DEFICIENT INTERLEUKIN 2 ACTIVITY IN MRL/Mp AND C57BL/6J MICE BEARING THE *lpr* GENE*

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MRL/Mp-*lpr*/*lpr* (MRL-*lpr*) mice spontaneously develop autoimmune disease characterized by antibodies to nucleic acids, immune complex glomerulonephritis, and death from renal failure (1, 2). They have certain unique features that make them particularly interesting murine models for systemic lupus erythematosus (SLE).[1] First, they develop massive generalized lymphadenopathy associated with proliferation of Lyt-1^+23^- T cells (3). Second, they exhibit excessive T helper function (4, 5). Finally, a single autosomal recessive gene, *lpr*, is responsible for both the lymphoproliferation and severe autoimmunity of MRL-*lpr* mice (1).

Several immunoregulatory abnormalities that might contribute to autoimmunity in MRL-*lpr* mice have been identified (1–11). These include defective feedback suppression in vitro (6), as well as T helper lymphoproliferation in vivo (3, 4). Studies in two laboratories (10, 11) have focused attention on the syngeneic mixed lymphocyte reaction (SMLR), which is decreased before the onset of clinical illness. Failure to develop an SMLR is an early and consistent feature in murine models of SLE and may represent a common predisposition to autoimmunity (10, 11).

To clarify the molecular basis for abnormal immunoregulation in MRL-*lpr* mice, we examined their ability to produce and respond to interleukin 2 (IL-2). IL-2, previously called T cell growth factor, is a lymphokine produced by mitogen- or antigen-stimulated T cells (12). It is also produced during the course of a normal SMLR (13). IL-2 has significant T cell regulatory function (14–18). It stimulates thymocyte proliferation (14, 15), provides helper activity for antibody production (14–16), facilitates the induction of cytotoxic T cells (14–18), and promotes the proliferation of helper and cytotoxic T lymphocytes in long-term culture (15, 19, 20). We found that MRL-*lpr* mice have a progressive defect in the ability to produce IL-2. Furthermore, spleen cells from MRL-*lpr* mice fail to respond normally to IL-2. IL-2 deficiency precedes the onset of clinical illness and becomes increasingly severe with age.

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; IL-1, IL-2, interleukin 1 and 2; PMA, phorbol myristic acetate; SLE, systemic lupus erythematosus; SMLR, syngeneic mixed lymphocyte reaction.

We have transferred the *lpr* gene from the MRL-*lpr* mice onto the C57BL/6J background by eight cycles of cross-intercross mating. We found that, like MRL-*lpr* mice, C57BL/6J-*lpr/lpr* (B6-*lpr*) mice develop an autoimmune disease characterized by lymphadenopathy, antinuclear antibodies, and early mortality. Moreover, they lose the ability to produce and respond to IL-2. In contrast, control C57BL/6J-+/+ (B6-+/+) mice lacking the *lpr* gene have normal IL-2 production and response. These findings suggest that homozygosity at the *lpr* locus results in both autoimmunity and deficiency of IL-2 activity. Our results further suggest that IL-2 deficiency may contribute to the immunoregulatory abnormalities that underlie murine lupus.

Materials and Methods

Mice. MRL-*lpr*, MRL/Mp-+/+ (MRL-+/+), and B6-*lpr* breeding pairs were obtained from The Jackson Laboratory, Bar Harbor, Maine, and were maintained at the University of California, San Francisco Vivarium, and the Veterans Administration Medical Center, San Francisco. MRL-*lpr* and MRL-+/+ are congenic strains with 99.6% genetic homology. MRL-+/+ mice lack the autosomal recessive gene responsible for accelerated autoimmunity and lymphoproliferation in MRL-*lpr* mice (1).

Culture Medium. RPMI 1640 medium (Cell Culture Facility, University of California, San Francisco, Calif.) was supplemented with 10% heat-inactivated fetal calf serum (Sterile Systems, Inc., Logan, Utah), 1 mM glutamine, 0.1 mM nonessential amino acids, 1 μM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin, and 5×10^{-5} M 2-mercaptoethanol. This medium was used in the production and testing of IL-2.

