The B cell defect in X-/- mice closely resembles that seen in IL-7 receptor–deficient mice (4) and, thus, is most likely due to a lack of IL-7 receptor signaling. Comparisons of γc deficiencies in mice and humans have suggested that IL-7 plays a greater role for mouse as compared with human B cell development, as X-linked severe combined immunodeficiency (XSCID) patients (γc-deficient) have normal numbers of B cells (11). This differential requirement for γc-dependent cytokine signaling during B cell development is further supported by the normal numbers of B cells found in a SCID Jak3-deficient patient (12).

The phenotype of X-/- thymuses is comparable with that observed for thymuses in IL-7 receptor–deficient (4) and γc-deficient (10, 13) mice. However, both the thymuses and the spleens of X-/- mice show an increase in the ratio of CD4⁺ to CD8⁺ cells. This observation suggests that cytokine signaling may be more critical for the maturation or survival of CD8 lineage T cells than CD4 lineage T cells.

Previous biochemical studies of Jak3 indicated an association with IL-2 receptor signaling (1, 2). Furthermore, inhibitors of IL-2 receptor signaling block T cell proliferative responses (14). Our results suggest that Jak3 is essential for IL-2 receptor signaling in primary T cells. However, Jak3 may not be the only tyrosine kinase required for IL-2 receptor signaling, because both Jak1 and p56kck associate with the IL-2R β chain (2, 15).

Splenic T cells from X-/- mice, when stimulated, produce much less IL-2 than wild-type splenic T cells. This reduction in IL-2 secretion may result from a nonresponsiveness, or anergy, induced in the X-/- T cells as a result of prior T cell receptor stimulation in the absence of Jak3. This possibility is compatible with previous studies showing that activation of Jak3 is associated with the prevention of anergy (16). Consistent with this, we also observed increased expression of activation markers on the vast majority of X-/- splenic T cells, suggesting a prior T cell receptor activation event. One possibility is that most T cells routinely encounter a T cell receptor stimulation signal, perhaps as a component of the mechanism inducing thymic emigration (17), and that X-/- T cells are deficient in returning to the normal resting state. These unusual aspects of T cell development and differentiation in X-/- mice indicate previously undescribed functions of cytokine receptor signaling pathways.

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6. Lineage targeted derivatives were transfected into J1 ES cells and selected with G418 and fluorodeoxy-

Mutation of Jak3 in a Patient with SCID: Essential Role of Jak3 in Lymphoid Development

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Males with X-linked severe combined immunodeficiency (XSCID) have defects in the common cytokine receptor γc chain (γc) gene that encodes a shared, essential component of the receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15. The Janus family tyrosine kinase Jak3 is the only signaling molecule known to be associated with γc so it was hypothesized that defects in Jak3 might cause an XSCID-like phenotype. A girl with immunological features indistinguishable from those of XSCID was therefore selected for analysis. An Epstein-Barr virus (EBV)-transformed cell line derived from her lymphocytes had normal γc expression but lacked Jak3 protein and had greatly diminished Jak3 messenger RNA. Sequencing revealed a different mutation on each allele: a single nucleotide insertion resulting in a frame shift and premature termination in the Jak3 JH4 domain and a nonsense mutation in the Jak3 JH2 domain. The lack of Jak3 expression correlated with impaired B cell signaling, as demonstrated by the inability of IL-4 to activate Stat6 in the EBV-transformed cell line from the patient. These observations indicate that the functions of γc are dependent on Jak3 and that Jak3 is essential for lymphoid development and signaling.

