Expression of Human λ Fusion Gene in Transgenic Mice. In the course of studies of Ig gene expression, we generated five strains of transgenic mice that express the λ construct shown in Fig. 1 *A*. This construct contains an intact human λ L chain gene and the intronic enhancer (Eμ) from the mouse Ig H chain gene. Such a construct might have been expected to be expressed specifically in lymphoid and hematopoietic tissues. However, while the highest levels of human λ mRNA expression are indeed found in these tissues (Fig. 1 *B*) (bone marrow, thymus, and spleen; note that the breast tissue preparation includes lymph node), transgene mRNA is detectable to an appreciable extent in all tissues tested (including liver, visible on a longer exposure). Since human λ protein can be detected in the serum and urine, as well as within lymphoid cells of transgenic mice (29, and see below), we conclude that this mRNA is functional.

The unanticipated ectopic transgene expression we observe is unlikely to be due to lymphoid cells that are fortuitously present in nonlymphoid tissue samples. RNA samples from diverse tissues derived from nontransgenic mice contain very little mouse κ mRNA (29) indicating levels of lymphocyte contamination that are too low to account for the levels of human λ expression seen in these transgenic mice (29). Further, all the λ transgenic strains display similar tissue expression patterns, suggesting that such widespread expression is not an idiosyncrasy of the site of insertion of the transgene (29). Although an instance of Ig H chain transgene transcription in the brain has been reported (4), such expression is generally limited to the lymphoid system (30–32). One interpretation of these results would be that the transgene used in these studies lacks putative negative regulatory elements normally encoded in the vicinity of rearranged λ L chain genes. It may be necessary to exclude these sequences to gain expression of this gene in nonlymphoid tissues.

Susceptibility to Infectious Disease. Hemizygous carriers of the fusion transgene in one of the λ strains (EG) displayed a striking phenotype involving susceptibility to infectious disease. The average life expectancy of this strain in an animal colony containing several endemic pathogens was 90 d, whereas wild-type FVB/N mice (held for cancer incidence studies) frequently live for 2 yr in this environment. Deaths in the EG strain were largely due to acute pneumonia caused by a combination of Sendai virus and bacterial or mycoplasma

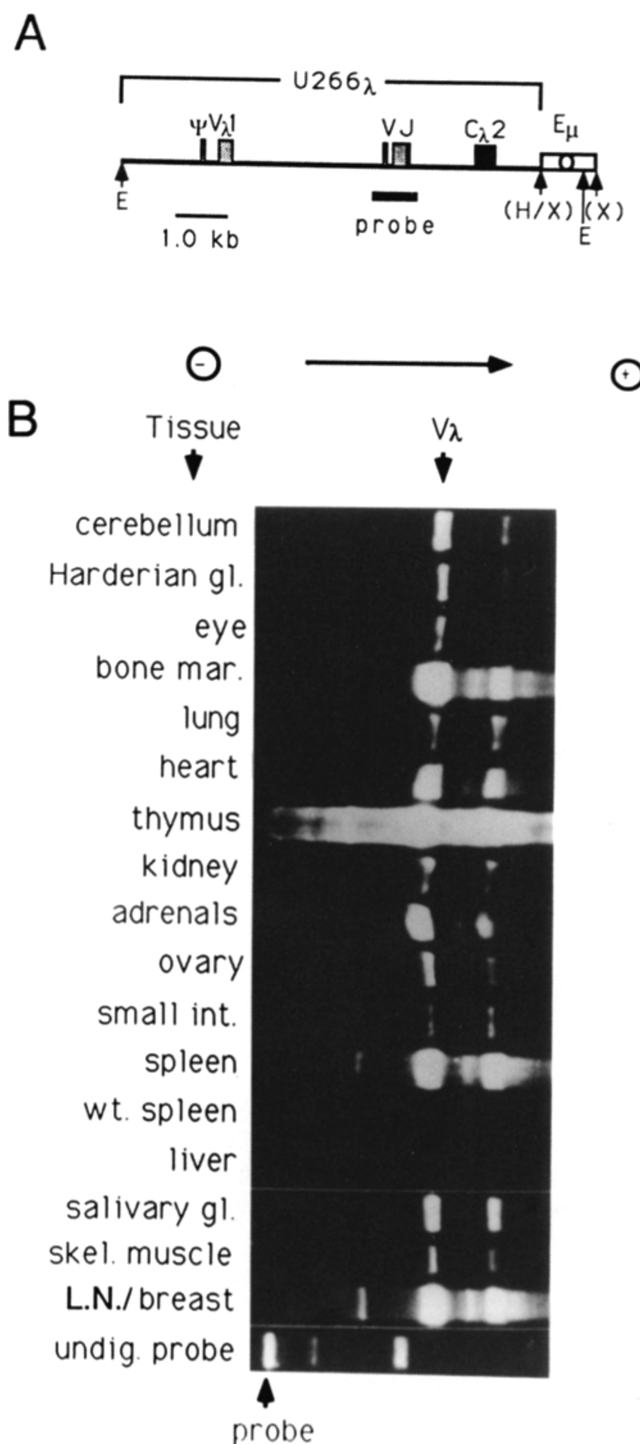


Figure 1. (*A*) Diagram of the DNA construct used to generate λ transgenics. (*B*) RNase protection analysis of human λ transcription in *B-less* transgenic mice. 10-μg samples of RNA were hybridized to a uniformly labeled RNA probe complementary to the variable region of U266λ mRNA, digested with RNase, and electrophoresed on a 5% sequencing gel. The negative control is wild-type spleen (*wt. spleen*). (*Bottom*) *Undigested probe*, 783 nucleotide. Direction of migration is to the right. 344-nucleotide protected Vλ fragment is indicated by (↑).

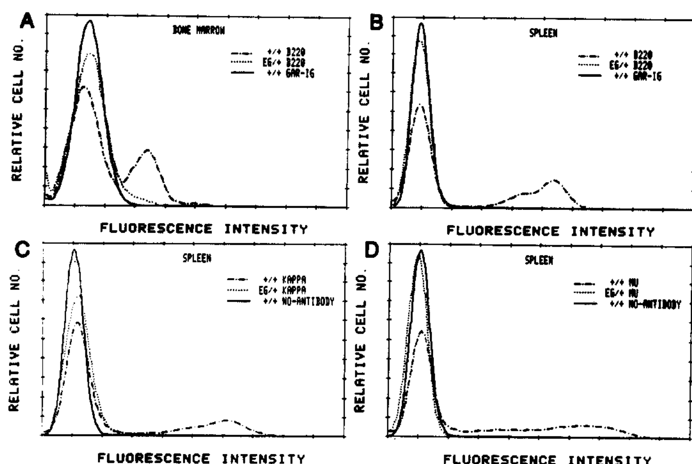


Figure 2. Flow cytometric analysis of B cells in bone marrow and spleen of wild-type and λ transgenic mice. (*A*) and (*B*) Ly-5 (B220) staining of bone marrow and spleen cells. (*C*) and (*D*) mouse κ and μ staining of spleen cells. See Table 1 for percent antigen-positive cells. Cells were labeled with the rat anti-mouse B220 antibody (6B2) and FITC-conjugated goat anti-rat antibody, or with FITC-conjugated rat anti-mouse κ (MRCox-20) or goat anti-mouse μ. Control cells received only goat anti-rat antibody (*A* and *B*) or no antibody (*C* and *D*). GAR-IG, goat anti-rat Ig.

