locomotor activity and muscular tone. Orphanin FQ was also administered IT (2.5 to 10 nmol per 4 μl per mouse), but again no analgesic effect was observed (*13*). We therefore conclude that, in the hot-plate test, orphanin FQ does not induce analgesia. However, when tested in the tail-flick assay, orphanin FQ induced hyperalgesia (0.3 to 10 nmol per 5 μl per mouse, ICV). At a dose of 1 nmol, the reaction time was reduced by ~75% relative to that of vehicle-injected mice (Fig. 4C). The hyperalgesic effect of orphanin FQ was not observed in the hot-plate test, possibly because this test is more dependent on muscular tone and locomotion than is the tail-flick assay (*14*).

Our results demonstrate that despite its structural similarity to the opioid peptides, orphanin FQ appears to be pharmacologically and physiologically distinct from them. Like other neuropeptides, orphanin FQ is presumably synthesized as part of a larger precursor protein (*15*). In view of the increasing number of orphan receptors being identified, it is likely that strategies similar to that used for the identification of orphanin FQ will lead to the description of numerous neurotransmitters or neuropeptides that are unknown today.

REFERENCES AND NOTES

1. Z. A. Hall, *Trends Neurosci.* **10**, 99 (1987); C. F. Stevens, *Nature* **328**, 198 (1987).
2. O. Civelli *et al., Mol. Neurobiol.* **1**, 373 (1987); J. R. Bunzow *et al., Nature* **336**, 783 (1988); F. Libert *et al., Science* **244**, 569 (1989).
3. J. R. Bunzow *et al., FEBS Lett.* **347**, 284 (1994); C. Mollereau *et al., ibid.* **341**, 33 (1994); J. B. Wang *et al., ibid.* **348**, 75 (1994); K. Fukuda *et al., ibid.* **343**, 42 (1994); Y. Chen *et al., ibid.* **347**, 279 (1994); M. J. Wick *et al., Mol. Brain Res.* **27**, 37 (1994); J. E. Lachowicz, Y. Shen, F. J. Monsma Jr., D. R. Sibley, *J. Neurochem.* **64**, 34 (1995). This opioidlike receptor has been named LC132, ORL-1, XOR-1, ROR-C, XOR, Hyp 8-1, and C3, respectively, by these authors.
4. C. J. Evans *et al., Science* **258**, 1952 (1992); B. L. Kieffer *et al., Proc. Natl. Acad. Sci. U.S.A.* **89**, 12048 (1992); K. Yasuda *et al., ibid.* **90**, 6736 (1993); Y. Chen *et al., Mol. Pharmacol.* **44**, 8 (1993).
5. Freshly frozen porcine hypothalamic tissue (4.5 kg) was extracted in 9 liters of a solution containing 0.5 M acetic acid, 10 mM ascorbic acid, and 1 mM EDTA. After centrifugation, the supernatant was adsorbed in batches onto C_{18} silica matrix; unbound material was removed by washing and bound material was eluted with 80% methanol. A total of 2 liters of methanolic eluate was concentrated by rotary evaporation to a final volume of 44 ml. Material with a molecular mass of <10 kD was obtained by ultrafiltration and applied in 10 individual runs to a cation-exchange HPLC column.
6. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and X, any amino acid.
7. R. K. Reinscheid, unpublished data.
8. Orphanin FQ (10 nM) was applied together with opioid agonists or antagonists (β-endorphin, dynorphin B, dynorphin A 2-13, naloxone, cyclazocine, bremazocine, nor-BNI, ICI 204.448, U 62.066, U 50.488, and U 69.593, all at 1 μM), and the effect on forskolin-stimulated cAMP accumulation in LC132-transfected cells was determined as described (*16*).
9. ^{125}I-labeled Tyr^{14}-orphanin FQ and nonradioactive iodinated peptide were synthesized by the chloramine T method [W. M. Hunter and F. C. Greenwood, *Nature* **194**, 495 (1962)] and purified by reversed-phase HPLC. The monoiodinated species was obtained as a single peak, so that the specific activity was estimated to be 2200 Ci/mmol on the day of synthesis.
10. C. H. Li, in *Biochemical and Clinical Aspects of Neuropeptides,* G. Koch and D. Richter, Eds. (Academic Press, Orlando, FL, 1983), pp. 17–31.
11. R. K. Reinscheid, unpublished data.
12. Immediately after administration, mice were placed in transparent boxes in groups of three, and behavioral signs were recorded. Emphasis was placed on signs indicative of depressant, stimulant, and autonomic effects [S. Irwin, *Psychopharmacologia* **13**, 222 (1968)]. Animal experimentation and care were done in accordance with the Swiss Federal Ordinance on the Protection of Animals.
