ANALYSIS OF T CELL FUNCTION IN AUTOIMMUNE MURINE STRAINS

Defects in Production of and Responsiveness to Interleukin 2*

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The availability of several murine strains manifesting autoimmune syndromes similar in nature to human systemic lupus erythematosus (SLE)[1] prompted extensive studies on the immunological abnormalities of these mice in an attempt to better understand autoimmune diseases. These studies demonstrated a wide range of abnormalities in various compartments of the immune system, including the thymus-derived (T) cell compartment (1, 2).

Other recent studies suggested a central role of the antigen-nonspecific lymphokine T cell growth factor (3, 4), more recently termed interleukin 2 (IL-2) (5), in the regulation of both cell-mediated and humoral T cell-dependent immune responses (6, 7). According to a generally accepted model (6, 7), IL-2, generated by ligand-activated T cells, regulates the clonal expansion of antigen-stimulated T cells in a hormonal manner by binding to specific surface receptors on such T cells. Because of this central role, believed to be played by IL-2 in regulating T cell responses, and in view of several abnormalities in T cell function manifested by SLE-prone mice (1, 2), we have analyzed in detail patterns of IL-2 production and consumption by T cells of autoimmune murine strains and their relationship to various cell-mediated immune responses manifested by such mice. The studies reported in this communication document defective patterns of cell-mediated immune responses in autoimmune murine strains, most marked in the MRL/Mp-*lpr/lpr* (MRL/l) mouse and, specifically, they demonstrate defects in the mode of production and consumption of IL-2 by T cells in relation to their proliferative and cytotoxic T lymphocyte (CTL) responses. The most severe defects were found in older MRL/l mice, which responded poorly to concanavalin A (Con A) or allogeneic stimulator cells, generated very low levels of IL-2 upon Con A stimulation and responded very weakly to exogenous IL-2.

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[1] *Abbreviations used in this paper:* αMM, α-methyl-D-mannoside; CML, cell-mediated lympholysis; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; CTLp, CTL precursors; CTLL2, interleukin-2-dependent CTL line; FCS, fetal calf serum; IL-2, interleukin 2; MLR, mixed lymphocyte reaction; MRL/l, MRL/Mp-*lpr/lpr* mice; MRL/n, MRL/Mp-+/+ mice; NTA, natural thymocytotoxic antibodies; SLE, systemic lupus erythematosus.

Whereas both spleen and lymph node cells of old MRL/l mice gave weak proliferative responses in an allogeneic MLR, the spleen (but not lymph node) cells generated normal levels of allogeneic CTL activity and these findings were corroborated by direct analysis of CTL precursor frequency.

Materials and Methods

Mice. The strains and sex of mice used in these experiments are listed in Results. All mice were obtained from the Scripps Clinic and Research Foundation mouse breeding colony. MRL mice of both substrains MRL/l and MRL/Mp-+/+ (MRL/n) and BXSB mice were developed by Dr. E. Murphy of The Jackson Laboratory, Bar Harbor, Maine. Unless otherwise stated, young donors were 4–6 wk old and "old" mice were 4–6 mo of age. Information about the derivation and mortality rates of these strains as well as their histoimmunopathologic and serologic characteristics has been detailed elsewhere (1, 2).

Cell Preparation. Lymph nodes and spleens were aseptically removed from donor mice and single cell suspensions were prepared by teasing the organs in a glass homogenizer with a loosely fitting pestle. Spleen cell suspensions were treated briefly with an ammonium chloride buffer to lyse erythrocytes and viable cells were enumerated using 0.1% trypan blue. Cells were resuspended at the desired concentration in RPMI 1640 medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 1% penicillin-streptomycin solution, 5% fetal calf serum (FCS) (all from Irvine Scientific, Santa Ana, Calif.), and 5×10^{-5} M 2-mercaptoethanol (Eastman Kodak Co., Rochester, N. Y.). Cells were cultured in this medium in a 5% CO_2 humidified incubator at 37°C.

IL-2 Production. Cells were resuspended in culture medium containing 2.5 or 5 μg/ml Con A (Miles-Yeda, Rehovot, Israel) at 5×10^6/ml and volumes of 10 ml were added to 25-cm^2 tissue culture flasks (3013; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). In some experiments, control cultures lacking Con A were also established. Cultures were harvested after 24, 48, or 72 h and the supernates, obtained by centrifugation at 1,500 *g* for 15 min, were sterilized in 0.45-μm filters (Millipore Corp., Bedford, Mass.) and kept at 4°C until assayed.

IL-2 Bioassay. IL-2 activity was quantitated in a bioassay (8) using cells of the long-term, IL-2-dependent CTL line, CTLL-2 (obtained from Dr. J. Watson, Irvine, Calif.) as indicator cells (4). This cell line was maintained in culture medium supplemented with 25–50% crude rat IL-2 and passed twice weekly at initial cell concentrations of 5×10^3–10×10^3/ml. CTLL-2 cells were washed once in IL-2-free medium and cultured in flat-bottomed microtiter plates (3040; Falcon Labware) at 1×10^4/0.2 ml of medium containing serial (three- or fourfold) dilutions of culture supernates to be tested for their IL-2 activity. A standard rat IL-2 preparation was used as a reference in most experiments. Cultures were pulsed after 20 h with 1 μCi [^{3}H]TdR (5 Ci/mmol; Amersham Corp., Arlington Heights, Ill.), harvested 4 h later on a Titertek cell harvester (Flow Laboratories, Rockville, Md.) and counted in a liquid scintillation counter. The response was quantitated by probit analysis (8) and one unit of IL-2 activity was defined as the amount of IL-2-containing crude culture supernate that produced 50% of the maximal proliferative response generated by the reference IL-2 preparation. The IL-2 activity in units per milliliter was derived from that amount.

Con A-induced Mitogenic Response. 100-μl aliquots of control or Con A-stimulated cultures were transferred after 24, 48, or 72 h of culture in 25-cm^2 flasks (established as described above) into flat-bottomed microtiter plates and pulsed for 4 h with 1 μCi [^{3}H]TdR. Alternatively, cultures were set in microtiter wells with various numbers of spleen or lymph node cells, usually 2.5×10^5/well, in 200 μl of culture medium without or with 2.5 μg/ml Con A. Cultures were pulsed after 24, 48, or 72 h and harvested 4 h later as described above.

