Deregulated T Cell Activation and Autoimmunity in Mice Lacking Interleukin-2 Receptor β

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In mice lacking the interleukin-2 receptor β chain (IL-2Rβ), T cells were shown to be spontaneously activated, resulting in exhaustive differentiation of B cells into plasma cells and the appearance of high serum concentrations of immunoglobulins G1 and E as well as autoantibodies that cause hemolytic anemia. Marked infiltrative granulocytopoiesis was also apparent, and the animals died after about 12 weeks. Depletion of $CD4^+$ T cells in mutant mice rescued B cells without reversion of granulocyte abnormalities. T cells did not proliferate in response to polyclonal activators, nor could antigen-specific immune responses be elicited. Thus, IL-2Rβ is required to keep the activation programs of T cells under control, to maintain homeostasis, and to prevent autoimmunity.

IL-2R is composed of at least three different subunits, α, β, and γ (*1*). Although signal transduction through IL-2R is not fully characterized, the intracytoplasmic tails of the β (*2*) and γ (*3*) chains are crucial for signaling. Recently, IL-2Rβ has been shown to bind IL-15 (*4*), which is produced by a variety of cells and tissues and has several biological functions in common with IL-2. Expressed on resting T cells at low density and up-regulated by stimulation of the T cell receptor (TCR), IL-2Rβ is also present on B cells (*5*), natural killer cells (*6*), monocytes (*7*), dendritic epidermal cells (*8*), neutrophils (*9*), and large granular lymphocyte–like cells in decidua during early pregnancy (*10*). Given that IL-2Rβ functions in more than one receptor and is expressed on various cell types, it may play multiple, as yet unidentified, immunomodulatory roles.

Mice defective in IL-2Rβ expression were generated by insertion of neomycin resistance cassette (Fig. 1A) into the IL-2Rβ gene at exon 6, which encodes a region of the extracellular domain proximal to the transmembrane region (*11*). Southern (DNA) blots and flow cytometric analysis were performed to confirm disruption of the gene (Fig. 1B) and lack of surface IL-2Rβ expression (Fig. 1C). Mice from two independent embryonic stem cell lines (D3 and E14) were separately bred homozygous for the defect, with both lineages showing identical gross appearances. The mice show normal growth until ~3 weeks after birth. After 4 weeks of age, they are generally smaller than normal or heterozygous littermates and have abnormal appearances characterized by fuzzy hair, slow movement, and poorly developed external genitals. Death occurs at ~12 weeks. Experiments were performed on mice generated from D3 embryonic stem cells.

IL-2R is expressed transiently on developing $CD4^-CD8^-$ thymocytes (*12*) and thus has been thought to play a role during thymic T cell development (*13*). In mutant mice, the thymus revealed normal phenotypic features at 1 and 3 weeks, including a normal expression pattern for IL-2Rα (*13*). By 6 weeks of age, absolute numbers of thymocytes had decreased [$(1.6 \pm 0.5) \times 10^7$ ($n = 3$)] compared with heterozygous littermates [$(11.4 \pm 1.5) \times 10^7$ ($n = 4$)]; there was also an apparent increase of $CD4^+$ and $CD8^+$ single-positive ($CD3^{hi}TCR\alpha\beta^{hi}$) cells and reduced numbers of $CD4^+CD8^+$ cells (Fig. 2A). Thymic irregularities similar to those of our mutant mice have been described in mice under a variety of stressed (pathological) conditions (*14*). To examine the irregular thymocyte profiles in older animals, fetal thymic organ cultures were done (*15*) and were found to be normal (Fig. 2B). Thus, the thymic pro-

