Deficit of Interleukin 2 Production Associated with Impaired T-Cell Proliferative Responses in *Mycobacterium lepraemurium* Infection

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C57BL/6 and BALB/c mice were infected intravenously with 10^7 *Mycobacterium lepraemurium* (MLM). At various times after infection, spleen cells were tested for their capacity to proliferate in vitro in response to concanavalin A (ConA) and to allogeneic cells. The generation of alloreactive cytotoxic T lymphocytes was also studied. The mitogen- and allogeneic-cell-induced blastogenesis of splenocytes from MLM-infected C57BL/6 and BALB/c mice was shown to be depressed during infection. The maximal decrease occurred 6 months after infection. Conversely, no reduction in the ability to generate alloreactive cytotoxic T lymphocytes was observed even after 6 months of infection. At the same time, interleukin 2 (IL2) activity generated by ConA stimulation of infected splenocytes was measured in both strains. IL2 activity in the ConA-stimulated culture supernatants was decreased as early as 1 month after MLM inoculation as compared with supernatants from age-matched control mice. Thus, IL2 production by infected-mouse spleen cells was shown to decline earlier than their proliferative responses to ConA and to allogeneic cells. ConA-induced T-cell blasts from infected mice showed a reduced ability to proliferate when incubated with an IL2-containing reference supernatant from ConA-stimulated normal spleen cells. These data suggest that a defect in IL2 production and utilization might contribute to the impairment of T cell-mediated immunity observed in MLM-infected mice.

Lepromatous leprosy in humans is characterized by an extensive bacillary invasion, a marked impairment of specific T cell-mediated immunity to *Mycobacterium leprae*, and a generalized state of anergy (4, 14). The infection of mice with *Mycobacterium lepraemurium* (MLM) induces a progressive form of murine leprosy which is, in many ways, comparable to human lepromatous leprosy and has been used as an experimental model to study immunity to mycobacterial infection (8, 9, 17, 19).

Susceptible mice infected either intravenously or intraperitoneally with large doses of MLM have been shown to display impaired specific and nonspecific T cell-mediated immunity both in vivo (23) and in vitro (21, 22) and impaired T cell-dependent antibody responses to sheep erythrocytes (5, 37).

Previous studies have suggested a major role for T-cell growth factor (13), an antigen-nonspecific lymphokine more recently termed interleukin 2 (IL2) (1), in the regulation of both cell-mediated and humoral T cell-dependent immune response (for a review, see 12, 28, 36).

IL2 is a genetically unrestricted soluble factor which is produced by T cells when stimulated with either T-cell mitogens (3) or allogeneic cells (34). IL2 has been reported to induce thymocyte proliferation after mitogenic stimulation (26), to promote the proliferation and the generation of cytotoxic T lymphocytes (CTL) from an alloantigen-stimulated T-cell population (34), and to augment antibody synthesis by splenocytes from nude mice (35). IL2 has also been shown to maintain antigen-specific activated helper T cells and specific alloreactive cytolytic T cells in long-term culture (35). IL2 production requires a macrophage-derived monokine termed lymphocyte-activating factor (20, 29) or IL1 (1). IL2 interacts with lectin/antigen-activated T cells via a receptor, through which it initiates the T-cell proliferative response (11, 24). Because of the important role believed to be played by IL2 in T-cell growth and differentiation and in view of several abnormalities in T-cell responsiveness manifested by MLM-infected mice, we analyzed patterns of IL2 production and utilization by spleen cells from infected BALB/c or C57BL/6 mice. Their relationships to various cell-mediated immune responses, such as mitogen-induced

T-cell proliferation, the mixed lymphocyte reaction (MLR), and the generation of CTL were studied during infection.

Our results suggest that IL2 production or recognition or both might contribute to the observed decline in T-cell functions in murine leprosy.

MATERIALS AND METHODS

Mice. Male BALB/c and female C57BL/6 mice were obtained from Iffa Credo (Fresnes, France). All animals were 7 to 8 weeks of age at the time of infection.

MLM. The strain of MLM, obtained from P. H. Lagrange (Institut Pasteur, Paris, France), was maintained by successive passage into susceptible albino rats. Purified suspensions were obtained from nonulcerated lepromata, 6 to 7 months after the subcutaneous injection of MLM. Bacilli were harvested, purified by differential centrifugation (200 to 3,000 × g), diluted to the appropriate concentration, and stored at −80°C until used.

Infection of mice. C57BL/6 and BALB/c mice were inoculated intravenously (in the tail vein) with 10^7 MLM. Age-matched, uninfected mice were used as controls in all experiments.