IL-2 Adsorption. Cells used for adsorption of IL-2 were 48-h Con A-stimulated spleen cells. The cells were washed three times in serum-free RPMI 1640 medium with 30 min incubation at 37°C between washes. The cells were then resuspended in 0.5 ml of mouse IL-2 preparations and this suspension was incubated for 2 h at 37°C with occasional shaking. The supernate, obtained by centrifugation at 1,500 *g* for 15 min, was assayed for residual IL-2 activity in the bioassay described above.

Mixed Lymphocyte Reaction (MLR). The MLR was performed as described before (9). Briefly, 2×10^5 responding and 2.5 or 5×10^5 irradiated stimulator spleen cells in 200 μl medium were cultured for a total of 72 h in flat-bottomed microtiter plates, pulsed with 1 μCi [^{3}H]TdR for the final 24 h, harvested, and counted as described above.

CTL Induction and Cell-mediated Lympholysis (CML) Assay. CTL were induced in a 5-d allogeneic MLR and assayed for their cytolytic activity in a CML assay as described before (9). Briefly, 2×10^6 each of responding and 3,000 rad-irradiated stimulator spleen cells were cultured in 1 ml medium in 35-mm tissue culture dishes on a rocking platform for 5 d. CML was assayed by adding 1×10^4 ^{51}Cr-labeled Con A-stimulated lymphoblasts to the cultures and harvesting the cultures 4 h later.

Determination of CTL Precursor (CTLp) Frequency. Determination of alloreactive CTLp frequency was performed using a limiting dilution assay, similar to those described by others (10–12). Limiting numbers of MRL/l or C3H/St lymph node or spleen responder cells were cultured (at 24 replicate wells per responder cell dose) with 5×10^5 2,000 rad-irradiated allogeneic C57BL/6 (*H-2^b*) spleen cells in 0.2 ml/well of extra high amino acids medium (13) supplemented with 10% FCS and 25% (vol:vol) of a rat IL-2-containing supernate. The Con A in this supernate was blocked by the addition of 20 mg/ml α-methyl-D-mannoside (αMM) (Sigma Chemical Co., St. Louis, Mo.). Preliminary experiments established that: (*a*) αMM effectively blocked the mitogenic effect of the residual Con A on fresh thymocytes or spleen cells; (*b*) the αMM itself had no effect on the induction of alloreactive CTL; (*c*) in the absence of exogenous IL-2, no CTL responses were generated by the low numbers of responder cells used; and (*d*) responder cells cultured with IL-2 only, in the absence of allogeneic stimulators, failed to generate CTL activity. Cultures were set in round-bottomed microtiter plates (76-011-05; Linbro Scientific, Inc., Hamden, Conn.) for 7 d, after which 100 μl of the spent culture medium was drawn from each well and 2×10^3–5×10^3 ^{51}Cr-labeled EL4 (*H-2^b*)target cells in 100 μl fresh RPMI 1640 plus 5% FCS were added to each well. After an additional 4 h incubation at 37°C, 100 μl of supernate was removed from each well for gamma counting. Spontaneous ^{51}Cr release was established in 24 cultures that contained only stimulator cells and IL-2. Cultures were considered positive for cytolytic activity when the ^{51}Cr release in a given well exceeded the mean spontaneous release value by at least three standard deviations of the mean. The frequency of the CTLp was calculated by plotting the percent of nonresponding cultures vs. the number of responder cells per well (10–12).

Results

Con A-induced Mitogenic Responses. Fig. 1 shows the Con A-induced mitogenic responses of the SLE strains and their normal counterparts. Among the young mice, MRL/l (panel A) and male BXSB (panel B) showed a defective response at 1.5 mo of age. All other young SLE (as well as normal) strains between 1 and 2.5 mo of age gave normal mitogenic responses.

The responses of the early, severe SLE-developing strains MRL/l (panel A) and male BXSB (panel B) were greatly reduced at 4–4.5 mo of age compared with age-matched immunologically normal strains of mice. In contrast, the responses of late, mild SLE-developing MRL/n (panel A) and female BXSB (panel B) mice at similar age were within normal limits. NZB and (NZB × NZW)F_1 hybrid (BWF_1) mice at 5–6.5 mo of age were normal in this respect (panel C); however, at 7.5 mo of age, the female (but not male) BWF_1 mice gave a markedly reduced mitogenic response (panel D). Similarly, the responses of MRL/n mice tested at 8–8.5 mo of age were greatly reduced, being only 25–30% of the normal ones (results not shown).

IL-2 Levels in Culture Supernates. The levels of IL-2 in unstimulated and Con A-stimulated culture supernates of spleen cells derived from various mouse strains were determined using the bioassay described in Materials and Methods (8). Culture supernates were harvested after 1, 2, or 3 d and serial dilutions were assayed for their

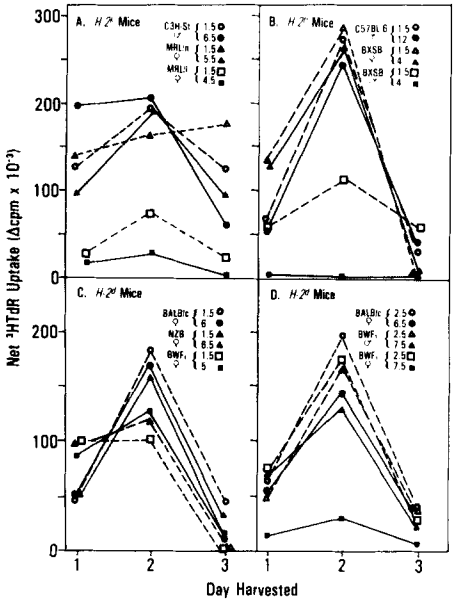

FIG. 1. Con A-induced mitogenic responses of spleen cells from normal and autoimmune murine-strains. Uptake was determined after 1, 2, or 3 d of stimulation after a 4-h pulse with 1 μCi [^{3}H]-TdR. Net [^{3}H]TdR uptake levels after subtraction of uptake in control and unstimulated cultures (12,000 cpm or less) are shown. Numbers in the figure panels represent the age of the cell donors in months.

IL-2 activity. Supernates of control cultures, not stimulated with Con A, displayed some IL-2 activity in some but not all cases; however, this activity, possibly induced by FCS stimulation, never exceeded 2% of the IL-2 activity in parallel, Con A-stimulated culture supernates. Furthermore, spleen cells of autoimmune mice did not spontaneously secrete into their culture supernates more IL-2 than did spleen cells of normal mice (data not shown).