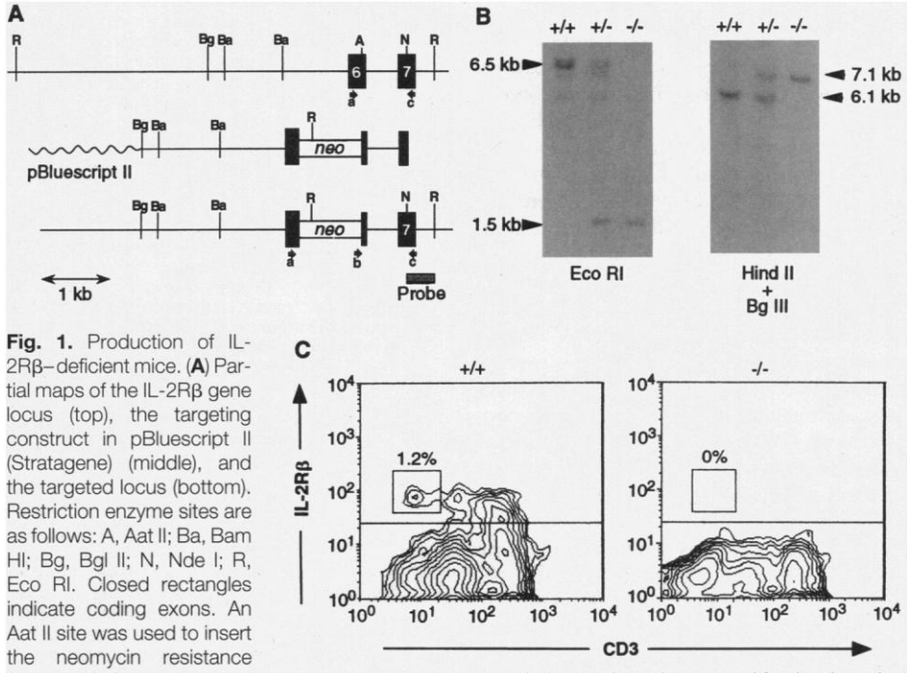

Fig. 1. Production of IL-2Rβ–deficient mice. (**A**) Partial maps of the IL-2Rβ gene locus (top), the targeting construct in pBluescript II (Stratagene) (middle), and the targeted locus (bottom). Restriction enzyme sites are as follows: A, Aat II; Ba, Bam HI; Bg, Bgl II; N, Nde I; R, Eco RI. Closed rectangles indicate coding exons. An Aat II site was used to insert the neomycin resistance (*neo*) gene. Small arrows (a, b, and c) indicate polymerase chain reaction primers used for the detection of the homologous recombination event. The IL-2Rβ flanking probe used in Southern blot analysis is shown as a stippled rectangle. (**B**) Southern blot analysis of mouse tail DNA. Tail DNA from IL-2Rβ$^{+/+}$, IL-2Rβ$^{+/-}$, and IL-2Rβ$^{-/-}$ mice was digested with Eco RI or Bgl II plus Hind III, and analyzed with the probe shown in (A). The 6.5-kb band produced by Eco RI digestion and the 6.1-kb band produced by digestion with Hind III and Bgl II correspond to the endogenous wild-type gene. A 1.5-kb band after Eco RI digestion and a 7.1-kb band after digestion with Hind III and Bgl II represent the targeted gene. (**C**) Expression of IL-2Rβ on splenocytes. Spleen cells from IL-2Rβ$^{+/+}$ and IL-2Rβ$^{-/-}$ mice were stained with monoclonal antibodies to both IL-2Rβ [conjugated to phycoerythrin (PE)] and to CD3 [conjugated to fluorescein isothiocyanate (FITC)]. Gene-targeted mice show no expression of IL-2Rβ.

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files of older mutant mice may reflect general stress-induced changes rather than intrinsic problems with thymocyte development. Although a role for IL-2Rβ on thymocytes has been postulated (*16*), taken together our results are consistent with the finding that IL-2Rβ is not required for thymocyte development (*8*).

By 3 weeks of age, homozygous mice suffered from splenomegaly and lymphoadenopathy. The spleen and lymph nodes contained large numbers of large blasting TCRαβ T cells (Fig. 2C). Flow cytometric analysis of lymph node cells revealed no significant difference in the ratio of $CD4^+$ and $CD8^+$ cells as compared with that in normal mice. By 3 weeks of age, lymph nodes of homozygous mice showed increased expression of the early T cell activation marker CD69 (*17*) relative to wild-type littermates (Fig. 2C). A fraction of peripheral T cells also showed up-regulated expression of IL-2Rα.

The presence of activated $CD4^+$ cells in mutant mice suggested that B cell activity may be affected. Serum immunoglobulin (Ig) titers revealed 10- to 100-fold increases in IgG1 and IgE in 3-week-old homozygous mice compared with heterozygous littermates (Fig. 3A). No significant differences were observed for serum concentrations of other Ig isotypes. Preliminary results indicate that $CD4^+$ T cells from mutant mice secrete greater amounts of both interferon γ and IL-4 as compared with controls, showing no bias toward either a T_H1-type or T_H2-type T helper cell response (*18*). The presence of high serum Ig concentrations and activated T cells indicated a risk for spontaneous autoimmunity. We therefore examined serum for antinuclear and anti-DNA antibodies by immunofluorescence staining and discovered that all homozygous mice contained high concentrations of these antibodies (Table 1). Mild anemia was observed in 3-week-old homozygous mice, which became more anemic with age. Anemia appeared to be the result of autoimmune hemolysis because anti-erythrocyte IgG was detected (*19*) and the number of reticulocytes was increased (Table 1), indicating an increased rate of erythropoiesis to compensate for ongoing hemolysis. In older mice, however, we noted a marked decrease in the number of reticulocytes before death (Table 1), caused, at least in part, by granulocytic infiltration and granulocytopoiesis displacing hematopoiesis from the bone marrow.