infections. Often there was evidence of hepatic necrosis and central nervous system demyelination, indicative of mouse hepatitis virus infection. In contrast, hemizygous EG/+ mice raised under specific pathogen-free (SPF) conditions display none of these pathologies and live nearly normal life spans. Notwithstanding the widespread expression of the transgene, all other organ systems (except the B cell compartments referred to below) of the pathogen-free EG mice appeared to be normal. Given this susceptibility to infectious disease, SPF EG/+ mice were used in the studies that follow. Furthermore, although the mice characterized below were generally 4–10 wk old, similar results were obtained with mice ranging from 3 wk to 2 yr of age.

Immunodeficiency in EG Transgenics. The pronounced sensitivity to infectious disease of the hemizygous EG animals suggested an immunodeficiency state. Accordingly, we evaluated the immune system in that strain in comparison with wild-type littermates. In addition to the EG mice, another of our λ transgenic lines (ET) has a reduced B cell population (see below). However, none of our other λ transgenic mice (nor other Ig transgenics reported to date [3]) are unusually susceptible to infection, nor do they display the severe immunodeficiency phenotype seen in the EG strain. Spleens of mature EG/+ mice are morphologically normal, but they are 50–75% smaller than normal, containing 1–5 × 10^7 cells as compared with 0.8–1.2 × 10^8 cells in the spleens of their wild-type littermates.

The gross findings were confirmed at the cellular level using flow cytometric analysis. Cells bearing the pre-B and B cell marker, B220 (LY5), were significantly reduced in EG bone marrow (27 vs. 6.5%) and spleen (40 vs. 2.1%) (see Table 1) (24, 33, 34). The spleens of EG/+ mice contain 95% fewer B220 $(Ly5)^+$ cells than spleens from wild-type control mice (Table 1), while spleens from homozygous EG/EG mice dis-

Table 1. *B Cell Surface Antigen-positive Cells in Lymphocytes from Spleen and Bone Marrow of Normal and B-less mice*

Tissue	wt (FVB/N)	EG (FVB/N)	EG (FVB/N × C57BL/6)
Spleen			
B220 (Ly5)	40 ± 7.1 (8)*	2.1 ± 0.97 (14)	11 ± 1.4 (2)
Mouse κ	37 ± 11 (11)	2.3 ± 2.0 (14)	7.4 ± 0.1 (2)
Mouse μ	39 ± 11 (8)	1.6 ± 0.93 (6)	13 ± 1.5 (2)
Bone marrow			
B220 (Ly5)	27.2 ± 2.4 (6)	6.5 ± 1.7 (6)	ND

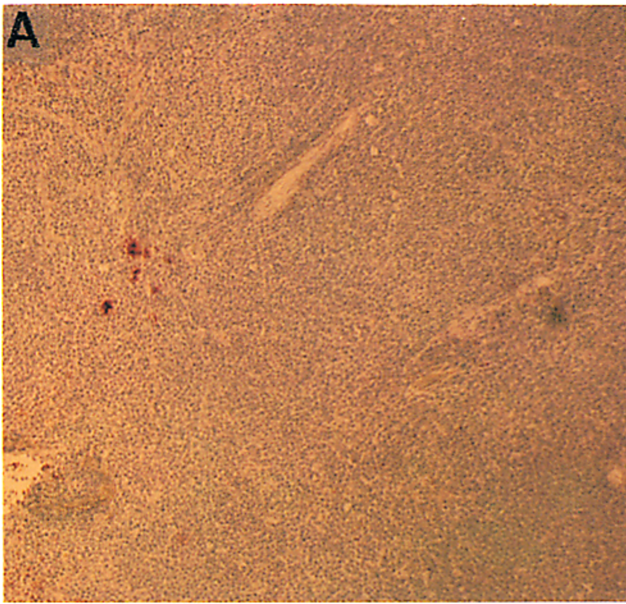
* Mean percentage ± SD (number tested).

Table 2. *Serum Ig (sIg) in Bone Marrow Chimeras*

Donor	Host	sIg	Time post-transplant
		mg/ml	*d*
wt	EG	1.0 ± 0.27	60
EG	wt	0.37 ± 0.21	60
wt*	wt	2.0 ± 0.8	60
EG	wt	1.0 ± 0.59	80
wt*	EG	4.5 ± 2.5	80

The mean and SD are shown. Each group contains either five or six animals. Controls: wt, 4.2 ± 1.4 mg/ml; EG, 0.033 ± 0.015 mg/ml.
* Phenotypically normal line containing a *lacZ* transgene.