IL-2 Production. Spleen cells were suspended at a density of 1×10^7/ml in culture medium supplemented with 10 μg/ml concanavalin A (Con A; Miles Laboratories, Inc., Elkhart, Ind.). The cells were incubated in Falcon 3008 multiwell tissue culture plates (Falcon Labware, Oxnard, Calif.) for 36 h at 37°C in a humidified atmosphere containing 5% CO_2. These conditions are optimal for production of IL-2 by murine spleen cells (21). Cells were removed from the culture supernatants by centrifugation at 1,500 *g* for 10 min. Cell-free supernatants were subsequently passed through 0.22-μm filters (Millipore Corp., Bedford, Mass.) and stored at −20°C.

IL-2 Assay. IL-2 activity was measured using a slight modification of the technique of Smith et al. (22). Normal spleen cells were cultured for 48 h with Con A (2 μg/ml) and subsequently washed three times in 10 mg/ml α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, Mo.). The cells were then incubated in 96-well microtiter plates (Linbro Scientific, Inc., Hamden, Conn.) with serial dilutions of the culture supernatant being tested for IL-2 activity. Each well contained 5×10^3 cells in a volume of 200 μl. Cultures were supplemented with 10 mg/ml α-methyl-D-mannoside to block the possible mitogenic effects of Con A in the test samples. They were maintained at 37°C in a humidified atmosphere of 5% CO_2. 1 μCi of [^{3}H]thymidine added during the last 24 h of a 72-h incubation yielded optimal thymidine incorporation. The cultures were then harvested onto glass fiber filter strips, and retained radioactivity was measured in a Packard liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.).

In preliminary experiments, it was determined that spleen cells from MRL-+/+, B6-+/+, and DBA/2 mice proliferated comparably in response to IL-2. Therefore, we used MRL-+/+ spleen cells in our assay of IL-2 activity.

IL-2 Response. The assay described above was also used to determine the ability of MRL-*lpr* spleen cells to respond to IL-2. This was accomplished by incubating Con A-activated MRL-*lpr* spleen cells with supernatants known to contain IL-2. The preparation of partially purified human IL-2 (Associated Biomedic Systems, Inc., Buffalo, N. Y.) used in these experiments has been described previously (23).

IL-2 Absorption. Fresh spleen cells from MRL-*lpr* and MRL-+/+ mice were stimulated with Con A (2 μg/ml) for 48 h, washed three times in α-methyl-D-mannoside (10 mg/ml), and incubated with a standard IL-2 preparation (5×10^7 cells/ml) for 2 h at 37°C. After incubation,

the cells were removed by centrifugation at 1,500 *g* for 10 min, and the remaining supernatants were tested for IL-2 activity.

Interleukin 1 (IL-1) Production. The IL-1 used in these experiments was generously provided by Dr. Robert Mishell of the University of California at Berkeley. Briefly, macrophage culture supernatants were derived from PU5-1.8 cells as described previously (24) and tested for IL-1 activity in the thymocyte proliferation assay of Farrar et al. (25). Culture supernatants retained 50% maximum activity in the IL-1 assay at a 1:32 dilution.

Results

IL-2 Production by MRL-lpr Spleen Cells. MRL-*lpr* mice had a marked defect in IL-2 production that was already prominent early in life and increased with age (Fig. 1). Culture supernatants derived from 2-mo-old MRL-*lpr* mice had relatively little IL-2 activity even at the highest concentration tested (1:2), and had no activity beyond a 1:4 dilution. In contrast, culture supernatants derived from age-matched MRL-+/+ mice retained activity through a 1:64 dilution (Fig. 1 a). By 5 mo of age, the defect in IL-2 production was virtually absolute (Fig. 1 b). The reduction in IL-2 activity was independent of the duration of culture or the dose of Con A used (Fig. 2).

Effect of MRL-lpr Spleen Cells on IL-2 Production by MRL-+/+ Spleen Cells. To determine whether suppressor cells were responsible for IL-2 deficiency in MRL-*lpr* mice, we co-cultured equal concentrations (5×10^6 cells/ml) of lymphocytes from 5-mo-old MRL-+/+ and MRL-*lpr* mice. Control cultures consisted of either MRL-+/+ or MRL-*lpr* cells alone. In four separate experiments, we found no evidence of cellular suppression of IL-2 production. A representative experiment is shown in Fig. 3. These results also imply that MRL-*lpr* spleen cells do not produce a soluble inhibitor of IL-2 activity. If such an inhibitor were produced, it would have inhibited IL-2 activity in the mixed cell cultures as well as in the cultures containing MRL-*lpr* cells only.

