Combining Fas Mutation with Interleukin-2 Deficiency Prevents Colitis and Lupus

IMPLICATING INTERLEUKIN-2 FOR AUTO-REACTIVE T CELL EXPANSION AND Fas LIGAND FOR COLON EPITHELIAL CELL DEATH*

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Both the lpr gene defect and interleukin 2-targeted mutation (IL-2 KO) in mice are lethal. Interestingly, mice bearing both mutations live significantly longer than mice with either of the single mutant genes, approximating the life span of normal controls. They do not display the major disease phenotypes of lpr and IL-2 KO mice. Systemic autoimmune response, the accumulation of the abnormal CD4⁺ CD8⁻ B220⁺ double-negative T cells, kidney disease pathology, anemia, colony damage, and lethality are prevented. Our data indicate that IL-2 is mandatory for the expansion of auto-reactive T cells in lpr mice and that CD95 (Fas) is the critical target for the development of anemia and ulcerative colitis in IL-2 KO mice in which CD178 (FasL) on intraepithelial T cells is the major effector responsible for colonic damage and lethality.

T cell tolerance to self-antigens is acquired during thymic education which eliminates high affinity auto-reactive clones and allows the maturation and emigration of selected clones to the periphery. There, they respond to foreign antigens with high affinity and self-antigens with low affinity below the activation threshold (1, 2). In addition to the “activation threshold” hypothesis, two major mechanisms have been put forth to explain how auto-reactive T cells are contained in the periphery. Auto-reactive T cells can be eliminated by activation-induced cell death (AICD) mediated by CD95 (Fas) or CD178 (FasL) interaction during T cell hyper-activation (3–5). Auto-reactive T cell development is also contained by regulatory T cells (Treg). One function of Treg is to inhibit the expansion of auto-reactive T cells (6, 7).

Fatal disease has been observed either in mice bearing defects in the Fas/FasL-mediated apoptotic pathway or in mice deficient in Treg cells (8–10). Mutations in the fas or fasl gene cause a severe autoimmune lymphoproliferative syndrome characterized by dramatic lymphadenopathy and a lupus-like autoimmune disease with renal failure late in life. This autoimmune disease is T cell-dependent. However, the thymic selection process is normal in lpr mice (11), and it is generally accepted that the disease in lpr mice is caused by the lack of Fas/FasL-mediated regulation in the periphery. Whether Treg cells play a role and how the low affinity auto-reactive T cells are expanded are unclear.

Mice whose Il2 gene has been eliminated by a targeted mutation (IL-2 KO or Il2−/−) develop mild lymphadenopathy, severe anemia, and ulcerative colitis (10). The development of ulcerative colitis in IL-2 KO mice depends on T cells and the presence of intestinal flora (12). Whether IL-2 KO mice have a defect in negative selection has not been resolved (13, 14). There was no obvious difference in the number of thymocytes between young IL-2 KO and B6 mice (15). However, IL-2 KO mice are deficient in CD4⁺CD25⁺ Treg cells. The expansion of organ-specific T cells due to the absence of Treg cells has been implicated as a major cause for ulcerative colitis (16, 17).

The molecular mechanism by which the intestinal flora-dependent T cells cause colon organ failure in IL-2 KO mice has not been established (16, 18). IL-2 KO mice have been shown to have a defect in FasL expression, an interpretation that would preclude a role of FasL in the pathology, end organ failure, and lethality observed in IL-2 KO mice (19). Unlike lpr mice, IL-2 KO mice do not produce anti-dsDNA antibodies and rheumatoid factors and do not exhibit immune complex-mediated glomerulonephritis (10). These differences suggest that the disease manifestations of lpr and IL-2 KO mice are the results of different mechanisms.