Figure 3. Immunohistochemical analysis of frozen sections of spleens from wild-type (*A*, *C* and *E*) and *B-less* (*B*, *D*, and *F*) animals. Sections were stained with either no primary antibody (*A* and *B*), anti-B220 (*C* and *D*), or anti-Thy-1.1 (*D* and *E*) followed by biotinylated goat anti-rat Ig and HRP avidin/biotin complex. Finally, the sections were incubated with AEC, which produced the red signal, and counterstained with hematoxylin.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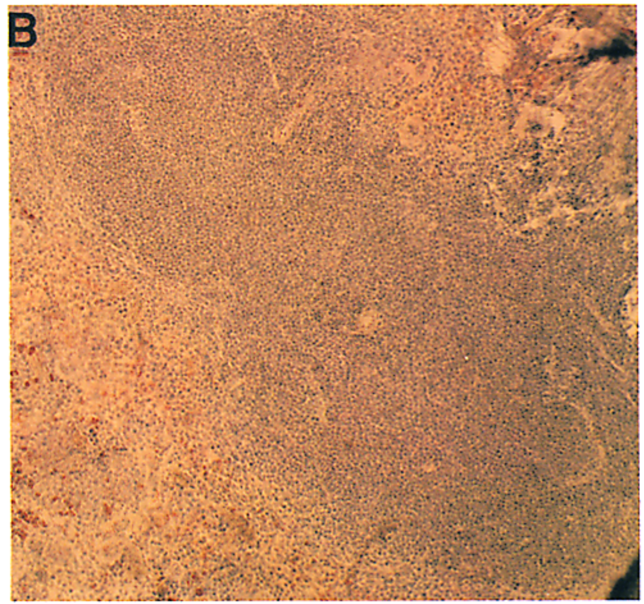
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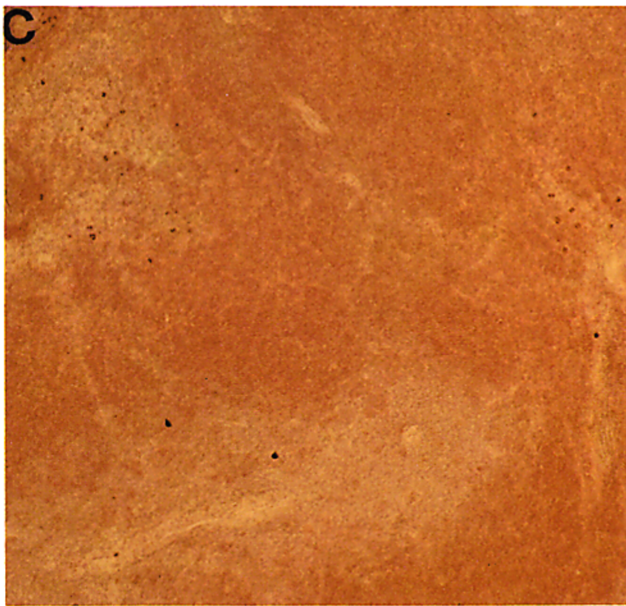
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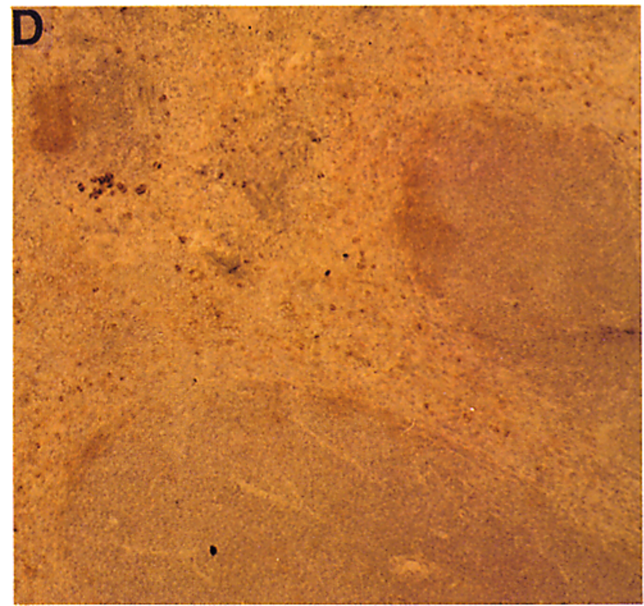
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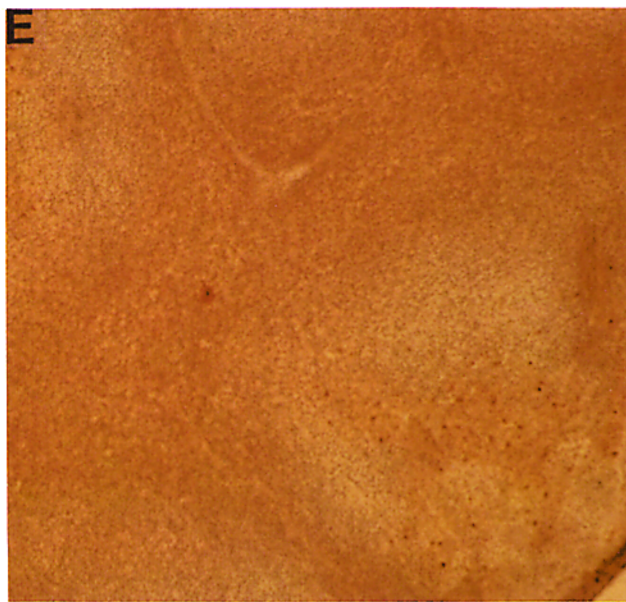
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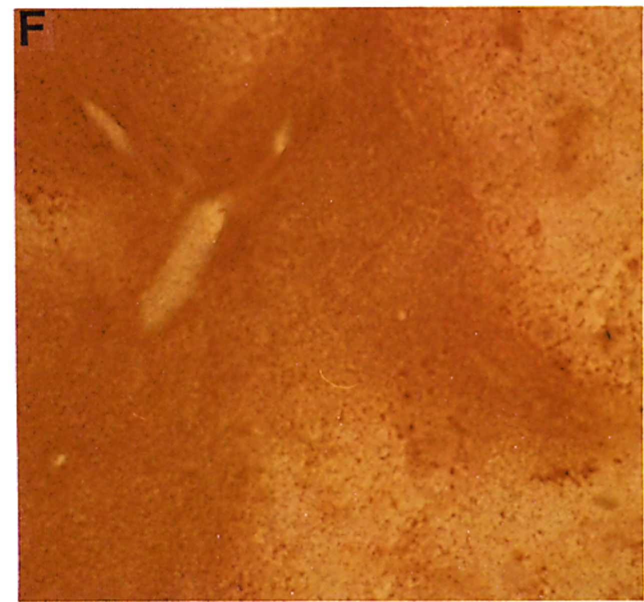
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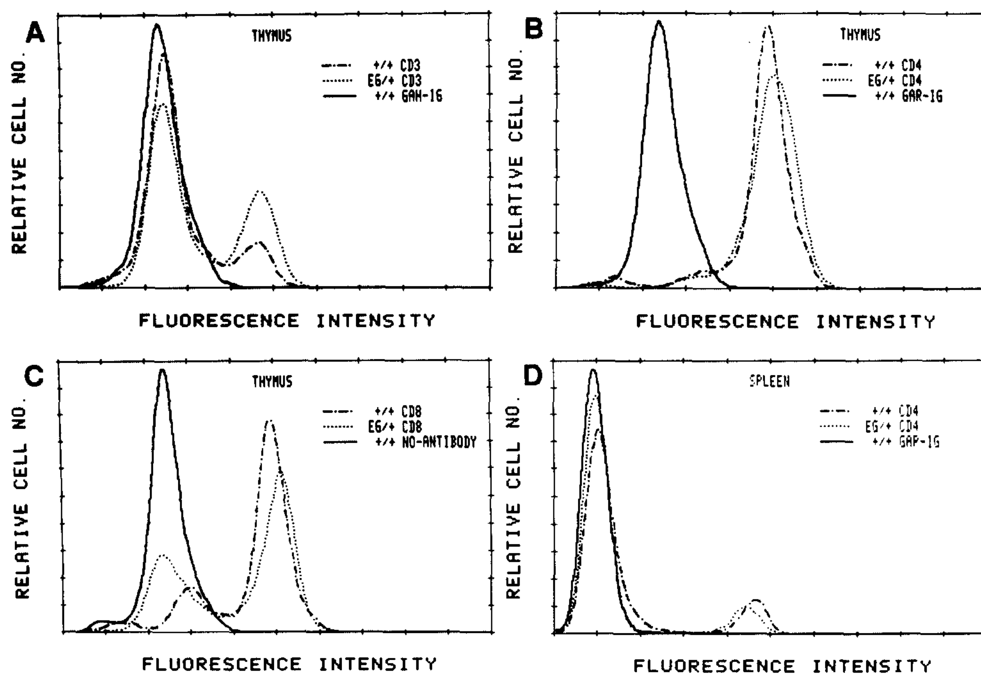