Effect of Macrophage-replacing Factors on IL-2 Production. IL-2 production is macrophage dependent (26). Farrar et al. have shown that the requirement for macrophages

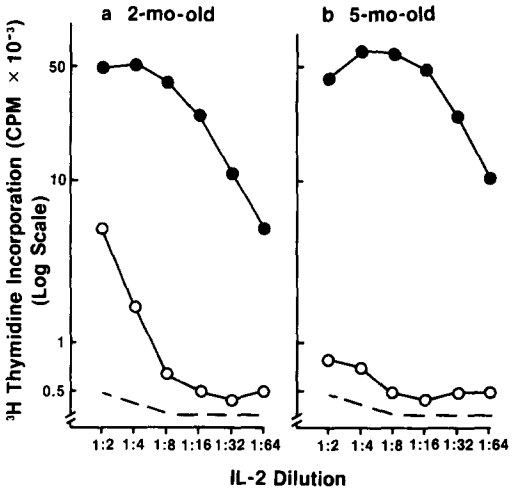

FIG. 1. IL-2 activity in culture supernatants derived from (a) 2-mo-old, and (b) 5-mo-old MRL-*lpr* (○) and MRL-+/+ (●) spleen cells. Each point represents the mean value of six individual mice. The dotted line shows the stimulatory effect of Con A (10 μg/ml) alone in the IL-2 assay.

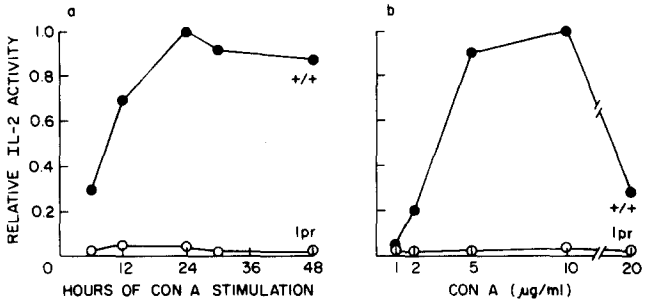

FIG. 2. IL-2 production by MRL-*lpr* (●) and MRL-+/+ (○) spleen cells as a function of (a) duration of Con A stimulation, and (b) concentration of Con A. Relative IL-2 activity was determined by probit analysis as described by Gillis et al. (12).

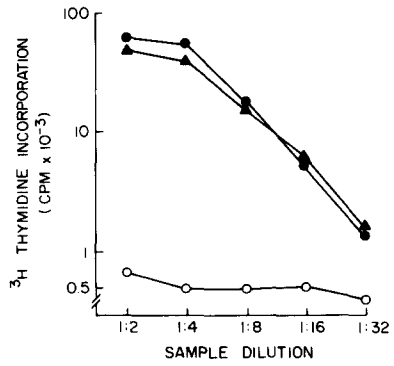

FIG. 3. Effect of MRL-*lpr* spleen cells on IL-2 production by MRL-+/+ spleen cells. IL-2 activity was measured in supernatants derived from cocultures (▲) of MRL-+/+ spleen cells (5×10^6/ml) and MRL-*lpr* spleen cells (5×10^6/ml). Control cultures consisted of either MRL-+/+ (●) or MRL-*lpr* (○) cells alone at a concentration of 5×10^6/ml.

can be replaced in vitro by either phorbol myristic acetate (PMA) or the macrophage product, IL-1 (26). To determine whether IL-2 deficiency in MRL-*lpr* mice reflects a macrophage defect rather than a primary T cell defect, we supplemented our cultures with these macrophage-replacing factors. Neither PMA nor IL-1 stimulated IL-2 production by Con A-activated MRL-*lpr* spleen cells, although they did enhance IL-2 production by control MRL-+/+ spleen cells (Fig. 4). Peak IL-2 production by MRL-+/+ spleen cells was associated with 10 ng/ml PMA or a 1:10 dilution of our IL-1 preparation. MRL-*lpr* spleen cells failed to produce IL-2 at PMA concentrations ranging from 5 to 20 ng/ml and IL-1 dilutions ranging from 1:2 to 1:32. Neither PMA nor IL-1 stimulated IL-2 production in the absence of Con A, nor did PMA or IL-1 cause significant thymidine incorporation in the IL-2 assay (data not shown).

