Immunopathology of Interleukin (IL) 2–deficient Mice: Thymus Dependence and Suppression by Thymus-dependent Cells with an Intact IL-2 Gene

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Summary

Interleukin (IL) 2–deficient mice develop a fatal immunopathology characterized by lymphadenopathy, splenomegaly, T cell infiltration of the bone marrow, loss of B cells, anemia, and inflammation of the gut. The thymus dependence of these disease symptoms was tested by introducing the IL-2 mutation into athymic mice. With the exception of an increase in $CD8^+$ intrahepatic α/β T cells, IL-2 deficiency had no detectable effect on leukocyte composition or health of athymic mice, indicating a key role for thymus-derived T cells in the initiation of disease and demonstrating that B cell development and survival are independent of IL-2. In adoptive transfer studies, lymph node and spleen cells from euthymic IL-2–deficient mice induced disease in athymic mice with an intact IL-2 gene, suggesting that thymus-independent IL-2–expressing cells are unable to control the development of immune pathology. Both $IL\text{-}2^+$ and $IL\text{-}2^{-/-}$ bone marrow cells repopulated the thymus and the peripheral T cell compartment of the recombination activator gene 2–deficient recipients, and chimeras that had received IL-2–deficient bone marrow developed immune pathology. Disease development was, however, fully or at least partially prevented when 30% of the bone marrow inoculum was derived from mice able to express IL-2. These results demonstrate that the IL-2 deficiency syndrome depends on the intrathymic differentiation of T cells carrying the IL-2 mutation, and that the abnormal activation of IL-2–deficient lymphocytes can be controlled by thymus-derived but not thymus-independent lymphocytes.

IL-2, originally described as T cell growth factor, has pleiotropic effects on the proliferation and differentiation of T, B, and NK cells. In addition, inhibition of T cell development by antibodies to the α chain of the IL-2 receptor (IL-2R) reported by some (1, 2) but not by other investigators (3) had suggested an essential role for IL-2 in intrathymic T cell maturation. Unexpectedly, however, mainstream T cell development in mice with a disrupted IL-2 gene is normal (4), and after 3–4 wk of age, peripheral T cells are even increased in number and display an activated phenotype. This marks the onset of a lethal syndrome characterized by splenomegaly and lymphadenopathy, an early burst of B cell activation followed by their virtual disappearance, T cell infiltration of the bone marrow, anemia, autoantibody production, and a severe colitis preceded by an infiltration of the gut epithelium with CD4 and CD8 α/β T cells (5–8). Hyperactivation of T cells and development of autoimmunity have also been observed in mice lacking the IL-2Rβ chain (9), supporting the concept that IL-2R–mediated signals are required for the containment of T cell activation in vivo. Among the mechanisms that could lead to the development of the symptoms listed above are an incomplete deletion of autoreactive clones in T cell ontogeny, unchecked lymphocyte activation caused by a disturbance of the cytokine network, physical or functional absence of specialized suppressor cells, and a requirement for IL-2 in limiting clonal growth by predisposing mature activated T cells toward apoptotic death (10). In addition, breakdown of the B cell compartment could either result from T cell–mediated destruction or from an impaired replenishment of the peripheral pool from precursors expanded by IL-2, a possibility suggested by the expression of the IL-2Rα chain on pro-B cells (11). So far, attempts at identifying defects in T cell repertoire selection have yielded negative results (12). With regard to a breakdown in peripheral regulation, the delayed onset of immunopathology in germfree IL-2–deficient mice suggests that an antigen trigger is required to drive the abnormal lymphoproliferation observed (6). Since the oligoclonal repertoire of athymic mice allows alloreactive but not self-MHC–restricted T cell responses to environmental antigens (13–16), the IL-2 mutation was introduced into *nu/nu* mice to ask whether thymus-processed T cells (with a diverse repertoire) are required to initiate disease, including the destruction of B cells and bone marrow cells. Furthermore, it remained to be formally proven that lack of IL-2 expression in bone marrow–derived cells suffices for the development of the IL-2 deficiency syndrome. Therefore, recombination activator gene

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(RAG)[1] 2–deficient mice repopulated with H-2–matched bone marrow from IL-$2^{-/-}$ mice were investigated for signs of disease. Finally, the simultaneous transfer of IL-2^+ and IL-2^- bone marrow into RAG-2–deficient recipients was used to address the question of whether autocrine production of IL-2 is required for the control of clonal expansion or whether IL-2–producing or –dependent bystander cells could interfere.

Materials and Methods

Mice. IL-$2^{+/-}$ mice from a mixed C57BL/6/H129OLA background (4) were back-crossed for four generations to C57BL/6 mice. After mating with C57BL/6 nu/nu mice, IL-$2^{-/-}$ mice were identified by PCR among the nude offspring of the F2 generation. Euthymic IL-$2^{+/+}$ and IL-$2^{+/-}$ mice (which are phenotypically identical and will be referred to as IL-2^+) were used as controls. RAG-$2^{-/-}$ H-2^b mice were originally generated by Shinkai and colleagues (17). These and B6 Ly5.2-congenic mice (following the original nomenclature used for derivation of congenic strains and mAbs [18]) were kindly provided by Dr. H. R. Rodewald, Basel Institute for Immunology, Basel, Switzerland). All animals were bred in a barrier-sustained facility and were found free of common pathogens.

[1] *Abbreviations used in this paper:* BSS, balanced salt solution; IEL, intraepithelial lymphocytes; RAG, recombination activator gene.