We hypothesize that IL-2 has a dual role in the development of auto-reactive T cells. As a powerful lymphokine, IL-2 is both a positive regulator required for the expansion of the low affinity auto-reactive T cells and a negative regulator necessary for the development of Treg cells and the induction of FasL for AICD in highly activated T cells. On the other hand, IL-2 is not required for the expansion of T cells that respond to intestinal flora stimulation, and IL-2 is not necessary for the induction of FasL on these activated T cells. To test our hypothesis, we generated mutant mice defective in both fas and Il2 genes, i.e. Il2−/−/lpr+/− mice (we use lpr+/− to refer to the lpr mutant genotype). Unlike Il2−/− mice, these mice do not die at a young age. Furthermore, they do not develop the lethal autoimmune lymphoproliferative syndrome characteristic of lpr mice. The data reported herein support our working hypothesis and im-

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¶ The abbreviations used are: AICD, activation-induced T cell death; FasL, Fas ligand; TdT, terminal deoxynucleotidyltransferase; TUNEL, TdT-mediated dUTP nick end labeling; dsDNA, double-stranded DNA; PBS, phosphate-buffered saline; Ab, antibody; mAb, monoclonal Ab; FITC, fluorescein isothiocyanate; RBC, red blood cell(s); PE, phycoerythrin; TUNEL, TdT-mediated dUTP nick end labeling; dsDNA, double-stranded DNA.
II2 and fasl Gene Interaction in Vivo

EXPERIMENTAL PROCEDURES

Breeding and Genotyping—C57BL/6Il2+/+ mice and B6 lpr (lpr+/+) mice were obtained from The Jackson Laboratories, Bar Harbor, ME. Il2+/+ mice (homozygous mice are sterile) were mated with lpr+/+ mice to obtain Il2+/− lpr+− F1 offspring. We intercrossed them to obtain Il2−/− lpr+− and Il2+/− lpr+− mice. We then bred the Il2−/− lpr+− × Il2+/− lpr+− pair to increase the frequency of Il2−/− lpr+− offspring. TAIL DNA was used for genotyping by PCR. The primers used for fas PCR analysis are as follows: fasF1 (Fas forward primer 1, 5′-GTAATATTGTGCGGTGTG-3′), fasR1 (Fas reverse primer 1, 5′-CAAATCTAG-CGATTACAGCT-3′). The primers used for PCR analysis of the Il2 gene are as follows: IL-2P1 (IL-2 primer 1, 5′-TCGATCGCAGCTG-3′), IL-2P2 (IL-2 primer 2, 5′-CTAGCCACAGATT-GAAAGATCT-3′), IL-2P3 (IL-2 primer 3, 5′-GTAGTGGAAATCTC-GCATCAGCC-3′). Amplification was performed under standard conditions for 55 cycles with 94 °C for 1 min to melt the DNA, 65 °C for 1 min to anneal the DNA, and 72 °C for 1 min to extend the DNA. PCR products were as follows: 218 bp for fas mutant lpr (fasF1 and fasR2), 182 bp for the normal fas (fasF1 and fasR1), about 500 bp for the Il2−/− lpr+− (IL-2P1 and IL-2P3), and 324 bp for the normal Il2 (IL-2P2 and IL-2P3). DNA samples from B6, Il−2 KO, and B6 lpr mice were used as controls.

General Examination of Mice—Mice were examined twice weekly and weighed once weekly. Blood samples were collected weekly once the mice were 4 weeks old. Ten μl of blood were collected and immediately diluted in 90 μl of cold PBS, pH 7.2. Aliquots were used to determine hemoglobin levels and white blood cell counts. Cell-free samples were collected by centrifugation and used for the determination of anti-dsDNA Ab and Ig levels. In some experiments mice were sacrificed, and various tissues were collected for analyses as described in specific experiments (see Figs. 2–7) in the text.

AntidNA Ab Assay—We coated plates with pcDNA3 plasmid DNA and conducted an enzyme-linked immunosorbent assay to determine the anti-dsDNA Ab levels of individual serum samples. Serum of a 6-month-old female mouse was used as a positive control. Bound anti-dsDNA Abs were measured with horseradish peroxidase-conjugated rabbit anti-mouse IgG using a 2,2′-azinobis(3-ethylbenz-thiazolone-6-sulfonic acid) substrate (Sigma). The results were expressed as absorbance at 405 nm.

