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Disruption of the *Jak1* Gene Demonstrates Obligatory and Nonredundant Roles of the Jaks in Cytokine-Induced Biologic Responses

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Summary

Herein we report the generation of mice lacking the ubiquitously expressed Janus kinase, Jak1. *Jak1*$^{-/-}$ mice are runted at birth, fail to nurse, and die perinatally. Although *Jak1*$^{-/-}$ cells are responsive to many cytokines, they fail to manifest biologic responses to cytokines that bind to three distinct families of cytokine receptors. These include all class II cytokine receptors, cytokine receptors that utilize the γ_c subunit for signaling, and the family of cytokine receptors that depend on the gp130 subunit for signaling. Our results thus demonstrate that Jak1 plays an essential and nonredundant role in promoting biologic responses induced by a select subset of cytokine receptors, including those in which Jak utilization was thought to be nonspecific.

Introduction

Cytokines that bind to class I or class II cytokine receptors employ the Jak-STAT signaling pathway to manifest many of their biologic effects on cells (Darnell et al., 1994; Ihle et al., 1995; Darnell, 1997; O'Shea, 1997). This pathway consists of four Janus family protein tyrosine kinases (Jaks) and a family of seven latent cytosolic transcription factors known as signal transducers and activators of transcription (STATs).

Recent work using mutagenized cell lines and gene-targeted mice has shown that the STATs play a major role in determining cytokine signaling specificity (Bach et al., 1997; Darnell, 1997; O'Shea, 1997). Distinct STAT proteins are recruited to individual ligated cytokine receptors, where they undergo activation and dimerization. Activated STAT dimers then translocate to the nucleus, where they bind to the promoter regions of specific genes and activate gene transcription. Although the Jaks are known to play a critical role in this process, the physiologic functions and fine specificities of these enzymes remain poorly defined (Ihle et al., 1995; O'Shea, 1997).

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A role for Jaks in cytokine signaling was initially identified using mutagenized human fibrosarcoma cell lines that lacked individual Jak proteins and displayed unique patterns of biologic unresponsiveness to IFNα or IFNγ (Velazquez et al., 1992; Müller et al., 1993; Watling et al., 1993). This work demonstrated that distinct combinations of Jaks were required for the induction of cellular responses to type I versus type II interferons. However, subsequent studies that sought to define a role for Jaks in signaling by other cytokine receptors monitored only whether particular Jaks became phosphorylated and/or activated following in vitro treatment of a cell with different cytokines. This approach often produced conflicting results and failed to define a causal relationship between cytokine-dependent Jak activation and the induction of a biologic response (Ihle et al., 1995). This problem has been most evident for the family of receptors that utilizes the gp130 receptor subunit (i.e., the receptors for IL-6, IL-11, leukemia inhibitory factor [LIF], oncostatin M [OSM], ciliary neurotrophic factor [CNTF], and cardiotrophin-1 [CT-1] [Kishimoto et al., 1995]). By monitoring the ability of ligands of the gp130 receptor family to induce Jak and STAT activation in cells engineered to overexpress particular Jaks, one group concluded that Jaks can associate in a nonselective manner with certain cytokine receptor subunits (Stahl et al., 1994). In contrast, using a Jak1-deficient tumor cell line, another group reported that Jak1 was indeed required for IL-6–dependent induction of the IRF-1 transcription factor in vitro (Guschin et al., 1995). However, no subsequent experiments were performed to determine whether Jak1-deficient cells were capable of developing a full biologic response. Thus, it remains unclear whether, under physiologic conditions, Jaks participate in a specific manner in effecting Jak-STAT pathway–dependent biologic responses.

One problem impeding the elucidation of the physiologic roles of these enzymes is the absence of mammalian models of Jak protein deficiency. To date, the only known Jak abnormality is that found in *Jak3* gene–targeted mice and Jak3-deficient human SCID patients (Nosaka et al., 1995; Park et al., 1995; Russell et al., 1995; Thomis et al., 1995). The analysis of these individuals has revealed that Jak3 associates exclusively with a single cytokine receptor subunit (γ_c) and functions predominantly to promote lymphopoiesis. Thus, due to its highly restricted nature, Jak3 deficiency does not provide a suitable system to evaluate the generalized physiologic functions of the Jaks.

In contrast, the other members of the Janus kinase family (Jak1, Jak2, and Tyk2) are ubiquitously expressed and interact with many different cytokine receptor subunits (Darnell et al., 1994; Ihle et al., 1995; O'Shea, 1997). We therefore decided to generate Jak1-deficient mice using homologous recombination techniques and determine whether Jak1 deficiency leads to distinct biologic response deficits. In this report, we demonstrate that Jak1 indeed plays an essential and nonredundant role in promoting biologic responses induced only by specific subsets of cytokine receptors, including those in which

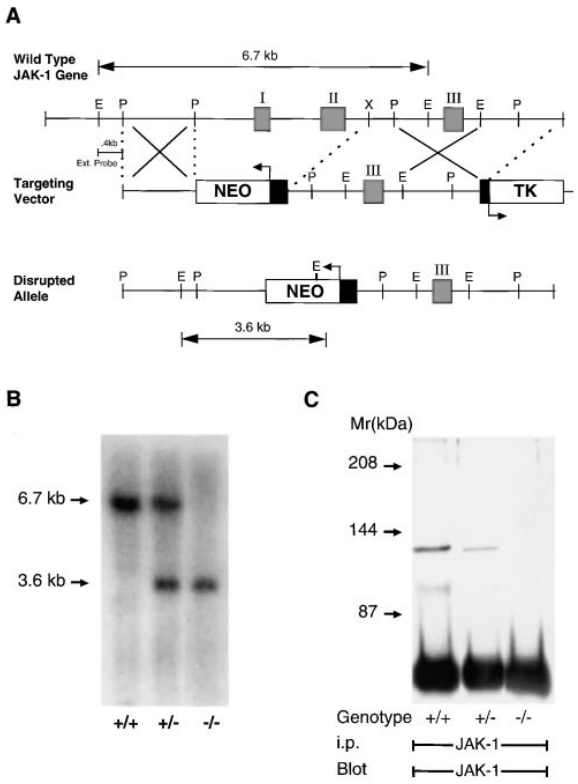

Figure 1. Targeted Disruption of the *Jak1* Gene Ablates Expression of Jak1 Protein

(A) The genomic locus of the wild-type *Jak1* gene, the targeting vector, and the disrupted *Jak1* gene. The first three exons (I, II, and III) encoding the 5′ region of the Jak1 protein, the neomycin resistance cassette (Neo), and the thymidine kinase cassette (TK) are marked by shaded boxes. The orientation of transcription of Neo and TK are indicated by arrows. The restriction fragment used as a probe external (Ext. Probe) to the targeting vector in Southern analyses is marked. The locations of restriction sites for Eco RI (E), Pst I (P), and Xba I (X) are indicated.

(B) Southern blot analysis of Eco RI digested DNA isolated from the tails of e16.5 embryos derived from the mating of heterozygote mice. The bands at 6.7 and 3.6 kb correspond to the endogenous and mutant *Jak1* genes, respectively.

(C) Two million EFs of each genotype were lysed and analyzed by immunoprecipitation and Western blotting for the presence of Jak1.

Jak utilization was originally thought to be nonspecific. These results thus demonstrate that the Jaks are utilized by cytokine receptors in a functionally dedicated manner to promote development of cytokine-specific biologic responses.

Results

Generation of Jak1-Deficient Mice

To ablate the mouse *Jak1* gene, a targeting construct was designed to replace the first two exons of the gene and 1.6 kb of 5′ upstream sequence with a neomycin resistance cassette (Figure 1A). Following electroporation and selection, 3 out of 235 ES cell clones were identified by Southern blot analysis as having undergone successful gene targeting. Chimeric animals from two of these clones transmitted the mutation germline to generate animals heterozygote for the targeted deletion. Heterozygote × heterozygote breedings produced wild-type, heterozygote, and homozygote embryos as identified by Southern blot analyses (Figure 1B). Immunoprecipitation and Western blot analyses using Jak1 antisera showed the presence of the 130 kDa Jak1 protein in lysates of embryonic fibroblasts (EFs) derived from wild-type embryos but not embryos homozygous for the disrupted *Jak1* gene (Figure 1C). EFs derived from heterozygote embryos contained reduced amounts of Jak1 relative to wild-type EFs.

Cells from Jak1-Deficient Mice Fail To Develop Biologic Responses to Ligands of Class II Cytokine Receptors

Previously published work using a randomly mutagenized human fibrosarcoma cell line indicated that Jak1 was required for both signaling and biologic response induction by IFNα and IFNγ (Müller et al., 1993). To extend these observations to primary cells with a targeted deletion of *Jak1*, we monitored the ability of the interferons to enhance MHC class I expression on EFs derived from wild-type and Jak1 null mice. Whereas wild-type EFs exposed to either IFNα or IFNγ expressed enhanced levels of H2-K^b, *Jak1*$^{-/-}$ EFs were unresponsive to either cytokine (Figure 2A). Similar results were obtained when antiviral activity was monitored as a second IFN-dependent biologic response (data not shown).

