T Lymphocyte Development and Function in Dogs with X-Linked Severe Combined Immunodeficiency[1]

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Canine X-linked severe combined immunodeficiency disease (XSCID) is characterized by a failure to thrive, thymic dysplasia, and a lack of a T lymphocyte mitogenic response. As in human XSCID, affected dogs in our colony have a mutation in the IL-2R-γ gene. This mutation dramatically altered T lymphocyte development, because XSCID thymi were severely reduced in size and cellularity, contained an increased proportion of immature $CD4^-CD8^-$ thymocytes, a decreased proportion of intermediate $CD4^+CD8^+$ thymocytes, and a normal proportion of $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes. XSCID thymi were also deficient in the percentage of CD3-L^+ thymocytes. Interestingly, several XSCID dogs had normal percentages of CD3-L^+ PBL. Although the mutation did not interfere with IL-2 production, PHA-activated XSCID PBL demonstrated severely diminished IL-2 binding and were nonresponsive to IL-2. These results indicate that the lack of a functional IL-2R-γ chain in dogs with XSCID primarily affects developing $CD4^-CD8^-$ thymocytes as they acquire the cell surface Ag CD3-L and interferes with the ability of peripheral T lymphocytes to bind and respond to IL-2. *The Journal of Immunology,* 1994, 153: 4006.

Genetic disorders of the immune system provide unique opportunities to determine the factors required for the progression of B and T lymphocyte development. Severe combined immunodeficiency disease (SCID)[3] represents a heterogeneous group of disorders that are characterized by a failure in humoral and cell-mediated immunity (1). The inheritance of SCID in humans can be either autosomal or X-linked recessive. In the United States, approximately 15 to 20% of children with SCID have an associated adenosine deaminase deficiency that is inherited as an autosomal recessive trait and is easily identified (2). Another 2 to 5% of patients have an autosomal recessive defect in the expression of histocompatibility Ags (3, 4). In the remaining SCID children, the genetic defect remains to be determined; however, inasmuch as the ratio of affected males to females in this group of patients is approximately 4:1, it is thought that the X-linked form of SCID (XSCID) represents the most common form in the United States (1, 5). Phenotypic analysis of PBL from children with XSCID has demonstrated increased percentages of B lymphocytes and markedly decreased percentages of mature T lymphocytes (6). Recent reports have shown that the IL-2R-γ gene is on the X chromosome at Xq13, linked to the XSCID locus, and mutations in the IL-2R-γ gene were identified in seven XSCID patients (7, 8). Noguchi et al. (7) implied that the IL-2R-γ subunit plays a pivotal role in thymocyte development, because human XSCID patients have absent or markedly reduced numbers of T lymphocytes.

The only animal in which SCID results from a defect inherited on the X chromosome is the dog (9, 10). Canine XSCID is characterized by a failure to thrive, the lack of a T lymphocyte mitogenic response, and thymic dysplasia (9–11). Cytogenetic and linkage studies have shown that the gene defect in our colony of XSCID dogs maps to proximal Xq and is tightly linked to the same genes as in humans (12). Furthermore, we have recently shown that these XSCID dogs have a mutation in the IL-2R-γ gene that precludes production of a functional protein (13). The purpose of these studies was to document the functional

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[3] Abbreviations used in this paper: SCID, severe combined immunodeficiency disease; XSCID, X-linked severe combined immunodeficiency disease; DN, $CD4^-CD8^-$; DP, $CD4^+CD8^+$; SP, $CD4^-CD8^+$ and $CD4^+CD8^-$; CD3-L, CD3-like antigen; PE, phycoerythrin; MFI, mean fluorescence intensity.

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lesion(s) present in XSCID T lymphocytes and to characterize the developmental abnormalities in T lymphocytes that result from a lack of a functional IL-2R-γ chain.

Materials and Methods

Animals

XSCID dogs were derived from a breeding colony established from a single carrier female as previously described (9, 10). Affected males were diagnosed at approximately 3 wk of age by a whole blood lymphocyte transformation test (9). This diagnosis was confirmed after necropsy by the thymic dysplasia and absence of peripheral lymph nodes. Studies were performed on 4- to 8-wk-old dogs that were free of any clinical signs of illness. Age-matched normal littermates were used as controls.

Cell preparation

Peripheral blood was collected by venipuncture into a heparinized syringe before the dogs were killed. PBMC were obtained by a discontinuous Hypaque-Ficoll density gradient centrifugation procedure specifically developed for the isolation of canine PBMC, as previously described (14). After the dogs were killed, the entire thymus was removed, trimmed of any fat, and weighed. The thymus was then placed into a petri dish with RPMI 1640 (Mediatech, Washington, DC) and minced into a single cell suspension with forceps. The resulting suspension was filtered through a fine mesh filter and washed twice with HBSS (Mediatech, Washington, DC). Splenocytes were obtained in a similar manner except that after filtration, cells were centrifuged and resuspended in ammonium chloride lysis buffer (Sigma Chemical Company, St. Louis, MO) to remove RBC. After a 5-min incubation on ice, an equal volume of HBSS was added and the cell suspension was adjusted to 10% with CPSR-2 (Controlled Product Serum Replacement-2; Sigma). The cells were then washed twice in HBSS. Bone marrow cells were collected from killed dogs by removing a segment of the femur, cracking open the bone, scraping the marrow into a petri dish containing RPMI 1640, and mincing it into a single cell suspension with forceps. The resulting bone marrow cell suspension was then processed as described for splenocytes.