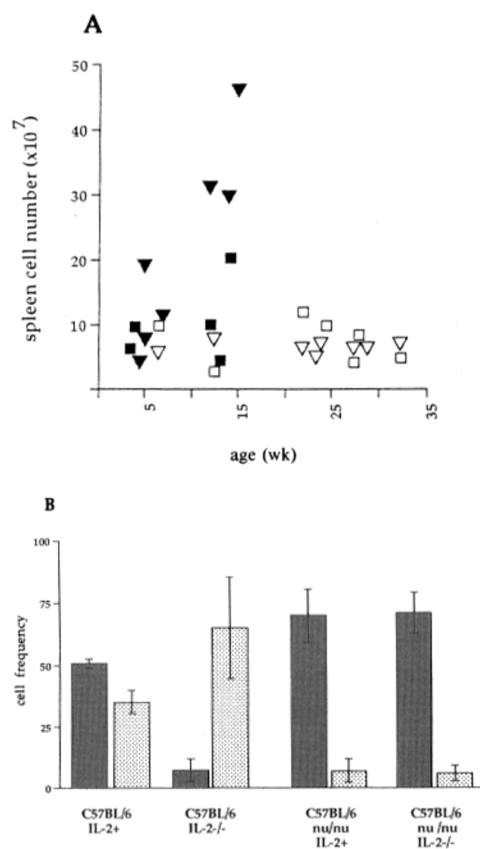

Figure 1. Influence of IL-2 deficiency on splenic cellularity and T/B cell content in euthymic and athymic mice. (*A*) Nucleated cells per spleen recovered from euthymic IL-2^+ (■), euthymic IL-$2^{-/-}$ (▼), athymic IL-2^+ (□), and athymic IL-$2^{-/-}$ (▽) C57BL/6 mice. (*B*) Proportion of splenic T cells (▒) and B cells (■). Spleens from six to seven mice of the groups indicated were analyzed for the fraction of Thy1- and B220-expressing cells at 8–12 (euthymic mice) or 8–31 wk (athymic mice).

Preparation and Transfer of Cells. Cells from thymus, lymph nodes, and spleen were prepared by grinding organs through a stainless steel sieve into ice-cold balanced salt solution (BSS). To prepare intraepithelial lymphocytes, the small intestine was opened longitudinally in PBS, and Peyer's patches were removed. Small pieces of the residual tissue were treated with Ca/Mg-free BSS/1 mM dithiothreitol for 20 min and with RPMI-1640/10% FCS for 40 min at 37°C. Filtered cells were enriched for lymphocytes by Percoll-gradient centrifugation. Hepatic mononuclear cells were prepared by injecting PBS into the portal vein to remove circulating cells from the liver before dissecting the animal. The tissue was then crushed and washed twice with BSS. Lymphocytes were enriched by Ficoll-gradient centrifugation. Bone marrow was ejected from femur and tibia of hind legs by injecting BSS. Bone marrow cells were washed twice with BSS. In the case of euthymic bone marrow donors, cells were further depleted of Thy-1^+ cells by a magnetism-activated cell sorter (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). To reconstitute RAG-2–deficient mice, 2×10^7 BM cells, and for transfer into nu/nu mice, 5×10^7 lymph node and spleen cells (1:1), were injected intravenously.

Antibodies and Flow Cytometry. The following antibodies were used: PE-conjugated anti-CD4 (clone H129.19; Becton Dickinson & Co., Mountain View, CA), biotinylated or FITC-conjugated anti-CD8α (clone 53-6.7; Boehringer Mannheim GmbH, Mannheim, Germany), PE-conjugated anti-B220 (clone RA3-6B2; Serva, Heidelberg, Germany), and FITC-conjugated anti-αβTcr (clone H57-597; PharMingen, San Diego, CA).. FITC-conjugated anti-Ly5.1 (clone 104.2.1), and biotinylated anti-Ly5.2 (clone A20-1.7) (18) were kindly provided by Dr. H. R. Rodewald.

For two- or three-color flow cytometry, 2×10^5 cells in 0.1 ml PBS/0.1% BSA/0.02% sodium azide were stained with the biotinylated antibody for 15 min on ice, washed, and incubated for 15 min on ice with streptavidin red^{670} (Life Technologies GmbH, Eggenstein, Germany), the FITC-labeled mAb, and/or the PE-conjugated mAb. All antibodies were used at saturating concentrations. Flow cytometry was performed with a FACScan® flow cytometer using Lysys II software (Becton Dickinson & Co.). Results are shown as log–log dot plots or as histograms.

Results

Thymus Dependence of the IL-2 Deficiency Syndrome. Both the nu and the IL-2^- mutation were bred to homozygosity into the C57BL/6 background. Athymic IL-2–deficient mice appeared healthy and, in contrast ot their euthymic littermates, showed no increased mortality. Even at >6 mo of age, a time point where few euthymic IL-$2^{-/-}$ mice survive, none of the pathological alterations regularly observed in IL-$2^{-/-}$ euthymic mice were found.

As shown in Fig. 1 *A*, cellularity of spleens remained normal in athymic IL-$2^{-/-}$ mice, whereas their euthymic littermates developed severe lymphadenopathy (not shown) and splenomegaly, as previously described (5, 6). Moreover, the loss of splenic B cells (Fig. 1 *B*) and mature B cells from the bone marrow (Fig. 2), which follows initial polyclonal T and B cell activation in euthymic IL-$2^{-/-}$ mice, was not observed in athymic IL-2–deficient mice, suggest-

ing that uncontrolled activation of thymus-derived T cells is crucial for B cell depletion. Similarly, infiltration of the bone marrow with mature T cells, which is likely to play a role in the loss of B cells and development of anemia in euthymic IL-$2^{-/-}$ mice, did not occur in IL-2–deficient athymic mice (Fig. 2). Importantly, adoptive transfer of peripheral lymphocytes from IL-$2^{-/-}$ but not from IL-2^{+} C57BL/6 mice into athymic IL-2^{+} C57BL/6 mice, caused T cell infiltration of the bone marrow (Fig. 2) and loss of B cells from bone marrow and spleen (not shown).