The results of such analyses, repeated at least twice for most strains studied, are summarized in Table I. Although the IL-2 levels in stimulated culture supernates derived from normal strains are highly variable, it is still evident that culture supernates of 4–5-mo-old MRL/l and male (but not female) BXSB and female BWF_1 mice at 5.5 mo of age contain markedly lower levels of IL-2 activity. Similarly, stimulated culture supernates of MRL/n mice at 8.5 (but not at 5.5; data not shown) mo of age show decreased levels of IL-2. On the other hand, normal IL-2 levels were found in supernates derived from 4.5–5-mo-old NZB mice. With respect to the young mice, decreased levels of IL-2 were consistently found in Con A-stimulated culture supernates of MRL/l mice and in 48- (but not 24-) h culture supernates of spleen cells from young (6-wk-old) male BXSB mice, whereas spleen cells of all other young autoimmune murine strains produced normal levels of IL-2.

A detailed analysis indicated that the defects in IL-2 production were evident in the spleens of young MRL/l mice at 1, 3, 4, and 5 wk of age and IL-2 levels were much lower than in culture supernates of C3H/St spleen cells of parallel ages (Fig. 2). This early reduction in IL-2 levels was not associated with lymphoproliferation

TABLE I

*IL-2 Levels in Con A-stimulated Spleen Cell Culture Supernates of Autoimmune and Normal Murine Strains**

Strain and sex	H-2 haplotype	IL-2 level (U/ml) in culture supernates‡			
		Day 1		Day 2	
		Young	Old	Young	Old
Normal strains					
C3H/St ♂	*k*	330	910	250	800
C57BL/6 ♂	*b*	270	120	55	270
BALB/c ♀	*d*	780	530	60	95
Autoimmune strains					
MRL/l ♀	*k*	100	[5] §	[<1]	[<1]
MRL/n ♀	*k*	390	[25]	160	[12]
BXSB ♂	*b*	280	[8]	[8]	[<1]
BXSB ♀	*b*	220	170	220	240
NZB ♀	*d*	130	335	50	230
BWF_1 ♀	*d/z*	140	[<1]	80	[32]

* Summary of five separate experiments. Young mice were 4–6 wk old and old mice were 4–6 mo old, except the MRL/n mice, which were 8.5 mo old.

‡ IL-2 levels in 3-d culture supernates were, in almost all cases, <1 U/ml.

§ Boxed numbers are markedly below the range of IL-2 levels in normal strains.

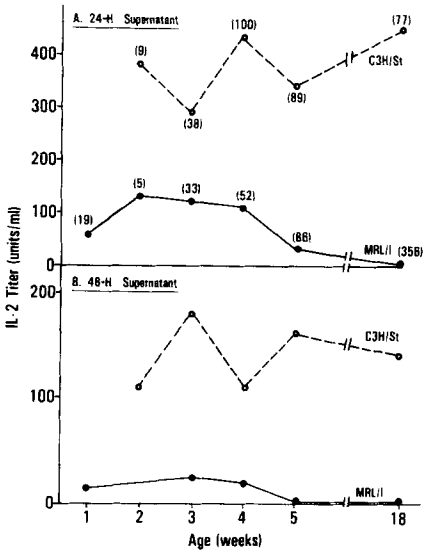

FIG. 2. II 2 activity in Con A-stimulated spleen cell culture supernates derived from C3H/St and MRL/! mice of various ages. Culture supernates were harvested after 1 or 2 d of stimulation with 2.5 μg/ml Con A in 16-mm wells (5×10^6 cells/ml per well) and assayed in a standard IL-2 assay. Numbers in parentheses indicate the yield of viable nucleated cells per spleen ($\times 10^6$).

because the numbers of nucleated cells recovered from the spleens of MRL/l mice up to 5 wk of age were very similar to those of age-matched C3H/St mice (Fig. 2).

Effect of Culture Conditions on Responses of MRL/l Mice. The defects in Con A-induced

mitogenic response and IL-2 production manifested by MRL/l mice even at a young age were somewhat surprising in view of the massive and selective T cell proliferation that this strain manifests (1, 2). Therefore, we decided to focus our subsequent studies on clarifying the mechanisms underlying the defective IL-2 response in this strain. One possible explanation for these defects was simply inadequate culture conditions, e.g., suboptimal concentrations of responding cells or Con A. However, results of several experiments (data not shown) revealed that the severe defects in mitogenic response and IL-2 production were consistent across a wide range of mitogen (1.25–20 μg/ml) and cell (0.625–10 × 10^6/ml) concentrations.

The Role of Suppressor Cells in Defective Responses of Old MRL/l Mice. Cultured spleen cells, either without or with Con A stimulation, were shown to generate suppressor cells that can manifest their suppressive activity in a variety of systems (14–16). However, examination, by cell mixture studies, of spleens of old MRL/l mice for cells that would suppress the Con A-induced proliferative responses and IL-2 production by functionally normal spleen cells from young C3H/St, MRL/n, or MRL/l mice, failed to reveal any significant suppressor cell activity (data not shown). Thus, it can be concluded that under our experimental conditions, suppressor cell activity does not appear to play a major role in defective IL-2 production by MRL/l T cells.

Search for a Suppressive Material in Stimulated Culture Supernates of Old MRL/l Mice. Soluble nonspecific suppressive molecules were reported to be induced upon stimulation of lymphoid cells with Con A (17). In addition, it has been suggested recently that such molecules may act as antagonists that regulate IL-2 action on activated T cells (18). This raises the possibility that the apparent deficiency of IL-2 in Con A-stimulated culture supernates of old MRL/l spleen cells may be due to abnormally high levels of some suppressive material(s), which inhibits IL-2 production or action. To study this possibility, we added increasing amounts of 24-h Con A-stimulated spleen cell culture supernates of old MRL/l (or control C3H/St) mice to a standard optimal dose (10% vol:vol) of an active C3H/St-derived IL-2 preparation. The IL-2 activity in these mixed supernates was measured in a standard IL-2 bioassay (Fig. 3). Direct titration of IL-2 activity clearly revealed that the MRL/l-derived