Splenic red pulp was enlarged and filled with myelopoietic cells, infiltration of mature and immature myeloid cells was observed in liver sinusoids and periportal tracts, and the pulp of lymph nodes contained increased numbers of plasma cells and neutrophils. These changes were al-

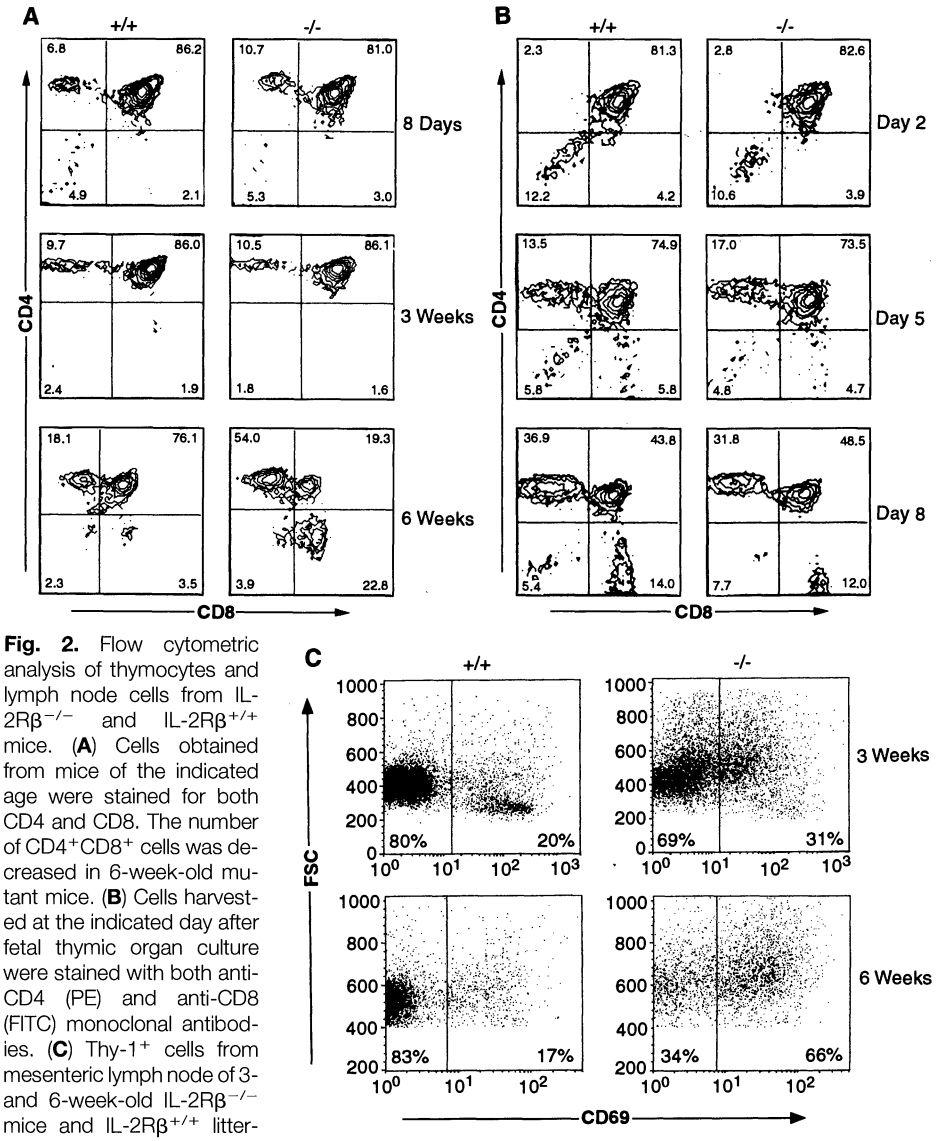

Fig. 2. Flow cytometric analysis of thymocytes and lymph node cells from IL-$2R\beta^{-/-}$ and IL-$2R\beta^{+/+}$ mice. (**A**) Cells obtained from mice of the indicated age were stained for both CD4 and CD8. The number of $CD4^+CD8^+$ cells was decreased in 6-week-old mutant mice. (**B**) Cells harvested at the indicated day after fetal thymic organ culture were stained with both anti-CD4 (PE) and anti-CD8 (FITC) monoclonal antibodies. (**C**) Thy-1^+ cells from mesenteric lymph node of 3- and 6-week-old IL-$2R\beta^{-/-}$ mice and IL-$2R\beta^{+/+}$ littermates were stained with monoclonal antibodies to Thy-1 and to CD69 and gated on Thy-1^+ cells. Many resting cells remain at 3 weeks.