13. At 10 nmol of orphanin FQ per mouse IT, all mice tested exhibited hindlimb paralysis and a decrease in locomotor activity (A. Bourson, unpublished data).
14. P. L. Wood, in *Analgesics: Neurochemical, Behavioral and Clinical Perspectives,* M. J. Kuhar and G. W. Pasternak, Eds. (Raven, New York, 1984), pp. 175–194.
15. D. R. Lynch and S. H. Snyder, *Annu. Rev. Biochem.* **55**, 773 (1986).
16. CHO dhfr$^-$ cells were transfected by calcium phosphate precipitation with the LC132 cDNA cloned into the eukaryotic expression vector pRcRSV (Invitrogen). Stable clones were selected with G418 and screened for expression of the corresponding mRNA in the reverse transcription polymerase chain reaction. One clone (LC-7) was chosen for further experiments. For determination of concentrations of cAMP, receptor-transfected CHO cells or CHO dhfr$^-$ wild-type cells were plated in 24-well plates and grown to confluency. After removal of the culture medium, portions of HPLC fractions or peptides dissolved in a total volume of 0.2 ml of Dulbecco's modified Eagle's medium [containing 10 mM Hepes (pH 7.4), 1 μM forskolin, and 1 μM phosphodiesterase inhibitor Ro 20-1724] were added and the cells were incubated for 10 min at 37°C. Reactions were stopped by addition of 0.5 ml of ice-cold ethanol and plates were frozen at −80°C for 12 hours. After centrifugation of the plates, portions of the supernatant were removed and dried for cAMP determination. cAMP assays (Biotrak SPA, Amersham) were done according to the manufacturer's instructions.
17. Membranes from LC132-transfected cells (12 to 16 μg of membrane protein per assay) were incubated with various concentrations of ^{125}I-labeled Tyr^{14}–orphanin FQ in a final volume of 0.2 ml of binding buffer [50 mM Hepes (pH 7.4), 10 mM NaCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1% bovine serum albumin, and 0.025% bacitracin] containing 1 mg of wheat germ agglutinin – coated SPA beads (Amersham). Assays were done in 96-well plates (OptiPlate, Canberra Packard), and the mixtures were agitated for 60 min at room temperature before counting. Under these conditions, membranes become linked to the bead surface through a lectin-glycoprotein interaction. Bound ^{125}I was detected by scintillation proximity [N. Nelson, *Anal. Biochem.* **165**, 287 (1987); N. Bosworth and P. Towers, *Nature* **341**, 167 (1989)] with a TopCount microplate scintillation counter (Canberra Packard). A conversion factor of 6.35 for the ratio of gamma to scintillation counts was obtained by measuring ^{125}I-labeled fluomicrospheres in both counting modes.
18. P. J. Munson and D. Rodbard, *Anal. Biochem.* **107**, 220 (1980).
19. Experiments on tail-flick analgesia were done on a contract basis at Institut Technique pour L'Etude du Médicament–Laboratoire de Recherche, Le Kremlin-Bicetre, France.

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Defects in B Lymphocyte Maturation and T Lymphocyte Activation in Mice Lacking Jak3

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Biochemical studies of signaling mediated by many cytokine and growth factor receptors have implicated members of the Jak family of tyrosine kinases in these pathways. Specifically, Jak3 has been shown to be associated with the interleukin-2 (IL-2) receptor γ chain, a component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15. Mice lacking Jak3 showed a severe block in B cell development at the pre-B stage in the bone marrow. In contrast, although the thymuses of these mice were small, T cell maturation progressed relatively normally. In response to mitogenic signals, peripheral T cells in Jak3-deficient mice did not proliferate and secreted small amounts of IL-2. These data demonstrate that Jak3 is critical for the progression of B cell development in the bone marrow and for the functional competence of mature T cells.

