Defective Lymphoid Development in Mice Lacking Jak3

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(GCC CAC) → Gly-Asp (GGC GAC); and Arg^{895}-Pro^{896}-Glu^{897} (CCG CCA GAG) → Arg-Gln-Ser (CGC CAG AGC). It should be noted that some transcripts from the patient contained insertions (67, 78, or 114 nucleotides) after nucleotide 1441. These insertions appeared to arise by alternative splicing because they represent intronic sequences beginning 230, 194, or 183 nucleotides 3′ of the exon-intron boundary at nucleotide 1441 and extend to a common endpoint. Although the insertions contain in-frame stop codons, the alternative splicing does not represent a disease-causing difference in AP as no mutations in the intron or surrounding exon sequence were found, and these splice variants were also found in other RNA samples including both of AP's parents and PBLs from a normal donor. The physiological effects, if any, of this alternative splicing are unknown.

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24. RNA was prepared with RNAzol, reverse-transcribed with Superscript II reverse transcriptase (Gibco BRL), and PCR-amplified in overlapping regions of 400 to 1200 base pairs from AP and normal controls (PN and RB) with Taq polymerase (Gibco BRL). Direct PCR sequencing was performed on both strands with the Applied Biosystems 373A DNA Sequencing System. PCR products were also subcloned with the TA Cloning kit (Invitrogen), and multiple clones were sequenced with Sequenase (USB, Amersham) to generate the gels shown in Fig. 2, B and C.

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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 (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 superfamily (*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, associates 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. To address this question and to determine whether Jak3 is critical for signaling in other lineages, we developed mice lacking Jak3.

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 (+/+) mice, 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*).

Morphologically, the lymphoid tissues of the −/− mice were markedly different from

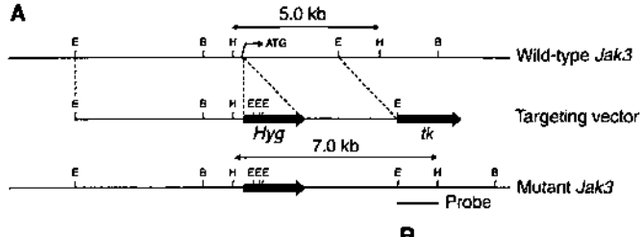

Fig. 1. Disruption of the *Jak3* gene by homologous recombination. (**A**) Maps of the *Jak3* locus (top), the targeting construct in pBluescript II (Stratagene) (middle), and the targeted locus (bottom). Restriction enzymes were Eco RI (E), Bam HI (B), and Hind III (H). Genomic *Jak3* clones were obtained from a 129-derived CCE ES cell genomic library. The hygromycin-resistance gene (*Hyg*) cassette, containing the same promoter as in 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 (*tk*) 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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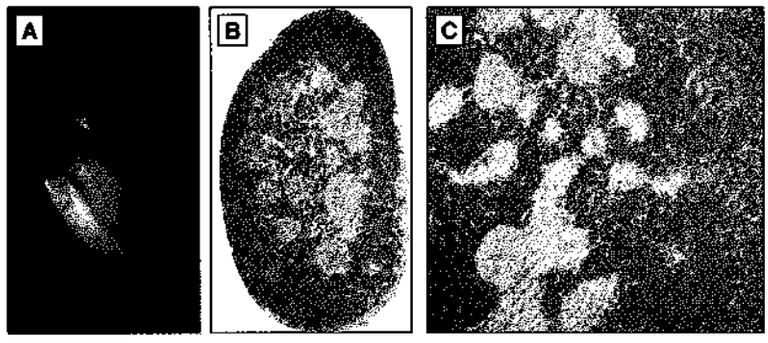

Fig. 2. Thymus size and morphology of −/− mice compared with normal littermate controls. (**A**) Freshly isolated thymuses (magnification, ×2.8) from a 9-day-old −/− mouse (top) and from a wild-type littermate (bottom). (**B** and **C**) Histological analysis of thymuses from a 4-week-old −/− mouse (B) and from a wild-type littermate (C) stained with hematoxylin and eosin (magnifications, ×22).

