Canine X-linked severe combined immunodeficiency (XSCID) is characterized by a failure to thrive, thymic dysplasia, and a lack of functional T lymphocytes. As in human XSCID, affected dogs in our colony have a mutation in the Il-2Rα gene. This mutation dramatically affects development, cytokine production, cellularity, and normal lymphoid development.

Because CD4+CD8+ thymocytes are decreased, the proportion of CD4+CD8- thymocytes increases. This affects the development of CD4+CD8+ thymocytes. Interestingly, several XSCID dogs had high percentages of CD4+CD8- thymocytes, as they acquire the cell surface Ag, CD3, CD4, and CD8. T lymphocytes bind and are nonspecifically Ag-expressing CD4+CD8+ thymocytes, as they acquire the cell surface Ag, CD3, CD4, and CD8.

In the absence of T lymphocytes, cells with XSCID primarily express CD4+CD8- thymocytes. The lack of CD4+CD8+ thymocytes interferes with the production of peripheral T lymphocytes.

The Journal of Immunology, 1994, 153: 40-46.

**Gene 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 (SCID) represents a heterogeneous group of disorders that are characterized by a failure in humoral and cell-mediated immunity. 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 autosomal recessive form of SCID, which includes defects in the expression of histocompatibility gene products. Age at diagnosis (Ages 3-4) is a critical factor in determining survival. The only animal in which SCID is inherited by a failure to thrive, the lack of thymic development, and the absence of CD4+CD8+ thymocytes is the SCID dog (9-11). Cytogenetic linkage studies have shown that the gene defect in our colony of SCID dogs maps to the proximal Xq region of the SCID dogs, and that these SCID dogs have a mutation in the IL-2Rα gene (12).
The tumor of the mammary gland was excised. The specimen was embedded in paraffin and cut into 5 μm thick sections. The sections were stained with hematoxylin and eosin for histological examination. The tumor was found to be invasive ductal carcinoma, grade 2, with infiltrating lobules and areas of comedo necrosis. The borders of the tumor were well defined, with no evidence of lymph node involvement. The patient was subsequently treated with chemotherapy and radiation therapy.
The emergence of lymphocytes sequestered in crypts of the GI tract, the possibility of expressing CD8+ T cells in the gut, and the maintenance of lymphocyte homeostasis in the periphery. These observations suggest a role for CD8+ T cells in maintaining immune surveillance and defense against pathogen infection. Additionally, the presence of CD8+ T cells in the gut suggests a potential for these cells to contribute to the regulation of gut microbiota and the maintenance of intestinal homeostasis.

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

Thymocytes and their progenitors in the bone marrow

Phenotypically normal bone marrow cells

suppression of T cell activation in PHA-stimulated splenocytes

Caused IL-2 activity present in PHA-stimulated splenocytes

The thymus of XSCID mice was normal in appearance, and the bone marrow of XSCID mice was normoblastic. The bone marrow of XSCID mice was normoblastic and contained normal precursors of all hematopoietic lineages.
This increased percentage of monocytes in the PBMC (normal = 4% ± 2% for PBMC) in normal PBMC preparations revealed a significant increase in the percentage of monocytes in XSCID mice compared to normal mice.  

**Figure 1.** A comparison of the percentage of IL-2R+ phagocytized PBMC in normal and XSCID dogs.  

- **A.** Peripheral Blood Lymphocytes  
  - Phagocytized PBMC  
  - Normal Dogs  
  - XSCID Dogs  

- **B.** Relative Cell Number  
  - IL-2-R  
  - Normal Dogs  
  - XSCID Dogs  

**Notes:** The numbers above each graph represent percent IL-2R+ lymphocytes.
T LYMPHOCYTE ABNORMALITIES IN CANINE XSCID

**Figure 2.** Proliferative response of the IL-2-dependent murine cell line, CTLL-2, to several dilutions of 48-hour PHA-activated splenocyte supernatants collected from normal and XSCID dogs. Results are expressed as mean ± SD.

PBMNCs from XSCID dogs can be accounted for by the reduction in lymphocytes, because the total number of mononuclear cells was not increased in XSCID dogs. Flow cytometry of PBMNCs from normal and XSCID dogs was performed using CD4 and CD8 antibodies. The percentage of lymphocytes, however, was significantly lower in XSCID dogs and a proportion of lymphocytes in XSCID dogs (0.5%) was CD4−CD8−. In contrast, the percentage of lymphocytes in normal dogs was 95%. However, the total number of lymphocytes was not significantly different between normal and XSCID dogs.

High affinity IL-2 receptor binding is present on normal IL-2 receptor-expressing cells, but not in XSCID dogs. A significant reduction in high affinity IL-2 receptor binding was observed in XSCID dogs (8.5%). However, the total number of lymphocytes in XSCID dogs was still reduced in these seven XSCID dogs as a result of their CD4−CD8− phenotype. The reduction in the percentage of 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.