B lymphocyte development in the bone marrow and T lymphocyte development in the thymus are dependent on signaling pathways mediated by a complex array of cell surface receptors. These signals include responses to lymphocyte–stromal cell interactions, as well as responses to soluble growth and differentiation factors. The Janus kinase (Jak) family tyrosine kinases, Jak1 and Jak3, have been implicated in signaling through receptors for IL-2, IL-4, IL-7, IL-9, and IL-15, all of which use the IL-2 receptor common γ (γ_c) chain (*1*, *2*). Unlike Jak1, expression of Jak3 is restricted to lymphoid and myeloid cell lines and to hematopoietic tissues such as thymus, bone

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marrow, fetal liver, and spleen (*1*, *3*). Because IL-7, in particular, is involved in lymphocyte development (*4*, *5*), it seemed likely that Jak3 might play a nonredundant role in B and T lymphocyte maturation. Therefore, we generated mice deficient in Jak3 expression by gene targeting in embryonic stem (ES) cells (*6*).

Insertion of the neomycin-resistance gene replaces sequences encoding subdomains I to IV of the Jak3 kinase domain, inactivating Jak3 enzymatic activity (Fig. 1A). Germline-transmitting chimeric mice generated from the targeted ES cells were bred to C57BL/6 mice, and progeny heterozygous for the Jak3 mutation were intercrossed. A Southern (DNA) blot showing the genotypes of the progeny from an intercross of two heterozygotes indicates the expected three types of progeny (Fig. 1B). Homozygous *Jak3*$^{-/-}$ mice were healthy and showed no gross differences in size or weight compared with their wild-type littermates. Analysis of protein lysates from bone marrow, thymus, and spleen of heterozygous (+/–) and homozygous (–/–) mutant mice demonstrated the absence of any Jak3 protein in mutant mice (Fig. 1C). Longer exposure of the protein immunoblot failed to show any partial fragments encoding the NH_2-terminal portion of Jak3 (*7*).

To examine the requirement for Jak3 in B cell development, we surface stained cells from the bone marrow of heterozygous and homozygous mutant mice with antibody to CD45R [anti-CD45R (B220)], anti-CD43, and antibody to immunoglobulin M (IgM) (*8*) (Fig. 2). Normal B cell development proceeds from CD43$^+$CD45R$^+$ (pro-B), to CD43$^-$CD45R$^+$ (pre-B), and then to the CD45R$^+$IgM$^+$ (immature and mature B) stage (*9*). The bone marrow from –/– mice contained similar total numbers of cells in comparison with bone marrow from wild-type mice and showed no significant decrease in CD43$^+$CD45R$^+$ cells, indicating normal development of pro-B cells. However, –/– bone marrow had decreased numbers of CD43$^-$CD45R$^+$ cells, and virtually no CD45R$^+$IgM$^+$ cells, reflecting an absence of immature and mature B cells (Fig. 2). Taken together, these findings indicate a block at the pre-B stage of maturation in –/– bone marrow. In contrast, no decrease in the numbers of Mac1$^+$ cells was seen, indicating that the myeloid progenitors assessed were normal (Fig. 2).

The thymuses of the –/– mice were much smaller than those of wild-type mice and ranged widely in total cell numbers, from ~0.5 to 10% of normal (Fig. 2). This wide range of thymocyte numbers did not correlate with age; all mice analyzed were 4 to 6 weeks of age, and –/– littermates analyzed in parallel often differed widely in thymocyte cellularity. However, in general, the CD4 versus CD8 staining profile of thymocytes from –/– mice appeared largely normal (Fig. 2). Similar to the γ_c-deficient mice (*10*), an increase in the percentage of CD4$^+$CD8$^-$ cells was often seen, particularly in the smallest thymuses. Anti-CD3 staining of –/– thymocytes was normal, with a slight increase in CD3^{hi} cells reflecting the increase in CD4$^+$ cells (Fig. 2). No differences in staining with antibodies to heat-stable antigen, CD44, or CD25 were detected (*7*). Overall, thymuses of homozygous mutant mice were small but normal in cell composition.