Flow cytometry

Cells were prepared for staining by additional washing in PBS, pH 7.4, containing 0.5% BSA and 0.01% sodium azide (PBS-BSA). In a volume of 100 μl, 10^6 cells in PBS-BSA were incubated with primary Ab for 30 min on ice. After washing three times in PBS-BSA, cells were resuspended in 100 μl of PBS-BSA, and 2.0 μl of the appropriate fluorochrome-conjugated secondary Ab was added followed by a 30-min incubation on ice. The cells were then washed three times with PBS-BSA, fixed in a 2% paraformaldehyde solution, and stored at 4°C until flow cytometric analysis was performed. Negative control samples received only the secondary Ab and were used to determine the threshold of specific staining. Analysis gates were adjusted to 2% positive staining with negative controls. Flow cytometric analysis was performed with the use of a Coulter ELITE flow cytometer (Coulter Electronics, Hileah, FL). FITC and phycoerythrin (PE) were excited by using 15 mW of 488 nm argon laser light. Before data acquisition, FITC- and PE-conjugated beads were run and the mean fluorescence intensity (MFI) was set at a fixed value. For each sample, forward angle light scatter, 90° light scatter, log FITC fluorescence, and log PE fluorescence were collected for 10,000 to 20,000 cells.

mAbs

F3–20–7, recognizing the canine Thy-1 Ag (15), was purchased from Harlan Bioproducts for Science (Indianapolis, IN). The murine mAbs 12.125, 4.78, and 8.358, recognizing canine CD4, CD8, and a CD3-like Ag (CD3-L), respectively, were kindly provided by Dr. Douglas Gebhard, North Carolina State University, Raleigh, NC (16). Anti-mouse IgG conjugated with PE and anti-mouse IgM conjugated with FITC were purchased from Fisher Scientific (Pittsburgh, PA). A FITC-conjugated heavy and light chain-specific $F(ab)_2$ reactive against canine Ig was purchased from Cappel (Durham, NC). The optimal amounts of each Ab were used as determined by titration experiments. The percentage of

Table I. *Proliferative responses of PBMC from normal and XSCID dogs* [a]

Stimuli	Normal (n = 10)	XSCID (n = 10)
PHA	21,613 ± 3,579	773 ± 235
PHA + IL-2	26,737 ± 4,008	724 ± 143
IL-2	2,765 ± 636	172 ± 25
Media	117 ± 16	99 ± 10

[a] Results are expressed as mean cpm ± SEM.

monocytes was determined by nonspecific esterase staining of cells from cytospin preparations (17). Two hundred cells were scored from each cytocentrifuged sample.

Proliferation assay

PBMC were cultured at 10^5/well in RPMI 1640 with 10% CPSR-2 and antibiotics (100 U/ml penicillin G, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B; Sigma) in 96-well flat-bottom microtiter plates (Costar, Cambridge, MA) in the presence of optimal concentrations for PHA (5 μg/ml; Sigma) in a final volume of 200 μl. Supernatant (60 μl) collected from PMA-activated EL-4 cells was used as a source of murine IL-2 (18). Triplicate cultures of PBMC were incubated for 72 h at 37°C in a humidified 5.0% CO_2 atmosphere. Sixteen to eighteen hours before harvesting, 0.5 μCi of [^{3}H]thymidine was added to each well, and cells were harvested onto glass filter strips by using a semi-automated cell harvester and counted in a liquid scintillation spectrometer. Results are expressed as mean counts per minute (cpm) ± SEM.

IL-2 assay

IL-2 activity in supernatants from PHA-activated splenocytes was assayed by the proliferation of the IL-2-dependent murine cell line CTLL-2 as described previously (19).

Detection of canine IL-2R

Cell surface expression of IL-2R by freshly isolated or PHA-activated (72 h) PBL was determined with the use of 50 nM human rIL-2 conjugated with PE and flow cytometry as described previously (20) with the use of the Fluorokine IL-2 Flow Cytometry Kit (R&D Systems, Minneapolis, MN). Briefly, IL-2R expression was determined by incubating 25 μl of washed cells at 4.0×10^6 cells/ml in PBS-BSA with 10 μl of human rIL-2 conjugated with PE or, as a control, anti-IgG-PE for 1 h on ice, and then washed twice with PBS-BSA. The final cell pellet was resuspended in 200 μl of PBS and kept on ice until analysis on a Coulter ELITE flow cytometer. Before data acquisition, PE-conjugated beads were run and the MFI was set at a fixed value so that the relative amount of bound IL-2 could be determined.

Statistics

Statistical analysis of the data was performed by using the two-tailed Student's *t*-test for unpaired samples. Values of $p < 0.05$ were considered to be significant.

Results

Defective proliferative response of XSCID PBMC

To examine the functional consequences of the IL-2R-γ gene mutation in XSCID dogs, the proliferative responses of normal and XSCID PBMC were compared. As shown in Table I, the proliferative response to PHA was severely reduced in XSCID dogs, and the addition of IL-2 did not result in any significant increase in proliferation. This is

consistent with the finding that XSCID PBMC were completely nonresponsive to IL-2 at concentrations that induced a significant proliferative response in freshly isolated normal PBMC.

Defective IL-2 binding and normal IL-2 production by XSCID lymphocytes

We and others have previously demonstrated that human rIL-2 can be used to examine the cell surface expression of IL-2R on canine T lymphocytes (20, 21). In fact, the IL-2R complexes on PHA-activated canine lymphocytes bound human rIL-2 with a similar affinity to activated human lymphocytes (21). Our IL-2R assay uses 50 nM of human rIL-2 conjugated with PE and subsequent flow cytometry to detect canine IL-2R. Previous results (20) demonstrated that this assay not only detects high affinity IL-2R on PHA-activated lymphocytes, but also detects IL-2R expression on freshly isolated PBL that lack high affinity IL-2R (21), and presumably express intermediate affinity IL-2R. The binding of human rIL-2 to PHA-activated PBL was inhibited by the addition of unlabeled human rIL-2 (20). The staining results of freshly isolated and PHA-activated PBL from normal and XSCID dogs are shown in Figure 1*A*, with representative histograms shown in Figure 1*B*. The percentage of resting PBL that expressed IL-2R was significantly reduced in XSCID dogs ($p < 0.024$). After PHA activation, the percentage of IL-2R$^+$ normal PBL increased nearly eightfold with a concomitant rise in MFI. By contrast, XSCID PBL did not demonstrate a significant increase in the percentage of IL-2R$^+$ PBL after PHA activation, and only a slight increase in MFI was observed. The ability of XSCID lymphocytes to produce IL-2, however, was not hindered, as evidenced by the increased IL-2 activity present in PHA-activated splenocyte supernatants (Fig. 2).