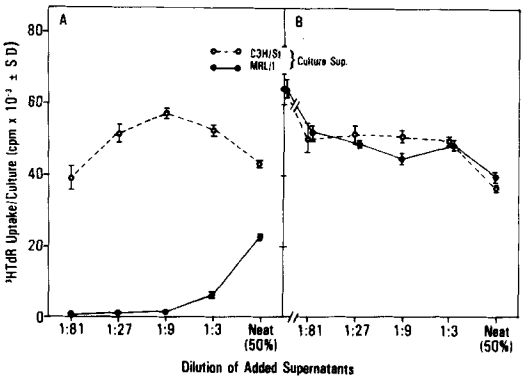

FIG. 3. Lack of soluble suppressor material(s) in Con A-stimulated culture supernates of old MRL/l-derived spleen cells. In panel A, a direct titration of IL-2 activity in C3H/St- and MRL/l-derived Con A-stimulated culture supernates is shown. Panel B: different dilutions of C3H/St- and MRL/l-derived culture supernates were added to a standard dose (10% vol:vol) of an active, C3H/St-derived IL-2 preparation.

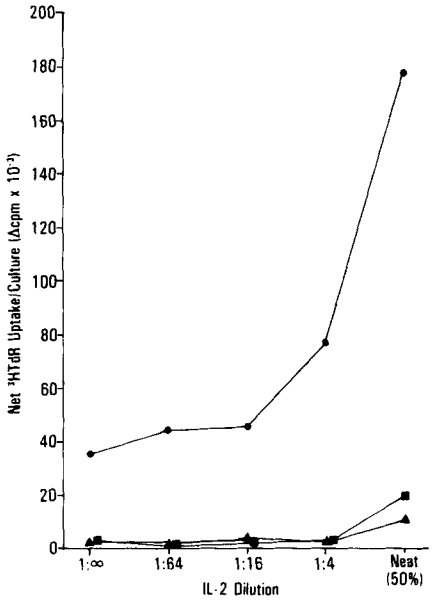

FIG. 4. The effect of exogenous IL-2 on the Con A-induced mitogenic response of C3H/St and MRL/l mice. 2.5 × 10^5 C3H/St spleen cells or MRL/l spleen or lymph node cells from 6-mo-old mice were stimulated with 2.5 μg/ml Con A in the absence (1:∞) or presence of various dilutions of a C3H/St-derived IL-2 preparation. [^{3}H]TdR uptake was determined 48 h after a 4-h pulse. [^{3}H]TdR uptake values by control, unstimulated cultures (C3H/St spleen: 10,073 cpm; MRL/l spleen: 1,793 cpm; MRL/l lymph nodes: 1,339 cpm) were subtracted to give the net levels of uptake shown. 6-mo-old responders: ●, old C3H/St spleen cells; ▲, old MRL/l spleen cells; ■, old MRL/l lymph node cells.

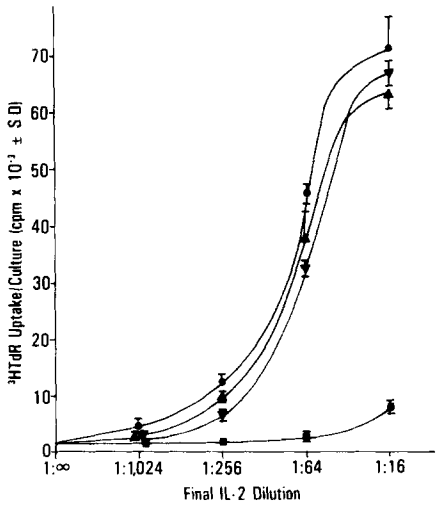

FIG. 5. Lack of IL-2 adsorption by Con A-stimulated MRL/l-derived spleen cells. A BALB/c-derived IL-2 preparation was titrated before or after adsorption for 2 h at 37°C on 7 × 10^7 fresh or Con A-stimulated spleen cells as shown. Spleen cells used for adsorption were obtained from 6 mo-old mice: ●, none; ▲, 7 × 10^7 fresh C3H/St; ■, 7 × 10^7 Con A-stimulated C3H/St; ▼, 7 × 10^7 Con A-stimulated MRL/l.

culture supernate was markedly deficient in IL-2 activity in comparison with the C3H/St-derived supernate (Fig. 3, panel A). As shown in panel B, the addition of MRL/l or C3H/St-derived culture supernates had very similar effects on the IL-2 activity of 10% (vol:vol) C3H/St-derived IL-2. Similar results were obtained with 2- or 3-d Con A-stimulated culture supernates (data not shown). These results demonstrate that culture supernates of stimulated old MRL/l-derived spleen cells do not contain unusual amounts of substances that act as a specific IL-2 antagonist(s).

Response of Old MRL/l Lymphocytes to Exogenous IL-2. The results presented so far strongly suggest an intrinsic defect in IL-2 production by T cells of MRL/l mice. Because IL-2 stimulates activated T cells to proliferate by binding to specific cell-surface receptors (7, 19, 20), we next asked whether spleen or lymph node cells of old MRL/l mice can respond normally to IL-2. We predicted that if cells bearing receptors for and capable of responding to IL-2 exist in MRL/l mice in normal proportions, then the addition of an exogenous active source of IL-2 would bypass the defective IL-2 production and allow a normal Con A response.

The results of such an experiment, using an active C3H/St-derived IL-2 preparation, are summarized in Fig. 4. Because this IL-2 preparation itself contained Con A (at 5 μg/ml), it was diluted in Con A-containing (5 μg/ml) fresh culture medium to keep the mitogen concentration constant (at a final dose of 2.5 μg/ml) in all cultures. Thus, the only variable is the amount of exogenous IL-2 added to the cultures. The results (Fig. 4) clearly demonstrate that although IL-2 was capable of strongly potentiating the already substantial Con A reactivity of C3H/St spleen cells, it did not restore the response of lymph node or spleen cells of 6-mo-old MRL/l mice to normal levels. Thus, T cells of MRL/l mice appear to be relatively incapable of responding to IL-2.

Taken together, the results presented so far clearly indicate that the deficient mitogenic response of old MRL/l T lymphocytes upon Con A stimulation reflects defects in both the production of and the response to IL-2.