Figure 4. Flow cytometric analysis of thymic and splenic T cells in *B-less* and wild-type mice. Thymus cells stained for: (*A*) CD3 (2C11), (*B*) CD4 (GK1.5), and (*C*) CD8 (Lyt-2), and (*D*) spleen cells labeled with anti-CD4 antibody. 2C11 is an unconjugated hamster antibody and was labeled with FITC-conjugated goat anti–hamster antiserum. The control in *A* received only the goat anti–hamster antiserum. CD4 and CD8 antibodies were directly conjugated and the controls in *B*, *C*, and *D* received no antibody. The difference in the CD3 staining of EG and wild-type thymus cells is probably not significant, as we have not consistently observed this shift in other experiments. (*GAH-IG*)Goat anti–hamster Ig. (*GAR-IG*)Goat anti-rat Ig.

play a >99% reduction in B cells (data not shown). Note that more mature B cells expressing surface Ig are also greatly reduced in the spleen (Fig. 2, *C* and *D*). Consistent with this, serum Ig levels in EG/+ mice are also reduced to <1% of that of wild-type (see legend of Table 2), while the level of Ig is below the limit of detection in homozygous EG/EG animals (data not shown).

To determine whether the splenic architecture of the affected transgenic mice is perturbed, we analyzed histological sections of EG and wild-type spleens. Although the gross morphology of EG spleens appears normal, immunohistochemical staining with a B cell–specific antibody (anti-B220) dramatically illustrates the extent of the B cell deficiency (Fig. 3, *C* and *D*). The reddish-brown stain marks the B cells that extensively populate the white pulp surrounding the central arterioles of the normal spleen (Fig. 3 *C*) (see reference 35 for a review of spleen structure). In contrast, EG spleens contain only small clusters of B220$^+$ cells at the margin of the white pulp (Fig. 3 *D*). However, the remainder of the white pulp stains strongly with the T cell marker thy-1 (Fig. 3, *E* and *F*). A similar picture emerges when spleen sections are stained with anti-mouse IgM (not shown). Controls treated with secondary antibody alone display no staining (Fig. 3, *A* and *B*; also see Fig. 2 *B*). As shown below, there is no human λ protein on the surface of EG splenocytes (Fig. 6 *B*), and flow cytometry reveals a large, unusual population of EG splenocytes bearing the Sca-1 antigen (described below, see Fig. 9 *A*).

The Defect Appears Restricted to the B Cell Lineage. We used flow cytometric analysis to assess the T cell population in hemizygous EG transgenic mice. Fig. 4, *A–D* illustrates that the thymic and splenic T cell populations are indistinguishable from those of wild-type mice with respect to cells bearing the CD3, CD4, and CD8 antigen markers (the somewhat larger numbers of CD3$^+$ cells in the EG thymus (Fig. 4 *A*) is not normally seen and may reflect an infectious state in that particular animal). To confirm the lack of functional B cells, and to determine whether the T cell compartment is functional, we performed mitogen stimulation assays on spleen cells. Stimulation of splenic lymphocytes with the B cell specific mitogen, LPS, indicates that there are few, if any, functional B cells in EG spleens (Fig. 5 *A*). However, Con A, a T cell–specific mitogen, induces a T cell response comparable with that of wild-type (Fig. 5 *B*), indicating that the T cell component is relatively intact. Thus the effect of the EG transgene appears to spare T cells while eliminating the B cells. The severe, specific pre-B and B cell deficiency in the EG transgenic strain has led us to call this mutation "*B-less*."

Human λ Protein Is Present in B-less Mice. The presence of human λ mRNA is not per se a measure of human λ L chain. To determine whether the transgenic human λ mRNA is translated, we performed sandwich immunosorbant assays to detect mouse and human Ig in *B-less* sera. Samples were found to contain 0.3–6.0 mg/ml of human λ protein, although H chain concentrations are <0.02 mg/ml (the limit of detection in this assay). Thus, λ chains in *B-less* serum are present primarily as free L chains. To determine whether the partic-

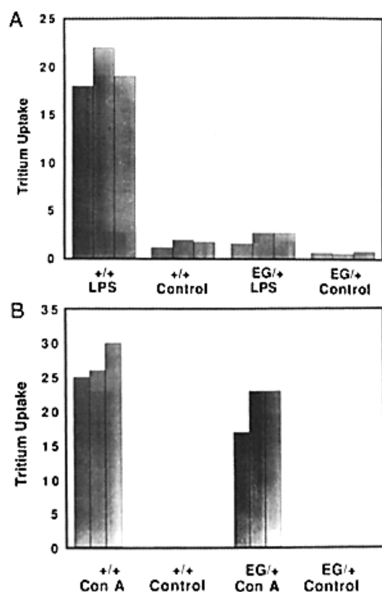

Figure 5. Mitogen stimulation of wild-type and *B-less* (EG) splenocytes. (*A*) LPS and (*B*) Con A. Tritium uptake is indicated in *cpm* ($\times 10^{-2}$ in *A* and $\times 10^{-3}$ in *B*) incorporated per 10^4 cells. Control samples received culture medium with no specific mitogen. Each bar represents a single culture.

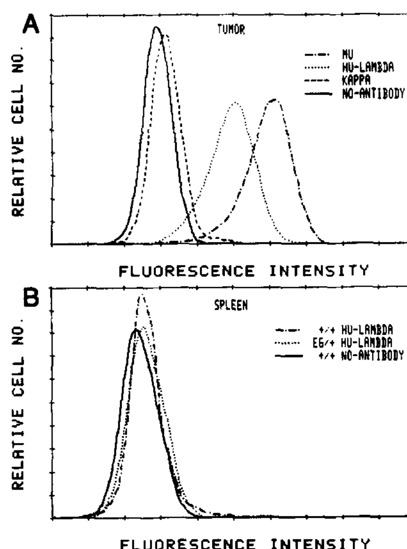

Figure 6. Flow cytometric analysis of cells labeled with anti–hu-λ mAb. (*A*) A tumor cell line, EG/Eμ-c-*myc*-EB10298, which arose in a mouse bearing both the λ and the Eμ-*myc* transgenes. (*B*) Wild-type and *B-less* splenocytes labeled with anti–hu-λ antibody. Human λ proteins are not present on the surface of splenocytes from *B-less* mice and the anti–hu-λ mAb does not cross-react with mouse κ. The hu-λ (*HU-LAMBDA*), m-κ (*KAPPA*), and m-μ (*MU*) antibodies are all directly conjugated with FITC, and the controls received no antibody.

ular human λ L chain encoded by the transgene is capable of forming stable complexes with mouse H chains, we analyzed a cell line that expresses both the mouse μ H chain, and the transgenic λ L chain. This line (EG/EB10298) was derived from a pre-B cell lymphoma that arose in a transgenic mouse bearing both the human λ transgene and an oncogenic Eμ-c-*myc* transgene (16). This cell line expresses comparable quantities of μ and λ chains as measured in whole cell extracts (see Table 3) and mouse μ and human λ chains are present on its surface (Fig. 6 *A*). Similar results were obtained using three other λ/Eμ-c-*myc* cell lines (data not shown). As would be expected, the mouse μ chain is immunoprecipitated from whole cell extracts of these coexpressing tumors using an antibody directed against the human λ chain (Fig. 7). Furthermore, none of these tumors express mouse κ chains (and all retain both κ alleles in the germline configuration, see Table 3), suggesting that the human λ–mouse μ complexes are functional to the extent that they mediate isotype exclusion.