Pathologic Features and IL-2 Production in B6-lpr Mice. The characteristics of B6-*lpr* mice are summarized in Table I. B6-*lpr* mice had the typical features of disease seen in MRL-*lpr* mice: lymph nodes were increased in size 10–20-fold by 4 mo of age; antinuclear antibodies were present at 6 mo of age; and the median survival was shortened to 284 d, as compared with 795 d in control B6-+/+ mice. B6-*lpr* mice developed a defect in IL-2 activity early in life that became progressively more severe with age. Although spleen cells from 2-mo-old B6-*lpr* mice produced IL-2 normally in

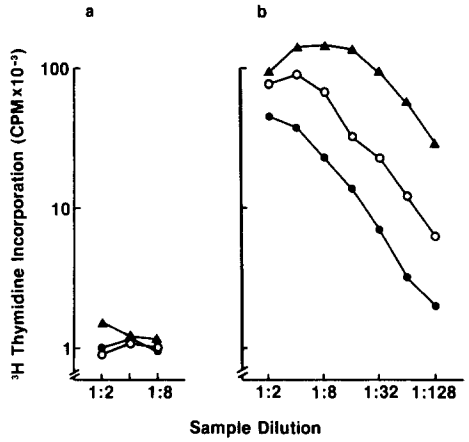

FIG. 4. Effect of 1:10 dilution of IL-1 preparation (○) or 10 ng/ml PMA (▲) on Con A-induced IL-2 production by (a) MRL-*lpr* and (b) MRL-+/+ spleen cells. These concentrations of IL-1 and PMA resulted in maximum IL-2 production by MRL-+/+ spleen cells. The closed circles (●) represent Con A-induced IL-2 production in cultures not supplemented with IL-1 or PMA.

TABLE I

Characteristics of C57BL/6J-lpr/lpr mice

Genotype	Axillary lymph node size at 4 mo	Antinuclear antibodies at 6 mo (number positive/total)*	Survival at 10 mo‡
	mg/g body wt ±SEM		%
C57BL/6J-*lpr/lpr*	2.99 ± 0.67	6/6§	37§
C57BL/6J-+/+	0.18 ± 0.01	0/8	96

* Sera (1:10 dilution) tested by indirect immunofluorescence using rat liver sections as substrate (1).

‡ Percent survival among 27 C57BL/6J-*lpr/lpr* mice and 24 C57BL/6J-+/+ mice.

§ $P < 0.005$ compared with C57BL/6J-+/+ mice (χ^2 analysis of 2 × 2 contingency table).

response to Con A, spleen cells from 3-mo-old mice showed a marked reduction in IL-2 activity (Fig. 5). There was virtually no IL-2 activity at 6 mo of age.

Spleen Cell Response to IL-2. Con A-stimulated spleen cells from MRL-*lpr* and B6-*lpr* mice proliferated significantly less in response to standard IL-2 preparations than did spleen cells from age-matched controls (Table II). Like the defect in IL-2 production, the defect in IL-2 response was present in 2-mo-old MRL-*lpr* mice ($P < 0.05$; *t* test for comparison of two independent means) and rose dramatically by 5 mo of age ($P < 10^{-5}$).

Absorption of IL-2 Activity by Activated T Cells. The failure of MRL-*lpr* spleen cells to respond normally to IL-2 suggested that they might be unable to bind the lymphokine. To investigate this possibility, we incubated a standard IL-2 preparation with Con A-stimulated lymphocytes from either 5-mo-old MRL-*lpr* or MRL-+/+ mice. After 2 h of incubation with activated MRL-+/+ spleen cells (5×10^7/ml), all of the IL-2