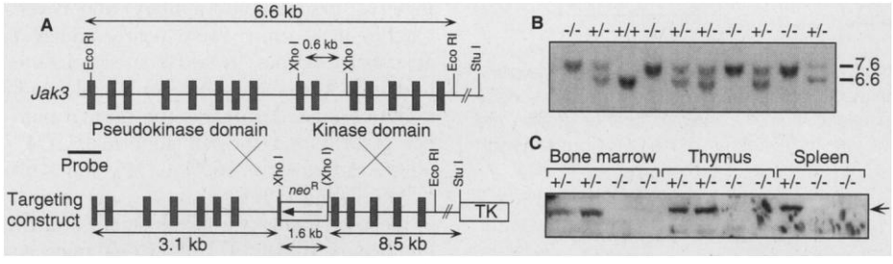

Fig. 1. Targeted inactivation of the gene *Jak3*. (**A**) Partial map of the *Jak3* locus, indicating the exons encoding the pseudokinase and the kinase domains. A 0.6-kb Xho I fragment, containing sequences encoding subdomains I to IV of the kinase domain, was replaced with the 1.6-kb *neo*^{R} cassette (*18*) shown in the targeting construct. The indicated probe used to screen for targeted insertions by Southern (DNA) blot analysis lies outside the region encompassed by the targeting vector. (**B**) Southern blot of Eco RI–digested tail DNA from a litter derived by heterozygous intercrossing, probed with a 0.35-kb Eco RI–Hind III fragment of the *Jak3* cDNA clone. Wild-type (+/+) individuals are homozygous for the 6.6-kb fragment, and –/– mutants are homozygous for the 7.6-kb fragment. (**C**) Protein immunoblot of total lysate derived from bone marrow, thymus, or spleen of +/– or –/– mice. The membrane was probed with a rabbit antiserum raised against a bacterial fusion protein containing residues 136 to 305 of the NH_2-terminus of Jak3.

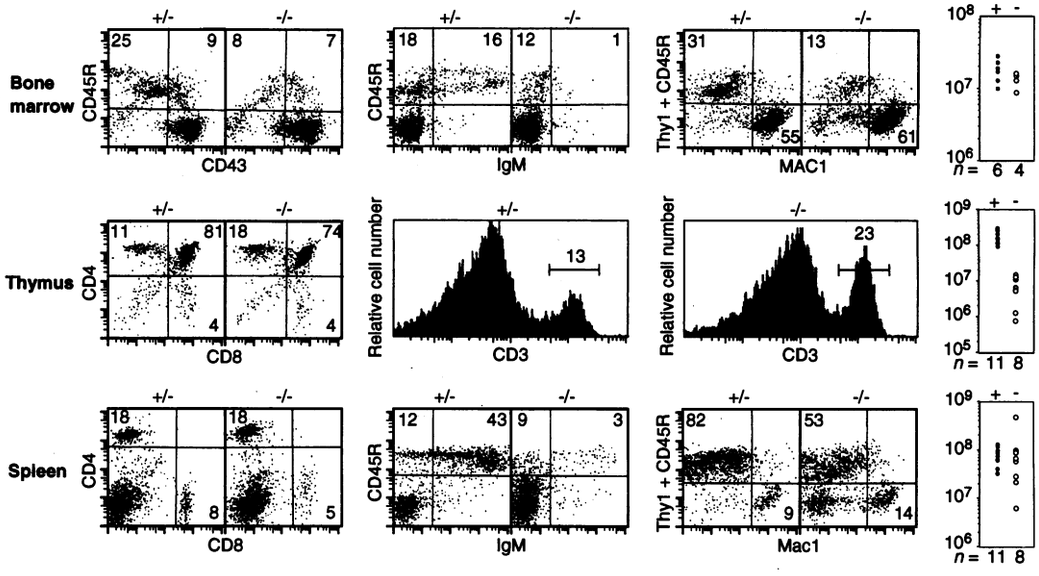

Fig. 2. Loss of IgM$^+$ B cells and low thymocyte cellularity in Jak3-deficient mice. The bone marrow, thymus, and spleen cells from +/– and –/– mutant mice were stained with the indicated antibodies and analyzed by flow cytometry. Numbers in the quadrants indicate the subpopulation percentages. The dot plots are representative of average staining profiles, although some individuals had greatly increased CD4$^+$/CD8$^+$ ratios in thymus and spleen. At the far right are shown the total cellularities for bone marrow, thymus, and spleen of each individual mouse analyzed (+, +/+ and +/– individuals; –, –/– individuals), with the numbers of indivuals analyzed indicated below. Note the large variation in thymocyte (>10-fold) and splenocyte (>100-fold) cellularity in –/– mice.