One of the key symptoms of the IL-2 deficiency syndrome is inflammatory bowel disease with fatal outcome (6). The onset of colitis is preceded by an infiltration of the gut epithelium with CD4 T cells, which represent a minority among normal intestinal intraepithelial lymphocytes (IEL). None of the athymic IL-$2^{-/-}$ mice developed overt colitis, and even at the more sensitive level of T cell subset composition, no significant effects of the IL-2 mutation were observed (Fig. 3). As shown above for the bone marrow, transfer of peripheral lymphocytes from euthymic IL-$2^{-/-}$ but not from IL-2^{+} mice resulted in infiltration of the gut epithelium with CD4 T cells (Fig. 3).

In summary, development of the IL-2 deficiency syndrome depends on an intact thymus, indicating that destruction of B cells and bone marrow cells as well as development of colitis are initiated by thymus-derived T cells, and that thymus-independent T cell lineages do not mediate immune pathology if deprived of IL-2. Furthermore, thymus-independent cells expressing an intact IL-2 gene are unable to control the abnormal activation of thymus-derived IL-$2^{-/-}$ T cells, at least under the conditions of adoptive transfer used here.

Intrahepatic T Cells in Athymic IL-$2^{-/-}$ Mice. Aging athymic mice contain variable numbers of T cells in their peripheral lymphoid organs, probably because of the expansion of few T cell clones from a small number of precursors (13–16). Based on phenotypic similarities such as a reduced level of TCR expression between hepatic T cells of euthymic mice (19) and peripheral T cells found in athymic mice (20, 21), the liver has been proposed as a site of extrathymic T cell differentiation (19, 22). On the other hand, the absence of RAG transcripts in the liver and influx of preapoptotic activated peripheral T cells that acquire the phenotype of liver T cells has argued against this view and has suggested a role for the liver in the elimination of activated T cells (23, 24). When T cell subsets were compared in spleens and lymph nodes of IL-2^{+} and IL-$2^{-/-}$ C57BL/6 nu/nu mice, no differences beyond the individual variability also found in IL-2^{+} athymic mice were observed (data not shown). Analyses of mononuclear cells from the livers of athymic mice, however, revealed a clear-cut effect of IL-2

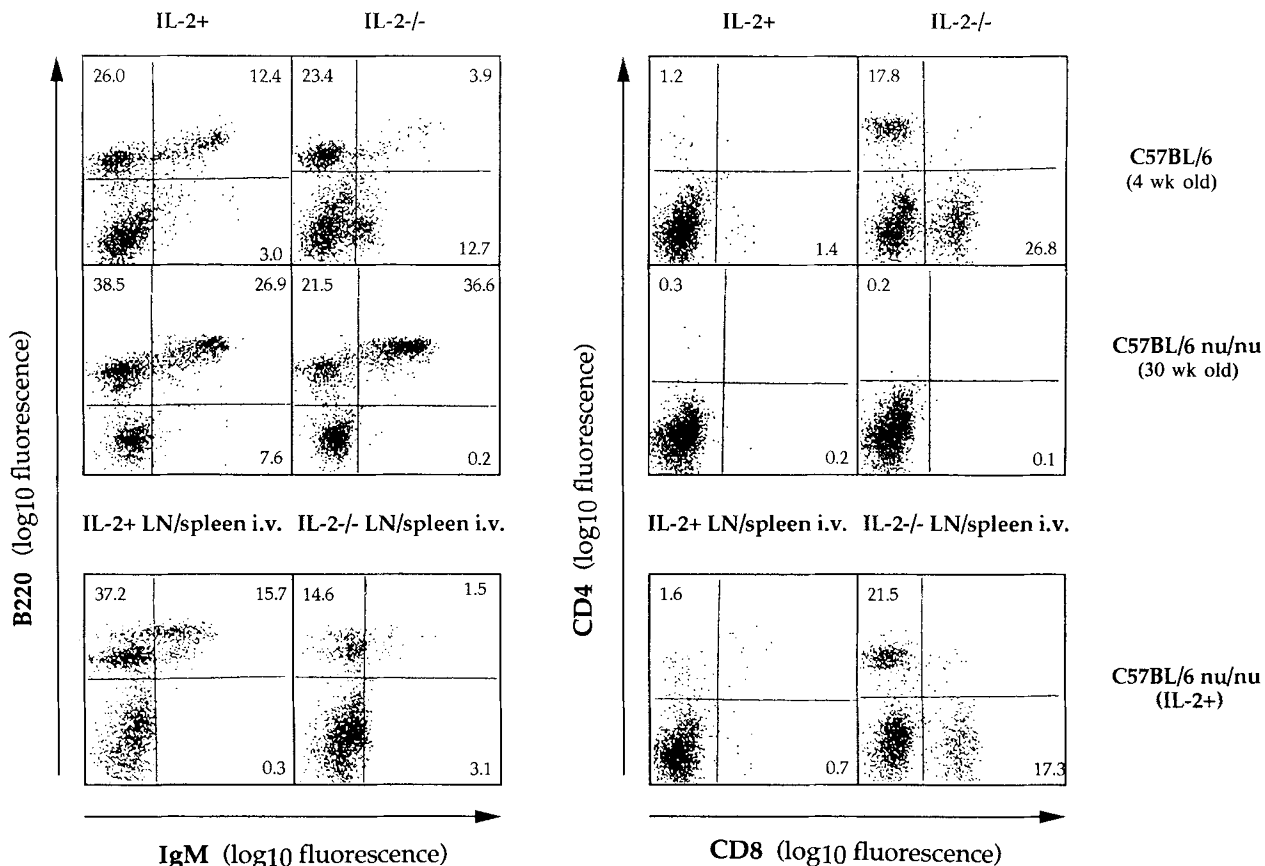

Figure 2. T cell infiltration and B cell destruction in the bone marrow of IL-2–deficient mice are thymus dependent. Flow cytometric analysis of bone marrow cells from IL-2^{+} and IL-$2^{-/-}$ euthymic (*top*) and athymic (*center*) C57BL/6 mice, and from IL-2^{+} athymic mice injected 6 wk previously with 5×10^{7} peripheral lymphocytes from 4-wk-old IL-2^{+} or IL-$2^{-/-}$ C57BL/6 mice.