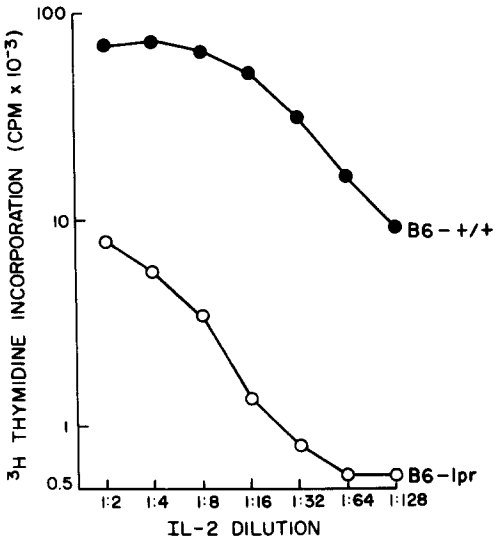

FIG. 5. IL-2 activity in culture supernatants derived from 3-mo-old B6-*lpr* (○) and B6-+/+ (●) mice. Each point represents the mean value of IL-2 activity in four individual mice.

TABLE II

Spleen Cell Response to IL-2

Spleen cell source	Number of mice tested	IL-2 source	Thymidine incorporation
			cpm
MRL-*lpr* (2-mo-old)	7	Murine*	25,075 ± 6,152‡
MRL-+/+ (2-mo-old)	7	Murine	45,454 ± 6,091
MRL-*lpr* (5-mo-old)	6	Murine	3,452 ± 1,660
MRL-+/+ (5-mo-old)	6	Murine	43,392 ± 4,417
MRL-*lpr* (5-mo-old)	4§	Human‖	1,010
MRL-+/+ (5-mo-old)	4	Human	17,773
B6-*lpr* (6-mo-old)	4	Human	2,590 ± 1,245
B6-+/+ (6-mo-old)	4	Human	24,595 ± 6,210

* Culture supernatant from Con A-stimulated MRL-+/+ cells.
‡ Mean ± SE.
§ Pooled specimen.
‖ Partially purified human IL-2.

activity had been removed from the supernatant (Fig. 6). In contrast, incubation with MRL-*lpr* cells removed very little, if any, IL-2.

Discussion

Culture supernatants derived from Con A-activated MRL-*lpr* but not MRL-+/+ spleen cells were deficient in IL-2 activity. In addition, MRL-*lpr* spleen cells responded poorly to IL-2. These defects were present early in life and became nearly absolute by 5 mo of age. These findings suggest that deficient IL-2 activity may contribute to impaired immunoregulation and to the development of autoimmune disease.

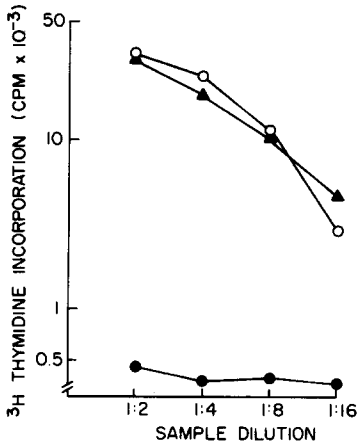

FIG. 6. Activity of a standard IL-2 preparation before (▲) and after absorption with Con A-stimulated MRL-*lpr* (○) or MRL-+/+ (●) spleen cells. Absorptions were performed at 37°C for 2 h using 5×10^7 cells/ml.

Because MRL-*lpr* and MRL-+/+ mice are congenic strains, we suspected that the *lpr* gene might code for a product that directly or indirectly caused deficient IL-2 activity and impaired immunoregulation. This hypothesis was supported by our finding that C57BL/6J mice bearing the *lpr* gene also developed early IL-2 deficiency and subsequently a lupus-like illness. Thus, in two mouse strains, the *lpr* gene is associated with both IL-2 deficiency and autoimmune disease.

We considered several mechanisms through which IL-2 deficiency might arise. Because production of IL-2 is macrophage dependent (26), a macrophage defect could result in decreased IL-2 production. Our observation that neither IL-1 nor PMA restored IL-2 production in MRL-*lpr* mice suggests that IL-2 deficiency is not due solely to a macrophage defect. In humans, Con A induces suppressor cells that suppress IL-2 production (27). We found no such suppressor cells in MRL-*lpr* mice, nor did MRL-*lpr* mice produce a soluble inhibitor of IL-2 activity.