To document that the unresponsive state was specifically due to the absence of Jak1, immortalized EFs from *Jak1*$^{+/+}$ and *Jak1*$^{-/-}$ embryos were analyzed for IFN responsiveness before and after reconstitution with Jak1. Immortalized *Jak1*$^{+/+}$ EFs responded to either IFNα or IFNγ in a dose-dependent manner and resisted the cytopathic effects of vesicular stomatitis virus (Figure 2B). In contrast, neither IFNα nor IFNγ protected *Jak1*$^{-/-}$ fibroblasts from viral infection. However, IFN responsiveness was restored to immortalized gene-targeted cells following stable transfection with a murine Jak1 expression plasmid. Reconstituted *Jak1*$^{-/-}$ EFs also recovered the ability to up-regulate MHC class I expression in response to IFNα or IFNγ (Figure 2A). Thus, the IFN unresponsiveness observed in fibroblasts of our gene-targeted mice is specifically due to the absence of Jak1.

We also tested whether Jak1 was required for development of IFN-dependent biologic responses in other cell types. Whereas macrophages from *Jak1*$^{+/+}$ embryos responded to the combination of LPS and either IFNα or IFNγ and produced nitric oxide, macrophages from *Jak1*$^{-/-}$ embryos did not (Figure 2C). *Jak1*$^{-/-}$ macrophages also failed to up-regulate ICAM-1 expression (Figure 2E) or induce expression of the third component of complement (data not shown) in response to IFNγ. However, *Jak1*$^{-/-}$ macrophages were not generally cytokine unresponsive because they up-regulated ICAM-1 following treatment with TNFα in a manner similar to that of wild-type macrophages. Thus, the absence of Jak1 results in a generalized cellular unresponsiveness to type I and type II interferons.

Based on the obligate requirement of Jak1 for biologic responses induced through the receptors for IFNα and

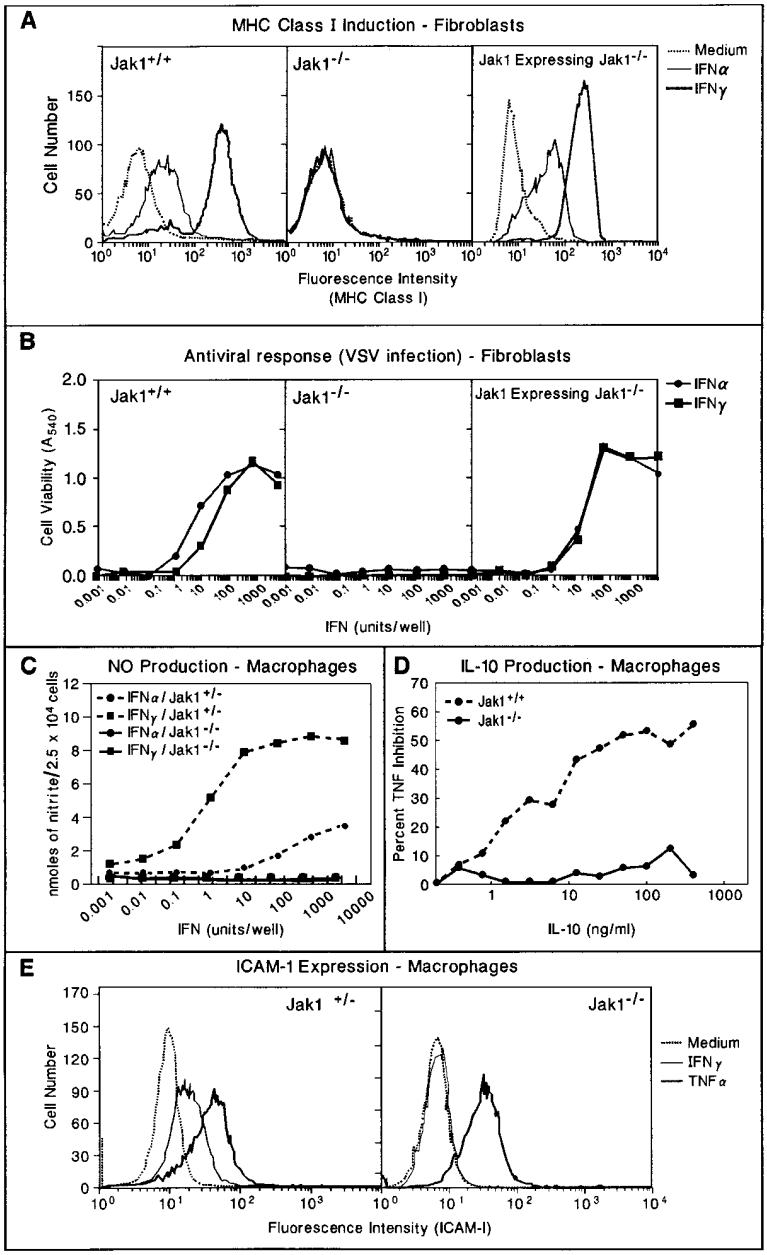

Figure 2. *Jak1*$^{-/-}$ Cells Are Unresponsive to Ligands that Utilize Class II Cytokine Receptors

(A) Primary *Jak1*$^{+/+}$ or *Jak1*$^{-/-}$ EFs, or immortalized *Jak1*$^{-/-}$ EFs stably transfected with a Jak1 expression plasmid (Jak1 expressing *Jak1*$^{-/-}$) were stimulated with IFNα (100 U/ml) or IFNγ (1000 U/ml) for 72 hr and analyzed for expression of MHC class I by flow cytometry.

(B) Immortalized EFs were stimulated with IFNα or IFNγ and cultured with vesicular stomatitis virus. After 48 hr, surviving cells were quantitated by vital dye staining.

(C) Fifty thousand in vitro differentiated macrophages from *Jak1*$^{+/-}$ or *Jak1*$^{-/-}$ mice were stimulated with LPS (5 ng/ml) plus different doses of IFNγ or IFNα for 48 hr and levels of nitrite determined by ELISA.

(D) Five thousand macrophages from *Jak1*$^{+/+}$ or *Jak1*$^{-/-}$ mice were incubated with IL-10 for 60 min followed by treatment with LPS (10 ng/ml). After 24 hr TNF in culture supernatants were quantitated by ELISA.

(E) Macrophages from either *Jak1*$^{+/-}$ or *Jak1*$^{-/-}$ mice were stimulated for 48 hr with IFNγ (1000 U/ml) or TNFα (100 ng/ml) and levels of ICAM-1 determined by flow cytometry.

IFNγ, we examined whether this enzyme is also required for biologic responses induced by the IL-10 receptor, the third known member of the class II cytokine receptor superfamily (Ho et al., 1995). Whereas macrophages derived from wild-type embryos responded to IL-10 in a dose-dependent manner by inhibiting LPS-induced TNFα production, macrophages from *Jak1*$^{-/-}$ mice did not (Figure 2D). Thus, Jak1 plays an obligate role in mediating biologic responses, which are manifest by each of the class II cytokine receptor family members.

Deficiency in Jak1 Results in Perinatal Lethality

Having confirmed the successful generation of *Jak1*$^{-/-}$ offspring through analysis of embryonic tissues, a large scale heterozygote × heterozygote breeding effort was initiated. By examining litters at different stages of embryonic development from e3.5–e19.5, we could identify viable *Jak1*$^{-/-}$ embryos in numbers that approximated Mendelian proportions throughout gestation. Moreover, viable *Jak1*$^{-/-}$ neonates were found in litters immediately after delivery. However, within 24 hr of birth, all *Jak1*$^{-/-}$ pups were dead, and no *Jak1*$^{-/-}$ mouse has survived more than 24 hr among the 326 mice produced by the breeding effort. Identical results were found when breeding was conducted using a second *Jak1* gene–targeted ES cell clone or when Jak1 heterozygote mice were generated on different genetic backgrounds. Thus, homozygous Jak1 deficiency results in a perinatal lethality.