We observed a wide range in total cellularity of –/– spleens, ranging from 10 to 500% of wild-type numbers (Fig. 2). As in bone marrow, the fraction of Mac1$^+$ cells in –/– spleens appeared normal (Fig. 2). Analysis of the spleens of –/– mice also confirmed the block in B cell maturation observed in the bone marrow, as extremely few CD45R$^+$IgM$^+$ cells were detected (Fig. 2). Corroborating this B cell defect, lipopolysaccharide (LPS) responses of spleen cells were substantially reduced (Table 1). In addition, peritoneal lavage cells from –/– mice contained significantly fewer CD45R$^+$IgM$^+$ cells than controls (7). In spite of this B cell deficiency, serum Ig concentrations in the mutant mice appeared roughly normal (7), although these results are difficult to interpret because of the contribution of maternal antibodies. Analyses of a small number of 3-month-old –/– mice showed decreased concentrations of serum IgG (7). The fraction of T cells in the spleen was generally normal (Fig. 2), although some –/– mice had substantially more CD4$^+$, and fewer CD8$^+$, cells (7). The –/– spleens also contained an increased percentage of Thy1$^-$ CD45R$^-$Mac1$^-$ cells of unknown origin (Fig. 2). Most notable, however, was the increased expression of activation markers on the majority of T cells in –/– spleens (Fig. 3). CD44, CD69, and CD25 were all up-regulated, whereas CD28 expression remained unchanged; in addition, forward scatter analysis indicated that –/– T cells were larger than control splenic T cells (Fig. 3). In contrast to the spleens, peripheral lymph nodes (such as inguinal, brachial, and axillary) and Peyer's patches in –/– mice were nearly undetectable. For example, in two homozygous mutant individuals analyzed, peripheral lymph nodes contained only 2% the normal number of cells and showed abnormal CD4$^+$/CD8$^+$ ratios, with >60% CD4$^+$ and <5% CD8$^+$ T cells (7).

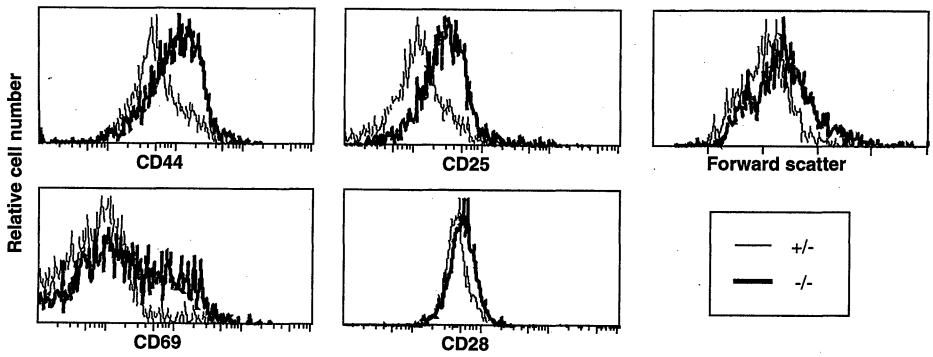

Fig. 3. Splenic T cells in Jak3-deficient mice have constitutively up-regulated activation marker expression. T cells from a +/– and a –/– spleen were stained with the indicated antibodies. For antibody surface staining, histograms show a logarithmic scale of fluorescence intensity; forward scatter profiles are shown on a linear scale. All profiles show gated CD4$^+$ cells, with the exception of the CD44 profile, which shows gated Thy1$^+$ cells.

Table 1. Reduced functional responses of Jak3-deficient thymocytes and spleen cells to mitogenic stimuli. Thymocytes or splenocytes (1×10^5) were cultured with the indicated mitogens. For proliferation assays, triplicate cultures were incubated for 48 hours, then pulsed overnight with [^{3}H]thymidine and counted. For IL-2 assays, supernatants from duplicate cultures stimulated with anti-CD28 plus anti-CD3 were harvested at 24 hours. IL-2 was quantitated by titration on HT-2 indicator cells and compared with a standard curve generated with recombinant IL-2 where 1 unit/ml corresponds to 1/10 maximal proliferation of the indicator cells. Both splenocyte (spl) and thymocyte (thy) populations of –/– mice contained equal or greater numbers of mature T cells as those from wild-type (+/+) populations. A reduced proliferative response of –/– T cells was observed in five independent experiments; IL-2 data (listed as the last line) in experiment 1 are derived from stimulation of 1×10^6 cells, and in experiment 2, from 1×10^5 cells. All values are means ± SD. LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; iono., ionomycin; Con A, concanavalin A; ND, not determined.