A structural abnormality might produce an IL-2 molecule lacking biological activity. However, this would not easily explain the increasing severity of IL-2 deficiency with age. Alternatively, spleen cells capable of producing IL-2 may be diminished in frequency due to proliferation of other lymphocyte subpopulations. We doubt that an alteration in cell ratios is solely responsible for the IL-2 deficiency because this defect is present in young MRL-*lpr* mice in whom lymphoproliferation is mild. Furthermore, the proliferating cells in MRL-*lpr* mice are Lyt-1^+23^- (3), as are the cells that normally produce IL-2 (28).

Two signals are necessary for normal lymphocytes to proliferate in response to IL-2 (22, 29). First, they must be stimulated by mitogen or antigen because receptors for IL-2 are expressed on activated T cells but not on resting T cells. Second, they must be stimulated by IL-2. We have shown that Con A-stimulated MRL-+/+ spleen cells can absorb IL-2, whereas Con A-stimulated MRL-*lpr* spleen cells cannot. These findings imply that the poor response of MRL-*lpr* spleen cells in the IL-2 assay reflects an actual or functional deficiency of surface receptors for IL-2. The relationship between the defect in production of IL-2 and the defect in generation of IL-2 receptors is a problem for further study. However, it does not appear that these defects simply

reflect a generalized failure of Con A-stimulated responses because certain Con A-induced functions, such as cellular suppression of immunoglobulin synthesis (5), are normal even in old MRL-*lpr* mice.

The relationship between IL-2 deficiency and autoimmunity is intriguing. Normal immunoregulation depends upon a complex set of cellular interactions (30) in which IL-2 appears to play an important role (14–18). IL-2 stimulates thymocyte proliferation (14, 15), provides helper activity for antibody production (14–16), and facilitates the induction of cytotoxic T cells (14–18). Diminished IL-2 production might alter vital immune functions and thereby contribute to autoimmunity. Indeed, the depressed SMLR, which is considered to be a model for abnormal cellular interaction in autoimmune mice (10, 11), may be due to the deficiency of IL-2. There is a temporal correlation between the disappearance of IL-2 activity and the loss of syngeneic reactivity in MRL-*lpr* mice. Both occur early in life, before the onset of overt clinical disease. The defective cell in the SMLR bears the Lyt-1^+23^- phenotype characteristic of IL-2-producing cells (11, 28). It has recently been shown that IL-2 is produced in the course of a normal SMLR (13). Its absence could contribute to the abnormal SMLR of MRL-*lpr* mice.

The coexistence of T cell proliferation and reduced IL-2 activity in MRL-*lpr* mice may appear paradoxical. However, it is possible that controlled proliferation of Lyt-1^+23^- T cells requires IL-2-dependent T cell differentiation. Uncontrolled proliferation of Lyt-1^+23^- T cells might then arise as a consequence of IL-2 deficiency. Alternatively, the stimulus for lymphoproliferation in MRL-*lpr* mice may be IL-2 independent.

The observation that IL-2 deficiency precedes the onset of overt autoimmune disease in two strains bearing the *lpr* gene (MRL, C57BL/6J) raises the possibility that the *lpr* mutation is causally related to murine lupus through defective T cell regulation arising as a consequence of the IL-2 abnormality.

Summary

Spleen cells from MRL-*lpr* and B6-*lpr* mice have a marked defect in the ability to produce interleukin 2 (IL-2) in response to concanavalin A stimulation. This defect precedes the onset of clinical illness, increases with age, and eventually becomes virtually absolute. It is not due to cellular suppression of IL-2 production, nor does it reflect the presence of a soluble inhibitor of IL-2 activity. Failure to restore IL-2 production with macrophage-replacing factors, such as interleukin 1 and phorbol myristic acetate, suggests that IL-2 deficiency reflects a primary T cell defect rather than a macrophage defect. MRL-*lpr* and B6-*lpr* spleen cells also have an age-dependent reduction in IL-2 response that apparently results from a deficiency of cell surface receptors for IL-2. Congenic MRL-+/+ and B6-+/+ mice, which lack the *lpr* gene responsible for accelerated autoimmunity and lymphoproliferation, have normal IL-2 activity. These findings suggest that a defect in IL-2 activity may contribute to impaired immunoregulation in mice bearing the *lpr* gene. The absence of such a defect in MRL-+/+ and B6-+/+ mice further suggests that a single autosomal recessive gene is responsible for the IL-2 deficiency.

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