Since there are so few B cells in *B-less* mice, it is not surprising that human λ chains are not detectable on the surface of splenic lymphocytes (Fig. 6 *B*), despite the high level expression of human λ mRNA observed in *B-less* spleens. Cell

Table 3. *Ig Concentrations in Whole Cell Extracts and Gene Rearrangements in A-MuLV transformed and Lymphoma Cell Lines Derived from Wild-Type and Transgenic Mice*

TG	Human λ *ng/mg*	Mouse μ *ng/ml*	Mouse μ genes (D–J)			κ genes
			R/R*	R/G	G/G	G/G
None	<20 (8)	23 ± 13 (7)*	100% (8)	0	0	100% (8)
EG	700 ± 1,100 (16)‡	16 ± 10 (16)‖	70% (19)	19% (5)	11% (3)	100% (27)
EG/EB¶	420 (1)	340 (1)	100% (3)	0	0	100% (3)

Numbers in parentheses represent number of cell lines tested.
* R, rearranged; G, germline, number of cell lines.
‡ One line contained 2,370 ng/ml of μ protein.
§ One line contained 5,400 ng/ml of human λ; two of these cell lines contained no measurable human λ protein despite the fact that they still contained the transgene.
‖ One of these lines contained 1,150 ng/ml of mouse μ protein; two contained 34 and 48 ng/ml, and the rest contained no detectable mouse μ protein.
¶ λ/Eμ-c-*myc* tumor cell lines (see Materials and Methods).

surface expression depends on expression of an Ig H chain which, in turn, occurs only in B cells. In another λ transgenic strain (ET) that produces appreciable numbers of B cells, 50–60% of the B220$^+$ splenic lymphocytes bear human λ and mouse μ chains, but no mouse κ chains (data not shown), again consistent with the functional integrity of the human λ transgene.

The B-less Defect Is Autonomous to Cells of the B Lineage. That the *B-less* phenotype is a B cell autonomous property was established using in vitro bone marrow cultures derived from transgenic and wild-type bone marrow stem cells and stromal feeder cells (17). We found that *B-less* bone marrow stroma supports the growth of feeder-dependent, wild-type B cells in culture up to levels of 2 × 10^6 cells/ml, and cultures of *B-less* bone marrow and fetal liver cells grown on wild-type feeder layers generate <10^5 cells/ml.

To confirm that the *B-less* defect is intrinsic to B cells, we generated bone marrow chimeras by injecting irradiated *B-less*

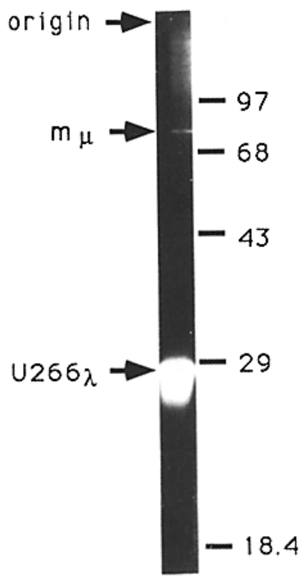

Figure 7. SDS-PAGE analysis of immunoprecipitated human λ protein from the λ-EG/Em-*myc* tumor cell line, EG/EB10298. Cells were labeled with [^{35}S]-methionine, lysed, and treated with anti-human antibodies. The positions of human λ, mouse μ, and size markers are indicated.