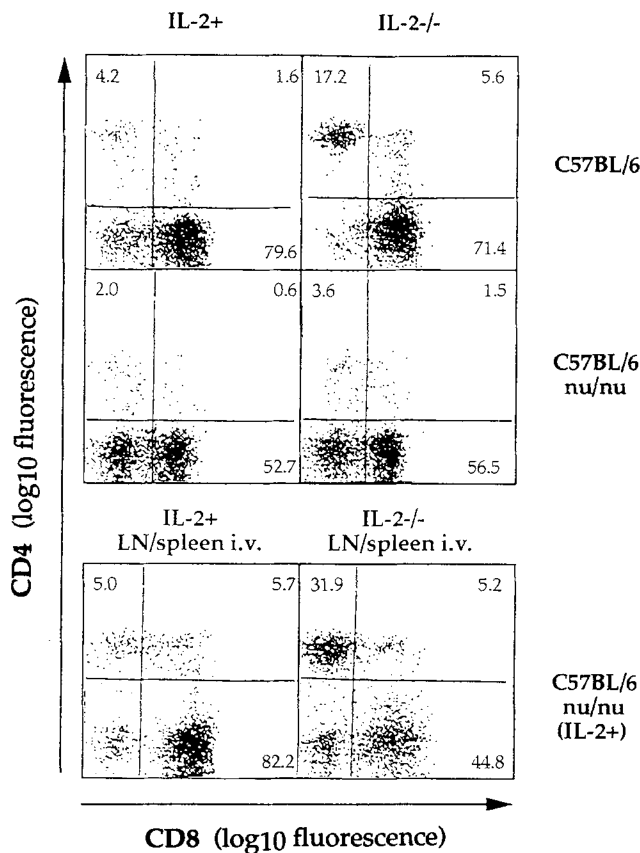

Figure 3. Thymus dependence of CD4 T cell infiltration in the epithelium of the small intestine of IL-2–deficient mice. Flow cytometric analysis of intestinal IEL from IL-2^+ and IL-$2^{-/-}$ euthymic (*top*) and athymic (*center*) C57BL/6 mice, and from IL-2^+ athymic mice injected 6 wk previously with 5×10^7 peripheral lymphocytes from 4-wk-old IL-2^+ or IL-$2^{-/-}$ C57BL/6 mice.

deficiency. Thus, the total number of recovered mononuclear cells (Table 1) and the frequency of α/β T cells with a reduced level of TCR expression (TCR^{int} cells; Fig. 4 *A*), which is characteristic of T cells in the liver, was strongly

Table 1. *Number of Intrahepatic Mononuclear Cells Isolated from the Livers of IL-2^+ and IL-$2^{-/-}$ Euthymic and Athymic Mice*

Mouse	Age	Mononuclear cells/ liver*
	wk	
C57BL/6 IL-2^+	3	1.0×10^5
C57BL/6 IL-2^+	3	0.7×10^5
C57BL/6 IL-$2^{-/-}$	3	1.2×10^6
C57BL/6 IL-$2^{-/-}$	3	2.6×10^6
C57BL/6 nu/nu IL-2^+	22	1.4×10^5
C57BL/6 nu/nu IL-2^+	30	2.0×10^5
C57BL/6 nu/nu IL-$2^{-/-}$	22	6.0×10^5
C57BL/6 nu/nu IL-$2^{-/-}$	30	7.2×10^5

*Mononuclear cells were isolated as described in Materials and Methods, and viable cells were counted by trypan blue exclusion.

increased in both euthymic and athymic C57BL/6 mice carrying the IL-2 mutation compared with their IL-2^+ littermates. This increase in T cell number was primarily caused by CD4^-8^+ cells (Fig. 4 *B*), of which the frequency among intrahepatic mononuclear cells increased 5–10-fold in IL-2–deficient mice, irrespective of the presence of a thymus. It is unclear at the present time whether the expanded CD4^-8^+TCR^{int} subset of intrahepatic T cells is caused by a delayed elimination of immigrant peripheral T cells or by local expansion (see Discussion).

Development of the IL-2 Deficiency Syndrome in RAG-2–deficient Mice Reconstituted with IL-2^+ and/or IL-$2^-/^-$ Bone Marrow. To further analyze the role of the thymus in the establishment of the IL-2 deficiency syndrome, RAG-2–deficient mice were injected with bone marrow cells expressing or lacking an intact IL-2 gene. To ensure that the bone marrow cells obtained from IL-2–deficient mice had not already been damaged before transplantation, IL-2–deficient athymic donors were chosen. As can be seen from Table 2 and Fig. 5, transfer of both IL-2^+ and IL-$2^{-/-}$ bone marrow cells led to a normal CD4/8 subset composition of the thymus in recipient RAG-$2^{-/-}$ mice when animals were analyzed 6 wk later. Whereas no pathologic alterations were observed in the control group that had received IL-2^+ bone marrow cells, all chimeras that had received IL-$2^{-/-}$ bone marrow exhibited lymphadenopathy and splenomegaly, loss of red cells from the bone marrow, and one animal died 5 wk after transfer. An elevated frequency of CD4 T cells was found in five of five and T cell infiltration of the bone marrow in four of five IL-$2^{-/-}$ $\rightarrow$ RAG-$2^{-/-}$ chimeras. Taken together with the results obtained in athymic mice, this indicates that expression of the IL-2 mutation in hematopoietic cells and a functional thymus are required for the development of the IL-2 deficiency syndrome.