Determination of Hemoglobin Level—Blood samples were diluted with 150-fold, 10 μl of dilution buffer (360 mm KF, 360 mm KCl, 1.5 mm GTP, 5 mm NaH2PO4, 0.1% Nonidet P-40). Releasable hemoglobin was converted to cyanohemoglobin, and the relative hemoglobin concentrations were determined photometrically at 546 nm (20).

Immunohistochemical and Immunofluorescence Staining—Mice of various groups were euthanized. Immune complex deposition on fixed kidney sections was determined using alkaline phosphatase-conjugated rabbit anti-mouse IgG antibodies. Frozen sections of kidneys were used for immunofluorescence staining. Immune complex deposits were determined using FITC-conjugated rabbit anti-mouse IgM (BD Biosciences). Sections of paraffin-embedded colon were stained with hematoxylin and eosin. FasL staining was carried out according to the manufacturer’s protocol using rabbit anti-mouse FasL Ab (N20, Santa Cruz Bio-technology, Santa Cruz, CA) followed by biotinylated goat-anti-rabbit Ig and horseradish peroxidase-avidin. The horseradish peroxidase-stained slides were counterstained with hematoxylin.

Apoptosis was determined using a terminal deoxynucleotidyltransferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay kit (ApopTag Plus Peroxidase in situ apoptosis detection kit, Intergen, Purchase, New York). We followed the manufacturer’s protocol and included the positive control provided by the manufacturer. Colon sections were freed from paraffin and dehydrated in graded alcohol.

Endogenous peroxidase activity was quenched in 3% hydrogen peroxide in PBS for 5 min. After rinsing, slides were incubated at 37 °C for 1 h with TdT in reaction buffer in a humidity chamber. Background staining was removed by incubation with buffer in the absence of TdT. Enzymatic reactions were stopped with a stop/wash buffer, and the slides were washed with PBS. Sections were then incubated with an anti-digoxigenin-peroxidase conjugate for 30 min. After washing with PBS, sections were incubated with a 0.05% 3,3-diaminobenzidine tetrahydrochloride to develop the TUNEL cells. Slides were counterstained with hematoxylin.

RESULTS

Mutual Protection from Lethality—We have generated 39 Il2−/− lpr+/− mice, 75 Il2−/− lpr−/− mice, and 120 B6 mice by breeding B6Il2+/+ mice and B6 lpr mice (all mice are in the B6 background). Fig. 1 shows a representative genotypic result from various progeny of (Il2−/− lpr+/− × Il2+/− lpr+/−)F1 and (Il2−/− lpr+− × Il2+/− lpr+/−)F1 breeding. The life spans of these mice were determined and compiled (Fig. 2a). Less than 50% of the Il2−/− lpr−/− mice were alive at 12 weeks of age, and none of them survived beyond 8 months. In sharp contrast, none of the Il2−/− lpr+− mice died before 8 months. The data demonstrate that Il2−/− lpr−/− mice, having the IL-2P2 and IL-2P3 gene, are critically dependent on host Fas expression. On the other hand, the Il2−/− gene also prolongs the life span of lpr+− mice. Those lpr+− mice bearing either an Il2−/− or Il2−/− genotype began to die about 6 months after birth, whereas no death was observed for Il2−/− lpr+− mice. At 1 year after birth, ~35% of lpr+− mice (mostly female) died, whereas more than 90% of the Il2−/− lpr+− mice remained alive.

Restoration of Body Weight by lpr+− Gene—A major clinical manifestation of Il2−/− mice is the lack of weight gain during growth (Fig. 2b). There were no major differences in weight until 5–6 weeks postnatal. Although a sharp increase in body weight was observed in B6 mice, there was a decline of body weight in the Il2−/− mice. By contrast, there was a significant