The γc chain is an essential signaling component of receptors for IL-2 (1), IL-4 (2, 3), IL-7 (4, 5), IL-9 (6, 7), and IL-15 (8). Defects in this chain cause XSCID (9, 10), a disease characterized by impaired function of B cells and a complete or almost complete deficiency of T cells (10). Thus, XSCID occurs as a result of inactivation of numerous cytokine signaling pathways, an observation compatible with the severe phenotype of Jak3 (9, 10) and of γc-deficient mice (11): IL-2, IL-4, IL-7, IL-9, and IL-15 all activate the same Janus family kinases, Jak1 and Jak3 (6, 12). In each case, Jak1 associates with the receptor chain that plays the major role in determining both the cytokine binding specificity (6, 13) and the STAT (signal transducers and activators of transduction) proteins that are activated (14, 15). Jak3 is primarily associated with γc, and as no other signaling molecules have been identified that associate with γc, we hypothesized that the phenotype resulting from defects in Jak3 might be indistinguishable from that resulting from defects in γc, and that the key role of γc may be to bring Jak3 into proximity with the primary binding chain and its associated signaling molecules (6). Although true XSCID (γc−) females
could be born [for example, to XSCID carrier (\(\gamma^{+/-}\)) females and healthy (\(\gamma^{-/-}\)) fathers in whom a \(\gamma\) mutation occurred during spermatogenesis], such individuals would be expected to be quite rare. Because the gene for Jak3 is on the X chromosome (16), we hypothesized that females with clinical and immunological features typical of XSCID might instead have defects in Jak3. A girl with features typical of XSCID was identified (Table 1, patient AP), and an EBV-transformed B cell line was generated from her peripheral blood lymphocytes (PBLs). We then examined protein lysates from these cells for \(\gamma\) expression (Fig. 1A). In contrast to EBV-transformed cells from a \(\gamma\)-deficient XSCID patient, AP had \(\gamma\) expression equivalent to the normal control EBV-transformed cell line from individual PN. AP also had normal expression of Jak1 (Fig. 1B); however, AP did not express detectable amounts of Jak3 protein (Fig. 1C).

Protein immunoblot analysis of lysates from EBV-transformed cells derived from the parents (GP and JF, both of whom were healthy) showed substantial Jak3 expression (Fig. 1D). The lack of Jak3 in AP was explained by the observation that Jak3 mRNA was undetectable in AP cells by Northern (RNA) blot analysis (Fig. 1E) in contrast to \(\gamma\) mRNA, which was normal (Fig. 1F).

Despite the absence of Jak3 mRNA as detected by Northern blot, trace amounts of Jak3 RNA were identified by reverse transcriptase–polymerase chain reaction (RT-PCR) after 40 cycles of amplification, allowing us to sequence the entire coding region of Jak3 from both AP and normal controls. Consistent with this girl’s form of SCID being an autosomal trait in which a different mutation was inherited from each parent, she had mutations at two positions (m1 and m2, Fig. 2A) (17). A single base insertion of a G nucleotide was found in the JH4 domain at nucleotide 1172 (amino acid 391) that resulted in a frame shift and subsequent stop codon at amino acid 408 (m1, Fig. 2B), and a point mutation was found at nucleotide 1695 (amino acid 565) in the JH2 domain that converts TGCC (encoding Cys) to TGA (stop codon) (m2, Fig. 2C). These observations may explain why Jak3 RNA was not detectable by Northern analysis, as premature stop codons frequently cause reduced RNA expression as is the case with the XSCID patient of Fig. 1, C and E (18) through a number of mechanisms (19). Thus, it was assumed that the amount of RNA derived from the mutant allele would be much less than that derived from the wild-type allele in AP’s parents, so genomic DNA from EBV-transformed cells from the mother (JP) and father (GP) was amplified. Direct sequencing indicated a homozygous TGCC codon in PN and GP, whereas AP and JF were both heterozygous for TGC and TGA at this position, indicating that the stop codon mutation (m2) was inherited from the mother, and the insertion mutation (m1) was inherited from the father (18).

The correlation between the lack of Jak3 expression and greatly diminished T cells in this patient indicates a role for Jak3 in T cell development. This diminished immunoglobulin synthesis makes sense in view of the diminished T cell help in the patient. To determine whether there was also an intrinsic B cell defect, we investigated whether Stat6 could be activated by IL-4 in the EBV-transformed B cell line from AP. Stat6 was activated by IL-4 in the PN but not in the AP EBV line (Fig. 3A), demonstrating an apparent defect in B cell signaling and that Jak3 was required for Stat6 activation in these cells. Stat6 is ubiquitously expressed (14), and the lack of Stat6 activation by IL-4 was not due to a lack of Stat6 protein in AP cells (Fig. 3B). We also observed the same defect in Stat6 activation by IL-4 in XSCID cell lines (18). Thus, the defects in B cell function seen in the patient AP and in XSCID patients are probably due to deficiencies in B cell signaling as well as to deficiencies in T cell help. It is interesting that B cell numbers are normal or increased in humans with XSCID and Jak3 deficiency, but are essentially absent in mice with \(\gamma\) deficiency (11) and in young (4- to 6-week-old) mice with Jak3 deficiency (20).