their littermates. The thymuses of the −/− mice were much smaller (Fig. 2), as were the mesenteric and peripheral lymph nodes and gut-associated lymphoid tissues. The spleens were slightly smaller in the youngest mice examined (1 week old) and were consistently smaller than those of normal littermates with age. All other organs were macroscopically normal. Peripheral blood smears from the −/− mice showed decreased numbers of lymphoid cells, but the numbers of granulocytes, erythroid cells, and monocytes were normal. Consistent with these findings, the numbers of Gr-1–positive granulocytic cells and Mac-1–positive cells were normal (*7*).

To further characterize the deficiencies, we examined the effect of age on the numbers, phenotype, and function of lymphoid cells. The total numbers of thymocytes in the −/− mice were 1 to 10% of those in the +/− or +/+ mice (Table 1). Histologically, the −/− thymuses were normal with identifiable cortical and medullary areas (Fig. 2B). Flow cytometric analysis showed decreased percentages of $CD4^+CD8^+$ cells in the youngest mice (1 week old) relative to control littermates; these percentages increased to normal with age (Table 1 and Fig. 3A). With age, the −/− mice showed a relative increase in the proportion of $CD4^+$ to $CD8^+$ thymocytes compared to that in controls (Table 1). In contrast to those from controls, thymocytes from −/− mice at all ages failed to respond to concanavalin A (Con A) or phorbol myristyl acetate (PMA) in combination with antibody to CD3 (anti-CD3); the results obtained with thymocytes from 4-week-old mice are shown in Fig. 4A. Thymocytes from the −/− mice also failed to respond to Con A in combination with IL-2 (*7*), which indicated that the lack of a response was not caused by an inability to produce IL-2.

The spleens of the −/− mice were consistently smaller. For example, in 2- to 4-week-old −/− mice, the total numbers of nonerythroid splenocytes were 10 to 25% of those of controls (Table 1). Flow cytometric analyses of splenocytes from 1-week-old −/− mice revealed a marked reduction in the number of $B220^+$ cells (Fig. 3B and Table 1) and decreased numbers of $CD4^+$ or $CD8^+$ cells relative to controls. In older −/− mice, there were detectable numbers of $B220^+sIgM^-$ cells (sIgM, surface immunoglobulin M) and of immature and mature B cells that are $B220^+sIgM^+$ cells (Fig. 3C and Table 1). In addition, $CD4^+$ and $CD8^+$ cells were detectable, although in the −/− mice the proportion of $CD4^+$ to $CD8^+$ cells was higher than in controls, comparable to the differences seen in thymocytes. The absolute numbers of single-positive T cells and $B220^+sIgM^+$ cells in the spleens of the −/− mice were 5 to 10% and 1.6 to 3.3% of those in controls, respectively.

Splenocytes and lymph node cells from 4-week-old −/− mice responded poorly to Con A, PMA plus anti-CD3, or lipopolysaccharide (LPS) (Fig. 4A). LPS-activated normal splenic B cells respond to IL-4 by acti-