To analyze whether the control of T cell activation that breaks down in IL-2–deficient mice requires autocrine regulation by IL-2 or can be exerted by IL-2–expressing cells (providing IL-2 in a paracrine fashion or acting as specialized suppressor cells), RAG-$2^{-/-}$ mice were coinjected with 70% IL-$2^{-/-}$ and 30% Ly5.2-marked IL-2^+ bone marrow cells. When analyzed 6 wk after transfer, the ratio of Ly5.1- and Ly5.2-expressing lymphocytes was similar (7:3) in the thymus and in peripheral lymphoid organs of the reconstituted animals. Importantly, three of four chimeras appeared healthy 6 wk after transfer, and only one had an abnormally increased size and cellularity of spleen and lymph nodes along with red cell destruction in the bone marrow. Neither T cell infiltration nor red cell destruction were observed in the bone marrow of the remaining animals (Table 2 and Fig. 5). Overrepresentation of CD4^+ cells among intestinal IEL was found in one additional mouse, which, however, remained free of diarrhea or overt colitis. These results show that to a large extent, T cells capable of producing IL-2 are able to contain the dysregulated activation of IL-2–deficient T cells that leads to the development of the IL-2 deficiency syndrome in RAG-$2^{-/-}$ mice restored with IL-$2^{-/-}$ bone marrow cells.

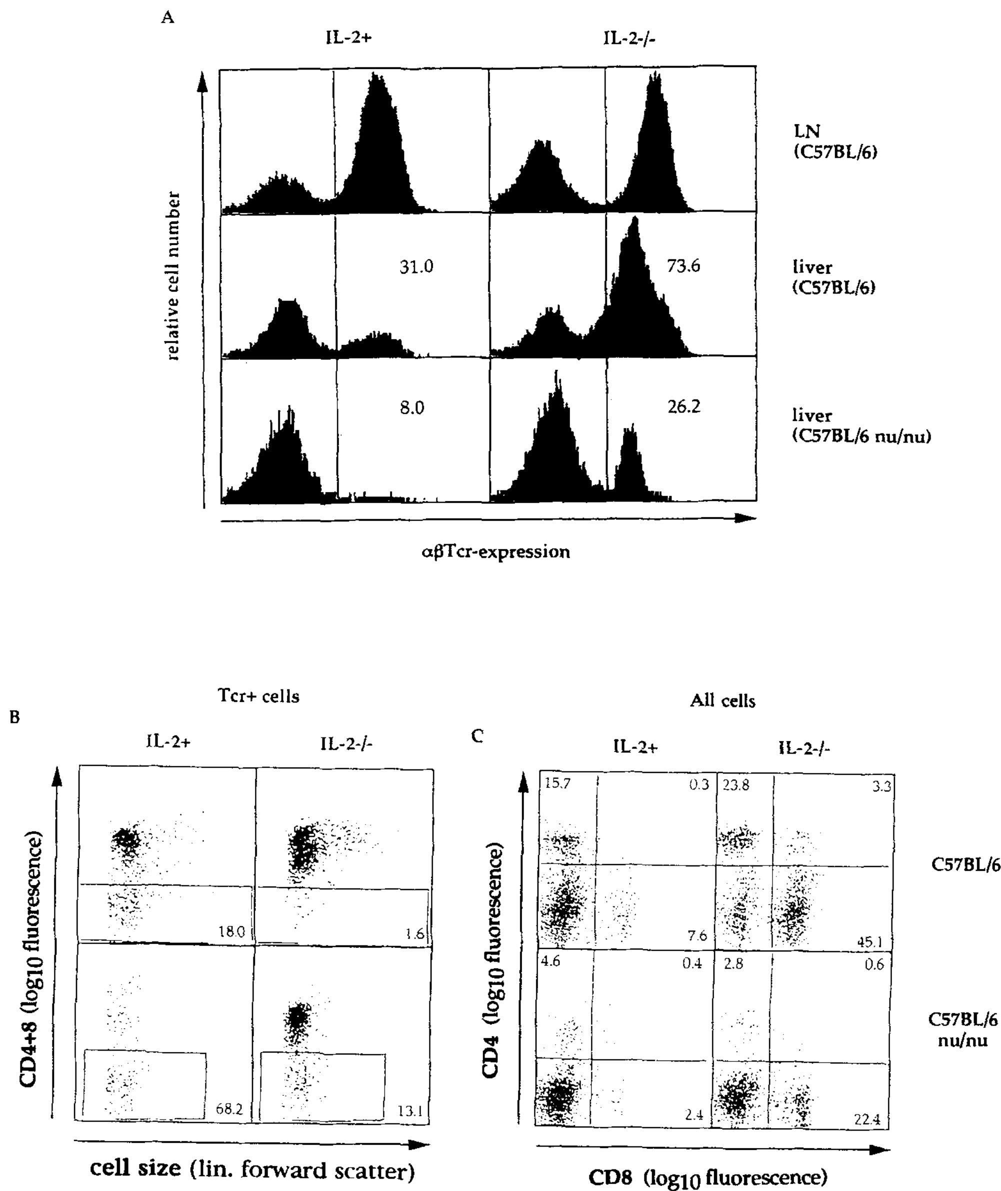

Figure 4. Increase of TCR^{int} $CD4^{-}8^{+}$ intrahepatic lymphocytes in both euthymic and athymic IL-$2^{-/-}$ mice. (*A*) TCR expression on mononuclear cells isolated from the lymph nodes (*top*) and liver (*center*) of 3-wk-old euthymic IL-2^{+} and IL-$2^{-/-}$ mice, and on intrahepatic mononuclear cells from 22-wk-old athymic IL-2^{+} and IL-$2^{-/-}$ mice (*bottom*). (*B*) Expression of CD4 and/or CD8 on TCR^{+} intrahepatic lymphocytes. (*C*) CD4/CD8 distribution of all intrahepatic mononuclear cells.