IL-2 Adsorption Studies. The deficient response of Con A-stimulated lymphocytes of old MRL/l mice to exogenous IL-2 (Fig. 4) could be explained by several mechanisms, among them failure to bind, or the degradation and inactivation of IL-2 by the cells before triggering can occur. In both cases, the final outcome would be manifested as a lack of proliferative response to exogenous IL-2. To determine whether Con A-incubated spleen cells of old MRL/l mice can remove IL-2 from a biologically active supernate or degrade IL-2 activity, we titrated IL-2 preparations for their residual biological activity after adsorption on fresh or Con A-stimulated old MRL/l (or control C3H/St)-derived spleen cells (Fig. 5).

As expected, fresh C3H/St spleen cells did not absorb IL-2 activity to any significant degree when compared with the unadsorbed IL-2 preparation (Fig. 5). Conversely, the Con A-stimulated C3H/St spleen cells removed almost completely the IL-2 activity from the supernate, in agreement with their ability to respond normally to Con A stimulation. In contrast, the IL-2 preparation retained its full biological activity after incubation with spleen cells of old MRL/l mice that had been exposed for 2 d to Con A. The lack of IL-2 adsorption by these cells was not unexpected because as shown above, T cells of older MRL/l mice fail to respond to Con A, either in the absence (Fig. 1) or presence (Fig. 4) of exogenous IL-2. The results clearly indicate, however, that spleen cells of older MRL/l mice do not degrade or otherwise

TABLE II

*Allogeneic MLR of MRL/l, MRL/n, and C3H/St-derived Spleen Cells against BALB/c (H-2^d) Stimulator Spleen Cells**

Strain and age	Net [^{3}H]TdR uptake‡	Stimulation index
	Δ *cpm*	
MRL/l, young	151,770	10.1
MRL/n, young	210,483	6.8
C3H/St, young	208,583	10.0
MRL/l, old	8,972 §	1.9
MRL/n, old	83,060	8.2
C3H/St, old	125,341	9.1

* 2 × 10^5 responder spleen cells were cultured with 2.5 × 10^5 irradiated syngeneic or allogeneic BALB/c spleen cells and [^{3}H]TdR uptake was determined on the 3rd d of culture. [^{3}H]TdR uptake by irradiated stimulator cells alone was ≤1,000 cpm.

‡ [^{3}H]TdR uptake by allostimulated cultures minus that in cultures containing syngeneic stimulator cells.

§ Boxed numbers represent a deficient response.

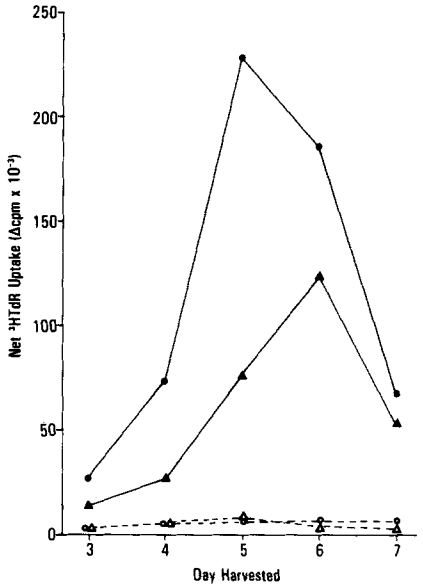

FIG. 6. Kinetics of the anti-C57BL/6 (*H-2^b*) MLR by spleen and mesenteric lymph node cells of 4-mo-old C3H/St and MRL/l mice. 2.5 × 10^5 responder cells were cultured with 5 × 10^5 irradiated (2,000 rad) syngeneic or allogeneic C57BL/6 spleen cells and harvested on the days indicated after a 16-h pulse with 1 μCi [^{3}H]TdR. Net levels of isotope uptake after subtraction of [^{3}H]TdR uptake in cultures with syngeneic stimulators are shown. C3H/St: ●, mesenteric lymph node cells; ▲, spleen cells. MRL/l: ○, mesenteric lymph node cells; △, spleen cells.

inactivate IL-2. Interestingly, fresh (not Con A-stimulated) proliferating spleen cells from the enlarged spleens of MRL/l mice also failed to adsorb IL-2 from a biologically active supernate (data not shown).

Alloantigen Reactivity of MRL/l Spleen Cells. As another parameter of T cell reactivity, we next studied the alloantigen reactivity of MRL/l T lymphocytes because the

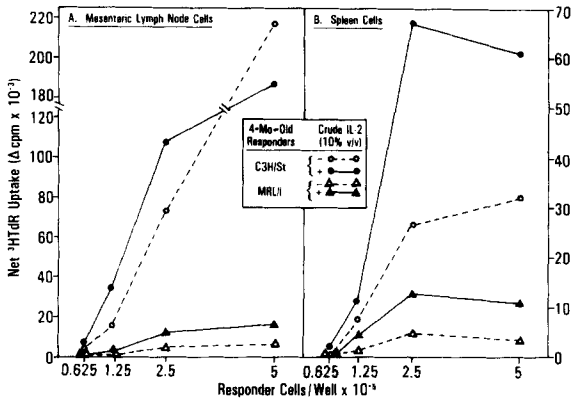

FIG. 7. Exogenous IL-2 does not reconstitute to normal levels the deficient MLR of MRL/l lymph node and spleen cells against C57BL/6 stimulator cells. Mesenteric lymph node or spleen cells of 4-mo-old C3H/St or MRL/l mice were cultured at the numbers indicated in the abscissa with 5×10^5 irradiated (2,000 rad) syngeneic or allogeneic C57BL/6 spleen cells in the absence or presence of 10% (vol:vol) crude IL-2 derived from rat spleen cell cultures stimulated for 48 h with 2.5 μg/ml Con A. The Con A in the IL-2 preparation was blocked by adding 20 mg/ml αMM, which completely blocked the mitogenic effect of this preparation on fresh thymocytes or spleen cells. Cultures were harvested after 4 d, following a 16-h pulse with 1 μCi [^{3}H]TdR. Net [^{3}H]TdR uptake after subtracting the levels of uptake in control cultures (with syngeneic stimulators) is shown.