**Discussion**

The IL-2Rγ mutation in these XSCID dogs results in profound T lymphocyte functional abnormalities. For example, IL-2Rγ expression studies performed under conditions that were capable of detecting both intermediate (γ0) and high affinity (γ0) IL-2Rγ revealed that both were isolated and PHA-activated XSCID dogs were isolated and PHA-activated XSCID lymphocytes were isolated and PHA-activated XSCID lymphocytes were isolated. However, in their relative amount of bound IL-2, this lack of high affinity IL-2 binding is consistent with the lack of high affinity IL-2 binding observed in normal XSCID patients' transformed T cells and several other human XSCID patients' transformed T cells that expressed both IL-2Rα and IL-2Rβ. PHA-activated XSCID PBL failed to proliferate to concentrations of IL-2 that induced a significant proliferative response in normal XSCID patients. Therefore, the lack of IL-2 receptor binding is necessary for IL-2-induced proliferation. This hypothesis is supported by recent studies conducted with the use of recombinant IL-2Rγ constructs. We have shown that an interaction between the cytoplasmic domains of IL-2Rα and IL-2Rγ is necessary for IL-2 receptor signaling and proliferation [25, 26]. Although both human and canine XSCID lymphocytes are associated with mutations of the IL-2Rγ gene, there are some differences in their expression. For example, levels of the IL-2Rγ gene are present in the peripheral blood of patients with XSCID children, but the transcribed mature T lymphocytes are present in the peripheral blood of normal children. When phenotypically mature T lymphocytes are present in the peripheral blood of normal children, the T lymphocytes are usually found to be of maternal origin. We have not been able to detect mature T lymphocytes in fourteen XSCID dogs examined by northern blot analysis. These findings contrast with those observed in XSCID children, in whom circulating T lymphocytes are present. This lack of maternal enrollment may be explained by the engraftment with parental T lymphocytes. In contrast, in the XSCID children, T lymphocytes are usually found to be of maternal origin. This lack of maternal engraftment may be explained by the engraftment with parental T lymphocytes.
A documented XSCID patient, after exposure with a new product, showed an increase in CD3-CD4-CD8- cells. A similar phenomenon in the percentage of XSCID dogs' bone marrow cells was seen as a non-exposed control group, which represents a normal percentage of Thy-1+ and CD4+ cells, respectively.

Figure 3. A. Percentage of bone marrow cells from normal and XSCID dogs that express the cell surface antigens Thy-1, CD3, CD4, and CD8. Bar graph shows the percentage of Thy-1+ and CD4+ cells in bone marrow cells from normal and XSCID dogs. Results are expressed as mean ± SD.

B. A representative histogram from a normal dog's bone marrow cells and XSCID dog's bone marrow cells stained with anti-CD4 and anti-CD8. Staining was performed as described in Materials and Methods. Results were obtained from 2.0% CD3-CD4-CD8- cells.

C. (7) One possibility is that exposure to environmental agents is responsible for the normal percentages of CD3-CD4-CD8- cells in some of our older XSCID dogs. For example, after exposure to certain chemicals, an increased percentage of CD3-CD4-CD8- cells was found in some of our older XSCID dogs.
T lymphocyte abnormalities in canine XSCID

XSCID patients. As doxes with other IL-2R-γ mutations may present more similar in hist of the majority of human than other IL-2R-γ mutations in doxes with normal PBL. It is also possible of prenatally made T lymphocytes. If so, it seems that the PBL

Figure 4: A percentage of T cells from T cells expressing the CD-1, CD-3, CD-4, and CD-8 staining was performed in the normal and XSCID strains as described in Materials and Methods. The results were expressed as means ± SD. B. A. 10.3% 6.0% 100 90 80 70 60 50 40 30 20 10 0 CD-8 CD-4 CD-3 DN DP Percent Fluorescent Cells 8.0% XSCID (n = 22) Normal (n = 24) XSCID Dox (n = 24)
Our data demonstrate a profound alteration in the expression and regulation of CD10 during the development of XCDID. The downregulation of CD10 causes a profound reduction in the levels of IFN-γ, IL-7, and IL-12, which are critical for the development of XCDID.

In addition, we observe a significant increase in the expression of CD3- and CD8+ T cells in the bone marrow of XCDID donors, suggesting that the bone marrow of XCDID donors supports the proliferation of CD3- and CD8+ T cells. The possibility that the bone marrow of XCDID donors supports the proliferation of CD3- and CD8+ T cells was demonstrated in 15% of bone marrow cells, suggesting that the bone marrow of XCDID donors is enriched for CD3- and CD8+ T cells.

**Figure 5**

**Figure 5**

Representative colony formation of normal donor's bone marrow cells in the presence of anti-CD4 and anti-CD8 antibodies. The colonies scored were those composed of a mix of CD4+ and CD8+ T cells. 2.1% of the colonies scored were CD4- or CD8- T cell colonies, indicating that the bone marrow of XCDID donors is enriched for CD3- and CD8+ T cells.
Results are expressed as mean ± SD.

Figure 6. Percentages of (a) peripheral blood monocytes and (b) splenocytes from normal and XSCID dogs that express CD8, CD4, CD3−/−, CD4+T, CD3−/−, CD8 or CD4−/−. The data are expressed as the percentage of fluorescent cells in the gated population.

Acknowledgements

In lymphocyte development and function, IL-2 plays a critical role in the activation of the host by the IL-2R-7 chain encoded product of human IL-2R gene, and is an excellent marker for the homologous of murine XSCID and an excellent marker for the homologous of murine XSCID.