Discussion

The hallmark of the inflammatory and autoimmune manifestations of the mouse IL-2 deficiency syndrome is a hyperactivation of T cells, which precedes all other symptoms described above and, according to the present results, is likely to be the key event in the development of immune pathology. Thus, athymic mice carrying the IL-2 mutation remain free of bone marrow atrophy and red cell destruction, maintain normal B cell numbers, and do not develop colitis. Consequently, a direct effect of IL-2 deficiency on the survival of bone marrow stem cells and on the generation and maintenance of their B cell and erythrocyte progeny can be excluded. Furthermore, the thymus-independent T cell lineages originating in the gut epithelium do not mediate the inflammatory response found at that site in euthymic IL-2–deficient animals. In contrast, peripheral T cells transferred into nude mice from IL-$2^{-/-}$ donors infiltrate the gut and the bone marrow, supporting the conclusion that thymus-derived T cells are responsible for disease development.

The only difference detected in IL-2–deficient athymic mice compared with their IL-2^{+} littermates was a numeric increase in hepatic T cells that displayed the TCR^{int} phenotype found on the majority of hepatic T cells in normal mice and on T cells in both lymphoid organs and liver of athymic mice. This increase was mainly caused by $CD4^{-}8^{+}$ cells, which were also severalfold expanded in the liver of euthymic IL-2–deficient mice compared with IL-2^{+} controls. We have previously reported that among the small subset of $\alpha/\beta TCR^{int}IL\text{-}2R\beta^{+}$ thymocytes, which in normal mice are predominantly $CD4^{-}8^{-}$, IL-2 deficiency also causes a marked skewing toward the $CD4^{-}8^{+}$ phenotype (25). Such $\alpha/\beta TCR^{int}IL\text{-}2R\beta^{+}$ T cells in the thymus and

Table 2. *Phenotype of RAG-2$^{-/-}$ Mice Reconstituted with IL-2$^+$ and/or IL-2$^{-/-}$ Bone Marrow Cells*

Intact IL-2 gene in bone marrow	Splenomegaly/ lymphadenopathy*	Red cell destruction‡	% T cells in bone marrow§	% CD4 in IEL§	Other
+	−	−	3.1	10.5	−
+	−	−	9.3	7.3	−
+	−	−	6.2	8.4	−
−	+	+	3.0	20.1	Diarrhea
−	+	+	36.2	20.7	−
−	+	+	30.0	16.4	−
−	+	+	52.9	12.6	−
−	+	+	18.3	19.3	−
−	?	?	?	?	Dead
+,− (3:7)	−	−	3.8	8.4	−
+,− (3:7)	+	+	4.8	24.0	−
+,− (3:7)	−	−	8.3	18.4	−
+,− (3:7)	−	−	5.0	8.8	−

RAG-2$^{-/-}$ mice were injected intravenously with a total of 2 × 10^7 bone marrow cells (for details see legend to Fig. 5) and analyzed 6 wk after transfer.

*More than twice the size of organs in age-matched C57BL/6 mice.

‡Only white cells in bone marrow.

§As determined by flow cytometry using fluorescent TCR- and CD4-specific mAbs.

α/βTCR^{int} cells in the liver could either represent a distinct lineage or the end stage of clonally expanded cells before apoptosis. In the former scenario, the development of the CD4$^-$8$^+$ subset of this lineage might be favored by the absence of IL-2; in the latter, IL-2 deficiency may retard the process of elimination of clonally expanded CD4$^-$8$^+$ cells more severely than that of other T cells. This view is compatible with recent evidence showing that the CD4$^-$8$^-$ α/β T cells present in the thymus (26) and those accumulating in Fas-deficient lpr/lpr mice are predominantly MHC class I dependent and, by implication, may be derived from the CD8 lineage (27–29). Furthermore, activated TCR-trans-

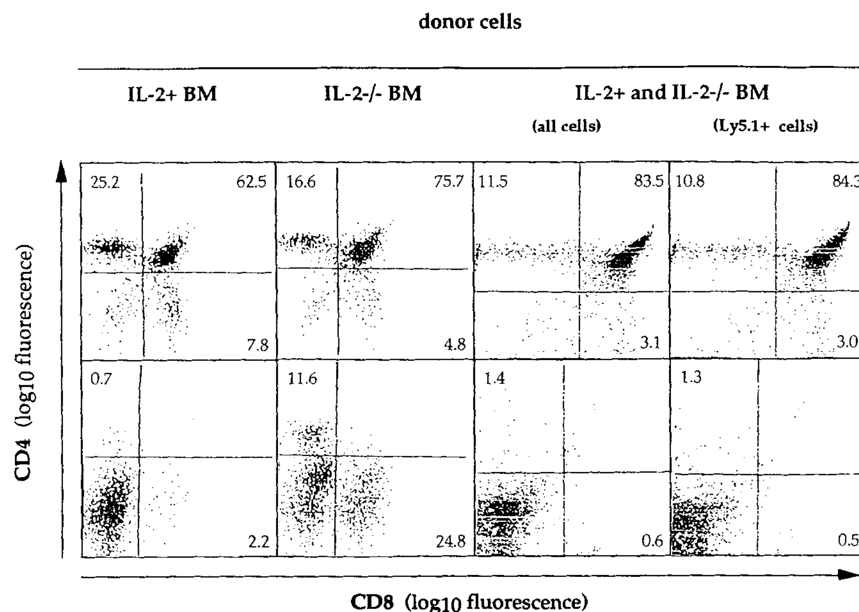

Figure 5. IL-2$^+$ cells suppress bone marrow infiltration by IL-2$^{-/-}$ CD4 T cells. Thymocytes (*top*) and bone marrow cells (*bottom*) from RAG-2$^{-/-}$ mice injected 6 wk previously with bone marrow cells from the following sources: C57BL/6, C57BL/6 nu/nu IL-2$^{-/-}$, or C57BL/6 nu/nu IL-2$^{-/-}$ (70%) and B6Ly5.2 (30%). Single chimeras were analyzed by two-color, double chimeras by three-color flow cytometry and gated on Ly5.1$^+$ lymphocytes to detect progeny of the IL-2$^{-/-}$ bone marrow.

genic CD8 T cells downregulate CD8 before undergoing apoptosis in the liver (24). Thus, IL-2 deficiency may retard the conversion of activated CD8 T cells to $CD4^-8^-$ T cells and their subsequent elimination by apoptosis, resulting in an accumulation of the intermediate $CD8^+TCR^{int}$ phenotype in the liver and a shift from $CD4^-8^-$ to $CD4^-8^+$ cells among IL-$2R\beta^+$ thymocytes.