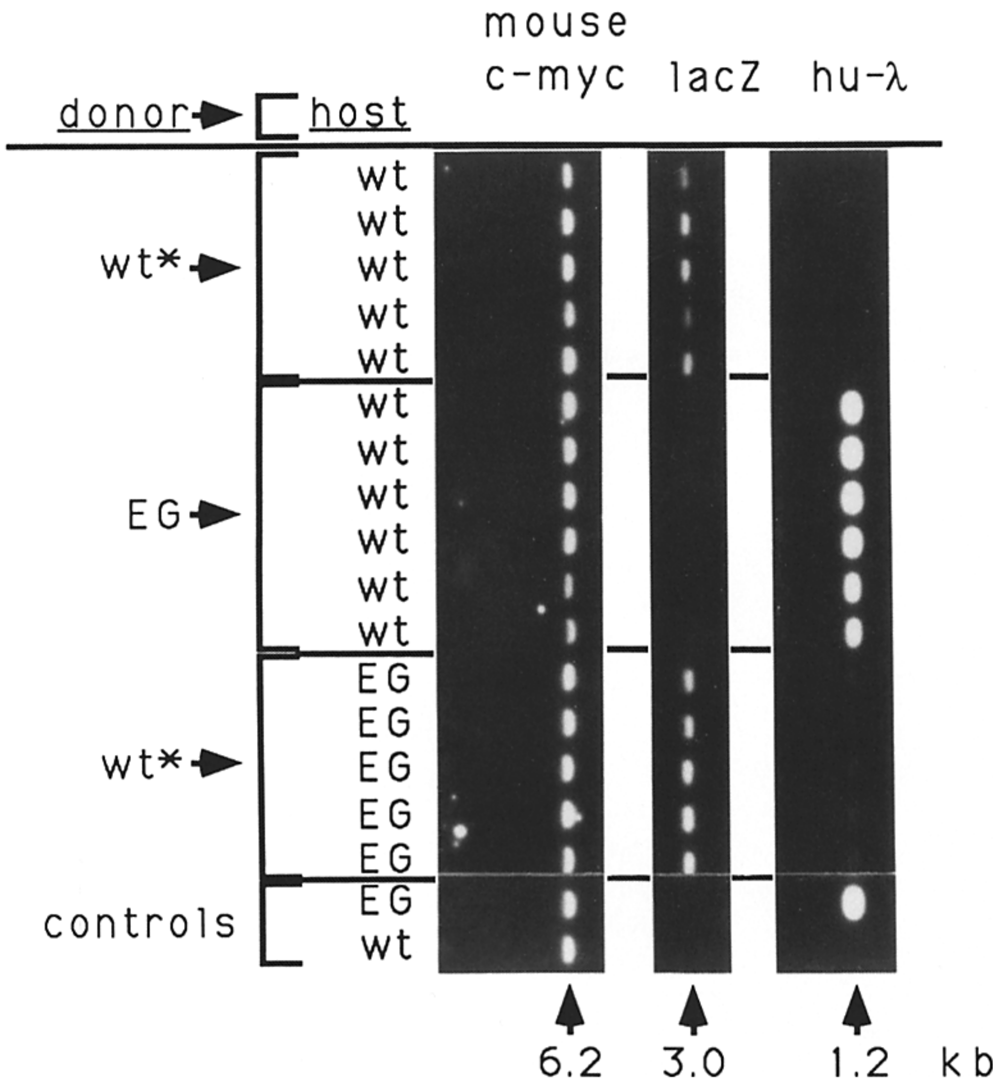

Figure 8. Southern blot analysis of PBL DNA from bone marrow transplant animals 60 d posttransplant. DNA was digested with BamHI. wt* animals were the TG.XA strain which is marked with a *lacZ* transgene. The blot was serially probed with human λ, *lacZ*, and mouse c-*myc* probes. The sizes of the relevant DNA fragments are indicated.

(EG) animals with wild-type bone marrow and *B-less* bone marrow into irradiated wild-type animals. To permit identification of the wild-type donor cells in reconstituted chimeric hosts, we used otherwise normal bone marrow donors bearing a *lacZ* transgene as a genetic marker (designated *wt**, Fig. 8). Control, wild-type, and *B-less* host mice do not contain *lacZ* crosshybridizing DNA, which can only be provided by the transplant (Fig. 8, *controls* lanes). PBLs from five wild-type mice reconstituted with wt* bone marrow are marked with the *lacZ* gene (Fig. 8, *wt*→wt* lanes) and thus are chimeras with contributions from the wt* donor. *B-less* recipients of wt* bone marrow also display the lacZ band (Fig. 8, *wt*→EG* lanes). All mice reconstituted with normal bone marrow (including the *B-less* recipients) display normal serum Ig levels 60 d after transplantation (Table 2). Although hematopoietic cells from the *B-less* donor populate the peripheral blood of the wild-type host mice reconstituted with *B-less* bone marrow, these cells (because they lack B cell precursors) fail to reconstitute the serum Ig to wild-type levels within 60 d (Table 2, Fig. 8, *EG→wt** lanes). The apparently normal levels of serum Ig seen 80 d post-transplant (Table 2) are likely to be due to host B cell recovery. Evidence of such minimal host B cell recovery is visible in the wt* to EG graft (see the faint human λ band in *wt*→EG* lanes [Fig. 8]).

A-MuLV Transformation Indicates a Profound Deficiency of Pre-B Cells. To determine the level at which B cell development is blocked in *B-less* mice, we used the A-MuLV transformation assay to quantitate early B cell precursors in *B-less* bone marrow and fetal liver. A-MuLV specifically transforms B cell precursors in these organs (18) and can be used to assess the number of pre-B cell targets in these populations. Transformation of *B-less* bone marrow and day 14–17 fetal liver yielded <2% of the normal number of transformed colonies, suggesting that the block in B cell development occurs very early in the B lineage pathway. For example, day 16 embryos from wild-type mice yielded 170 (± 100, n = 9) transformed colonies per 10^6 fetal liver cells, and *B-less* embryos yielded only 3.0 (± 2.7, n = 7).

To further characterize the rare A-MuLV-transformed clones that arise from infection of *B-less* fetal liver cells, several transformed cells were cultured, the arrangements of their Ig genes were determined, and their Ig protein production was assayed (Table 3). Although the rare transformed cells obtained from *B-less* fetal liver are apparently B cell precursors by these criteria, they are distinct from those obtained from wild-type animals. When assessed after 3 mo in culture, 30% of the *B-less*–derived cell lines retain one or both H chain alleles in the germline configuration, while the wild-type cell lines generally rearrange both their H chain alleles. Further, 14 of 16 transformed *B-less* cell lines express large quantities of human λ protein, while only three express mouse μ chains (Table 3). All the transformed lines retained their κ genes in the germline configuration.

Appearance of Sca-1–bearing Cells among B-less Splenocytes. To determine whether B cell precursors are altered in *B-less* mice, we examined cells for the expression of several antigens characteristic of hematopoietic progenitors. Among those tested,

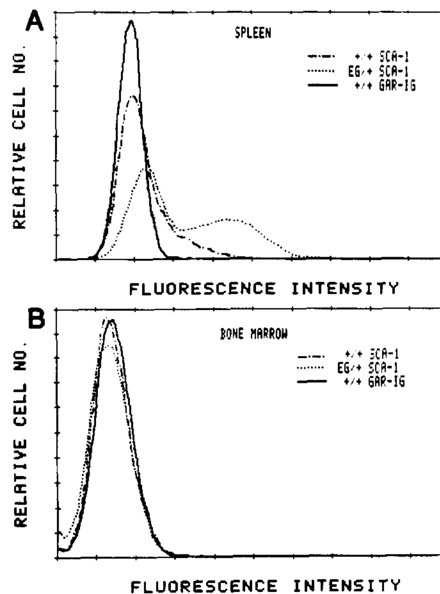

Figure 9. Flow cytometric analysis of Sca-1 on spleen and bone marrow cells labeled with antibody to Sca-1. (*A*) Spleen and (*B*) bone marrow cells. FITC-conjugated goat anti–rat antiserum (*GAR-IG*) was used as the second antibody to label the Sca-1 Ab.

a dramatic difference is detectable with an antibody to the Ly-6A.2 antigen (the stem cell antigen-1 [Sca-1]). The labeling profile of *B-less* splenocytes with the Sca-1 antibody reveals a relatively increased population of strongly positive cells (Fig. 9 *A*), though such cells are not seen in the bone marrow (Fig. 9, *A* and *B*, Table 4). Other antigens, such as AA4, BP1, and Mac1, showed no differences.

The significance of the appearance of cells bearing this antigen is not clear. Certain T cells and endothelial cells normally express Sca-1 antigen at low levels, and a rare population of bone marrow cells (~0.01%) express high levels of this antigen and represent pluripotent hematopoietic progenitor cells (36). Since hematopoietic progenitors are not normally found in the spleens of healthy adult mice, T cells and endothelial cells constitute the Sca-1$^+$ population normally found in the peripheral lymphoid organs. A portion of the Sca-1$^+$ population we observe may include B cell precursors (or other hematopoietic precursors). Alternatively, they may represent a cell type induced to hyperproliferation in response

Table 4. *Sca-1$^+$ Cells in Spleen and Bone Marrow*

Tissue	wt*	EG*
Spleen		
Sca-I (Ly-6)	9.8 ± 6.4 (3)	40.1 ± 10.2 (4)
Bone marrow		
Sca-I (Ly-6)	1.4 (1)	1.2 (1)

* Mean percentage ± SD (no. tested).

to the B cell deficiency, perhaps because of the action of a lymphokine. Alternatively, they may be bone marrow cells induced to migrate to the periphery because of the deficiency. As a rough test of this precursor notion, we determined that *B-less* splenocytes produce 5–10-fold more spleen colonies (measured 12 d posttransplant) than do wild-type splenocytes when injected into lethally irradiated wild-type recipients (CFU-S assay, data not shown).