Gross external examination of *Jak1*$^{-/-}$ mice failed to

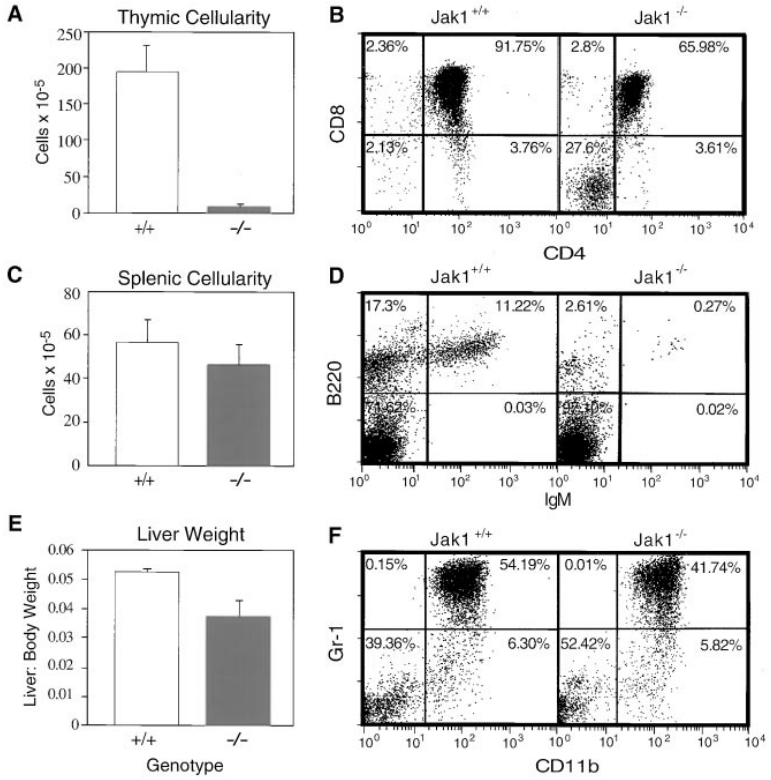

Figure 3. *Jak1*$^{-/-}$ Mice Exhibit Deficits in Lymphopoiesis but Not Myelopoiesis

(A, C, and E) Cellular recoveries from thymi (A) spleens (C) and weight of livers (E) derived from newborn *Jak1*$^{+/+}$ (open boxes) and *Jak1*$^{-/-}$ (filled boxes) mice.

(B) Thymocytes derived from newborn *Jak1*$^{+/+}$ (left panel) and *Jak1*$^{-/-}$ (right panel) mice were stained with FITC anti-CD4 and PE anti-CD8 and analyzed by flow cytometry.

(D) Splenocytes derived from newborn *Jak1*$^{+/+}$ (left panel) and *Jak1*$^{-/-}$ (right panel) mice were stained with FITC anti-IgM and PE anti-B220 and the gated lymphoid population analyzed by flow cytometry.

(F) Fetal liver cells derived from e18.5 *Jak1*$^{+/+}$ (left panel) and *Jak1*$^{-/-}$ (right panel) animals were stained with FITC anti-Mac-1 and PE anti-Gr-1 and the gated myeloid populations analyzed by flow cytometry.

The percentage of the total gated cells analyzed is given within each quadrant.

reveal significant developmental abnormalities compared to Jak1-sufficient newborns. However, newborn *Jak1*$^{-/-}$ mice (0.99 ± 0.11 g) weighed 40% less than their heterozygote (1.61 ± 0.16 g) or wild-type (1.65 ± 0.09 g) littermates. Moreover, Jak1-deficient neonates failed to nurse despite a normal nurturing behavior of the mothers even when these mice were part of very small litters or when all Jak1 sufficient siblings were removed from the litter (data not shown).

Severely Impaired Lymphocytic Development in Jak1-Deficient Mice

Blood from newborn *Jak1*$^{-/-}$ mice contained normal numbers of erythrocytes, mononuclear phagocytes, neutrophils, and platelets (data not shown). Moreover, upon necropsy of newborn Jak1 null pups, no gross abnormalities were found in several organs, such as heart, liver, lungs, kidney, and brain. In contrast, thymi from newborn *Jak1*$^{-/-}$ mice were of severely reduced size compared to those isolated from control newborn littermates. Histologic examination of comparable cross sections of Jak1-sufficient and Jak1-deficient littermates revealed the presence of abundant developing thymocytes in wild-type mice but a severely reduced number of thymocytes in Jak1 null mice. Both types of animals displayed normal thymic epithelial architecture.

To examine the thymocyte deficiency in detail, the cellularities of thymi from wild-type and Jak1 null mice were compared (Figure 3A). An average thymus from a newborn wild-type mouse contained 182 ± 85 × 10^5 (mean ± SD, n = 12) thymocytes. In contrast, an average thymus from a *Jak1*$^{-/-}$ mouse contained only 0.7 ± 0.6 × 10^5 thymocytes (n = 17), which represents a 260-fold reduction in cellularity. Nevertheless, the distribution of CD4- and CD8-positive cells within the wild-type and *Jak1*$^{-/-}$ thymocyte populations was similar (Figure 3B). The thymi of gene-targeted mice contained a slightly higher percentage of $CD4^-CD8^-$ cells relative to controls. However, these cells most likely represent stromal cells, which contaminate the limited number of thymocytes that can be isolated from newborn *Jak1*$^{-/-}$ mice. Thus, Jak1 deficiency results in a primary defect in thymocyte production rather than a definitive block in thymocyte maturation.

To determine whether B cell production and/or development was also dependent on Jak1, the B cell lineages present in the spleens and livers of wild-type and gene-targeted mice were quantitated. No significant differences in total splenocyte numbers were observed between newborn wild-type and *Jak1*$^{-/-}$ mice (Figure 3C). This result is consistent with the fact that spleens of newborn mice contain only a limited number of lymphoid cells. However, when the B cells present in the spleens of Jak1-sufficient and Jak1-deficient mice were quantitated by monitoring expression of the B220 and IgM cell surface markers, Jak1 null mice were found to display profound abnormalities in the B cell compartment. Compared to spleens of wild-type littermate controls, *Jak1*$^{-/-}$ spleens contained 40-fold fewer double positive B cells and 7- to 10-fold fewer $B220^+$, IgM^- B cell precursors (Figure 3D). Identical results were obtained using B cells from fetal livers (data not shown). Since the $B220^+$, IgM^- cell population in normal mice contains pro– and pre–B cells, NK cells, and a subpopulation of T cells, we analyzed it for expression of CD43, a marker that is expressed on pro–B cells but not on pre–B cells (Hardy

et al., 1991). Whereas 49.7% of the wild-type cells in this population expressed the CD43 marker, more than 90% of the B220^{+}, IgM^{-} splenocytes derived from Jak1 null mice were CD43 positive (data not shown). Thus, Jak1 deficiency leads to a developmental blockade of B lymphocyte differentiation at the pro–B to pre–B cell transition step and results in a profound deficit in the generation of mature B lymphocytes.

In contrast to the lymphoid compartment deficiencies, no significant abnormalities were observed in total liver weight or in Mac-1^{+} and/or Gr-1^{+} myeloid cells derived from fetal liver or newborn spleen of Jak1 null mice (Figures 3E and 3F). Thus, the hematopoietic defects of *Jak1*$^{-/-}$ mice are limited exclusively to the lymphoid compartment. To date, we have been unable to repopulate the lymphoid compartment of irradiated *Jak1*$^{+/+}$ mice with *Jak1*$^{-/-}$ fetal liver cells despite successful reconstitution with wild-type fetal liver cells (data not shown).

Abnormal Lymphoid Cell Development Is Due to IL-7 Receptor Dysfunction Caused by the Absence of Jak1

A number of cytokines that promote hematopoiesis, including IL-3, IL-5, GM-CSF, G-CSF, and IL-7, have been reported to activate Jak1 using different in vitro signaling assay systems (Ihle et al., 1995). To obtain insights into the molecular basis of the lymphopoietic defects in the Jak1 null mice, we utilized methylcellulose colony forming assays to determine whether any of the receptors for the known hematopoietic cytokines display an obligatory requirement for Jak1 for growth and/or survival of hematopoietic precursor cells (Figure 4A). Fetal liver cells from Jak1-sufficient and Jak1-deficient embryos formed equivalent numbers of colonies in response to M-CSF, G-CSF, GM-CSF, and IL-5. IL-3 also induced colony formation in Jak1 null fetal liver cells; however, these colonies were somewhat reduced in number and significantly reduced in size (Figure 4A and data not shown). In contrast, whereas wild-type and heterozygote fetal liver cells formed colonies when incubated with IL-7 at 10 ng/ml, no colonies were observed in cultures of IL-7–treated *Jak1*$^{-/-}$ cells. Similar experiments performed with higher doses of IL-7 (50 ng/ml) or using cells isolated from newborn spleen instead of fetal liver yielded identical results (data not shown).