![Fig. 1. Genotyping of (Il2−/− lpr+/− × Il2+/− lpr+/−)F1 and (Il2−/− lpr+− × Il2+/− lpr+/−)F1 mice. Tail DNA samples of individual mice were analyzed by PCR as described under “Experimental Procedures.” Samples from Il2−/−, B6, and lpr+/− mice in lane 1, lane 2, and lane 3, respectively, were used as references. Individual samples from progeny of breeding were loaded (from lane 4 to lane 18). The fast lane band in each panel represents the PCR product of wild type gene. This is true for both Il2 and fas genes. In this experiment, 4, 15, and 17 were typed as Il2− lpr−, Il2− lpr+−, and Il2− lpr+−, respectively.](attachment:figure1.png)
gain in body weight by the IL2−/−lpr+/+ mice. The body weight gain was noticeably lower than that observed in B6 and lpr+/+ mice, suggesting that lpr+/+-dependent protection, although considerable, remained incomplete.

Prevention of Anemia in IL2−/− Mice by the lpr+/+ Gene—Anemia and ulcerative colitis have been implicated as causes for the lack of weight gain and the high mortality of IL-2 KO mice (10). If so, an association between these phenotypes should be observed. This is indeed the case. Severe anemia developed early in life with great variability in IL2−/− mice. In contrast, severe anemia failed to occur in the IL2−/−lpr+/+ mice, although mild anemia was occasionally detected in a few individuals (Fig. 2c).

The reasons for the severe anemia observed in IL2−/− mice have not been resolved. Certain IL2−/− strains, especially Balb/c.II2−/− mice, develop potent immune responses to RBC (21). The autoimmune anti-RBC response erodes red blood cells and RBC differentiation defects are potential causes for the severe anemic condition. We tested RBC for coated antibodies and for the ability to coat RBC but could not detect an anti-RBC response in IL2−/−lpr+/+ or IL2−/− mice. We could, however, detect antibodies on the RBC of old NZB mice that were used as a positive control (Fig. 2d).

Protection from Colon Damage by lpr+/+ Gene—If lpr+/+ gene prolongs the life span of IL2−/− mice, then one must conclude that FasL-mediated cytotoxicity functions in vivo in the absence of IL-2 and is responsible for the susceptibility of IL2−/− mice to lethal ulcerative colitis. Both IL2−/− and the IL2−/−lpr+/+ mice developed colitis according to histological criteria, which include infiltration of leukocytes in the lamina propria (Fig. 3). The 4-month old IL2−/− mouse showed a severe destruction of colon architecture with atrophic epithelial cells, dilated mucous glands, and a marked reduction of mucin-secreting cells. By contrast, in the colon of the 4-month old IL2−/−lpr+/+ mouse, the glandular structure was preserved with apparently normal goblet cells and epithelial linings, albeit with some reduction in mucin-secreting cells. The mild inflammation was associated with mucosal hypertrophy. A clear dissociation of inflammation from end organ failure is suggested from studies of a 12-month-old IL2−/−lpr+/+ mouse. Although the colon was more heavily inflamed and the mucosal layer more thickened, the general architecture was maintained, and the intestinal epithelial cells remained intact. These results suggest that the Fas-mediated apoptotic pathway plays a critical role in the death of colon epithelial cells, leading to the destruction of colon structure, end organ failure, and lethality of IL2−/− mice.

FasL Expression in the Inflamed Colon of IL2−/− and IL2−/−lpr+/+ Mice—To provide direct evidence that the inflamed colon expresses FasL, colon sections were stained for FasL (Fig. 4a). Infiltrating leukocytes of IL2−/−lpr+/+ mice were strongly stained with affinity-purified rabbit anti-FasL Ab. Weak staining of epithelial cells was also observed. The staining is specific because sections from B6 and lpr+/+ controls displayed essentially undetectable staining. Moreover, normal rabbit IgG failed to stain colon sections of IL2−/−lpr+/+ mice. Similar results were observed with IL2−/− mouse colon samples, although the extent of staining was weaker than that observed in IL2−/−lpr+/+ mice. As reported in other colitis models (22, 23), the FasL-expressing leukocytes contain CD4+ T cells, as determined by double staining using fluorescent Abs and confocal microscopy (Fig. 4b). The results provide evidence that IL2−/− mice are capable of producing FasL at least in the inflamed areas of colon.