Table 1. Autoantibodies, hematocrit, and reticulocyte number in IL-$2R\beta^{-/-}$ mice. Two independent mice (1 and 2) for each genotype (IL-$2R\beta^{+/-}$ or IL-$2R\beta^{-/-}$) were analyzed at the indicated ages. Immunofluorescence analysis of anti-nuclear and anti–native DNA antibodies was performed according to the protocol of the manufacturer (Sigma Diagnostics, St. Louis, Missouri), with the exception that FITC-conjugated antibodies to mouse IgG were used instead of FITC-conjugated antibodies to human Ig. Serum from individual mice was diluted as indicated and staining was judged with a fluorescence microscope. Peripheral blood was also centrifuged in heparin-coated capillary tubes for 5 min and the ratio of cell volume to total blood volume was measured. Peripheral blood cells were stained with new methylene blue and the number of reticulocytes per 1000 red blood cells (RBCs) counted.

IL-2Rβ genotype	Age (weeks)	Anti-nuclear (serum dilution)		Anti-DNA (serum dilution)		Hematocrit (%)		Reticulocytes (per 1000 RBCs)	
		1	2	1	2	1	2	1	2
+/−	3	− (1:10)	− (1:10)	− (1:10)	− (1:10)	48	43	52	52
+/−	5	− (1:10)	− (1:10)	− (1:10)	− (1:10)	54	50	31	12
+/−	8	− (1:10)	− (1:10)	− (1:10)	− (1:10)	52	46	63	44
−/−	3	+ (1:40)	+ (1:40)	± (1:10)	+ (1:40)	33	33	164	137
−/−	5	+ (1:80)	+ (1:80)	+ (1:80)	+ (1:80)	29	25	86	2
−/−	8	+ (1:20)	+ (1:40)	− (1:10)	+ (1:40)	9	18	4	1

ready apparent in 4-week-old homozygous mice and became more severe with increasing age. Cytospin preparations from 8-week-old mice showed that granulocytopoietic cells accounted for >90% of cells in the spleen. A study showing that IL-2–deficient mice develop chronic inflammatory bowel disease (*20*) prompted us to examine the intestines of IL-2Rβ$^{-/-}$ mice by histology and immunohistochemistry. Although abnormal infiltration by granulocytes was apparent, no evidence of bowel disease before death was detected.

The ratio of B cells to T cells in lymph nodes of 3-week-old animals was similar in mutant mice and littermate controls but declined markedly by 6 weeks of age in the homozygous animals (Fig. 3B). At 8 weeks of age, B220$^+$ and IgM$^+$ populations had almost completely vanished from the spleen (Fig. 3C). Colony-formation assays with lipopolysaccharide (LPS) and the pre–B cell growth factor IL-7 indicated that B cell development was partially compromised in 1-week-old mutant mice, whereas 8-week-old mice showed negligible B cell colony formation (Fig. 3D). The loss of B220$^+$ and IgM$^+$ populations in older mice was probably attributable to deteriorated B cell lymphopoiesis (resulting from granulocytic infiltration) in the bone marrow combined with exhaustive activation of B cells in the periphery.

Because activation of CD4$^+$ T cells preceded the disappearance of B220$^+$ B cells, we tested the effects of early CD4$^+$ T cell depletion on the developing phenotype of mutant mice. Beginning 4 days after birth, homozygous and heterozygous littermates were injected weekly with monoclonal antibodies to CD4 (*21*), which reduced the number of CD4$^+$ T cells below the limit of detection (Table 2). At 8 weeks, treated homozygous mice developed lymphoadenopathy and splenomegaly and showed increased numbers of Gr-1$^+$ cells in both spleen and bone marrow, similar to untreated mutant animals (Table 2). However, the number of B cells in the spleen as well as serum IgG1 and IgE concentrations re-

Table 2. B cells, granulocytes, and serum antibodies in IL-2Rβ$^{-/-}$ mice treated with anti-CD4 antibodies or in nude mice injected with IL-2Rβ$^{-/-}$ T cells. IL-2Rβ$^{+/-}$ and IL-2Rβ$^{-/-}$ mice with (ΔCD4) or without anti-CD4 treatment were analyzed at 8 weeks of age. FACScan analysis was performed to determine the relative numbers of CD4$^+$, CD8$^+$, B220$^+$, and Gr-1$^+$ cells. Total cell numbers in spleen were calculated with a hemacytometer. Serum concentrations of immunoglobulins and anti-nuclear antibodies were determined as described in Fig. 3A and Table 1, respectively. T cells from IL-2Rβ$^{+/-}$ (+/−) or IL-2Rβ$^{-/-}$ (−/−) mice were also injected into C57BL/6$^{nu/nu}$ (nude) mice. Nine weeks after T cell injection, spleen cells were analyzed by FACScan for expression of B220, Gr-1, and Thy-1, and serum immunoglobulins and antinuclear antibodies were assayed.