This is probably because IL-7 is an essential pre-B cell growth factor in mice but not in humans (21).

Table 1. Comparison of clinical and immunological parameters of patient AP and normal control individuals.

| Serum Ig | Patient | Controls |
|----------|---------|----------|
| IgG      | n.d.    | 192–515 mg/dl |
| IgA      | n.d.    | 12–31 mg/dl  |
| IgM      | 50 mg/dl| 39–92 mg/dl  |
| IgE      | 2 IU/ml | 0–222 IU/ml  |

| Lymphocyte subtype abundance (cells/mm³) | Patient | Controls |
|------------------------------------------|---------|----------|
| CD3                                     | 204     | 1,014–5,784 |
| CD4                                     | 204     | 567–3,725  |
| CD8                                     | 58      | 390–2,673  |
| CD16                                    | 29      | 87–1,189   |
| CD20                                    | 2,592   | 121–1,072  |

| Lymphocyte proliferation (cpm)          | Patient | Controls* |
|-----------------------------------------|---------|-----------|
| Medium                                  | 632     | 693 ± 895 |
| PHA                                     | 8,221   | 222,330 ± 62,592 |
| Con A                                   | 12,357  | 208,534 ± 53,751 |
| PWM                                     | 2,621   | 135,520 ± 35,077 |
| Autologous cells                        | 11,191  | 3,183 ± 2,874 |
| Allogenic cells                         | 15,337  | 59,818 ± 38,445 |

*The values are given as mean ± SD, n = 167.
Fig. 1. Defective expression of Jak3 in an EBV-transformed B cell line from AP. Lysates from 10⁶ EBV-transformed cells from a normal control (PN), the patient (AP), and a patient with XSCID [Pt. 2 from (9)] were immunoblotted with (A) antiserum to γc [Ref78 (3, 4)], (B) a monoclonal antibody to Jak1 (Transduction Laboratories), or (C) antiserum to the COOH-terminus of Jak3 (27). D Lysates from EBV-transformed cell lines from a normal control (RS) and AP’s father (GP) and mother (JP) were also immunoblotted for Jak3. (E and F) Northern blot of total RNA (30 μg per lane) from the EBV-transformed cell lines derived from a normal control (FB), from AP, and from COS-7 cells. The blot was hybridized with a DNA probe corresponding to a 5′ region of the coding sequence for Jak3 (nucleotides 275 to 480, where nucleotide 1 corresponds to the Jak3 translation initiation codon; see GenBank accession number U03667) that shared no homology with other Jak kinases (E), or with a full-length γc complementary DNA (cDNA) probe (F). RNA was isolated with RNAzol (Tel-Test, Friendswood, Texas).

The identification of defective Jak3 expression in a case of SCID allows a number of conclusions to be drawn. First, the diminished number of T and natural killer (NK) cells indicates the important role played by Jak3 in the development of these lineages. Second, because the phenotype of Jak3-deficient SCID is indistinguishable from that of XSCID, most if not all γc-dependent signals may be dependent on Jak3. Third, the observation that the Jak3 deficiency was not more severe than XSCID suggests that Jak3 may not be essential for signaling from cytokines other than those whose receptors contain γc. The cell type-restricted expression of Jak3 (22), and the fact that Jak3 appears only to be required for signaling through γc-containing receptors, is in contrast to other Jak family kinases, which are ubiquitously expressed and associate with multiple cytokine receptors. Fourth, the lack of Stat6 activation by IL-4 in AP cells indicates that Jak3 plays an important role in STAT protein activation; indeed, it is possible that the loss of STAT protein activation significantly contributes to the phenotype in XSCID and Jak3-deficient SCID. Because STAT activation is only one type of signal induced by IL-2, IL-4, IL-7, IL-9, and IL-15, it will be interesting to determine the role of Jak3 in activating the other signaling pathways.

The possibility of using pharmacological agents that block γc-Jak3 association as immunosuppressants has been suggested (6). The current study further suggests that any agents that inactivate Jak3 function may be potent immunosuppressants. Moreover, the identification of Jak3 deficiency as the molecular basis for some autosomal recessive cases of SCID will allow the development of diagnostic procedures for identification of Jak3-deficient SCID patients and carriers and investigation into the possibility of gene therapy for Jak3-deficient SCID patients, analogous to that being developed for XSCID (23).