The lack of IL-7–induced *Jak1*$^{-/-}$ cell colony formation was confirmed using cell proliferation assays in liquid culture (Figure 4B). Whereas Jak1-sufficient thymocytes proliferated when cultured with IL-7 plus phorbol ester, Jak1 null thymocytes did not. Since the IL-7 receptor belongs to the family of cytokine receptors that also includes the receptors for IL-2, IL-4, IL-9, and IL-15 that share a common subunit (the γ_c chain), the proliferation of Jak1-sufficient and Jak1-deficient thymocytes in response to IL-2 and IL-4 was also examined. As was the case for IL-7, IL-2 and IL-4 induced proliferation of Jak1-sufficient thymocytes but not Jak1 null thymocytes. Interestingly, *Jak1*$^{-/-}$ thymocytes were also unresponsive to the combination of PMA and ionomycin, a result that suggests that *Jak1*$^{-/-}$ thymocytes may have an inherent survival defect. FACS analysis revealed that thymocytes

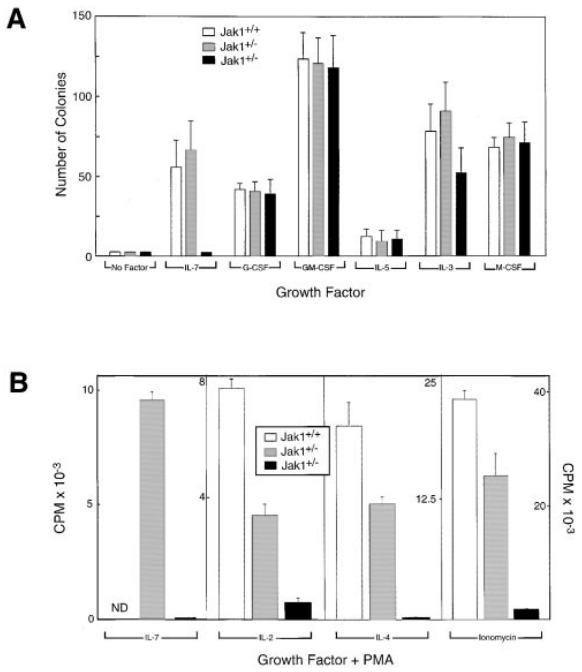

Figure 4. *Jak1*$^{-/-}$ Hematopoietic Cells Exhibit Selective Unresponsiveness to Lymphopoietic Cytokines that Use Receptors that Contain the γ_c Receptor Subunit

(A) Fetal liver cells from e15.5–e17.5 Jak1-sufficient or Jak1-deficient embryos were plated in duplicate methylcellulose cultures supplemented with either no factor, IL-7 (10 ng/ml), GM-CSF (12 ng/ml), IL-3 (16 ng/ml), IL-5 (100 ng/ml), G-CSF (13.3 U/ml), or M-CSF (33.3 U/ml). After 7–8 days of culture, total colonies (>50 cells) visible at 10× magnification were enumerated.

(B) Duplicate cultures of 1×10^5 thymocytes from newborn *Jak1*$^{+/+}$ (open bars), *Jak1*$^{+/-}$ (light shaded bars), or *Jak1*$^{-/-}$ (dark shaded bars) mice were stimulated with medium, PMA, or PMA in combination with muIL-7 (100 ng/ml), muIL-2 (25 ng/ml), muIL-4 (25 ng/ml), or ionomycin, and proliferative responses were determined by quantitating ^{3}H-thymidine uptake.

from Jak1 null mice expressed the γ_c subunit and the IL-4 receptor α subunit at levels comparable to wild-type cells (data not shown). Thus, Jak1 deficiency leads to the ablation of lymphopoiesis in response to many if not all cytokine receptors that utilize the γ_c, including the IL-7 receptor. Since IL-7 is known to act early and specifically during lymphocyte development to promote lymphocyte survival (Akashi et al., 1997), the lymphopoietic deficiencies in the *Jak1*$^{-/-}$ mouse can be linked to the absence of IL-7 receptor function.

Signaling via the IL-6 and LIF Receptors Is Deficient but Not Abolished in Jak1 Null Cells

The aforementioned data showed that Jak1 is used in a highly specific and restricted manner by particular cytokine receptors. We therefore asked whether Jak1 played a similar dedicated role in mediating signaling and biologic response induction by the gp130 family of cytokine receptors that has been reported to use the Jaks in a nonspecific manner (Stahl et al., 1994).

To test whether Jak1 is required for gp130 receptor family signaling, macrophages, EFs, and cardiomyocytes from Jak1-sufficient and Jak1-deficient mice were

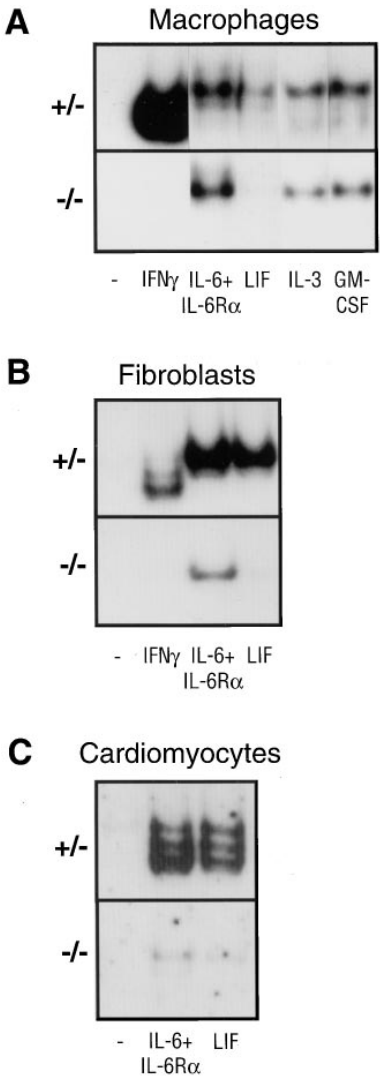

Figure 5. Diminished but Detectable STAT Activation following Stimulation of *Jak1*$^{-/-}$ Cells with Ligands that Utilize gp130 and/or LIFRβ

Five million macrophages (A) derived from *Jak1*$^{+/-}$ or *Jak1*$^{-/-}$ mice, one million EFs (B) derived from *Jak1*$^{+/-}$ or *Jak1*$^{-/-}$ mice, or one million cardiomyocytes (C) derived from *Jak1*$^{+/-}$ or *Jak1*$^{-/-}$ mice were stimulated with either buffer alone, human IL-6 (400 ng/ml) and human IL-6Rα (500 ng/ml), murine LIF (400 ng/ml), murine IL-3 (200 ng/ml), or murine GM-CSF (200 ng/ml) for 15 min or murine IFNγ (1000 u/ml) for 10 min. Cells were lysed and DNA binding activity quantitated by EMSA.

stimulated in vitro with different cytokines and Stat activation assessed using an electrophoretic mobility shift assay (Figure 5). All cell types derived from Jak1-sufficient animals activated Stat3 (and in some cases Stat1) in response to either IL-6 or LIF. In contrast, Stat3 activation was diminished but detectable in *Jak1*$^{-/-}$ cells stimulated with IL-6 (50%, 85%, and >95% reduction for macrophages, EFs, and cardiomyocytes, respectively) and was almost completely abolished (>95%) but still detectable in *Jak1*$^{-/-}$ cells treated with LIF. Whereas these results are consistent with the observations of others (Stahl et al., 1994; Guschin et al., 1995), they clearly point to a major quantitative contribution of Jak1 to the signaling response. As expected, STATs were activated in wild-type but not *Jak1*$^{-/-}$ macrophages, and EFs were exposed to IFNγ. Moreover, *Jak1*$^{-/-}$ macrophages retained the ability to activate STATs in response to IL-3 and GM-CSF (Figure 5A), and *Jak1*$^{-/-}$ fetal liver cells activated STATs when treated with GM-CSF, IL-3, or EPO (data not shown).

Lack of Biologic Response Induction to gp130 Receptor Family Ligands in *Jak1*$^{-/-}$ Mice

Since Jak1 null cells displayed only a limited capacity to activate the Jak-STAT signaling pathway when challenged with ligands for the gp130 receptor family, we asked whether partial signaling was of sufficient magnitude to induce biologic responses in the treated cells. Cardiomyocytes from control newborn mice manifest a hypertrophic response when incubated with either IL-6, LIF, or CT-1 (Figure 6), similar to that previously reported for normal rat cardiomyocytes (King et al., 1996). In contrast, no hypertrophy was induced in cells from *Jak1*$^{-/-}$ mice. Thus, the quantitative signaling deficiency observed in the *Jak1*$^{-/-}$ cardiomyocytes translates into a qualitative biologic response deficit.