Phenotypically normal bone marrow cells in XSCID dogs

Because bone marrow is a major source of stem cells for the hematopoietic lineage, phenotypic analysis of XSCID bone marrow was performed to determine if the IL-2R-γ mutation manifests itself at these early stages of lymphocyte development. Although light scatter analysis of bone marrow cells from XSCID dogs revealed a significant increase in the proportion of large, granular cells ($p < 0.0219$), the cell surface Ag expression of these cells was similar to that of cells isolated from normal dogs. Flow cytometric evaluation of bone marrow cells from normal and XSCID dogs revealed similar percentages of sIg$^+$, Thy-1$^+$, CD3-L$^+$, and CD8$^+$ cells (Fig. 3*A*). Whereas the increased percentage and MFI of Thy-1$^+$ bone marrow cells from XSCID Dog 13 was not a consistent finding in all XSCID dogs, a significantly increased percentage ($p < 0.0261$) and MFI ($p < 0.001$) of CD4$^+$ bone marrow cells was (Fig. 3*B*). As CD4 is also expressed on neutrophils in dogs (22), the possibility exists that the increase observed in XSCID dogs might be caused by neutrophils. In fact, the peripheral blood of most XSCID dogs examined showed increased percentages of neutrophils (data not shown).

Defective thymocyte development in XSCID dogs

Although XSCID dogs, on average, weighed approximately 30% less than their normal littermates, the average weight of their thymus was reduced by >10-fold (normal = 10.1 ± 2.8 grams; XSCID = 0.9 ± 0.6 grams) and the thymocyte yield was reduced by 40- to 1,300-fold (normal = $2.0 \times 10^{10} \pm 6.3 \times 10^9$; XSCID = $7.2 \times 10^7 \pm 1.2 \times 10^8$). Considerable variation in thymocyte yield between individual XSCID dogs was observed, such that nearly three-fifths of XSCID thymi yielded $<1.5 \times 10^7$ thymocytes, one-fifth yielded between 5.0×10^7 and 15.0×10^7 thymocytes, and the remaining one-fifth yielded between 15.0×10^7 and 47.0×10^7 thymocytes.

Analysis of the light scatter characteristics of XSCID thymocytes revealed a striking increase in the proportion of large thymocytes (normal = 12.4 ± 15.0%; XSCID = 28.8 ± 14.6%; $p < 0.0001$). These large thymocytes were negative for nonspecific esterase activity and thymic epithelial cell markers (data not shown). The flow cytometric evaluation of normal and XSCID thymocytes, gated to include both small and large thymocytes, revealed a significantly decreased percentage of Thy-1$^+$, CD3-L$^+$, and DP thymocytes ($p < 0.0001$), and significantly increased percentage of DN thymocytes ($p < 0.0001$) in XSCID thymi (Fig. 4*A*).

Two distinct populations of CD3-L$^+$ thymocytes, CD3-L^{bright} and CD3-L^{dim}, were present in normal dog thymi in proportions indicated in Figure 4*B*. A variable pattern of CD3-L expression was observed in individual XSCID thymi, ranging from a near normal pattern to a general decrease in one or both populations of CD3-L$^+$ thymocytes (Fig. 4*B*). Overall, XSCID thymi contained normal percentages of CD3-L^{bright} thymocytes (normal = 12.6 ± 4.4%; XSCID = 10.6 ± 10.0%) and significantly reduced percentages of CD3-L^{dim} thymocytes (normal = 76.9 ± 9.3%; XSCID = 42.6 ± 24.3%; $p < 0.0001$).

CD4 and CD8 expression by normal canine thymocytes revealed four distinct phenotypic subsets; DP, DN, and SP thymocytes, as observed in other species (Fig. 5). A variable pattern of CD4 and CD8 expression was observed in individual XSCID thymi, such that a few XSCID thymi had near normal proportions of DP and DN thymocytes (XSCID Dog 13), although the vast majority of XSCID thymi had pronounced deficiencies in the proportion of DP thymocytes.

Decreased percentage of Thy-1$^+$ and CD8$^+$ PBL in XSCID dogs

The percentage of lymphocytes, as determined by their characteristic forward angle light scatter and 90° light