Protection of Colon Epithelial Cell Apoptosis by lpr+/+ Gene—We used a TUNEL assay to determine the apoptosis of colon epithelial cells in IL2−/− mice and the protection of colon

Fig. 2. Determination of the mortality, body weight, hemoglobin level, and anti-RBC Ab response of various mouse strains. a, wild type (Wt), IL2−/−, lpr−/−, and IL2−/−lpr−/− mice were analyzed for survival rates (Wt, n = 10; IL2−/−lpr−/−, n = 20; IL2−/−, n = 75; lpr−/− n = 15). b, body weight measurements were recorded monthly (n ≥ 10, except for IL2−/− at the age of 6 months, where the mouse number is 4). c, blood samples were collected from tail veins. The relative hemoglobin concentrations were determined as described under “Experimental Procedures” (n ≥ 10, except for IL2−/− at the age of 6 months, where the mouse number is 4). Values are mean ± S.D. d, anti-RBC response was determined by staining freshly isolated RBC (2 × 10^6 cells) of various mouse strains with 0.5 μg of FITC-conjugated rabbit anti-mouse IgG and 0.5 μg of FITC-conjugated rabbit anti-IgM antibodies.
epithelial cell apoptosis in $Il2^{-/-}$ mice (Fig. 5). TUNEL$^+$ epithelial cells were evident and frequent in the $Il2^{-/-}$ samples. By contrast, TUNEL$^-$ epithelial cells were not observed in the $Il2^{-/-} lpr^{+/-}$ colon, indicating that Fas-mediated apoptosis is responsible for the colon epithelial cell death. TUNEL$^-$ leukocytes were observed not only in $Il2^{-/-}$ mice but also in $Il2^{-/-} lpr^{+/-}$ mice. The latter observation demonstrates the Fas-independent apoptosis of infiltrating leukocytes. The staining is DNA nick-specific because TUNEL$^+$ cells could not be detected in control mice or in $Il2^{-/-}$ mice in the absence of TdT. A role of FasL in intestinal epithelial cell apoptosis has been implicated in intestinal graft versus host disease (24).
The data provide strong evidence that FasL expressed on the infiltrating leukocytes is the key effector molecule responsible for colon damage, end organ failure, and lethality in IL2−/− mice. Significantly, the protection occurs concurrently with severe inflammation, emphasizing that Fas-mediated apoptosis is the critical component for lethality and a good target candidate for therapy.

Protection from lpr+/+ -imposed Lymphadenopathy by IL2−/− Gene—Interestingly, IL2−/− also protected lpr+/+ -imposed lethality and this coincided with an inhibition of the autoimmune syndrome associated with lpr+/+ mice. There was a remarkable difference in the size of lymph nodes between old lpr+/+ and old IL2−/− lpr+/+ mice. Without exception, IL2−/− lpr+/+ mice failed to develop lymphadenopathy. Fluorescent Ab staining of the lymph node leukocytes revealed that the former, but not the latter, is dominated by the presence of the abnormal DN T cells

Inhibition of Anti-dsDNA Ab Response by IL2−/− Gene—The T cell-dependent anti-dsDNA Ab is the prominent auto-Ab species in lpr mice of various strains. IL2+/+ mice are also aged and the age progressed (Fig. 6b). Anti-dsDNA Ab levels in the sera of IL2−/− mice were comparable with those of normal B6 mice. The same low levels of anti-dsDNA Ab were observed in the sera of the IL2−/− lpr+/+ mice, indicating that IL-2 is required for the anti-dsDNA response in lpr+/+ mice. Although B cell deletion occurs as IL2−/− mice age (26), this mechanism seems unlikely because anti-dsDNA was absent both in young and old IL2−/− lpr+/+ mice. Our data are consistent with the hypothesis that auto-reactive T cells cannot expand in the absence of IL-2 to provide help for anti-dsDNA Ab+ B cells.