Ligands of the gp130 receptor family play important roles in neuronal development and survival (Dechiara et al., 1995; Li et al., 1995; Ware et al., 1995). We therefore examined whether *Jak1*$^{-/-}$ neurons were capable of responding to neurotrophic cytokines. Neurons from dorsal root ganglions (DRGs) of control newborn mice cultured in the absence of growth factor died after 72 hr in culture, as evidenced by the absence of immunostaining for Neurofilament M (Figure 7A). However, neuronal viability was maintained if the cells were cultured in the presence of either nerve growth factor (NGF) or LIF. Viability of neurons from newborn *Jak1*$^{-/-}$ mice was also preserved by NGF. However, almost no viable *Jak1*$^{-/-}$ neurons were detected following 3 day culture in the presence of LIF.

Using this assay system, the response of DRG sensory neurons to a wider range of gp130 receptor family ligands was quantitated. Whereas LIF, CNTF, OSM, CT-1, and the combination of IL-6 + IL-6Rα individually preserved the viability of Jak1-sufficient neurons to approximately 75% of that induced by NGF (Figure 7B, top panel), none of these cytokines was able to maintain viability of Jak1-deficient neurons in culture (Figure 7B, bottom panel). In contrast, NGF was capable of preventing apoptosis of *Jak1*$^{-/-}$ neurons. In situ hybridization revealed the presence of LIF receptor β chain mRNA in DRG neurons of either wild-type or *Jak1*$^{-/-}$ mice (data not shown). These results thus demonstrate a central role for Jak1 in neuronal development and/or survival and strongly suggest that gp130 receptor family members display an obligate requirement for Jak1 to induce at least a subset of their cellular responses.

Interestingly, the total number of Jak1 null neurons surviving 3 day culture with NGF was 33%–50% lower than that of Jak1-sufficient neurons. This result suggested that *Jak1*$^{-/-}$ mice might have an inherent neuronal deficit. To examine this possibility, we quantitated recovery of DRG sensory neurons from wild-type, heterozygote, or Jak1 null mice examined 2, 24, or 72 hr

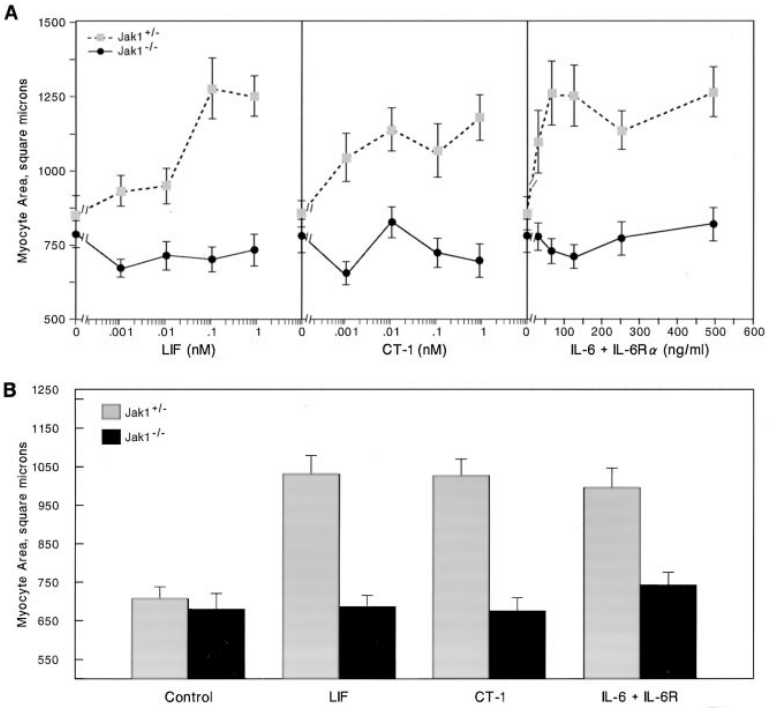

Figure 6. *Jak1*$^{-/-}$ Cardiomyocytes Fail To Undergo a Hypertrophic Response to LIF, CT-1, or IL-6

(A) One representative dose response experiment of *Jak1*$^{+/-}$ (open symbols) or *Jak1*$^{-/-}$ (closed symbols) cardiomyocytes plated in duplicate cultures and stimulated with the indicated amounts of cytokine for 48 hr. Cultures were fixed, stained, and the surface areas of 30 cells/dose quantitated and averaged.

(B) Mean values of three independent experiments of *Jak1*$^{+/-}$ (open boxes) and *Jak1*$^{-/-}$ (shaded boxes) cardiomyocyte surface areas following treatment with LIF (1 nM), CT-1 (1 nM), or IL-6 + IL-6Rα (500 ng/ml).

after isolation and culture in NGF. At each time point, significantly lower numbers of neurons were found in the *Jak1*$^{-/-}$ cultures compared to cultures established with Jak1-sufficient cells (Figure 7C and data not shown). These results indicate that Jak1 may be necessary in vivo to promote survival of at least certain subsets of neurons.

Discussion

Although Jak1 is a globally expressed Janus kinase, which is activated by a wide variety of cytokines, its requirement in the induction of cytokine-specific biologic responses remains largely undefined (Ihle et al., 1995; O'Shea, 1997). The purpose of this study was to identify those cytokine receptors that demonstrate an obligate dependency on Jak1 for induction of cellular responses. Our results show that Jak1 plays an indispensable role in mediating biologic responses induced by three major cytokine receptor subfamilies: class II cytokine receptors (i.e., the receptors for IFNα/β, IFNγ, and IL-10), cytokine receptors that utilize the γ_c receptor subunit (i.e., the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15), and receptors that utilize the gp130 subunit (i.e., receptors for IL-6, IL-11, LIF, OSM, CNTF, and CT-1). These results demonstrate that under physiologic conditions, the Jaks play dedicated and nonredundant roles in effecting cytokine specific biologic responses.

The results from analyses of cells and tissues derived from *Jak1*$^{-/-}$ mice demonstrate that there is an absolute requirement for Jak1 in mediating biologic responses to IFNα and IFNγ. This result is thus in agreement with earlier studies showing that mutant human tumor cell lines which lack Jak1 are IFN-unresponsive (Müller et al., 1993) and that induction of all biologic responses to the IFNs requires Jak-STAT pathway signaling (Durbin et al., 1996; Meraz et al., 1996). However, the demonstration that Jak1 is needed for induction of IL-10–dependent responses has not been previously demonstrated. This result thus establishes that Jak1 is the common Jak-STAT signaling pathway component, which is shared by all members of the class II cytokine receptor family.

Jak1 deficiency in mice led to severely reduced numbers of thymocytes, pre–B cells, and mature T and B lymphocytes but did not lead to alterations in the development of other hematopoietic lineages. This result thus demonstrates that in hematopoiesis, Jak1 is required only by cytokine receptors that selectively induce development of lymphoid cell populations. Through the use of colony forming assays and proliferation assays, Jak1 was shown to be required for IL-7–dependent lymphopoietic responses. Cells from *Jak1*$^{-/-}$ mice thus show a hematopoietic deficit similar to that observed in mice that lack IL-7, the IL-7 receptor α chain, or Jak3 (Peschon et al., 1994; Nosaka et al., 1995; Park et al., 1995; Thomis et al., 1995; von Freeden-Jeffry et al., 1995). These mice exhibit substantial reductions in T and B cell numbers but do not display an absolute block in lymphocyte development. Recently, the deficits expressed in IL-7 unresponsive mice have been shown to be a result of the absence of a survival signal provided to developing cells through the IL-7 receptor (Akashi et al., 1997). Our results reveal that IL-7 receptor–induced responses require Jak1 as well as Jak3 and demonstrate that Jak1 is the obligate partner for Jak3 for inducing the survival signal. In addition, thymocytes from Jak1 null mice failed to respond to other lymphopoietic cytokines such as IL-2 and IL-4 that associate with distinct ligand binding receptor subunits but also incorporate γ_c as part of their activated receptor complexes (Leonard et al., 1995). These results demonstrate the generality of the pairing