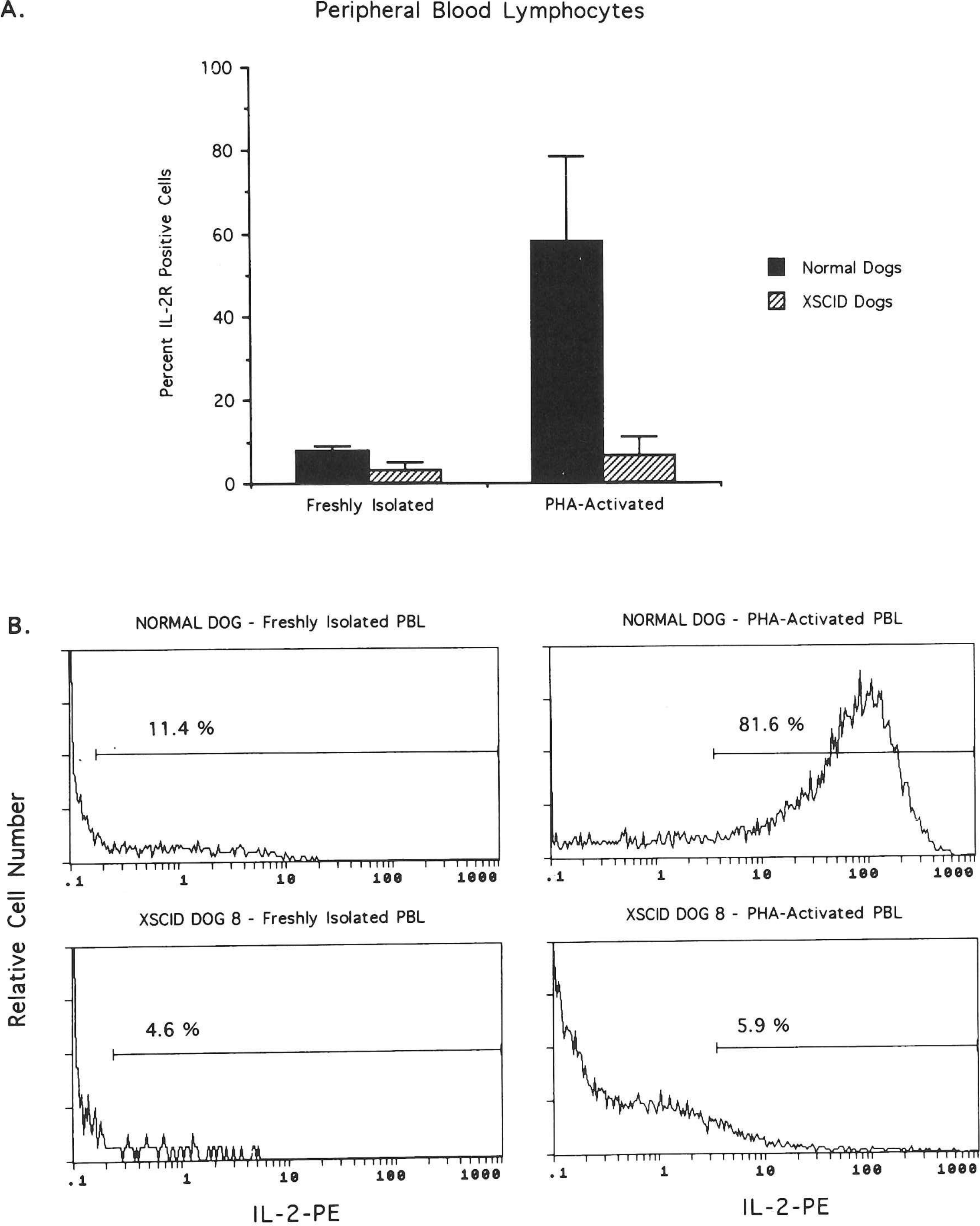


FIGURE 1. *A*. Comparison of the percentage of IL-$2R^+$ freshly isolated ($n = 4$) and PHA-activated ($n = 8$) PBL from normal and XSCID dogs. Staining was performed as described in *Material and Methods*. Results are expressed as mean ± SD. *B*. Representative histograms from a normal dog's PBL and an XSCID dog's PBL stained with human rIL-2 conjugated with PE before and after 72-h PHA activation. Analysis gates were adjusted to 2.0% positive staining with goat anti-mouse IgG-PE negative control. Numbers above analysis gates represent percent IL-$2R^+$ lymphocytes.

scatter, in PBMC preparations from XSCID dogs was significantly less than the percentage of lymphocytes in normal PBMC preparations (normal = 62.9 ± 13.9%; XSCID = 37.9 ± 12.0%; $p < 0.0001$). Nonspecific esterase staining of PBMC from cytospin preparations revealed a significant increase in the percentage of monocytes in XSCID PBMC (normal = 14.8 ± 4.3%; XSCID = 34.7 ± 8.4%; $p < 0.0001$). This increased percentage of monocytes in the

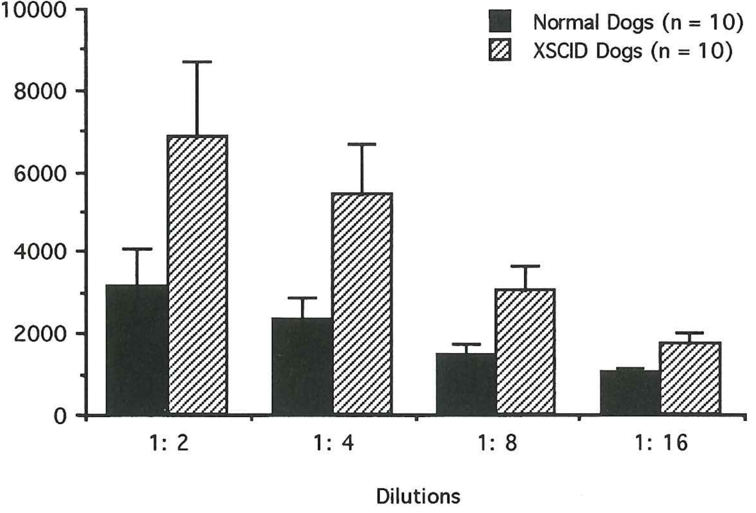


FIGURE 2. Proliferative responses of the IL-2-dependent murine cell line, CTLL-2, to serial dilutions of 48-h PHA-activated splenocyte supernatants collected from normal and XSCID dogs. Results are expressed as mean ± SD.

PBMC from XSCID dogs can be accounted for by the reduction in lymphocytes, because the total number of monocytes was not increased in XSCID dogs.

Flow cytometry of PBMC from normal and XSCID dogs, gated on lymphocytes, revealed no significant difference in the percentage of sIg^+, CD3-L^+, and $CD4^+$ lymphocytes; however, significantly fewer Thy-1^+ and $CD8^+$ lymphocytes were observed (Fig. 6*A*; $p < 0.006$ and $p < 0.0007$, respectively). Whereas the percentage of CD3-L^+ lymphocytes was severely reduced in six of thirteen XSCID dogs (16.0 ± 13.0%), seven XSCID dogs had normal percentages of CD3-L^+ lymphocytes (70.5 ± 10.5%). However, the total number of T lymphocytes was still reduced in these seven XSCID dogs as a result of their low lymphocyte count. The reduction in the percentage of $CD8^+$ lymphocytes in XSCID dogs resulted in a CD4 to CD8 ratio of 7:1, as compared with a ratio of 3:1 for normal dogs.