TABLE III

Allogeneic CTL Responses of MRL/l and C3H/St Mice

Responder spleen cells	Stimulator spleen cells (3,000 rad)	CML activity, percent specific lysis ± SE*
Young MRL/l	BALB/c	83.7 ± 2.6
Old MRL/l	BALB/c	81.3 ± 2.1
Old C3H/St	BALB/c	87.0 ± 0.6
Young MRL/l	C57BL/6	81.2 ± 3.1
Old MRL/l	C57BL/6	91.2 ± 1.4
Old C3H/St	C57BL/6	78.6 ± 3.3
Young MRL/l	SJL	89.8 ± 1.1
Old MRL/l	SJL	87.9 ± 3.0
Old C3H/St	SJL	87.5 ± 3.9
Young MRL/l	BALB/c (Anti-Thy-1.2 plus complement treated)‡	74.5 ± 7.6
Old MRL/l	BALB/c	77.9 ± 6.6
Old C3H/St	BALB/c	77.3 ± 6.1

* CML activity was assayed against target cells syngeneic to the stimulator cells in each case. Spontaneous ^{51}Cr release values were 23.4, 26.1, and 29.5% for the BALB/c, C57BL/6, and SJL target cells, respectively.

‡ 2×10^8 stimulator cells treated with 1 ml anti-Thy-1.2 ascites fluid (for 30 min at 4°C), followed by guinea pig complement (1:6 dilution, for 40 min at 37°C). These cells were reduced in their capacity to respond to Con A by 94% relative to the unfractionated starting population.

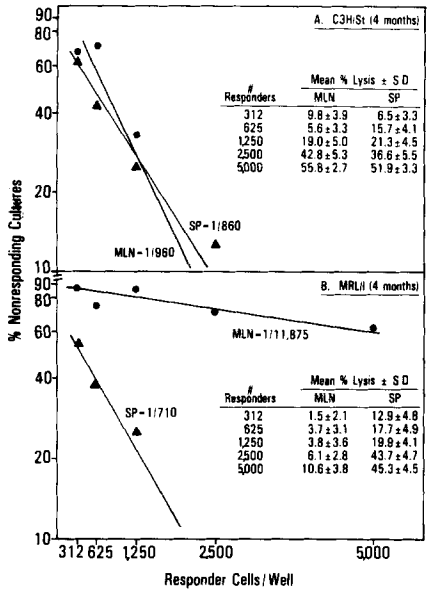

FIG. 8. Analysis of anti-C57BL/6 ($H\text{-}2^b$) CTLp frequency in mesenteric lymph nodes or spleens of 4-mo-old C3H/St and MRL/l mice. Various numbers of responder cells as shown were cultured with 5×10^6 irradiated C57BL/6 spleen cells and 25% (vol:vol) αMM-blocked Con A-stimulated rat spleen cell culture supernate as a source of IL-2 activity. ^{51}Cr release was determined after 7 d by adding 2×10^3 labeled EL4 ($H\text{-}2^b$) target cells and harvesting the supernates 4 h later. Spontaneous ^{51}Cr release was 17.1 ± 1.5%.

proliferative response of T lymphocytes in an allogeneic MLR is believed to involve the production and action of IL-2, and, furthermore, IL-2-like helper factor(s) produced in an MLR appear to be required for optimal induction of alloreactive CTL (21–26). Both alloantigen-induced proliferation and the induction of CTL were analyzed and a comparison was made between young and old MRL/l, MRL/n, and C3H/St mice.

The proliferative allogeneic responses of the various strains tested in one of three representative experiments are summarized in Table II. It is evident that although spleen cells of young (8-wk-old) MRL/l mice, as well as spleen cells of young or old MRL/n mice, manifested normal MLR reactivity in comparison with C3H/St spleen cells (as well as other normal strains; data not shown), the spleen cells of old MRL/l mice were highly deficient. The experiment summarized in Fig. 6 demonstrates that this deficient proliferation in the allogeneic MLR was consistent when cultures of either lymph node or spleen responder cells were harvested daily between 3 and 7 d after culture initiation. This indicates that the deficient MLR reactivity manifested by T cells of old MRL/l mice is not due simply to altered kinetics of the response.

The deficient MLR reactivity of spleen or lymph node cells from old MRL/l mice could not be reconstituted to normal levels by adding to the cultures an exogenous rat-derived IL-2. As shown in Fig. 7, although IL-2 was capable of augmenting substantially the MLR responses of C3H/St lymph node and particularly spleen cells, it failed to restore to normal levels the low responses of either lymph node or spleen cells of 4-mo-old MRL/l mice.

Table III summarizes the CTL responses generated in cultures of C3H/St or

MRL/l spleen or lymph node cells stimulated with irradiated allogeneic spleen cells. These results demonstrate that spleen cells derived from old (as well as young) MRL/l mice generated CTL responses, comparable to those of old C3H/St cells, against stimulators of three different *H-2* haplotypes. This finding was somewhat surprising in view of the deficient IL-2 production and MLR reactivity by spleen cells of old MRL/l mice. Several investigators (21–26) have demonstrated that the generation of allogeneic CTL responses requires helper T cells acting via release of IL-2, which in turn provides an amplifying signal to the CTL precursors. Because IL-2 production is known to be radiation resistant (26, 27), it was possible that T cells within the irradiated stimulator spleen cell population generated the IL-2 required for CTL generation. This possibility is most unlikely because irradiated stimulator BALB/c spleen cells depleted of T lymphocytes stimulated all three responder cell types (Table III).

The final possibility was that because these CTL responses were generated in bulk cultures, CTLp were present in excess and therefore we were measuring CTL activity at a high effector to target cell ratio, possibly in the plateau region of the dose-response curve. Thus, we determined the frequency of alloreactive CTLp by limiting dilution. As shown in Fig. 8, although the frequency of anti-C57BL/6 ($H\text{-}2^b$) CTLp in the spleens of 4-mo-old MRL/l mice was similar to that in C3H/St lymph nodes or spleen (1/710 vs. 1/960 and 1/860, respectively), the CTLp frequency in the mesenteric lymph nodes of MRL/l mice was by far lower (1/11,875). These findings are interesting for two reasons. First, the CTLp frequency in the spleen of old MRL/l mice is normal despite the fact that it contains three to four times higher numbers of total nucleated cells and a two to three times higher proportion of T cells than found in a normal spleen (28). Second, MRL/l spleen cells generate normal alloreactive CTL responses despite their marked defect in alloantigen-induced proliferation (Table II; Fig. 6).