Mitogen	Incorporation of [^{3}H]thymidine (cpm)			
	+/+ thy	–/– thy	+/+ spl	–/– spl
	Experiment 1			
Medium	84 ± 13	70 ± 16	476 ± 111	217 ± 56
LPS	ND	ND	64,348 ± 1,770	7,196 ± 267
PMA + iono.	13,581 ± 555	2,414 ± 311	95,232 ± 9,832	2,643 ± 199
Con A	5,387 ± 724	3,620 ± 275	72,709 ± 3,624	349 ± 36
Con A + IL-2	97,949 ± 7,012	3,693 ± 427	104,217 ± 2,826	365 ± 108
Anti-CD3	5,184 ± 1,928	1,209 ± 84	86,330 ± 3,989	643 ± 136
Anti-CD3 + IL2	71,978 ± 3,604	1,362 ± 181	120,722 ± 9,204	769 ± 110
Anti-CD3 + anti-CD28	62,629 ± 898	28,792 ± 8,954	139,550 ± 4,596	1,954 ± 24
IL-2 secreted (units/ml)	4.1 ± 1.1	1.6 ± 0.5	37.6 ± 1.2	8.0 ± 2.1
	Experiment 2			
Medium	92 ± 47	111 ± 43	573 ± 83	1,011 ± 558
LPS	ND	ND	76,815 ± 342	3,327 ± 1,505
PMA + iono.	ND	ND	ND	ND
Con A	11,162 ± 1,527	997 ± 264	62,508 ± 739	1,665 ± 217
Con A + IL-2	97,249 ± 9,876	937 ± 50	70,623 ± 5,915	1,464 ± 171
Anti-CD3	24,812 ± 3,519	614 ± 80	69,298 ± 5,792	1,040 ± 229
Anti-CD3 + IL-2	51,738 ± 2,091	634 ± 78	74,422 ± 5,855	1,006 ± 138
Anti-CD3 + anti-CD28	78,515 ± 2,157	24,613 ± 2,847	100,657 ± 446	1,940 ± 347
IL-2 secreted (units/ml)	0.3 ± 0.1	<0.1	8.3 ± 1.1	0.2 ± 0.1

Because thymocyte development and the numbers of splenic T cells in –/– mice appeared roughly normal, we were particularly interested in the functional capabilities of –/– T cells. When thymocytes and spleen cells from wild-type and –/– mice were cultured under a variety of mitogenic conditions, responses of –/– cells were generally reduced to less than 1/10 those of wild-type mice (Table 1). For splenic T cells, stimulation with phorbol 12-myristate 13-acetate (PMA) plus ionomycin, concanavalin A (Con A), anti-CD3, or anti-CD3 plus anti-CD28 failed to induce a proliferative response from –/– cells (Table 1). In comparison with wild-type cells, thymocytes from –/– mice generally proliferated only ½ to ⅓ as efficiently with anti-CD3 plus anti-CD28 stimulation (Table 1), although in one experiment –/– thymocyte responses were significantly more reduced (7). Neither the defective responses to anti-CD3 nor to Con A could be restored by the addition of exogenous IL-2 (Table 1). We also measured the IL-2 secreted by stimulated thymocytes and splenic T cells. Stimulation of splenic T cells with anti-CD3 plus anti-CD28 resulted in substantially less IL-2 secretion from mutant compared with wild-type cells (Table 1).

In summary, –/– mice have a profound block in B cell maturation at the pre-B stage, resulting in a significant reduction in the numbers of peripheral IgM$^+$ B cells. Thymuses of –/– mice are small, but relatively normal in subset distribution. Both thymocytes and splenic T cells from –/– mice show greatly reduced proliferative responses and secrete less IL-2 in response to mitogenic stimulation.

The B cell defect in –/–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 γ_c 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 (γ_c-deficient) have normal numbers of B cells (*11*). This differential requirement for γ_c-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 –/– thymuses is comparable with that observed for thymuses in IL-7 receptor–deficient (*4*) and γ_c-deficient (*10*, *13*) mice. However, both the thymuses and the spleens of –/– mice show an increase in the ratio of $CD4^+$ cells 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 $p56^{lck}$ associate with the IL-2R β chain (*2*, *15*).