The resemblance of the IL-2 deficiency syndrome to some features of GVHD has prompted an earlier search for a defect in negative repertoire selection in these animals (12). Neither central deletion of Vβ-defined T cell subsets by endogenous superantigens nor its acute induction by peptide administration in an MHC class I–restricted TCR-transgenic model, however, revealed any obvious effects of the IL-2 mutation. We thus favor the view that disease is initiated by an uncontrolled peripheral activation of T cells with a "normal" repertoire, rather than an increased escape of autoreactive T cells from intrathymic deletion. This uncontrolled activation is most likely driven by environmental antigens since athymic mice, which do contain functional T cells, albeit with a very restricted repertoire (13–16), do not develop disease. This conclusion is further supported by the delayed onset of inflammatory bowel disease in mice with a reduced antigenic environment (6).

As shown by cotransfer of IL-$2^{-/-}$ and IL-2^+ bone marrow into RAG-2–deficient mice, the simultaneous presence of lymphocytes with an intact IL-2 gene can greatly attenuate the development of the IL-2 deficiency syndrome. Therefore, IL-2–deficient mice either lack a specialized immunoregulatory cell population that depends on IL-2 for its development or function, or IL-2 provided in an autocrine or paracrine fashion is required for the termination of clonal expansion by apoptosis, as has been suggested by Lenardo (10). Indeed, recent results indicate that activated T cells from IL-2–deficient mice are more resistant to Fas-induced apoptosis than those from IL-2 wild-type mice (30). On the other hand, IL-2 could also be required for the induction of cytotoxic activity in CD8 T cells, which, as suggested by recent experiments by Jiang and colleagues (31), might eliminate clonally expanded CD4 T cells (which are the main population that expands in peripheral lymphoid organs of IL-2–deficient mice). Although antiviral CTL responses have been observed in IL-2–deficient mice (32), this seems to be a viable explanation since in a TCR-transgenic model, peptide immunization has revealed a stringent requirement for IL-2 in the induction of CTL effector function (12), raising the possibility that the proposed TCR-specific CTL responses required for the containment of CD4 T cell expansion are suboptimal in IL-2–deficient mice.

Whatever the mechanism by which IL-2R–mediated signals limit T cell activation in vivo, it seems unlikely from the present experiments that thymus-independent leukocyte populations are, by themselves, able to control the excessive activation of IL-2–deficient thymus-dependent T cells. Thus, despite the presence of NK cells and extrathymically derived T cells in athymic mice, IL-$2^{-/-}$ T cells initiated the characteristic symptoms of the IL-2 deficiency syndrome upon cell transfer. Further experiments will be aimed at identifying the subset of thymus-derived T cells with the most "curative" effect and to elucidate whether they contain T cell hyperactivation via paracrine IL-2 delivery or through the action of a specialized suppressor cell.

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References

1. Jenkinson, E.J., R. Kingston, and J.J.T. Owen. 1987. Importance of IL-2 receptors in intrathymic generation of cells expressing T-cell receptors. *Nature (Lond.).* 329:160–162.
2. Tentori, L., D.L. Longo, J.C. Zuniga-Pflücker, C. Wing, and A.M. Kruisbeek. 1988. Essential role of the interleukin 2–interleukin 2 receptor pathway in thymocyte maturation in vivo. *J. Exp. Med.* 168:1741–1747.
3. Plum, J., and M. De Smedt. 1988. Differentiation of thymocytes in fetal organ culture: lack of evidence for the functional role of the interleukin 2 receptor expressed by prothymocytes. *Eur. J. Immunol.* 18:795–799.
4. Schorle, H., T. Holtschke, T. Hünig, A. Schimpl, and I. Horak. 1991. Development and function of T-cells in mice rendered interleukin-2 deficient by gene targeting. *Nature (Lond.).* 352:621–624.
5. Schimpl, A., H. Schorle, T. Hünig, I. Berberich, and I. Horak. 1992. Lymphocyte subsets and immunoreactivity in IL-2 deficient mice. *In* New Advances on Cytokines. S. Romangnani, T.R. Mosmann, and A.K. Abbas, editors. Raven Press, New York. pp. 87–95.
6. Sadlack, B., H. Merz, H. Schorle, A. Schimpl, A.C. Feller, and I. Horak. 1993. Ulcerative colitis-like disease in mice

with a disrupted interleukin-2 gene. *Cell.* 75:253–261.