Discussion

Mice with SLE-like syndromes manifest a wide spectrum of abnormalities in T and B cell distribution and function (1, 2). However, the pathogenic importance of some of these abnormalities remains questionable. Moreover, with the single exception of a generalized B cell hyperactivity observed in all of these mice, no other universally expressed lymphoid cell abnormality has been described. In this study, we have analyzed various parameters of production of and response to the T cell-derived immunoregulatory lymphokine, IL-2, in SLE-prone mice. IL-2 appears to play a central role in the control of activated T cell proliferation (6, 7) and, therefore, abnormalities in its production or the response to it should be reflected in abnormal patterns of cell-mediated immunity as well as antibody responses that require T helper cells. In addition, we have analyzed some other cell-mediated functions in these mice, namely, the mitogenic responses to Con A and allogeneic stimulator cells, as well as the induction of alloreactive CTL responses.

Our results demonstrate a decline of Con A-induced mitogenesis and consequently IL-2 production in most SLE strains studied. The concomitant decrease of Con A responsiveness and of IL-2 production is not surprising because the proliferation of T cells appears to be mediated by IL-2, which in turn is secreted by Con A-activated T cells (6, 7). In general, there appears to be a correlation between the severity and time

of onset of the SLE syndrome and reduction in Con A activation and IL-2 production. Thus, mice expressing late-onset disease (BWF_1, female BXSB, and MRL/n) have a delayed reduction occurring at or just before the clinical onset of disease. In contrast, mice that develop early and severe disease (MRL/l and male BXSB) exhibit the defect early in life. The pathogenic importance of this abnormality in mice developing late disease remains questionable because generalized B cell hyperactivity and autoantibody production are observed in such mice much earlier than the decline in Con A responsiveness and IL-2 production. The decline of these T cell functions in NZ mice may merely reflect the altered composition of their lymphoid compartment and the reduced numbers of T cells observed with age in such mice (28–31). Furthermore, reductions in Con A-induced mitogenesis and IL-2 production do not appear to be due to direct or indirect effects of natural thymocytotoxic antibodies (NTA) observed in the sera of some SLE strains (30). This conclusion is based on the lack of correlation between appearance of NTA and the reduced Con A responsiveness and IL-2 production. The first appears very early in the life of NZ mice (32), whereas the other two appear late. In addition, MRL/l mice, which to a large degree lack detectable levels of NTA (32), exhibit the most pronounced deficit in Con A responsiveness and IL-2 production.

We are left, therefore, with two SLE strains in which the pathogenic importance of reduced Con A reactivity and decline in IL-2 production needs to be considered, i.e., the MRL/l and male BXSB mice. In both MRL/l and male BXSB mice, the reduction in these T cell functions is observed relatively early in life, before any clinical signs of disease. The decline becomes much more evident at or a little before the full clinical expression of disease. In the male BXSB mice, a decline in these T cell functions may be explained in part by the dilution of Con A-responding T cells by other, non-T cells. Older male BXSB mice exhibit a considerable degree of lymphoid hyperplasia due to B cell proliferation. As a result, there is an approximately threefold decrease in the frequency of T cells in the spleen or lymph nodes (28). However, the defects in mitogenic response and IL-2 production manifested by spleen cells of older male BXSB mice appear to be too profound to be explained entirely on the basis of the reduction in T cell frequency. Furthermore, increasing the spleen cell concentration in culture to relatively high densities did not overcome these defects (results not shown). Whether a selective loss (or decrease) of a particular T cell subset(s) occurs in older male BXSB mice is not yet known. Despite the profound decrease in Con A responsiveness and IL-2 production in older male BXSB mice, T cells of such mice perform normally in other ways such as alloreactivity, generation of cytotoxic cells after viral infection, and clearance of *Listeria monocytogenes* after systemic infection (33), as well as antigen-specific and antigen-nonspecific T cell suppression (1, 2).

The defective Con A-induced mitogenic responses and IL-2 levels manifested by MRL/l mice are somewhat surprising in view of the massive T cell proliferation that occurs in these mice (2, 28, 34). In fact, if IL-2 is the "universal" biologic mitogen for activated T cells, as has been suggested (6, 7), then one might expect an abnormally high level of IL-2 production by MRL/l T cells. However, the data presented here clearly demonstrate that this is not the case.

The possibility that lymphoid cells in older MRL/l mice suppress IL-2 production and/or proliferative responses was excluded because the addition of spleen cells from older MRL/l mice to spleen cell cultures of young syngeneic, congenic (MRL/n), and

histocompatible normal mice (C3H/St) did not result in decreased IL-2 production by the latter cell types. Similarly, our experiments failed to demonstrate a soluble suppressor material(s) in Con A-stimulated culture supernates of older MRL/l mice. Therefore, the combined results exclude the possibility that the reduced IL-2 levels manifested by MRL/l mice result from cells or substances that can inhibit IL-2 action or inactivate it.

These studies also established that, in addition to defective IL-2 production, T cells of MRL/l mice fail to respond in a normal manner to exogenous IL-2 when incubated together with Con A or allogeneic cells. This relative lack of response to IL-2 could be explained by several mechanisms, among them the relative lack of surface receptors for IL-2 or the degradation of IL-2, e.g., by some enzyme(s). Adsorption studies demonstrated that IL-2-containing culture supernates retained their full biologic activity after incubation with fresh or Con A-stimulated MRL/l splenocytes; this contrasted with the ability of Con A-activated C3H spleen cells to adsorb all IL-2 activity from the same preparation. Taken together with the above co-culture experiments, this finding strongly suggests that unstimulated or mitogen-stimulated spleen cells of older MRL/l mice have a relative lack of IL-2 receptors and do not inactivate IL-2 molecules. The fact that the massively proliferating T cells found in MRL/l mice do not respond to or adsorb exogenous IL-2 may suggest that the in vivo proliferation of these cells is IL-2 independent, as has recently been suggested in the case of leukemic T cells (35).

In addition to reduced Con A reactivity, older MRL/l mice display a similar defective proliferative response to allogeneic stimulator cells in both spleen and lymph nodes. Whether this reduced alloreactivity reflects decreased IL-2 production during the allogeneic MLR has not been directly tested in our experiments, but it is evident that the response to exogenous IL-2 in the MLR is defective. In contrast, spleen cells of older MRL/l mice appear to develop normal allogeneic CTL responses, both on the population level and in terms of CTLp frequency, in accord with our previous studies (2, 9, 33, 36). On the other hand, the CTLp frequency (against $H\text{-}2^b$ alloantigens) in the mesenteric lymph nodes of these mice is greatly reduced, being about 12-fold lower than in normal C3H/St mice. The differences in cytolytic activity between spleen and lymph node cells may reflect differences in T cell subset compositions in these two organs, as discussed below.