Changes in $CD4^+$ and $CD8^+$ splenocyte populations in XSCID dogs

Although light scatter analysis of splenocytes from XSCID dogs revealed a significant increase in the proportion of large, granular cells ($p < 0.0033$), the cell surface Ag expression of these cells was similar to that of cells isolated from normal dogs. Flow cytometric analysis of cell surface Ag expression by splenocytes from XSCID dogs revealed an increased percentage of $CD4^+$ splenocytes ($p < 0.0011$), and a decreased percentage of $CD8^+$ splenocytes ($p < 0.0002$), with no significant alterations in the percentage of CD3-L^+, Thy-1^+, or sIg^+ splenocytes (Fig. 6*B*). The percentage of monocytes in the splenocyte preparations from affected dogs was within the normal range.

Discussion

The IL-2R-γ mutation in these XSCID dogs results in profound T lymphocyte functional abnormalities. For example, IL-2R expression studies, performed under conditions that were capable of detecting both intermediate ($\beta\gamma$) and high affinity ($\alpha\beta\gamma$) IL-2R, revealed that both freshly isolated and PHA-activated XSCID lymphocytes were severely deficient in the percentage of IL-$2R^+$ lymphocytes and in their relative amount of bound IL-2. This lack of high affinity IL-2 binding is consistent with a missing IL-2R-γ chain and is in agreement with the lack of high affinity IL-2 binding observed with several human XSCID patients' transformed B cell lines that expressed both IL-2R-α and IL-2R-β (23, 24). Both freshly isolated and PHA-activated XSCID PBL failed to proliferate to concentrations of IL-2 that induced a significant proliferative response in normal lymphocytes, suggesting that IL-2R-γ is necessary for IL-2-induced proliferation. This hypothesis is supported by recent studies conducted with the use of chimeric receptor constructs that have shown that an interaction between the cytoplasmic domains of IL-2R-β and IL-2R-γ is necessary and sufficient for T lymphocyte signaling and proliferation (25, 26).

Although both human and canine XSCID are associated with mutations of the IL-2R-γ gene, there are some differences in the resulting phenotypic abnormalities observed in the peripheral blood. For example, seven of the thirteen XSCID dogs examined had normal percentages of CD3-L^+ peripheral blood T lymphocytes. These findings contrast with those observed in XSCID children, in whom few, if any, circulating mature T lymphocytes are found (6). When phenotypically mature T lymphocytes are present in XSCID children, the T lymphocytes are usually found to be of maternal origin (6). We have failed to detect maternal engraftment in XSCID dogs with normal percentages of CD3-L^+ lymphocytes by using a PCR-based assay for the presence of a normal IL-2R-γ gene (13, unpublished observations). This lack of maternal engraftment may be explained by the endotheliochorial type of placentation in the dog, in which an extra tissue layer further separates the maternal and fetal circulation, in comparison with human placentation