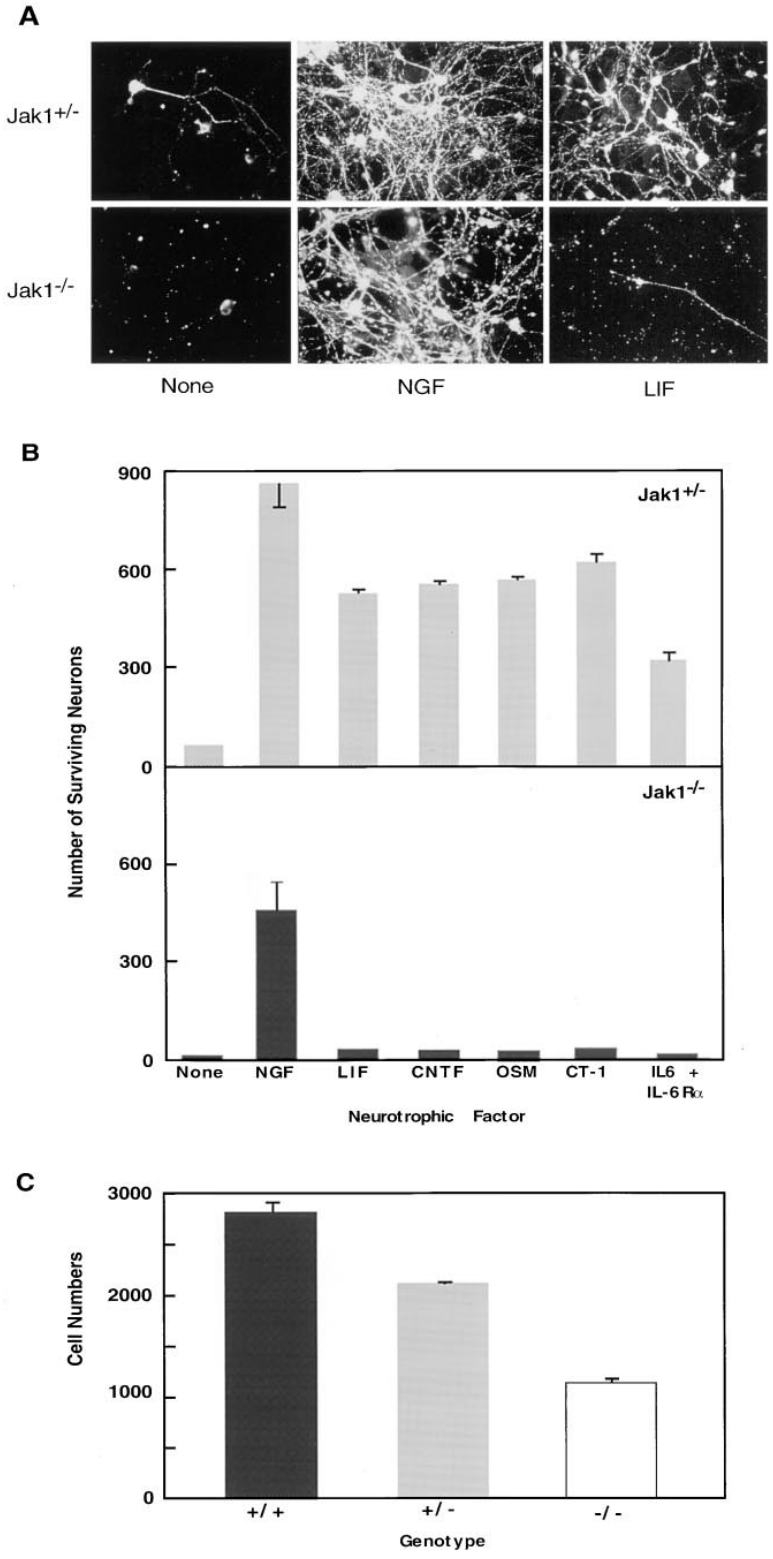

Figure 7. Ligands that Utilize the gp130 Receptor Family Fail To Prevent Apoptosis of *Jak1*$^{-/-}$ Sensory Neurons

(A) DRG neurons from newborn *Jak1*$^{+/-}$ or *Jak1*$^{-/-}$ mice were cultured in the presence of anti-NGF alone (None), NGF alone (50 ng/ml) (NGF), or anti-NGF plus LIF (10 ng/ml) (LIF) for 72 hr. Cultures were fixed, stained for Neurofilament M, and photographed at 20× magnification.

(B) DRG neurons isolated from newborn *Jak1*$^{+/-}$ (top panel) or *Jak1*$^{-/-}$ (bottom panel) mice were cultured for 72 hr with anti-NGF (none), NGF (50 ng/ml), or anti-NGF and LIF (50 ng/ml), CNTF (100 ng/ml), OSM (100 ng/ml), CT-1 (125 ng/ml), or IL-6 + IL-6Rα (125 ng/ml). Viable cells were stained with crystal violet and/or Neurofilament M and quantitated.

(C) Number of neurons recovered from spinal cord DRGs of newborn *Jak1*$^{+/+}$, *Jak1*$^{+/-}$, and *Jak1*$^{-/-}$ mice 2 hr after plating.

of Jak1 and Jak3 for biologic responses induced by all lymphopoietic receptors that utilize the γ_c receptor subunit. Moreover, they show that Jak1 is not required by other hematopoietic receptors, such as those that bind GM-CSF or G-CSF, which have been reported to activate both Jak1 and Jak2 in in vitro assays (Nicholson et al., 1994; Ihle et al., 1995; Kouro et al., 1996).

The most surprising aspect of this study is the finding that ligands for receptors that signal through the gp130 receptor subunit fail to induce at least certain biologic

responses in Jak1-deficient cells. These results thus directly address the controversy concerning the dedicated usage of Jaks by this family of cytokine receptors. Extensive work using cultured tumor cell lines and overexpression approaches led to the concept that Jaks may be used in a nonspecific manner by the receptors for IL-6, IL-11, LIF, OSM, CNTF, and CT-1 to initiate signaling (Stahl et al., 1994). Although this receptor family is known to control a wide variety of important developmental, immune, and inflammatory responses (Kishimoto et al., 1995), no biologic responses were examined in the earlier studies. This concept was subsequently challenged by an independent study showing that Jak1 was necessary for IL-6–dependent induction of an immediate early gene (IRF-1) in a human fibrosarcoma cell line (Guschin et al., 1995). Our results show that ligands that bind to gp130 receptor family members, although capable of inducing weak activation of the Jak-STAT pathway in different Jak1-deficient cell types, fail to provoke a biologic response in primary nontransformed cardiomyocytes or neurons. Importantly, in the case of cardiomyocytes, the discordance between initiation of signaling and the induction of biologic responses was noted in the same cell type. Thus, although other Jaks may indeed be able to compensate to some degree for the absence of Jak1 to initiate the signaling process, this compensation is not sufficient to result in the development of a full cellular response. These observations establish that development of biologic responses induced via the Jak-STAT pathway occur only when the level of signaling exceeds some, as yet undefined, threshold value.

Interestingly, the phenotype of *Jak1*$^{-/-}$ mice is distinct from that displayed by mice with targeted disruption of gp130 (Yoshida et al., 1996) or Jak2 (Neubauer et al., 1998; Parganas et al., 1998 [both in this issue of *Cell*]). Deficiency in gp130 results in malformation of the ventricular walls of the heart and leads to embryonic lethality between e14.5–e17.5 p.c. *Jak1*$^{-/-}$ mice exhibited no developmental heart abnormalities and survived to the first postnatal day of life. Moreover, no heart abnormality was found in *Jak1*$^{-/-}$ pups at the time of their perinatal death. These results show that Jak1 is not required for at least certain gp130-dependent events that lead to cardiac development and thereby lend support to the concept that gp130 can activate multiple signal transduction pathways that give rise to distinct biologic responses (Kishimoto et al., 1995). Jak2 deficiency results in an embryonic lethality due to a lack of definitive erythropoiesis. Importantly, cells from *Jak2*$^{-/-}$ mice display cytokine response deficits that are nearly complementary to those observed in our *Jak1*$^{-/-}$ mice. Like *Jak1*$^{-/-}$ cells, *Jak2*$^{-/-}$ cells do not respond to IFNγ. However, unlike *Jak1*$^{-/-}$ cells, *Jak2*$^{-/-}$ cells manifest biologic responses to IL-6 and IFNα/β and can repopulate the lymphoid compartment of irradiated lymphocyte-deficient mice but fail to respond to erythropoietin, IL-3, or thrombopoietin. Thus, the combined data obtained through the analysis of Jak1- and Jak2-deficient mice unequivocally demonstrate the specific utilization of the Jaks by cytokine receptors.

In contrast, the phenotypes of the *Jak1*$^{-/-}$ mice and mice lacking the LIFRβ subunit are remarkably similar (Li et al., 1995; Ware et al., 1995). Both types of gene-targeted mice are significantly smaller than their littermates at birth, fail to nurse, and die perinatally. In addition, neurons from both types of mice are unable to respond to the ligands of the gp130 receptor family and die by apoptosis. In the absence of the LIFRβ subunit, newborn mice display decreased numbers of motor neurons. Although we have not yet quantitated motor neurons in the *Jak1*$^{-/-}$ mice, it is striking that the numbers of sensory neurons isolated from these mice were reduced compared to those isolated from Jak1-sufficient mice. Thus, it is possible that a neuronal defect in the Jak1 null mice secondary to a lack of signaling through receptors that employ the LIFRβ subunit is the cause of the perinatal lethality. Work is currently underway to examine this possibility.