Inhibition of Glomerulonephritis by IL-2 −/− Gene—A critical factor for end organ failure and the lethality in lpr mice is the development of immune complex-induced glomerulonephritis. Therefore, we determined whether glomerulonephritis was inhibited in the IL2−/− lpr+/+ mice. Only kidneys from old lpr+/+ mice were positively stained for immune complex deposits on glomeruli using either enzyme-based staining with alkaline phosphatase-conjugated anti-IgG Ab or immunofluorescence staining with FITC-anti-IgM Ab. Staining with nonspecific Ab was negative (data not shown). Moreover, no significant staining was observed in the kidneys from B6, IL2−/−, and IL2−/− lpr+/+ mice (Fig. 6c).

Role of Treg Cells—In addition to AICD, peripheral tolerance is maintained by the presence of Treg cells (6, 7). Therefore, the expression of two major classes of Treg cells in these mice, CD4+CD25+ Treg cells and IL-10-producing T cells was determined. An increase in IL-10-producing CD4+ T cells was observed in IL2−/−, lpr+/+, and IL2−/− lpr+/+ mice relative to B6 control mice (Fig. 7a). Moreover, a significant reduction in CD4+CD25+ T cells was observed in both IL2−/− and IL2−/− lpr+/+ mice (Fig. 6b). This reduction correlates with the absence of IL-2, which is required for the development of the CD4+CD25+ Treg cells (Fig. 7a). The data indicate that changes in the expression of Treg cells are not responsible for the apparently healthy phenotype of the IL2−/− lpr+/+ mice. The data are also consistent with the interpretation that Treg cells act on the induction phase of inflammation by regulating the expansion of inflammatory T cells, whereas FasL on inflammatory T cells is the main effector responsible for colon damage.

DISCUSSION

Our data have significance in understanding how gene interaction regulates disease development as well as target organ failure. By using specific mutant genes, we conclude that the phenotypes observed are consequences of specific gene interactions. These interactions can be interpreted based on our current knowledge of the mechanisms and functions of these genes. Both the IL-2/IL-2 receptor and the Fas/FasL systems have been shown to have multiple functions operating via multiple mechanisms. Some of these mechanisms are based on in vitro studies and their in vivo relevance may not have been tested. In this respect our in vivo study is particularly significant because it provides evidence as to what particular mechanisms are critical to a healthy immune system under in vivo conditions.

As a result of lacking a cognate Fas/FasL interaction, lpr mice develop a lupus-like autoimmune disease. Many breeding experiments have been used to understand how the develop-
ment of the autoimmune disease in lpr mice is regulated, and useful information has been generated. These studies have shown that various immune regulatory mechanisms, particularly those involved in T cells, are critical. In most cases, a single gene defect in the immune system usually lessens the severity of the disease but rarely prevents the disease from developing (27–30). For example, the breeding of Jκ KO mice with lpr mice has revealed that the disease and pathology of lpr mice are only partially dependent on B cells and antibodies and breeding lpr mice with mice bearing targeted mutations in various cytokine genes often resulted in a partial response with respect to disease development (31).

Our study is unique in that we have identified a single lymphokine whose expression is required for the development of the autoimmune disease in lpr mice, and in its (IL-2) absence, the major phenotypes of the disease are alleviated.

As a result of thymic education, high affinity auto-reactive clones are deleted. Positively selected clones with high affinities for foreign antigens but low affinities for self-antigens are released to the periphery. Expansion of these low affinity “auto-reactive T cells” requires a high activation threshold. Changes in the activation threshold required for the expansion of low affinity auto-reactive T cells in lupus have been implicated in several studies (32, 33). Our study demonstrates that IL-2 is critically required for the expansion of low affinity auto-reactive T cells in lpr mice because only IL-2, but not other lymphokines such as IL-4 and IL-15 that are not affected by the targeted mutation, can overcome the threshold of activation. This interpretation is strongly supported by the demonstration that the development of the abnormal DN T cells in lpr mice is strongly inhibited in the absence of IL-2. It has been shown that the precursor cells for the abnormal DN T cells belong to a group of mis-selected T cells released from the thymus. Normally, these cells are activated in the periphery by “self-antigens” and then effectively deleted by Fas/FasL-mediated apoptosis (25). In this regard, the expansion of these abnormal DN T cells is similar to that of auto-reactive T cells, and our study shows that both are critically dependent on IL-2. In