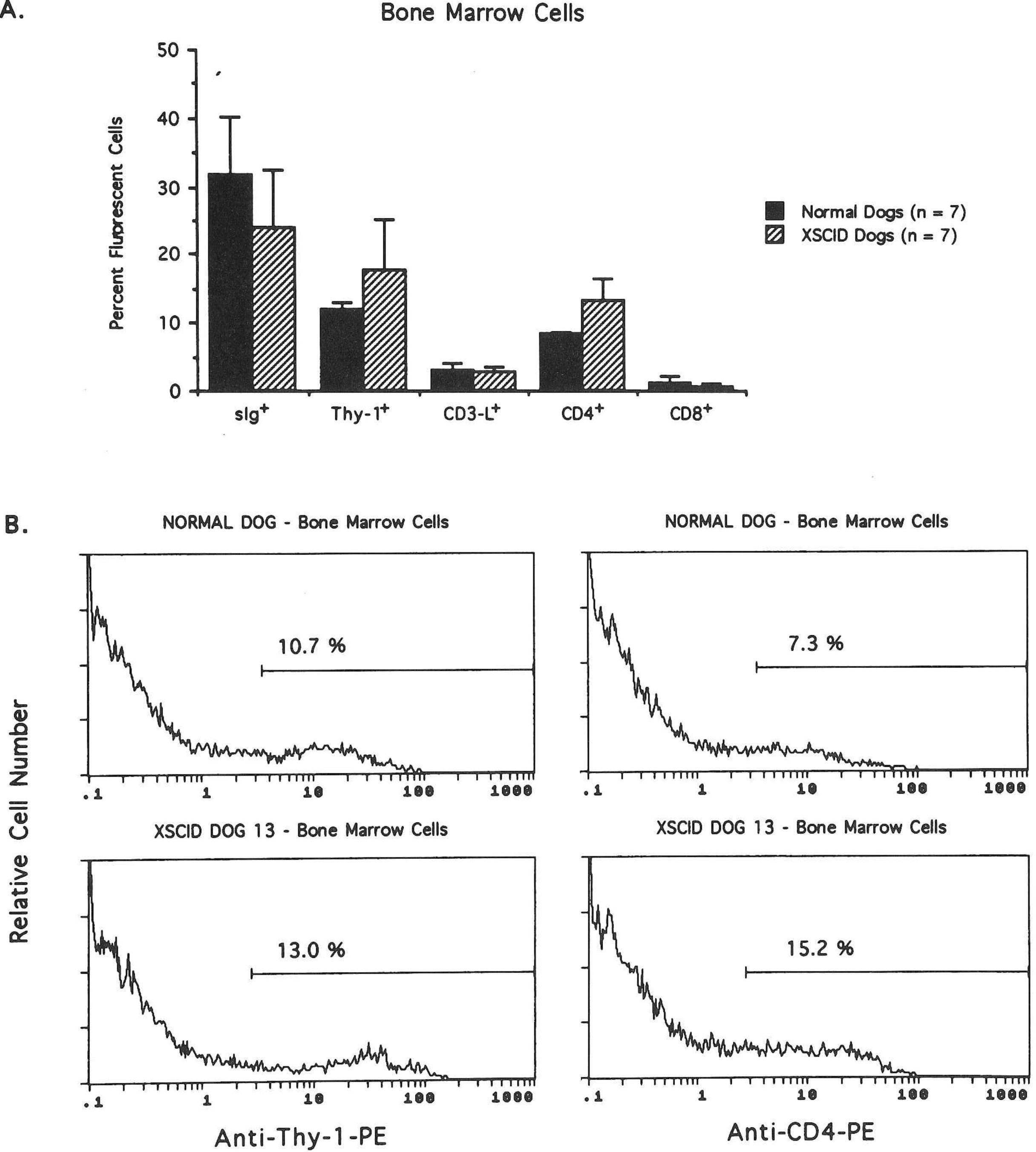
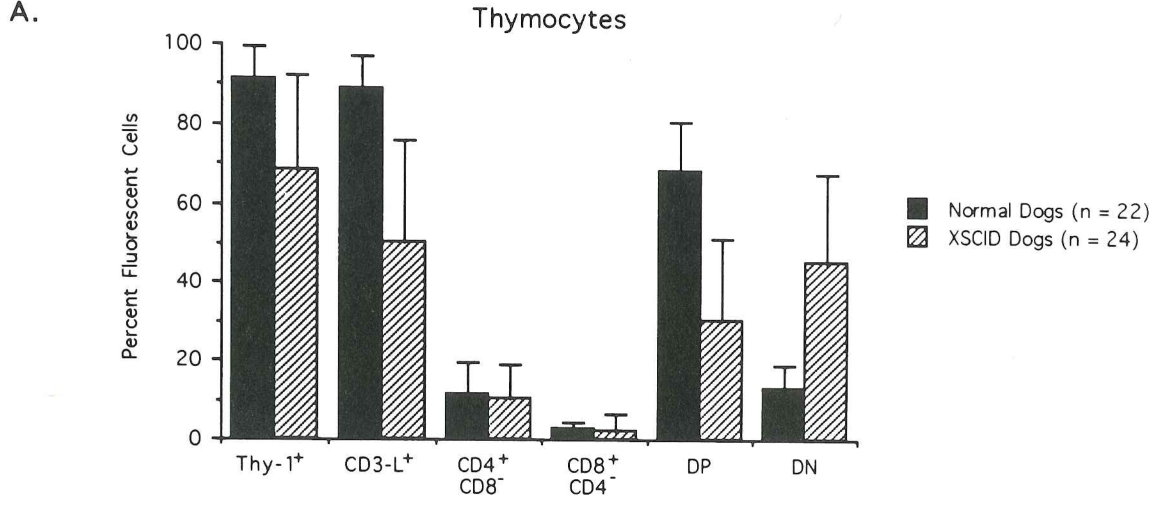


FIGURE 3. *A*. Percentage of bone marrow cells from normal and XSCID dogs that express the cell surface Ags sIg, Thy-1, CD3-L, CD4, and CD8. Staining was performed as described in *Material and Methods*. Results are expressed as mean ± SD. *B*. Representative histograms from a normal dog's bone marrow cells and an XSCID dog's bone marrow cells stained with anti-Thy-1 and anti-CD4. Staining was performed as described in *Materials and Methods*. Analysis gates were adjusted to 2.0% positive staining with goat anti-mouse IgG-PE-negative control. Numbers above analysis gates represent percent of Thy-1$^+$ and CD4$^+$ cells, respectively.

(27). One possibility is that exposure to environmental Ags is responsible for the normal percentages of CD3-L$^+$ PBL found in some of our older XSCID dogs. For example, after exposure to several microbial Ags, an increased percentage of CD3-L$^+$ PBL occurred in two gnotobiotic XSCID dogs, whereas the percentage of CD3-L$^+$ PBL in two other XSCID dogs, in a similar nonexposed chamber, remained constant. A similar fluctuation in the percentage of peripheral blood T lymphocytes (10 to 40%) was observed in David the "bubble boy," a documented XSCID patient, after exposure with a