The Lyt phenotype of T cells in the lymph nodes of MRL/l mice has been analyzed by ourselves (28) and others (37). The most conspicuous findings in these studies are the almost complete disappearance of the Lyt-23^+ subset, the great reduction of Lyt-123^+ cells, the normal frequencies of strongly Lyt-1^+, cells and the massive increase of an Lyt-"null" as detected by cytotoxicity testing (28), or a weakly Lyt-1^+ (by fluorescence-activated cell sorter analysis) (37) T cell subset. The exact nature of the weakly Lyt-1^+ cells and their position in the T cell differentiation scheme are not clear. Because the CTL precursors are included within the pool of Lyt-123^+ or Lyt-23^+ T cells (38, 39), the marked reduction in CTLp frequency in the mesenteric lymph nodes of older MRL/l mice can be explained by the great dilution of these T cell subsets by the proliferating weakly Lyt-1^+ cells. A similar analysis of Lyt subsets in MRL/l splenocytes is lacking. However, the proliferation of T cells in older MRL/l mice is more apparent in lymph nodes than in spleens with ~30:1 and 10:1 T to B cell ratios in these two organs, respectively (2, 28). Thus, although the overall

production of and response to IL-2 in the spleens of older MRL/l mice are markedly reduced, the available IL-2 in this organ, in contrast to lymph nodes, may be enough for the generation of CTL against a given alloantigen, considering the fact that only 1 in 710 T cells would require the amplifying signal of IL-2 in order to differentiate into $H\text{-}2^b$-specific CTL.

The phenotype of IL-2-producing and MLR-responding T cells has been determined to be largely Lyt-1^+ (38, 40). Whether the defect in IL-2 production involves exclusively the rapidly proliferating, immature, weakly Lyt-1^+ cells of the older MRL/l mice or whether there exists in addition a defect of the mature, strongly Lyt-1^+ cells in these mice remains to be determined. The reduced Con A response and IL-2 production even in young MRL/l mice (6 wk old), which do not as yet manifest lymphoid hyperplasia, would suggest that even the strongly Lyt-1^+, nonproliferating cells of these mice exhibit, to some degree, the above defects.

An alternative way to explain the defective IL-2 production by T cells of MRL/l mice is related to the role of macrophages in IL-2 production. It has been shown that a soluble product, lymphocyte-activating factor (41) or interleukin 1 (5), secreted by activated macrophages, is required for IL-2 production (42, 43). However, if a macrophage defect (primary or secondary, due to the dilution by the proliferating T cells) was the underlying reason for the reduced IL-2 production, the addition of exogenous IL-2 should have bypassed this defect and allowed normal proliferative responses. However, our studies indicated otherwise.

Recently, it was reported that IL-2-dependent lines of T cells from MRL/l mice could be established in the absence of deliberate exposure to antigen (37). This suggests the existence of IL-2-responsive cells in MRL/l mice. However, our findings that only a minority of T cells in older MRL/l mice respond to IL-2 in vitro suggest that the lines established by Lewis et al. (37) were highly selected and are not necessarily representative of the abnormally proliferating T cells of older MRL/l mice.

To summarize, the studies reported here show that the overall response to Con A and production of IL-2 in most SLE mice are defective at one time or another in their life. Because such defects appear relatively late, after various autoantibodies have appeared in some of these strains of mice such as BWF_1 and MRL/n, we conclude that these defects probably are not of primary importance in the disease of these strains. Similar defects that appear early in male BXSB mice, although possibly due, at least in part, to a dilution of the responding T cells by the proliferating B cells, may be attributed to some yet undetermined changes in T cell subsets. The most severe defects appear in MRL/l mice. Because these defects appear, although to a lesser extent, before evidence of abnormal T cell proliferation, it is clear that such defects are not entirely caused by the lymphoproliferative disorder.

It is of interest to note that despite the continuous stimulation of the autoimmune mice by a plethora of autoantigens expected to occur in vivo, in no instance did we detect an increased spontaneous (i.e., without mitogen stimulation) IL-2 production in vitro, compared with normal mice. The in vivo significance of these findings and, moreover, the role of the defects in IL-2 production-response circuit in the etiopathogenesis of murine lupus, in at least some of these strains, are questions that might be answered by future experimentation.

Summary

In the studies reported here, we have analyzed the production and consumption of T cell growth factor, more recently termed interleukin 2 (IL-2), as well as some cell-mediated immune functions, in murine strains [MRL, BXSB, NZB, and (NZB × NZW)F_1] manifesting systemic lupus erythematosus (SLE)-like syndromes. Young (4–6 wk) or old (4–8 mo) autoimmune or normal mice were studied and compared with regard to the following T cell functions in vitro after stimulation with concanavalin A (Con A): (*a*) mitogenic response; (*b*) IL-2 levels in culture supernates; and (*c*) the ability to respond to and adsorb IL-2. In addition, proliferative activity in the allogeneic mixed leukocyte culture and frequency of alloreactive cytotoxic T lymphocyte precursors (CTLp) were analyzed in some of these strains. Reduced Con A-induced mitogenic responses and IL-2 production appeared at 3–6 wk of age in the early, severe SLE developing strains MRL/Mp-*lpr/lpr* (MRL/l) and male BXSB and progressed thereafter. Similar defects appeared at a later stage in MRL/Mp-+/+ and (NZB × NZW)F_1 hybrid mice, which develop late disease. Detailed analysis of cells from the enlarged lymph nodes and spleens of older MRL/l mice demonstrated that such cells: (*a*) responded poorly to Con A or allogeneic stimulator cells, even in the presence of exogenous IL-2; (*b*) did not suppress IL-2 production by normal spleen cells; (*c*) were relatively incapable of adsorbing or inactivating IL-2; and (*d*) had a markedly reduced anti-$H\text{-}2^b$ CTLp frequency in the mesenteric lymph nodes but a normal one in spleen. These results indicate that the proliferating Thy-1.2^+, Lyt-1^+ T cells in MRL/l mice are defective in their responses to mitogenic stimuli, in IL-2 production, and in expression of acceptor sites for IL-2. The relevance of these defects to the MRL/l disease as well as to the role of IL-2 in autoimmunity in general remains to be determined.

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