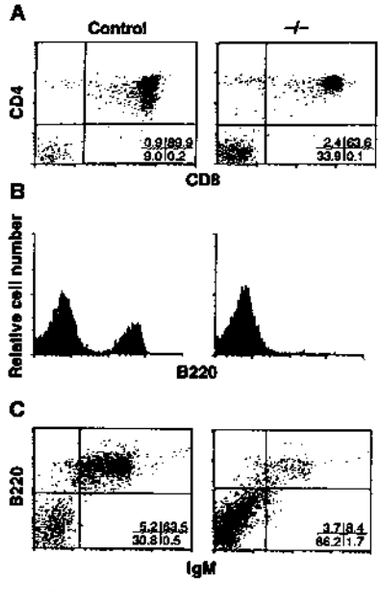

Fig. 3. Examples of flow cytometric analyses of thymocytes and splenocytes from −/− mice (right) and from +/+ or +/− control mice (left). (**A**) Thymocytes from 7-day-old mice stained with phycoerythrin (PE)–anti-CD4 (H129.19) and fluorescein isothiocyanate (FITC)–anti-CD8 (53-6.7). (**B**) Splenocytes from 7-day-old mice stained with PE–anti-B220 (RA3-6B2). (**C**) Splenocytes from 4-week-old mice stained with PE–anti-B220 and FITC–anti-IgM (R6-60.2). Cells were stained with monoclonal antibodies for 30 min at 4°C, washed with phosphate-buffered saline, and analyzed by double-color flow cytometry on a FACScan (Becton Dickinson). Antibodies were purchased from Pharmingen.

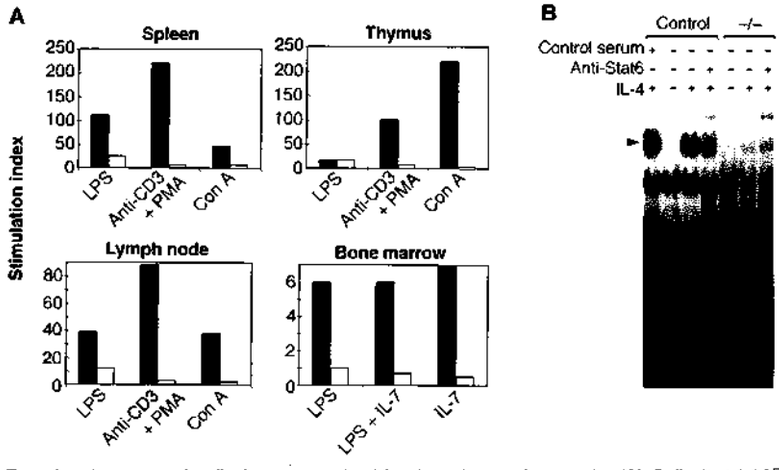

Fig. 4. Functional assays of cells from 4-week-old −/− mice and controls. (**A**) Cells (1×10^5) were stimulated for 72 hours with Con A (20 μg/ml), with anti-CD3ε (145-2C11) (10 μg/ml) plus PMA (10 ng/ml), or with LPS (10 μg/ml) and were then pulsed with 0.5 μCi of [^{3}H]thymidine for 12 hours, and incorporation of [^{3}H]thymidine was measured. Assays were done in triplicate [open bars, −/− mice; solid bars, control littermates (+/−)]. Bone marrow cells (1×10^5) were stimulated with LPS (10 μg/ml), IL-7 (5 ng/ml), or both. (**B**) Splenocytes were cultured in media containing LPS (25 μg/ml) for 24 hours and were then stimulated for 30 min with IL-4 (50 ng/ml). Extracts were prepared and analyzed for Stat6 DNA-binding activity as described (*8*). The arrowhead indicates the position of the Stat6-DNA complex.

Table 1. Numbers and phenotype of lymphoid cells in −/− mice. Thymus and spleen cellularities were determined for 13 −/− mice at ages from 7 to 28 days; representative values are compared with values for control (C) littermates (+/− or +/+). The phenotypes were determined by fluorescence-activated cell sorting; the percentages staining positive are given (–, not determined).