Experimental Procedures

Cytokines, Reagents, and General Procedures

Purified recombinant cytokines were obtained from the following sources: MuIFNγ and MuCT-1 from Genentech (South San Francisco, CA); HuIFN$\alpha_{A/D}$ from Dr. Michael Brunda, Hoffmann-LaRoche (Nutley, NJ); HuIL-6 and IL-6Rα chain was kindly provided by Dr. Tetsuya Taga of Osaka University (Osaka, Japan); GM-CSF was provided by Dr. Satwant Narula, Schering-Plough Research Institute (Kenilworth, NJ); HuM-CSF, MuIL-3, and human and murine IL-7 from Genzyme Corporation (Cambridge, MA); MuIL-5, HuEPO, and MuOSM from R&D Systems (Minneapolis, MN); LIF from GIBCO/BRL (Gaithersburg, MD); NGF from Harlan Bioproducts (Madison, WI); and rat CNTF was provided by Dr. Richard Scott and Dr. Sheryl Meyer, Cephalon (West Chester, PA). A cDNA-encoding murine Jak1 was provided by Dr. S. Nagata of Osaka Bioscience Institute (Osaka, Japan). All experiments presented were performed at least three times.

Targeting Vector

Genomic clones containing portions of the murine *Jak1* gene were isolated from a Lambda Fx II murine 129/Sv genomic library (Stratagene, LaJolla, CA). To generate the Jak1 targeting vector, a 5.5 kb Xba-Xba fragment containing exon 3 was inserted into the targeting vector pTK.NEO.UMS as previously described (Meraz et al., 1996) such that its 3′ end was adjacent to the polyoma enhancer promoter driven herpes simplex virus thymidine kinase gene. The 5′ end of the targeting construct was generated from a 1.6 kb PstI-PstI fragment upstream of exon I (Figure 1).

Transfection of ES Cells and Generation of Jak1-Deficient Mice

The GS-1 embryonic stem cell line was cultured as described (Huang et al., 1993). ES cells were electroporated with 20 μg of Sac II linearized targeting construct per 1×10^7 cells and grown under double selection as described (Meraz et al., 1996). Two hundred and thirty five colonies were analyzed for homologous recombination by PCR using a sequence derived from the external probe (Figure 1). Three positive clones (C4, L3, and 1A15) were identified by PCR, confirmed by Southern blotting, and microinjected into blastocysts. Two clones (C4 and 1A15) transmitted the mutation germline and thus produced two independent lines of *Jak1*$^{-/-}$ mice.

Genotyping by Southern Blot and PCR

Southern blot analysis was performed on 25 μg of Eco RI digested genomic DNA as described (Meraz et al., 1996). Filters were probed with a ^{32}P-labeled external probe (Figure 1). To genotype mice by PCR, 0.5 μg of genomic DNA was amplified with one set of primers derived from the neomycin gene (5′ sequence: CACGACGGGCGTT CCTTGCGCAG, 3′ sequence: CCTGATGCTCTTCGTCCAGATCAT) and a second set of primers derived from the second exon of the *Jak1* gene deleted by the targeting construct (5′ sequence: GAGGAC TGCAATGCCATGGCGTTC, 3′ sequence: CACTCCTGGGCGGCCC

TGATG). The samples were amplified in the following manner: 1 cycle at 94°C for 1 s; 25 cycles at the following conditions: 94°C for 30 s, 62°C for 45 s, and 72°C for 45 s; and finally 1 cycle at 72°C for 2 min. A 200 bp fragment derived from exon 2 and a 250 bp fragment derived from the neomycin gene were resolved on a 2% TBE agarose gel.

Jak1 Western Blots

Immunoprecipitation and Western blotting were performed on lysates of EFs as described (Meraz et al., 1996) using different Jak1-specific antisera obtained from Upstate Biotechnology Institute (Lake Placid, NY) and Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Culture and Transfection

Immortalized normal and *Jak1*$^{-/-}$ EFs were generated using the method of Todaro and Green (1963). *Jak1*$^{-/-}$ EFs were reconstituted by cotransfecting 1×10^6 EFs with 20 μg of a murine Jak1 expression plasmid and 5 μg of the pMON Hygromycin B resistance plasmid by electroporation at 400 V, 320 μF on a Biorad gene pulser (Hercules, CA). Transfected cells were selected in 0.1 mg/ml Hygromycin for 3 weeks and then cloned by limiting dilution. Macrophages derived from e16.5–18.5 fetal liver were prepared in a manner similar to that used for growing bone marrow derived macrophages (Celada et al., 1984).

Induction of Cytokine-Dependent Cellular Responses on EFs, Macrophages, and Lymphocytes

EFs and macrophages were stimulated with designated doses of cytokines and assayed for expression of cell surface proteins, antiviral activity, iNOS induction, or inhibition of LPS-induced TNF production as previously described (Meraz et al., 1996). Thymocytes isolated from newborn mice were stained with FITC anti-CD4 and PE anti-CD8 (Pharmingen, San Diego, CA) as described (Dighe et al., 1995). Fetal liver cells and newborn splenocytes were stained after blocking Fc receptors with FITC anti-IgM and PE anti-B220 (Pharmingen, San Diego, CA). The capacity of thymocytes to proliferate in response to IL-2, IL-4, IL-7, or ionomycin in the presence of PMA was determined as described (Park et al., 1995).

Colony Forming Assays Performed in Methylcellulose

Cytokine-dependent fetal liver cell colony formation was assessed as described (Suda et al., 1989) using a commercial assay system (Stem Cell Technologies, Vancouver, Canada). The following analyses were used: 1.0×10^5 cells/plate with M-CSF, 1.25×10^5 cells/plate with GM-CSF or IL-3, 3×10^5 cells/plate with G-CSF, and 5×10^5 cells/plate with IL-5. For IL-7 cultures, 5×10^5 cells/plate were cultured either in pre–B cell medium (Stem Cell Technologies), which contained 10 ng/ml HuIL-7, or in normal basal methylcellulose medium supplemented with up to 50 ng/ml MuIL-7.

Electrophoretic Mobility Shift Assay

Cells in 1 ml PBS/10% FCS were stimulated with different cytokines and analyzed by an EMSA that used a ^{32}P-labeled 18 base pair GRR probe (Greenlund et al., 1994).

Cardiomyocyte Hypertrophy

This assay was performed as described for rat cardiomyocytes (King et al., 1996). Cardiomyocytes isolated from the hearts of newborn mice were plated overnight at a density of 1.25×10^4 cells per well in 96-well tissue culture plates in DMEM/F12 medium containing insulin, transferrin, and aprotinin. Cultures were stimulated with LIF or CT-1 or mixtures of IL-6 plus soluble IL-6 receptor α chain. Forty eight hours later, cells were fixed, stained with crystal violet, and cell surface areas quantitated by digital microscopy (King et al., 1996).

Neuronal Cultures

These assays employed an adaptation of the culture system reported by others (Dechiara et al., 1995). DRG from individual newborn mice were dissected, placed in L-15 medium (Sigma Chemical Company, St. Louis, MO), and dissociated with trypsin (0.05%) for 60 min. Medium (MEM supplemented with 10% FCS, 2 mM L-glutamine, 100 μg/ml penicillin and streptomycin, 20 mM fluorodeoxyuridine, and 20 mM uridine) was then added and the neurons triturated using a Pasteur pipette. Dissociated ganglia were plated into collagen-coated 24-well tissue culture dishes or collagen-coated glass chamber slides in MEM media supplemented with cytokine and/or goat anti-murine NGF at a final dilution of 1/500. After 72 hr, cells were fixed in 4% formalin, stained with crystal violet or anti-Neurofilament M (Chemicon International, Temecula, CA), and the number of viable cells enumerated by microscopy (Gribaudo et al., 1985).

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References

Akashi, K., Kondo, M., von Freeden-Jeffry, U., Murray, R., and Weissman, I.L. (1997). Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. Cell *89*, 1033–1041.

Bach, E.A., Aguet, M., and Schreiber, R.D. (1997). The IFNγ receptor: a paradigm for cytokine receptor signaling. Annu. Rev. Immunol. *15*, 563–591.

Celada, A., Gray, P.W., Rinderknecht, E., and Schreiber, R.D. (1984). Evidence for a gamma-interferon receptor that regulates macrophage tumoricidal activity. J. Exp. Med. *160*, 55–74.

Darnell, J.E., Jr. (1997). STATs and gene regulation. Science *277*, 1630–1635.

Darnell, J.E., Jr., Kerr, I.M., and Stark, G.R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science *264*, 1415–1421.

Dechiara, T.M., Vejsada, R., Poueymirou, W.T., Acheson, A., Suri, C., Conover, J.C., Friedman, B., McClain, J., Pan, L., Stahl, N., et al. (1995). Mice lacking the CNTF receptor, unlike mice lacking CNTF, exhibit profound motor neuron deficits at birth. Cell *83*, 313–322.