Mouse	10^6 cells in spleen					Serum antibodies (μg/ml)			Anti-nuclear (serum dilution)
	Thy-1	CD4	CD8	B220	Gr-1	IgM	IgG1	IgE	
IL-2Rβ$^{+/-}$		9.8	9.2	31.5	4.0	250	500	<1	− (1:10)
IL-2Rβ$^{-/-}$		25.4	13.7	0.8	96.3	230	14000	40	+ (1:40)
IL-2Rβ$^{+/-}$ ΔCD4		0	13.5	22.0	5.6	250	350	<1	− (1:10)
IL-2Rβ$^{-/-}$ ΔCD4		0	3.5	26.3	96.7	380	780	<1	− (1:10)
IL-2Rβ$^{-/-}$ ΔCD4		0	8.2	34.7	70.8	440	750	<1	− (1:10)
Nude (+/−)	11.4			35.7	4.4	360	450	<1	− (1:10)
Nude (−/−)	13.0			4.8	24.8	400	3700	5	+ (1:10)

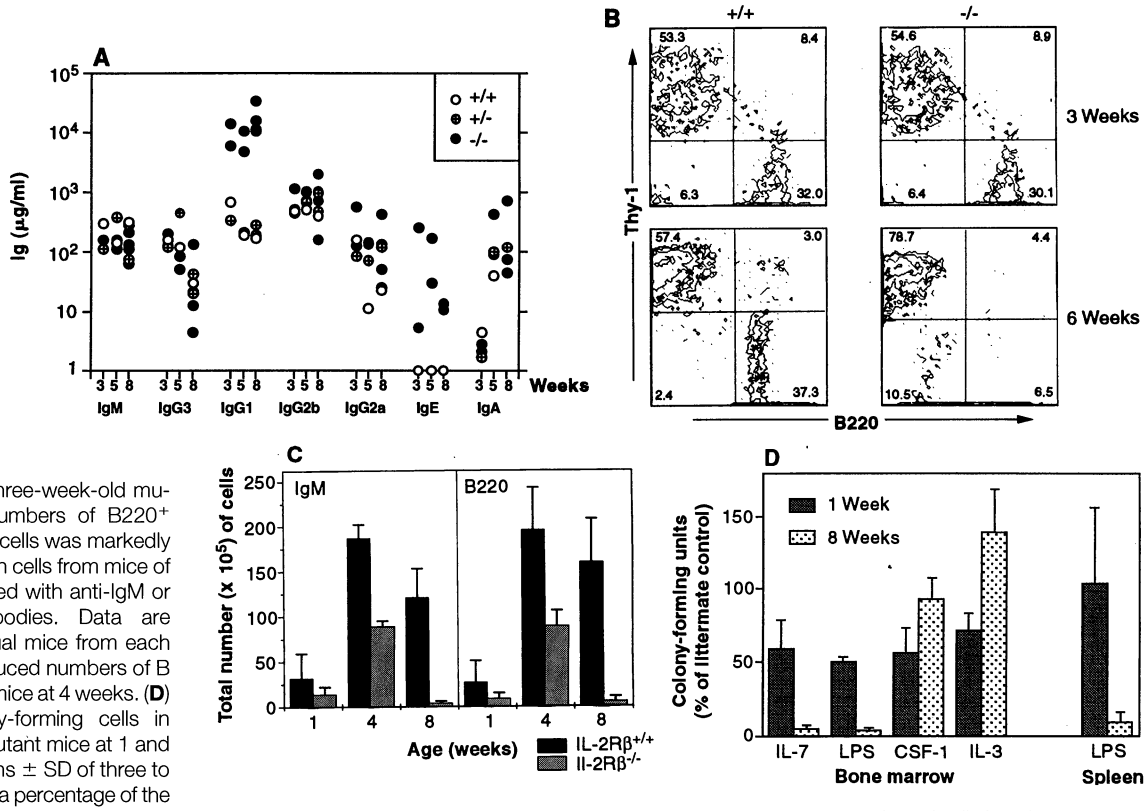

Fig. 3. (**A**) Serum Ig of the indicated isotypes in IL-2Rβ$^{+/+}$, IL-2Rβ$^{+/-}$, and IL-2Rβ$^{-/-}$ mice at 3, 5, and 8 weeks determined by enzyme-linked immunosorbent assay with isotype-specific antibodies. Absolute antibody concentrations were calculated from absorbance standard curves. Concentrations of IgG1 and IgE were markedly increased at all ages in homozygous mutant mice. (**B**) Lymph node cells stained with anti–Thy-1 and anti-B220 monoclonal antibodies and analyzed by FACScan. Three-week-old mutant mice showed normal numbers of B220$^+$ cells, but the number of these cells was markedly reduced at 6 weeks. (**C**) Spleen cells from mice of the indicated ages were stained with anti-IgM or anti-B220 monoclonal antibodies. Data are means ± SD of three individual mice from each group at each time point. Reduced numbers of B cells were apparent in mutant mice at 4 weeks. (**D**) Absolute numbers of colony-forming cells in spleen and bone marrow of mutant mice at 1 and 8 weeks of age. Data are means ± SD of three to five mice and are expressed as a percentage of the values for control littermates. Absolute numbers were calculated by multiplying the frequency of colony formation by the total number of cells in spleen or two femurs. CSF-1, colony-stimulating factor–1.

mained normal, and antinuclear antibodies or hemolytic anemia were not detected. Treated mice also had a healthy appearance as compared with untreated mutant animals. Thus, $CD4^+$ T cells appeared to cause the B cell pathology and autoimmune disease.

B cell responses in young mice were evaluated with vesicular stomatitis virus (VSV), which induces high titers of neutralizing antibodies in wild-type mice. IgM responses to VSV usually peak around 4 to 6 days after infection and are independent of T cell help; within 8 to 12 days, an Ig class switch to neutralizing IgG is observed, which is strictly dependent on T cell help (*22*). Three-week-old homozygous mutant mice showed neither detectable neutralizing IgM nor IgG responses after VSV infection (Fig. 4A), despite normal numbers of $B220^+$ and IgM^+ cells in lymph nodes (Fig. 3B). Although autoantibody production was the result of the $CD4^+$ T cell defect, B cells also had an intrinsic defect because they were unable to mount T cell–independent IgM responses.