	Cellularity (%) and genotype											
	Total (10^6 cells)		$CD4^+CD8^+$		$B220^+$ (total)		$B220^+sIgM^+$		$CD4^+$		$CD8^+$	
Cells	−/−	C	−/−	C	−/−	C	−/−	C	−/−	C	−/−	C
					Day 7							
Thymus	1.5	46.3	11.6	93.1	–	–	–	–	1.3	0.7	0.1	0.2
Spleen	29.5	35.0	–	–	2.6	22.0	–	–	0.7	2.9	0.1	1.0
					Day 9							
Thymus	0.8	57.0	83.6	93.2	–	–	–	–	9.0	4.7	0.3	0.5
Spleen	21.0	24.0	–	–	2.1	23.2	–	–	0.6	4.9	0.1	1.3
					Day 12							
Thymus	7.6	136.0	86.4	94.8	–	–	–	–	10.5	3.9	0.4	0.6
Spleen	5.8	41.0	–	–	11.6	62.5	9.4	60.2	6.6	9.7	0.4	3.2
					Day 25							
Thymus	12.8	151.0	81.2	88.2	–	–	–	–	11.4	9.3	1.3	1.7
Spleen	22.0	81.0	–	–	12.1	68.7	8.4	63.5	7.0	12.3	0.4	4.8

vating Jak3 (*2*) and inducing the tyrosine phosphorylation and activation of the DNA-binding activity of the signal transducer and activator of transcription 6 (Stat6) (*8*). Gel shift analysis showed that although IL-4–induced Stat6 DNA-binding activity was detectable in splenocytes from the +/+ and +/− mice (Fig. 4B), only a faint activity that was not affected by antisera to Stat6 was induced in splenocytes from the −/− mice. The absence of IL-7 or the IL-7 receptor α chain results in profound effects on lymphoid differentiation (*9*). Because the IL-7 receptor uses the γ_c chain and activates Jak3 as well as Lyn and Fyn (*10*), we examined the response of bone marrow cells to IL-7 (Fig. 4A). No stimulation was observed with bone marrow cells from the −/− mice.

The phenotype of −/− mice is similar to that of mice lacking the γ_c chain (*11*), the IL-7 receptor α chain, or IL-7 (*9*), consistent with the hypothesis that the interaction of IL-7 with its receptor and the activation of Jak3 are critical, nonredundant functions in lymphoid development. In all cases some lymphoid development proceeds, as assessed by phenotypic markers. In −/− mice, these cells are nonfunctional, even in response to mitogens that do not require Jak3 activation; this suggests a possible spectrum of effects on functional differentiation. The difference in the differentiation of $CD4^+$ versus $CD8^+$ T cells is readily demonstrable, and although the basis of this difference is unknown, it is also seen in γ_c-deficient mice.

Jaks are critical for signaling through cytokine receptors in a variety of lineages. The expression of Jak3 in myeloid cells suggests an importance in these lineages. The phenotype of the −/− mice clearly demonstrates that although Jak3 is critical for normal lymphoid development, it is not required (or is functionally redundant) for myeloid lineages. Therefore, the phenotype of mice that are deficient in other Jaks will be of interest. Because the phenotype of the −/− mice is that of SCID, our results support the hypothesis that mutations of *Jak3* and the absence of Jak3 in humans with SCID are sufficient to account for their immunodeficiency (*12*). Although the extent to which *Jak3* contributes to non–X-linked SCID is not currently known, this deficiency may be an excellent setting for gene therapy. The availability of a mouse model will be of value in verifying this hypothesis and conducting preclinical studies.

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Superior Parietal Cortex Activation During Spatial Attention Shifts and Visual Feature Conjunction

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Positron emission tomography was used to measure changes in the regional cerebral blood flow of normal people while they searched visual displays for targets defined by color, by motion, or by a conjunction of color and motion. A region in the superior parietal cortex was activated only during the conjunction task, at a location that had previously been shown to be engaged by successive shifts of spatial attention. Correspondingly, the time needed to detect a conjunction target increased with the number of items in the display, which is consistent with the use of a mechanism that successively analyzes each item in the visual field.

A central issue for theories of attention is whether a particular visual object is detected by the simultaneous analysis of all objects across the visual field (parallel search) or by the successive analysis of each object (serial search). To investigate this issue, psychologists have used visual tasks in which people search an array of items for the presence of a prespecified target item (*1*). Depending on conditions, the accuracy and speed shown in detection of a target can be independent of the number of distractor objects in the field (a flat search

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