The B-less Phenotype Is Suppressed on the C57BL/FVB Hybrid Background. In the course of experiments designed to determine whether humoral immunity plays a role in tissue graft rejection, we generated F_1 mice bearing the *B-less* transgene on the hybrid background C57BL/6 × FVB/N. By comparison to mice carrying the transgene in the FVB/N background, these mice displayed a less dramatic reduction in B cell number (~25% of the normal level) with surface Ig levels that were only one-third to one-fourth normal (Table 1). In addition, these mice were quite healthy, displaying no increased susceptibility to infection. The fact that hybrid (C57BL/6 × FBV/N)F_1 transgene carriers are less severely affected than the inbred FVB/N mice suggests the presence of a dominant suppressor(s) in the C57BL/6 genome.

The Possible Basis of the B Lineage Defect. We have shown that the immunodeficiency syndrome we observe is cell autonomous. That is, *B-less* bone marrow is not capable of establishing B cell cultures on wild-type stromal feeder layers in tissue culture. Further, wild-type bone marrow is capable of reconstituting lethally irradiated *B-less* mice with normal (donor type) B cells and normal levels of serum Ig (Fig. 8, and Table 2). It is thus likely that certain classes of B cell progenitors are deficient in *B-less* mice. The reduction in $B220^+$ cells in the bone marrow is consistent with this interpretation. It is also (though not exclusively) consistent with the finding that the A-MuLV target cell population is reduced to <2% of that of wild-type levels, a finding strongly suggestive of the absence of pre-B cells.

The *B-less* transgene has a dramatic effect on B cell populations in hemizygous EG/+ mice, suggesting a dominant genetic function. At least two models can be invoked to explain these results. In a relatively indirect model, the EG transgene might create a loss-of-function mutation by virtue of its insertion into a gene both essential for B cell development and sensitive to gene dosage. If this model were correct, *B-less* mice should display a dosage-dependent phenotype. As we have noted, homozygous animals do indeed have a more severe phenotype than hemizygotes. Nevertheless, the possibility that this phenotype is brought about by the insertional mutation of an endogenous gene is unlikely since we have produced an additional transgenic mouse strain bearing the same transgene construct which develops a similar, though less severe, B cell deficiency. Since the common element between these strains is the transgene, and is not likely to be the site of its insertion, the insertional mutagenesis model becomes far less likely.

Alternatively, a second model holds that expression of the human λ transgene is itself detrimental to B cell development, again in a dose-dependent manner. Variation in expression may be due to gene dosage or the spacial and/or temporal profile of transgene expression which may be influenced by copy number or the insertion site environment. Furthermore, U266λ may be functionally distinct from other Ig transgenes in its ability to interfere with B cell development. The observation that previously reported Ig transgenics display some reduction in B cell populations (3) and that another of our λ transgenic mice displays a phenotype similar to that of *B-less* is most consistent with this model. If this is correct, we must consider more detailed mechanisms by which aberrant expression of H or L chains could alter B cell development.

One such mechanism could depend upon allelic exclusion that normally regulates B cell development by preventing the expression of multiple idiotypes in any single B cell. Since antibody production and expression play a role in inducing expansion of cells of the B lineage, impairing expression of the endogenous antibody repertoire could interfere with this process. This mechanism would require expression of the interfering Ig transgene in early B cell precursors to foreclose expansion of the immune repertoire.

Another possible mechanism is suggested by the fact that the *B-less* phenotype eliminates cells of early B lineage. The prematurely expressed λ chain (or perhaps specifically the human U266λ chain) might interact with a cellular component(s) needed for very early B cell development. Such a cellular component might include one of the λ-like proteins (such as V_{preB} or ω [λ_5] [37–39]) essential for intracellular Ig traffic. This dominant-negative mechanism is consistent with the fact that the human λ L chain transgene is expressed in a variety of tissues, but is deleterious only in the B cell lineage. We can only speculate that the more severe effect seen in the *B-less* strain is due to either a higher level of λ expression in precursor cells and/or expression at an earlier time of B cell development than in those strains which fail to show the full phenotype. Such variation in expression, if it occurs, could be a consequence of the site of integration of the transgene.

Significance and Use of B-less Mice. *B-less* mice display a B cell deficiency that is similar in some respects to that observed in X-linked agammaglobulinemia (XLA) in humans (10). A notable difference is that bone marrow derived from patients with XLA contains normal numbers of pre-B cells. Both diseases result in virtually complete absence of mature, functional B cells. Thus, in addition to complementing the available murine immunodeficiency models, the *B-less* mouse provides a model system for the study of the importance of B cells in antigen presentation and T cell maturation (40, 41). It is particularly interesting to speculate on which other cells may substitute for B cells in the context of B cell immunodeficiencies (11). The *B-less* mouse should also provide insight into earlier stages of B cell development, and its study should illuminate the functions of B cells in cell-mediated immunity. Furthermore, the amplification of a novel cell population ($Sca-1^+$) suggests that the absence of B cells in *B-less* transgenic mice may stimulate proliferation of other novel cell types, perhaps under the influence of aberrantly produced growth factors for which the *B-less* mouse may ultimately provide a convenient source.