$CD8^+$ cytotoxic T lymphocyte (CTL) and $CD4^+$ T helper cell responses were assessed with lymphocytic choriomeningitis virus (LCMV) (*23*). Injection of LCMV into footpads results in extensive local replication with no visible pathology because the virus is noncytopathic. However, 8 days after infection, the ensuing immune response causes an immunopathologic two-phase swelling reaction in the infected footpad. The early phase occurs on day 8 and is mediated exclusively by $CD8^+$ CTLs; a later and more moderate phase on day 10 is mediated independently by $CD4^+$ T cells (*24*). In homozygous mutant mice, neither the $CD8^+$- nor the $CD4^+$-mediated phase of the swelling reaction was observed (Fig. 4B). Because polyclonal activation of T cells in mutant mice might render them nonresponsive to additional stimuli, we analyzed these mice at 3 weeks of age, when most T cells are not activated (Fig. 2C). Because of the high sensitivity of the in vivo footpad assay, which results in normal swelling with as few as 10% of the normal number of functional $CD8^+$ T cells (*25*), we concluded that no functional $CD8^+$ cell response could be generated against LCMV in mutant mice.

$CD8^+$ CTL activity was also analyzed in vitro by ^{51}Cr-release assays. Mice were killed 14 days after infection (5 weeks old) with LCMV, and spleen cells were restimulated in vitro with LCMV-infected macrophages and tested for LCMV-specific cytotoxicity (Fig. 4, C and D). LCMV-specific CTLs from the spleen of heterozygous mice were readily restimulated, even in the absence of exogenous cytokines. In contrast, similar restimulation of spleen cells from IL-2Rβ–deficient mice generated no CTLs, even in the presence of concanavalin A (Con A) supernatant, which contains a mixture of cytokines secreted by T cells. We also investigated the ability of T cells from mutant mice to proliferate in response to polyclonal T cell activators, including Con A, staphylococcal enterotoxin B, and phorbol 12-myristate 13-acetate (PMA) plus Ca^{2+} ionophore (Fig. 4E). T cells from mutant animals did not respond to any of these activators nor did they proliferate in response to cross-linking with antibodies to TCRαβ or to CD3. Dose-response curves with IL-2 showed a complete lack of stimulation of mutant cells at all IL-2 concentrations (*26*). T cell activation through the TCR can occur in the absence of cell proliferation, which is stimulated in T cells predominantly via the IL-2 signaling pathway (*27*). PMA plus Ca^{2+} ionophore bypasses signaling through the TCR but does not activate the IL-2R pathway (*27*). Therefore, the failure of IL-2Rβ–deficient T cells to proliferate in response to these mitogens confirms that independent pathways exist for IL-2 and TCR signaling and that IL-2Rβ is required for T cell proliferation. Residual T cell proliferation in response to Con A is apparent in IL-2–deficient mice (*28*), indicating that other cytokines, such as IL-15, may act through IL-2Rβ receptors to facilitate T cell proliferation.