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Defective Lymphoid Development in Mice Lacking Jak3

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The Janus tyrosine kinases (Jaks) play a central role in signaling through cytokine receptors. Although Jak1, Jak2, and Tyk2 are widely expressed, Jak3 is predominantly expressed in hematopoietic cells and is known to associate only with the common γ(c) chain of the interleukin (IL)-2, IL-4, IL-7, IL-9, and IL-15 receptors. Homozygous mutant mice in which the Jak3 gene had been disrupted were generated by gene targeting. Jak3-deficient mice had profound reductions in thymocytes and severe B cell and T cell lymphopenia similar to severe combined immunodeficiency disease (SCID), and the residual T cells and B cells were functionally deficient. Thus, Jak3 plays a critical role in γ(c) signaling and lymphoid development.

The Jaks have been implicated in the function of receptors of the cytokine receptor superfamiliy (1). After ligand binding, the Jaks are activated by tyrosine phosphorylation, and in turn they phosphorylate one or more of the receptor chains as well as cellular substrates. Jak3 is predominantly expressed in hematopoietic cells, associated with the γ(c) chain, and is activated by the cytokines IL-2, IL-4, IL-7, IL-9, and IL-15 that use the γ(c) chain (1-3). These cytokines control lymphoid differentiation and functions. Mutations of the γ(c) chain are associated with human X-linked SCID (4) and account for approximately half of the cases of human SCID (5). The γ(c) mutations affect receptor-mediated ligand activation of Jak3 (3), although it is unknown whether this effect is critical for the SCID phenotype.

We constructed a Jak3 targeting vector to disrupt the first coding exon, which created a null allele for expression (Fig. 1A). The construct was electroporated into E14 embryonic stem (ES) cells, and four independent clones with normal karyotypes were injected into C57BL/6 blastocysts to create chimeric mice. Chimeric mice from two clones transmitted the targeted allele, and heterozygous mice from the two clones were separately bred to create homozygous mutant (−/−) mice. Genotyping of 59 progeny yielded 16 wild-type (+/+), 30 heterozygous (+/−) mice, and 13 −/− mice; this correspondence to the expected ratios (1:2:1) indicated the lack of an effect on embryonic development. The −/− mice were indistinguishable from their littermates and thrived comparably under specific pathogen-free conditions. No Jak3 protein was detectable in splenic, thymic, or bone marrow extracts from the −/− mice (6).

Fig. 1. Disruption of the Jak3 gene by homologous recombination. (A) Maps of the Jak3 locus (top), the targeting construct in pbLueScript II (Stratagene) (middle), and the targeted locus (bottom). Restriction enzymes were Eco RI (E), Bam HI (B), and Hind III (H). Genomic Jak3 clones were obtained from a 129-derived CCE ES cell genomic library. The hygromycin-resistance gene (Hyg) cassette, containing the same promoter as pMC1neo (13), was inserted in an exonic Stu I site located 0.18 kilobase (kb) downstream of the first ATG of the Jak3 gene. The Herpes simplex virus thymidine kinase (k) gene cassette, containing the same promoter as in the Hyg cassette, was inserted into the 3′ end of the targeting vector for negative selection. The targeting vector contained 5.8- and 3.2-kb Jak3 fragments. Electroporation of the linearized plasmid into 129-derived E14 ES cells and screening by Southern (DNA) blot analysis for homologous recombination were as described (14); the 3′ flanking probe used in Southern blot analysis is shown as a bar. The efficiency of homologous recombination was 96%. (B) Southern blot analysis of mouse tail DNA. Genomic DNA from +/+ mice (lane 1), +/− mice (lanes 2 through 5, 7, and 9), and −/− mice (lanes 6 and 8) was digested with Hind III and probed with a 1.4-kb Eco RI–Hind III fragment. The 5.0- and 7.0-kb bands represent the wild-type and mutated alleles, respectively. When the blot was rehybridized with a Hyg probe, only the 7.0-kb band was detected (15). Bam HI digests probed with another 3′ probe and Eco RI digests probed with a 0.85-kb Sst I complementary DNA probe containing the first ATG further confirmed appropriate homologous recombination (15).

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