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References

1. Korsmeyer, S.J., P.A. Hieter, J.V. Ravetch, D.G. Poplack, T.A. Waldmann, and P. Leder. 1981. Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B-cells. *Proc. Natl. Acad. Sci. USA.* 78:7096.
2. Alt, F.W., G.D. Yancopulos, T.K. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore. 1984. Ordered rearrangement of immunoglobulin heavy chain variable region segments. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1209.
3. Storb, U. 1987. Transgenic mice with immunoglobulin genes. *Annu. Rev. Immunol.* 5:151.
4. Nussenzweig, M.C., A.C. Shaw, E. Sinn, D.B. Danner, K.L. Holmes, H.C. Morse III, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin μ. *Science (Wash. DC).* 236:816.
5. Nussenzweig, M.C., A.C. Shaw, E. Sinn, J. Campos-Torres, and P. Leder. 1988. Allelic exclusion in transgenic mice carrying mutant human IgM genes. *J. Exp. Med.* 167:1969.
6. Herzenberg, L.A., A.M. Stall, J. Braun, D. Weaver, D. Baltimore, L.A. Herzenberg, and R. Grosschedl. 1987. Depletion of the predominant B-cell population in immunoglobulin μ heavy-chain transgenic mice. *Nature (Lond.).* 329:71.
7. Hagman, J., D. Lo, L.T. Doglio, J. Hackett, Jr., C.M. Rudin, D. Haasch, R. Brinster, and U. Storb. 1989. Inhibition of immunoglobulin gene rearrangement by the expression of a λ2 transgene. *J. Exp. Med.* 169:1911.
8. Era, T., M. Ogawa, S. Nishikawa, M. Okamoto, T. Honjo, K. Akagi, J. Miyazaki, and K. Yamamura. 1991. Differentiation of growth signal requirement of B lymphocyte precursor is directed by expression of immunoglobulin. *EMBO (Eur. Mol. Biol. Organ.) J.* 10(2):337.
9. Lamers, M.C., M. Vakil, J.F. Kearney, J. Langhorn, C.J. Paige, M.H. Julius, H. Mossmann, R. Carsetti, and G. Kohler. 1989. Immune status of a μ, κ transgenic mouse line. Deficient response to bacterially related antigens. *Eur. J. Immunol.* 19:459.
10. Campbell, A.C., A.G. Bird, S.J. Carlton, A.M. Galvin, and J. Faulkner. 1984. T, B, and NK cell abnormalities in primary hypogammaglobulinaemia. *In* Progress in Immunodeficiency Research and Therapy. C.G. Griscelli and J. Vossen, editors. Elsevier Science Publishers B.V., Amsterdam. Vol. 1., 95–102.
11. Bird, G. 1988. Antibody deficiency states. *In* B lymphocytes in human disease. G. Bird and J.E. Calvert, editors. Oxford University Press, New York. 257–294.
12. Yamada, G., M. Ogawa, K. Akagi, H. Miyamoto, N. Nakano, S. Itoh, J.-I. Miyazaki, S.-I. Nishikawa, K.-I. Yamamura, and T. Taniguchi. 1991. Specific depletion of B-cell population induced by aberrant expression of human interferon regulatory factor 1 gene in transgenic mice. *Proc. Natl. Acad. Sci. USA.* 88:532.
13. Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature (Lond.).* 350:423.
14. Vasicek, T.J., and P. Leder. 1990. Structure and expression of the human immunoglobulin λ genes. *J. Exp. Med.* 172:609.
15. Muller, W.J., E. Sinn, P.K. Pattengale, R. Wallace, and P. Leder. 1988. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell.* 54:105.
16. Schmidt, E.V., P. Pattengale, L. Weir, and P. Leder. 1988. Transgenic mice bearing the human c-myc gene activated by an immunoglobulin enhancer: a pre-B cell lymphoma model. *Proc. Natl. Acad. Sci. USA.* 85:6047.
17. Whitlock, C.A., D. Robertson, and O.N. Witte. 1984. Murine B cell lymphopoiesis in long term culture. *J. Immunol. Methods.* 67:353.
18. Rosenberg, N., and D. Baltimore. 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. *J. Exp. Med.* 143:1453.
19. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 545 pp.
20. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and J.W. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources method in ribonucleases. *Biochemistry.* 18:5294.
21. Melton, D.A., P.A. Kreig, M.R. Rebagliati, T. Maniatis, K. Zinn, and R.R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035.
22. Lane, D.P., and E.B. Lane. 1981. A rapid antibody assay system for screening hybridoma cultures. *J. Immunol. Methods.* 47:303.

23. Coffman, R.L. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol. Rev.* 69:5.
24. Kincade, P.W., G. Lee, T. Watanabe, L. Sun, and M.P. Scheid. 1981. Antigens displayed on murine B lymphocyte precursors. *J. Immunol.* 127:2262.
25. Aihara, Y., H.-J. Buhring, M. Aihara, and J. Klein. 1986. An attempt to produce "pre-T" cell hybridomas and to identify their antigens. *Eur. J. Immunol.* 16:1391.
26. Leo, O., M. Foo, D.H. Sachs, L.E. Samelson, and J.A. Bluestone. 1987. Identification of monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. USA.* 84:1374.
27. Till, J.E., and E.A. McCulloch. 1961. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.* 14:213.
28. Mishell, B.B., and S.M. Shiigi, editors. 1980. Selected Methods in Cellular Immunology. W.H. Freeman, San Francisco. 486 pp.
29. Vasicek, T.J. 1990. Structure of the human immunoglobulin λ locus and B-cell deficiency in mice expressing λ transgenes. Ph.D. thesis. Harvard University, Cambridge, MA. 208 pp.
30. Grosschedl, R., D. Weaver, D. Baltimore, and F. Costantini. 1984. Introduction of a μ immunoglobulin gene into the mouse germ line: specific expression in lymphoid cells and synthesis of functional antibody. *Cell.* 38:647.
31. Yamamura, K.-I., A. Kudo, T. Ebihara, K. Kamino, K. Araki, Y. Kumahara, and T. Watanabe. 1986. Cell-type-specific and regulated expression of a human $\gamma 1$ heavy-chain immunoglobulin gene in transgenic mice. *Proc. Natl. Acad. Sci. USA.* 83:2152.
32. Storb, U., R.L. O'Brien, M.D. McMullen, K.A. Gollahon, and R.L. Brinster. 1984. High expression of cloned immunoglobulin κ gene in transgenic mice is restricted to B lymphocytes. *Nature (Lond.).* 310:238.
33. McKearn, J.P., and N. Rosenberg. 1985. Mapping cell surface antigens on mouse pre-B cell lines. *Eur. J. Immunol.* 15:295.
34. Cooper, M.D., D. Mulvaney, A. Coutinho, and P.-A. Cazenave. 1986. A novel cell surface molecule on early B-lineage cells. *Nature (Lond.).* 321:616.
35. Weiss, L. 1983. The spleen. *In* Histology Cell and Tissue Biology. L. Weiss, editor. Elsevier Biomedical, New York. 545–568.
36. Spangrude, G.J., S. Helmfeld, and I.L. Weissman. 1988. Purification and characterization of mouse hematopoietic stem cells. *Science (Wash. DC).* 241:58.
37. Kudo, A., and F. Melchers. 1987. A second gene, V_{preB} in the λ_5 locus of the mouse, which appears to be selectively expressed in pre-B lymphocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2267.
38. Pillai, S., and D. Baltimore. 1987. Formation of disulphide-linked $\mu_2\omega_2$ tetramers in pre-B cells by 18k ω-immunoglobulin light chain. *Nature (Lond.).* 329:172.
39. Sakaguchi, N., and F. Melchers. 1986. λ_5: a new light-chain-related locus selectively expressed in pre-B lymphocytes. *Nature (Lond.).* 324:579.
40. Lanzavecchia, A. 1986. Antigen presentation by B lymphocytes: a critical step in T-B collaboration. *Curr. Top. Microbiol. Immunol.* 130:66.
41. Chestnut, R.W., and H.M. Grey. 1986. Antigen presentation by B cells and its significance in T-B interactions. *Adv. Immunol.* 39:51.