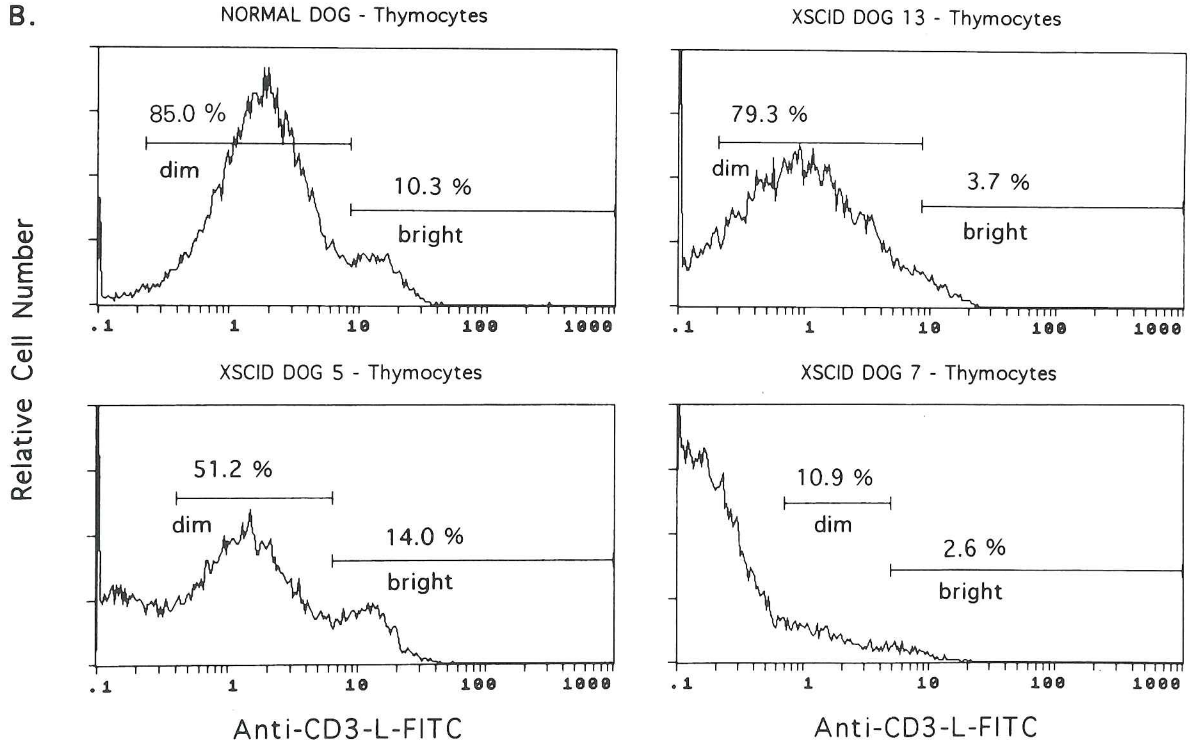


FIGURE 4. *A.* Percentage of thymocytes from normal and XSCID dogs that express the cell surface Ags sIg, Thy-1, CD3-L, CD4, and CD8. Staining was performed as described in *Material and Methods.* Results are expressed as mean ± SD. *B.* Representative histograms from a normal dog's thymocytes and three XSCID dog's thymocytes stained with anti-CD3-L demonstrating a bimodal distribution in normal thymocytes. Staining was performed as described in *Materials and Methods.* Analysis gates were adjusted to 2.0% positive staining with goat anti-mouse IgM-FITC negative control. A second analysis gate, placed according to the inflection of normal thymocytes staining profile, divides the CD3-L^{bright} from the CD3-L^{dim} thymocytes. Numbers above analysis gates represent percent of CD3-L^{dim} and CD3-L^{bright} thymocytes, respectively.

variety of microorganisms (28). Thus, it seems that the PBL phenotype in both human and canine XSCID can be altered by external factors, suggesting that mechanisms acting independently of the IL-2R-γ chain can result in the appearance of phenotypically mature T lymphocytes. It is also possible that other IL-2R-γ mutations in dogs might produce a PBL phenotype more similar to that of the majority of human XSCID patients. As dogs with other IL-2R-γ mutations are

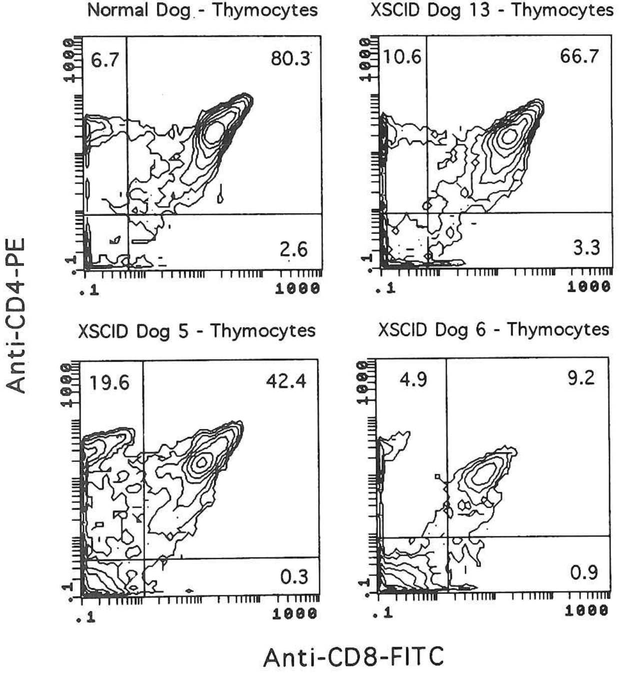


FIGURE 5. Representative contour plots of a normal dog's thymocytes and three XSCID dog's thymocytes stained with anti-CD4 and anti-CD8. Staining was performed as described in *Materials and Methods*. Analysis gates were adjusted to 2.0% positive staining with goat anti-mouse IgM-FITC and goat anti-mouse IgG-PE negative controls. Numbers in the corners represent the percentage of thymocytes within that quadrant.

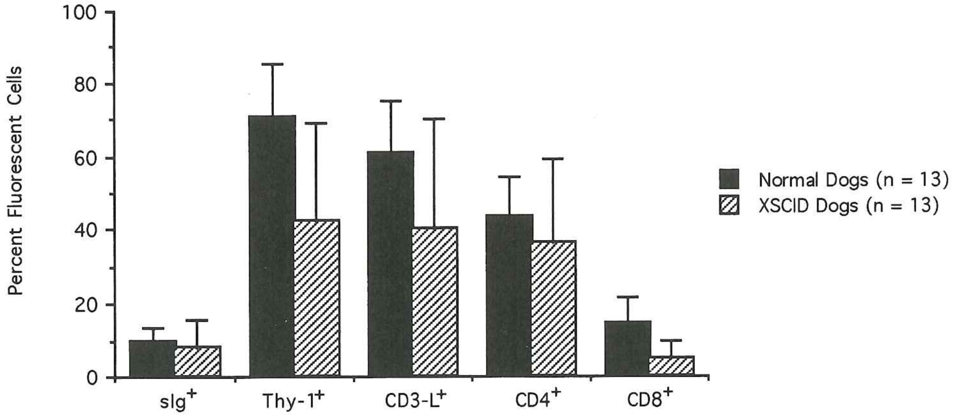
identified, we will examine their PBL phenotype to determine if they more closely resemble human XSCID patients' PBL phenotype.