Thus, homozygous mutant mice have dysfunctional T and B cell compartments as well as a myeloproliferative disorder. However, whereas $CD4^+$ T cell depletion revealed a causal relation between the activated T cells and the B cell pathology, it did not help explain the myeloproliferative disorder. We attempted to clarify whether the general abnormalities could be induced by defects in other T cell lineages by adoptively transferring 10^8 purified T cells (*29*) from 4-week-old mutant and heterozygous mice into C57BL/6 nude mice. The nude mice injected with homozygous T cells developed granulocyte abnormalities both in spleen (Table 2) and peripheral blood; the number of B cells also decreased. Adoptive transfer of T cells from heterozygous mice did not

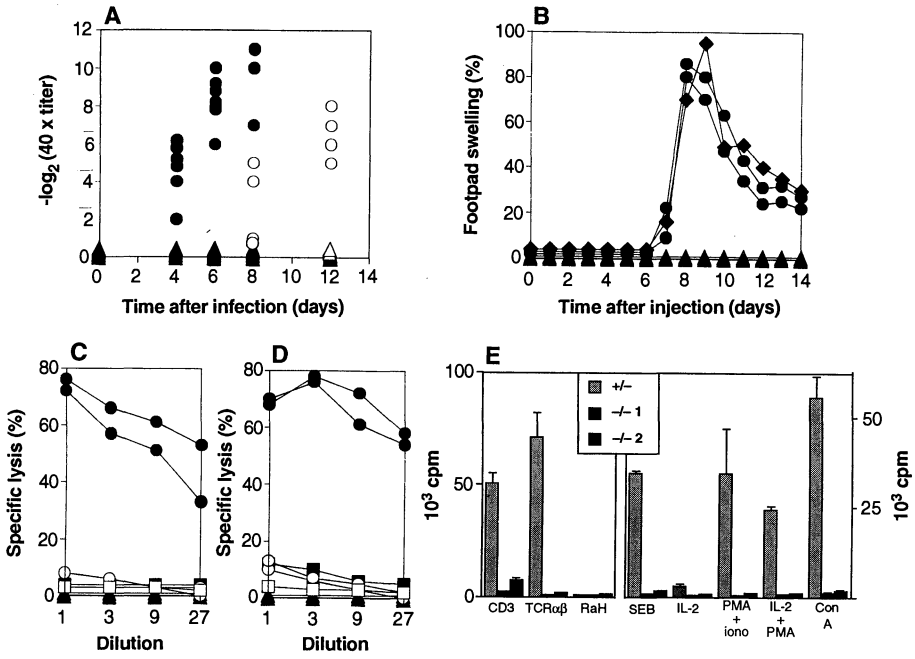

Fig. 4. (**A**) Antibody response to VSV was absent in 3-week-old mutant mice. Mice were infected intravenously with 2×10^5 plaque-forming units of VSV (New Jersey). (▲) IL-2Rβ$^{-/-}$ IgM; (●) IL-2Rβ$^{+/-}$ IgM; (△) IL-2Rβ$^{-/-}$ IgG; (○) IL-2Rβ$^{+/-}$ IgG. (**B**) T cell response to LCMV was absent in 3-week-old mutant mice. LCMV (2000 plaque-forming units) (Armstrong) was injected in footpads, and swelling (infected footpad thickness minus thickness before infection) was expressed as a percentage of footpad thickness before infection. (▲) IL-2Rβ$^{-/-}$; (●) IL-2Rβ$^{+/-}$; (◆) IL-2Rβ$^{+/+}$. (**C**) Fourteen days after infection with LCMV, spleen cells from mice in (B) were restimulated in vitro for 5 days with LCMV-infected macrophages and then assessed for specific cytotoxicity. (**D**) As in (C), with the exception that Con A supernatant was added to the 5-day culture. Cultures were harvested and tested for cytotoxicity against target cells (EL-4, H-2^b) labeled with major histocompatibility complex class I–binding LCMV peptides [(▲) IL-2Rβ$^{-/-}$; (●) IL-2Rβ$^{+/-}$; (■) unprimed IL-2Rβ$^{+/-}$] or against nonlabeled EL-4 targets [(○) IL-2Rβ$^{+/-}$; (□) unprimed IL-2Rβ$^{+/-}$]. (**E**) Lymph node T cells were purified on enrichment columns and seeded with autologous irradiated splenic feeder cells (1×10^5 T cells and 5×10^5 feeder cells per well). Activators: CD3, rabbit anti–hamster Ig (RaH) plus anti-CD3 antibodies; TCRαβ, RaH plus anti-TCRαβ antibodies; RaH, RaH alone; SEB, staphylococcal enterotoxin B; PMA + iono, PMA plus Ca^{2+} ionophore. Cells were pulsed after 3 days with 1 mCi of [^{3}H]thymidine per well for 10 hours. Data are means ± SD from triplicate cultures.