Fig. 5. Colon epithelial cells in II2−− but not II2−−lpr−− mice are apoptotic. Apoptosis of colon epithelial cells of mice with various genotypes was determined by a TUNEL assay (top and middle panels) as described under “Experimental Procedures.” Control staining (bottom panel) was conducted identically except that no TdT was added. The panels are from wild type (top left), lpr−− (top right), II2−− (middle and bottom left), and II2−−lpr−− (middle and bottom right) mice. TUNEL− epithelial cells (red arrows), TUNEL− leukocytes (brown arrows) are identified.
contrast to auto-reactive T cells and the abnormal DN T cells, the high affinity T cells specific for intestinal flora can be easily activated in the absence of IL-2 (12).

It is generally believed that an AICD defect is the major factor contributing to the development and expansion of auto-reactive T cells. In this regard, it is highly significant that in the presence of a Fas defect and absence of AICD, mice lacking IL-2 failed to develop a lupus-like autoimmune disease. Our study demonstrates that a critical step upstream of Fas/FasL-mediated AICD is the expansion of auto-reactive T cells and that this step requires IL-2. Previous studies implicate the inability to express FasL as a major factor for the "auto-immune" disease associated with IL-2 KO mice (34). Our study argues against this interpretation because the critical function of IL-2 is for the expansion of activated T cells, and this step is upstream of the IL-2-dependent induction of FasL expression.

Our data further suggest that IL-2 is not essential for the induction of FasL in intraepithelial T cells that infiltrate the colon in IL-2 KO mice. In vitro, IL-2 has been shown to be a major factor for inducing a strong FasL expression. However, T cell activation in vivo can be very different. In the inflamed areas in which T cells are chronically and repeatedly subjected to stimulation, the dependence on IL-2 for optimal FasL production can be overcome. We have previously shown that T cells from IL-2 KO and IL-2Rβ KO mice could be induced to undergo AICD when excess IL-4, IL-7, or IL-15 was present (35). The ability of IL-2 KO mice to produce FasL, resulting in the subsequent pathology and lethality, rules out the hypothesis that a defect in FasL production is the critical reason for the disease associated with IL-2 KO mice.

The reduction of anemia by a Fas mutation is intriguing although it is consistent with the interpretation that IL-2 KO mice are capable of producing FasL. A previous study suggests that B cells are required for the development of anemia but not ulcerative colitis in IL-2 KO mice that have a mixed B6/SV129 genetic background (36). Our study has identified a Fas-mediated, Ab-independent mechanism that is responsible for anemia in B6.IL-2 KO mice. Because anemia develops in young IL-2 KO mice in the absence of ulcerative colitis (10) and B6.IL-2 KO mice lack an anti-RBC response, a Fas-based erythropoiesis defect in this strain is suggested. Additional studies are needed to understand how IL-2 negatively regulates this Fas-based biological process. A Fas/FasL-based mechanism has been implicated in the negative regulation of erythropoiesis from studies using anti-Fas Ab treatment of erythroblasts.
Recent studies show that Treg cells are important regulators of autoimmune disease. In experimental colitis models, Treg cells could prevent the development of disease even after the disease has been established (39). In our study, Il2/lpr mice lack Treg cells and develop strong inflammation in their colons. Yet their colon epithelial cells remain intact, and their life span is dramatically prolonged. Our study helps to identify the critical pathological consequence of ulcerative colitis by dissociating the inflammation phase from the effector phase that induces the apoptosis of colon epithelial cells. Our study further shows that FasL-mediated apoptosis of colon epithelial cells is responsible for the lethality associated with that disease.

Finally, the prevention of lupus development and the inhibition of ulcerative colitis, each by a single gene, have therapeutic implications, particularly in view of the fact that treated mice have a life span that is close to that of normal mice and that they lived without the severe pathology observed in mice with the disease. Our study, therefore, raises the possibility that IL-2 and Fas could be considered critical targets of immunotherapy for lupus and ulcerative colitis, respectively.
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