7. Schimpl, A., T. Hünig, A. Elbe, I. Berberich, S. Krämer, H. Merz, A.C. Feller, B. Sadlack, H. Schorle, and I. Horak. 1994. Development and function of the immune system in mice with targeted disruption of the interleukin 2 gene. *In* Transgenesis and Targeted Mutagenesis in Immunology. H. Bluethmann and P. Ohashi, editors. Academic Press, San Diego. pp. 191–201.
8. Sadlack, B., J. Löhler, H. Schorle, G. Klebb, H. Haber, E. Sickel, R.J. Noelle, and I. Horak. 1995. Generalized autoimmune disease in interleukin-2 deficient mice is triggered by an uncontrolled activation and proliferation of $CD4^+$ T cells. *Eur. J. Immunol.* In press.
9. Suzuki, H., T.M. Kündig, C. Furlonger, A. Wakehan, E. Timms, T. Matsuyama, R. Schmits, J.J.L. Simard, P. Ohashi, H. Griesser, et al. 1995. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor β. *Science (Wash. DC).* 268:1472–1476.
10. Lenardo, M.J. 1991. Interleukin-2 programs mouse α/β T-lymphocytes for apoptosis. *Nature (Lond.).* 353:858–861.
11. Rolink, A., U. Grawunder, T.H. Winler, H. Karasuyama, and F. Melchers. 1994. IL-2 receptor α chain expression defines a crucial stage in pre-B cell development. *Int. Immunol.* 6:1257–1268.
12. Krämer, S., C. Mamalaki, A. Schimpl, I. Horak, D. Kioussis, and T. Hünig. 1994. Thymic selection and peripheral activation of TCR-transgenic CD8 T-cells in IL-2 deficient mice: requirement for IL-2 is restricted to the induction of cytotoxic effector function. *Eur. J. Immunol.* 24:2317–2322.
13. Hünig, T., and M.J. Bevan. 1980. Specificity of cytotoxic T cells from athymic mice. *J. Exp. Med.* 152:688–702.
14. Hünig, T. 1983. T-cell function and specificity in athymic mice. *Immunol. Today.* 4:84–87.
15. MacDonald, H.R., and R.K. Lees. 1984. Frequency and specificity of precursors of interleukin 2-producing cells in nude mice. *J. Immunol.* 132:605–610.
16. Maleckar, J.R., and L.A. Sherman. 1987. Composition of the T cell receptor repertoire in nude mice. *J. Immunol.* 138: 3873–3876.
17. Shinkai, Y., G. Rathbun, K.P. Lam, E.M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A.M. Stall, and F.W. Alt. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell.* 68:855–867.
18. Shen, F.-W. 1981. Monoclonal antibodies to mouse lymphocyte differentiation alloantigens. *In* Monoclonal Antibodies and T Cell Hybridomas. G. Hämmerling, U. Hämmerling, and J.F. Kearney, editors. Elsevier Science Publishers B.V., Amsterdam. pp. 25–31.
19. Ohteki, T., R. Okuyama, T. Abo, K. Sugiura, A. Kusumu, T. Ohmori, H. Watanabe, and K. Kumagai. 1992. Age-dependent increase of extrathymic T-cells in the liver, and their appearance in the periphery of older mice. *J. Immunol.* 149: 1562–1570.
20. Lawetzky, A., and T. Hünig. 1988. Analysis of CD3 and T-cell antigen-receptor expression on subpopulations of T-cells from aged athymic mice. *Eur. J. Immunol.* 18:409–416.
21. MacDonald, H.R., R.K. Lees, C. Bron, B. Sordat, and G. Miescher. 1987. T cell antigen receptor expression of athymic (nu/nu) mice. Evidence for an oligoclonal β chain repertoire. *J. Exp. Med.* 166:195–209.
22. Seki, S., T. Abo, K. Suguira, T. Ohteki, T. Kobata, H. Yagita, K. Okumara, H. Rikiishi, T. Masuda, and K. Kumagai. 1991. Reciprocal T cell responses in the liver and thymus of mice injected with syngeneic tumor cells. *Cell. Immunol.* 137:46–60.
23. Crispe, N.I., and L. Huang. 1994. Neonatal, moribund and undead cells: the role of the liver in T cell development. *Semin. Immunol.* 6:39–41.
24. Huang, L., G. Soldevila, M. Leeker, R. Flavell, and I.N. Crispe. 1994. The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. *Immunity.* 1:741–749.
25. Hanke, T., R. Mitnacht, R. Boyd, and T. Hünig. 1994. Induction of interleukin-2 receptor β chain expression by self-recognition in the thymus. *J. Exp. Med.* 180:1629–1636.
26. Bix, M., M. Coles, and D. Raulet. 1993. Positive selection of $V\beta8^+$ $CD4^-CD8^-$ thymocytes by class I molecules expressed by hematopoietic cells. *J. Exp. Med.* 178:901–908.
27. Ohteki, T., S. Iwamoto, S. Izui, and H.R. MacDonald. 1995. Reduced development of $CD4^-CD8^-B220^+$ T cells but normal autoantibody production in *lpr/lpr* mice lacking MHC class I molecules. *Eur. J. Immunol.* 25:37–41.
28. Maldonado, M.A., R.A. Eisenberg, E. Roper, P.L. Cohen, and B.I. Kotzin. 1995. Greatly reduced lymphoproliferation in lpr mice lacking MHC class I. *J. Exp. Med.* 181:641–648.
29. Mixter, P.F., J.Q. Russell, F.H. Durie, and R.C. Budd. 1995. Decreased $CD4^-8^-$ $TCR^-\alpha\beta^+$ cells in *lpr/lpr* mice lacking β2-microglobulin. *J. Immunol.* 154:2063–2074.
30. Kneitz, B., T. Herrmann, S. Yonehara, and A. Schimpl. 1995. Normal clonal expansion but impaired Fas-mediated cell death and anergy induction in IL-2 deficient mice. *Eur. J. Immunol.* 25:2572–2577.
31. Jiang, H., R. Ware, A. Stall, L. Flaherty, L. Chess, and B. Pernis. 1995. Murine $CD8^+$ T cells that specifically delete autologous $CD4^+$ T cells expressing Vβ8 TCR: a role of the Qa-1 molecule. *Immunity.* 2:185–194.
32. Kündig, T.M., H. Schorle, M.F. Bachmann, H. Hengartner, R. Zinkernagel, and I. Horak. 1993. Immune responses in interleukin-2 deficient mice. *Science (Wash. DC).* 262:1059–1061.