induce any changes in recipient mice. It seemed unlikely that the adoptive transfer of contaminating granulocytes was responsible for the granulocyte abnormality, because mice injected with mutant spleen cells containing >90% granulocytes did not develop an abnormal phenotype in any cell lineage. However, the expression of IL-2Rβ on myeloid cells necessitates cautious interpretation of these data; we cannot yet exclude the possibility that receptor deficiency may play some direct role in the observed myeloid disorder.

Taken together, our results suggest that IL-2Rβ, in addition to its known role as a mediator of activation signals for T cell proliferation, is also a central component of the regulatory system in T cells. It appears that a complete lack of signaling through the IL-2Rβ chain can result in deregulation of these lymphocytes and consequent loss of homeostasis and autoimmunity.

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29. Cells from spleen and lymph nodes were passed twice through a T cell enrichment column (R&D Systems, Minneapolis, MN). After confirming that T cells were >90% pure by staining with antibodies to Thy-1, 10^8 cells in 0.5 ml of RPMI 1640 medium were injected into 6-week-old C57BL/6$^{nu/nu}$ mice via the caudal vein.
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Identification of a Graft Versus Host Disease–Associated Human Minor Histocompatibility Antigen

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Minor histocompatibility antigen disparities between human leukocyte antigen (HLA)–matched bone marrow donors and recipients are a major risk factor for graft versus host disease (GVHD). An HLA-A2.1–restricted cytotoxic T cell clone that recognized the minor histocompatibility antigen HA-2 was previously isolated from a patient with severe GVHD after HLA-identical bone marrow transplantation. The HLA-A2.1–bound peptide representing HA-2 has now been identified. This peptide appears to originate from a member of the non–filament-forming class I myosin family. Because HA-2 has a phenotype frequency of 95 percent in the HLA-A2.1–positive population, it is a candidate for immunotherapeutic intervention in bone marrow transplantation.

In the 1970s, human bone marrow transplantation (BMT) became available as a therapy for severe aplastic anemia, leukemia, and immune deficiency disease (*1*). The long-term results of allogeneic BMT have greatly improved for a variety of reasons, including the participation of HLA-matched siblings as marrow donors, advancements in pretransplant chemoradiotherapy, the prophylactic use of potent immunosuppressive drugs, and better antibiotics and isolation procedures. Nonetheless, the selection of major histocompatibility complex (MHC)–identical donors and recipients does not guarantee avoidance of GVHD, nor does it ensure disease-free survival, even when the donor and the recipient are closely related (*2*). Up to 80% of cases of GVHD result from allogeneic BMT between unrelated HLA-matched adults (*3*, *4*). Disparities in minor histocompatibility antigens (mHags) between the donor and the recipient constitute a significant risk for graft failure or GVHD. These conditions necessitate lifelong pharmacological immunosuppression of organ and BMT recipients (*3*, *5*, *6*).

Cytotoxic T lymphocytes (CTLs) that are specific for host mHags have been detected in patients who develop GVHD after BMT from donors who are genotypically HLA-identical (*7*). Immunogenetic analyses with CTL clones have identified five non–sex-linked mHags, designated HA-1 through -5, that are recognized in a classical MHC-restricted fashion (*8*) and are products of single genes that segregate in a Mendelian fashion (*9*). In a prospective study, mismatching of these mHags significantly correlated with GVHD (*6*). These mHags show defined phenotype frequencies (*8*) and tissue distribution patterns (*10*). HA-1 and HA-2 are uniquely expressed on hematopoietic-derived cells, including leukemic cells (*11*), whereas HA-3 and HA-4 are present on other cell types as well (*10*).

Two unusual mHags have been charac-

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