Currently, few reagents are available for the identification of stem cells in canine bone marrow; however, previous studies on the distribution of Thy-1 in the dog have demonstrated that 15% of bone marrow cells, >95% of thymocytes, and 100% of peripheral T lymphocytes, but not B lymphocytes, were Thy-1^+ (15). Unfortunately, in these studies the percentage of mature T lymphocytes in the bone marrow cell preparations was unknown, leaving the possibility that the Thy-1^+ bone marrow cells were mature T lymphocytes. Our finding of approximately 15% Thy-1^+ cells in bone marrow cell preparations that contained only 3% CD3-L^+ lymphocytes provides evidence that Thy-1 is not exclusively expressed on mature T lymphocytes, and may in fact be expressed on T lymphocyte precursors, as hypothesized by McKenzie and Fabre (15). If the majority of Thy-1^+ bone marrow cells are, in fact, pre-T lymphocytes, then the normal percentage of Thy-1^+ cells in the bone marrow of XSCID dogs suggests that their development is not adversely affected.

Studies on human and murine thymocytes have revealed an ordered expression of cell surface Ags on developing thymocytes (29–32) as well as a controlled mitogenesis of thymocytes at discrete stages of development (33, 34). In normal dogs, these thymocyte phenotypic subsets were found in proportions similar to those described in humans, suggesting that the same ordered maturation process is occurring in dogs. However, the development of thymocytes in XSCID dogs is severely impaired, as evidenced by a marked reduction in total thymocyte number, an increased proportion of immature DN thymocytes, and a decreased proportion of intermediate DP thymocytes and CD3-L^+ thymocytes. The phenotype and number of XSCID thymocytes suggest a developmental block at the DN thymocyte stage before the acquisition of CD3-L. These findings indicate that a normal functioning IL-2R-γ chain is crucial for the proliferation and differentiation of DN thymocytes. Interestingly, the progression of thymocytes from CD3-L^{dim} to CD3-L^{bright} and from DP to to SP does occur in XSCID thymocytes that are able to progress to the DP stage, as evidenced by the normal percentages of CD3-L^{bright} thymocytes and SP thymocytes. The possibility does exist, however, that the SP thymocytes in XSCID thymi are not mature CD3-L^+ thymocytes but are immature CD3-L^- SP thymocytes on their way to becoming DP thymocytes (35, 36). Only three-color flow cytometric analysis of XSCID thymocytes will determine whether the SP thymocytes also express CD3-L.

Although similar alterations of thymocyte subsets and reduction in total thymocyte number have been observed in $p56^{lck}$ "knockout" mice and TCR-β knockout mice (37, 38), they both have normal numbers of DN thymocytes, unlike the severely reduced numbers of DN thymocytes found in XSCID dog thymi. This suggests that the IL-2R-γ chain may act on DN thymocytes or a subset of DN thymocytes before TCR-β-chain rearrangements and $p56^{lck}$ activity. This hypothesis is supported by the lack of TCR-β rearrangement found in thymocytes from XSCID children (39). Because IL-7 is a cofactor for the induction of TCR-β rearrangement (40) and IL-2R-γ is a functional component of IL-7R (41), perhaps the lack of a functional interaction between IL-7 and IL-2R-γ may be responsible for the thymocyte abnormalities. In mice, blockade of IL-7 signaling by treatment of fetal thymic organ cultures with anti-IL-7 (42), or treatment of pregnant mice with anti-IL-7 (43) or anti-IL-7R (44), resulted in similar thymocyte phenotypic alterations to that observed in XSCID dogs. Taken together, these findings suggest that the lack of or reduction in IL-7-induced signaling via the IL-2R-γ chain may account for the majority of thymocyte abnormalities in XSCID dogs.

Our data demonstrate a profound alteration in the expansion and differentiation of DN thymocytes in XSCID thymi and provide direct evidence that a functional IL-2R-γ chain is vital for thymocyte development to occur. Specifically, the lack of a functional IL-2R-γ chain in XSCID dogs results in the lack of stable binding with IL-2,



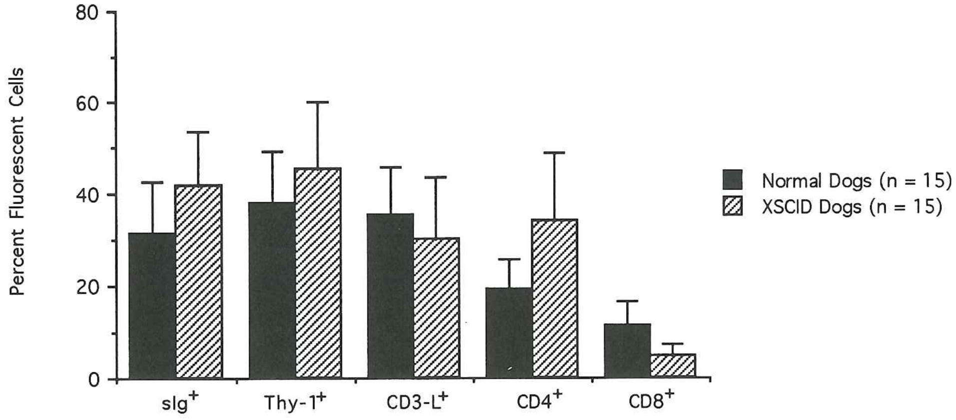


FIGURE 6. Percentage of (*A*) peripheral blood lymphocytes and (*B*) splenocytes from normal and XSCID dogs that express the cell surface Ags sIg, Thy-1, CD3-L, CD4, and CD8. Staining was performed as described in *Materials and Methods*. Analysis gates were adjusted to 2.0% positive staining with goat anti-mouse IgM-FITC and goat anti-mouse IgG-PE negative controls. Results are expressed as mean ± SD.

a severely impaired proliferative response to PHA, an undetectable proliferative response to IL-2, yet a normal capacity for IL-2 production. Canine XSCID represents the true homologue of human XSCID and is an excellent model for the examination of the role of the IL-2R-γ chain in thymocyte development and its function in mature T lymphocytes.

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