Dighe, A.S., Campbell, D., Hsieh, C.-S., Clarke, S., Greaves, D.R., Gordon, S., Murphy, K.M., and Schreiber, R.D. (1995). Tissue specific targeting of cytokine unresponsiveness in transgenic mice. Immunity *3*, 657–666.

Durbin, J.E., Hackenmiller, R., Simon, M.C., and Levy, D.E. (1996). Targeted disruption of the mouse *Stat1* gene results in compromised innate immunity to viral infection. Cell *84*, 443–450.

Greenlund, A.C., Farrar, M.A., Viviano, B.L., and Schreiber, R.D. (1994). Ligand induced IFNγ receptor phosphorylation couples the receptor to its signal transduction system (p91). EMBO J. *13*, 1591–1600.

Gribaudo, G., Cofano, F., Prat, M., Baiocchi, C., Cavallo, G., and Landolfo, S. (1985). Natural murine interferon-gamma: evidence for post-translational proteolytic processing. J. Biol. Chem. *260*, 9936–9940.

Guschin, D., Rogers, N., Briscoe, J., Witthuhn, B., Watling, D., Horn, F., Pellegrini, S., Yasukawa, K., Heinrich, P., Stark, G.R., Ihle, J.N., and Kerr, I.M. (1995). A major role for the protein tyrosine kinase Jak1 in the Jak/STAT signal transduction pathway in response to interleukin-6. EMBO J. *14*, 1421–1429.

Hardy, R.R., Carmack, C.E., Shinton, S.A., Kemp, J.D., and Hayakawa, K. (1991). Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J. Exp. Med. *173*, 1213–1225.

Ho, A.S.-Y., Wei, S.H.-Y., Mui, A.L.-F., Miyajima, A., and Moore, K.W. (1995). Functional regions of the mouse IL-10 receptor cytoplasmic domain. Mol. Cell. Biol. *15*, 5043–5053.

Huang, S., Hendriks, W., Althage, A., Hemmi, S., Bluethmann, H., Kamijo, R., Vilcek, J., Zinkernagel, R., and Aguet, M. (1993). Immune response in mice that lack the interferon-γ receptor. Science *259*, 1742–1745.

Ihle, J.N., Witthuhn, B.A., Quelle, F.W., Yamamoto, K., and Silvennoinen, O. (1995). Signaling through the hematopoietic cytokine receptors. Annu. Rev. Immunol. *13*, 369–398.

King, K.L., Lai, J., Winer, J., Luis, E., Yen, R., Hooley, J., Williams, P.M., and Mather, J.P. (1996). Cardiac fibroblasts produce leukemia inhibitory factor and endothelin, which combine to induce cardiac myocyte hypertrophy in vitro. Endocrine *5*, 85–93.

Kishimoto, T., Akira, S., Narazaki, M., and Taga, T. (1995). Interleukin-6 family of cytokines and gp130. Blood *86*, 1243–1254.

Kouro, T., Kikuchi, Y., Kanazawa, H., Hirokawa, K., Harada, N., Shiiba, M., Wakao, H., Takaki, S., and Takatsu, K. (1996). Critical proline residues in the cytoplasmic domain of the IL-5 receptor alpha chain and its function in IL-5-mediated activation of JAK kinase and STAT5. Int. Immunol. *8*, 237–245.

Leonard, W.J., Shores, E.W., and Love, P.E. (1995). Role of the common cytokine receptor γ chain in cytokine signaling and lymphoid development. Immunol. Rev. *148*, 97–114.

Li, M., Sendtner, M., and Smith, A. (1995). Essential function of LIF receptor in motor neurons. Nature *378*, 724–727.

Meraz, M.A., White, J.M., Sheehan, K.C.F., Bach, E.A., Rodig, S.J., Dighe, A.S., Kaplan, D.H., Riley, J.K., Greenlund, A.C., Campbell, D., et al. (1996). Targeted disruption of the STAT1 gene in mice reveals unexpected physiologic specificity in the Jak-STAT signaling pathway. Cell *84*, 431–442.

Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A.G., Barbier, G., Witthuhn, B.A., Schindler, C., et al. (1993). The protein tyrosine kinase JAK1 complements a mutant cell line defective in the interferon-α/β and -γ signal transduction pathways. Nature *366*, 129–135.

Neubauer, H., Cumano, A., Müller, M., Wu, H., Huffstadt, U., and Pfeffer, K. (1998). Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell *93*, this issue, 397–409.

Nicholson, A.E., Oates, A.C., Harpus, A.G., Ziemiecki, A., Wilks, A.F. and Layton, J.E. (1994). Tyrosine kinase Jak1 is associated with the granulocyte-colony-stimulating factor receptor and both become tyrosine-phosphorylated after receptor activation. Proc. Natl. Acad. Sci. USA *91*, 2985–2988.

Nosaka, T., van Deursen, J.M.A., Tripp, R.A., Thierfelder, W.E., Witthuhn, B.A., McMickle, A.P., Doherty, P.C., Grosveld, G.C., and Ihle, J.N. (1995). Defective lymphoid development in mice lacking Jak3. Science *270*, 800–802.

O'Shea, J.J. (1997). Jaks, STATs, cytokine signal transduction, and immunoregulation: are we there yet? Immunity *7*, 1–11.

Parganas, E., Wang, D., Stravopodis, D., Topham, D.J., Marine, J.-C., Teglund, S., Vanin, E., Bodner, S., Colamonici, O.R., van Deursen, J.M., Grosveld, G., and Ihle, J.N. (1998). Jak2 is essential for signaling through a variety of cytokine receptors. Cell *93*, this issue, 385–395.

Park, S.Y., Saijo, K., Takahashi, T., Osawa, M., Arase, H., Hirayama, N., Miyake, K., Nakauchi, H., Shirasawa, T., and Saito, T. (1995). Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity *3*, 771–782.

Peschon, J.J., Morrissay, P.J., Grabstein, K.H., Ramsdell, F.J., Maraskovsky, E., Gliniak, B.C., Park, L.S., Ziegler, S.F., Williams, D.E., Ware, C.B., et al. (1994). Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J. Exp. Med. *180*, 1955–1960.

Russell, S.M., Tayebi, N., Nakajima, H., Riedy, M.C., Roberts, J.L., Aman, M.J., Migone, T., Noguchi, M., Markert, M.L., Buckley, R.H., O'Shea, J.J., and Leonard, W.J. (1995). Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science *270*, 797–800.

Stahl, N., Boulton, T.G., Farruggella, T., Ip, N.Y., Davis, S., Witthuhn, B.A., Quelle, F.W., Silvennoinen, O., Barbiere, G., Pellegrini, S., Ihle, J.N., and Yancopoulos, G.D. (1994). Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. Science *263*, 92–95.

Suda, T., Okada, S., Suda, J., Miura, Y., Ito, Y., Sudo, T., Hayashi, S., Nishikawa, S., and Nakauchi, H. (1989). A stimulatory effect of recombinant murine interleukin-7 (IL-7) on B-cell colony formation and an inhibitory effect of IL-1α. Blood *74*, 1936–1941.

Thomis, D.C., Gurniak, C.B., Tivol, E., Sharpe, A.H., and Berg, L.J. (1995). Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science *270*, 794–797.

Todaro, G.J., and Green, H. (1963). Quantitative studies of the growth of mouse embryo cells in culture and their development in established lines. J. Cell Biol. *17*, 299–313.

Velazquez, L., Fellous, M., Stark, G.R., and Pellegrini, S. (1992). A protein tyrosine kinase in the interferon α/β signaling pathway. Cell *70*, 313–322.

von Freeden-Jeffry, U., Vieira, P., Lucian, L.A., McNeil, T., Burdach, S.E., and Murray, R. (1995). Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J. Exp. Med. *181*, 1519–1526.

Ware, C.B., Horowitz, M.C., Renshaw, B.R., Hunt, J.S., Liggit, D., Koblar, S.A., Gliniak, B.C., McKenna, H.J., Papayannopoulou, T., Thoma, B., et al. (1995). Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural, and metabolic defects and results in perinatal death. Development *121*, 1283–1299.

Watling, D., Guschin, D., Müller, M., Silvennoinen, O., Witthuhn, B.A., Quelle, F.W., Rogers, N.C., Schindler, C., Stark, G.R., Ihle, J.N. and Kerr, I.M. (1993). Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-γ signal transduction pathway. Nature *366*, 166–170.

Yoshida, K., Taga, T., Saito, M., Suematsu, S., Kumanogoh, A., Tanaka, T., Fujiwara, H., Hirata, M., Yamagami, T., Nakahata, T., et al. (1996). Targeted disruption of gp130, a common signal transducer for IL-6-family cytokines, leads to myocardial and hematological disorders. Proc. Natl. Acad. Sci. USA *93*, 407–411.