Splenic T cells from –/– 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 –/– 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 –/– 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 –/– T cells are deficient in returning to the normal resting state. These unusual aspects of T cell development and differentiation in –/– mice indicate previously undescribed functions of cytokine receptor signaling pathways.

REFERENCES AND NOTES

1. J. A. Johnston *et al.*, *Nature* **370**, 151 (1994); T. Musso *et al.*, *J. Exp. Med.* **181**, 1425 (1995); S. M. Russell *et al.*, *Science* **266**, 1042 (1994); B. A. Witthuhn *et al.*, *Nature* **370**, 153 (1994).
2. T. Miyazaki *et al.*, *Science* **266**, 1045 (1994).
3. M. Kawamura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6374 (1994); R. A. Kirken, H. Rui, M. G. Malabarba, W. L. Farrar, *J. Biol. Chem.* **269**, 19136 (1994); S. G. Rane and E. P. Reddy, *Oncogene* **9**, 2415 (1994); T. Takahashi and T. Shirasawa, *FEBS Lett.* **342**, 124 (1994); C. B. Gurniak and L. J. Berg, in preparation.
4. J. J. Peschon *et al.*, *J. Exp. Med.* **180**, 1955 (1994).
5. U. von Freeden-Jeffry *et al.*, *ibid.* **181**, 1519 (1995).
6. Linearized targeting vectors were transfected into J1 ES cells and selected with G418 and fluorodeoxyiodoara-U as described [E. Li, T. H. Bestor, R. Jaenisch, *Cell* **69**, 915 (1992)]. Clones were analyzed by Southern (DNA) blot analysis, and targeted ES clones (frequency of one-eighth) were injected into BALB/c or C57BL/6 blastocysts. Four germline-transmitting progeny were derived and were intercrossed to generate homozygous *Jak3*$^{-/-}$ mice. Homozygous mutant mice were found at the expected frequency and appeared grossly normal.
7. D. C. Thomis, A. H. Sharpe, L. J. Berg, data not shown.
8. For flow cytometry, 5×10^5 cells were stained with antibodies to IgM, CD43, Mac1, CD8, CD3, CD69, CD44 (Pharmingen), CD45R (antibody B220), CD4, CD25 (Gibco-BRL), Thy1.2 (Caltag), or CD28 [J. A. Gross, E. Callas, J. P. Allison, *J. Immunol.* **149**, 380 (1992)] and analyzed on a FACScan (Becton Dickinson). All profiles shown were gated on viable cells by forward and side scatter parameters, unless otherwise noted.
9. R. R. Hardy, C. E. Carmack, S. A. Shinton, J. D. Kemp, K. Hayakawa, *J. Exp. Med.* **173**, 1213 (1991).
10. X. Cao *et al.*, *Immunity* **2**, 223 (1995).
11. M. Noguchi *et al.*, *Cell* **73**, 147 (1993).
12. S. M. Russell *et al.*, *Science* **270**, 797 (1995).
13. J. P. DiSanto, W. Müller, D. Guy-Grand, A. Fischer, K. Rajewsky, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 377 (1995).
14. B. Bierer, *Chem. Immunol.* **59**, 128 (1994).
15. M. Hatakeyama *et al.*, *Science* **252**, 1523 (1991).
16. V. A. Boussiotis *et al.*, *ibid.* **266**, 1039 (1994).
17. R. Scollay and D. I. Godfrey, *Immunol. Today* **16**, 268 (1995).
18. R. M. Mortensen, D. A. Conner, S. Chao, A. A. T. Geisterfer-Lowrance, J. G. Seidman, *Mol. Cell. Biol.* **12**, 2391 (1992).

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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 γ chain (γ_c) 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 γ_c, 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 γ_c 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 γ_c are dependent on Jak3 and that Jak3 is essential for lymphoid development and signaling.

The γ_c 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 XSCID (*9*, *10*) and of γ_c-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 γ_c, and as no other signaling molecules have been identified that associate with γ_c, we hypothesized that the phenotype resulting from defects in Jak3 might be indistinguishable from that resulting from defects in γ_c, and that the key role of γ_c may be to bring Jak3 into proximity with the primary binding chain and its associated signaling molecules (*6*).

Although true XSCID ($\gamma_c^{-/-}$) females