Counting of bacilli in the spleens of infected mice. At different times after infection, five mice were sacrificed, and their spleens were homogenized with an Ultra-Turrax in 5 ml of saline. Appropriate dilutions of homogenates in saline were used to make 1-cm^2 smears on slides (Bioměrieux, Lyon, France), which were stained with auramin and counted with a Leitz fluorescent microscope by the Shepard technique (27). The number of stained acid-fast bacilli (AFB) per spleen and the arithmetic mean per group were calculated.

Cell preparation. At various times after infection, inoculated and uninfected control mice were killed by cervical dislocation, and their spleens were removed under sterile conditions. Spleen cells were washed twice in Hanks balanced salt solution (HBSS) and suspended in RPMI 1640 (Boehringer, Mannheim, Germany) medium supplemented with 2 mM glutamine, 100 IU of penicillin per ml, 100 μg of streptomycin solution (Boehringer) per ml, 1% gentamicin (1 mg/ml; Boehringer), 5×10^{-5} M 2-mercaptoethanol, and the appropriate concentration (see below) of fetal calf serum (FCS) (GIBCO, Glasgow, Scotland).

Cells were cultured in this medium in a 5% CO_2, humidified incubator at 37°C. All cultures were done in triplicate or quadruplicate.

ConA-induced mitogenic responses. A total of 2.5 × 10^5 spleen cells were incubated in 0.2 ml of medium containing 10% FCS and concanavalin A (ConA; Pharmacia Fine Chemicals, Uppsala, Sweden) (5 to 1.25 μg/ml) in flat-bottomed microtiter plates (no. 3042; Falcon Plastics, Oxnard, Calif.). Similar cultures without ConA were used as controls. Cultures were pulsed with 1 μCi of tritiated thymidine ([³H]TdR) (TRA 310; Amersham Corp., Arlington Heights, Ill.; specific activity, 2 Ci/mmol) after a 24-h incubation and harvested 24 h later onto Titertek filter papers with a Skatron cell harvester (Flow Laboratories, Inc., Hamden, Conn.). Filters were dried and counted in a liquid scintillation counter.

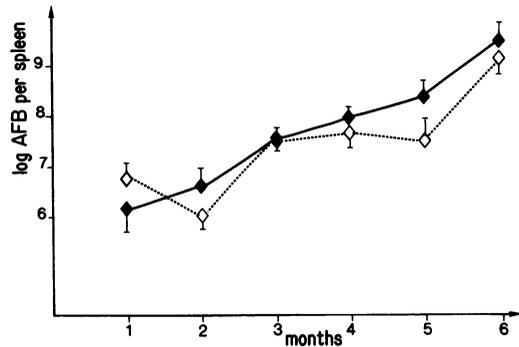**MLR.** A total of 2.5 × 10^5 responder spleen cells

FIG. 1. Kinetics of growth of MLM in the spleen of C57BL/6 or BALB/c mice after intravenous injection of 10^7 mycobacteria. Results are expressed as the arithmetic mean ± the standard deviation of bacilli counts from five spleens. Symbols: ♦, C57BL/6 mice; ◊, BALB/c mice.

from C57BL/6 (H-2^b) or BALB/c (H-2^d) mice were cocultured with 5×10^5 2,000-rad-irradiated allogeneic spleen cells from DBA/2 (H-2^d) or C57BL/6 (H-2^b) mice, respectively, in 0.2 ml of medium containing 10% FCS, in Falcon flat-bottomed microtiter plates. Similar cultures of responder cells with irradiated syngeneic spleen cells were set up as controls. On day 3, cultures were pulsed with 1 μCi of [³H]TdR and harvested 24 h later as described above.

In vitro generation and assay of CTL. Responder and stimulator cells were used at the same concentration as for studying the proliferative response in the MLR. C57BL/6 responder cells (2.5 × 10^6) were cultured with irradiated (2,000 rads) DBA/2 spleen cells (5 × 10^6) in 2 ml of medium supplemented with 10% FCS in multiculture plates (Linbro, Hamden, Conn.) for 5 days. The cytotoxic activity of the spleen cell culture was assayed by a microcytotoxicity assay employing ⁵¹Cr-labeled DBA/2 (H-2^d) P815 mastocytoma target cells as described by Thorn et al. (31). Briefly, labeled target cells were obtained by incubating P815 cells with ⁵¹Cr for 1 h at 37°C. Graded numbers of viable sensitized cells (from 2.5 × 10^4 to 5 × 10^5) harvested on day 5 were incubated at 37°C for 4 h with 10^4 ⁵¹Cr-labeled target cells. Each effector cell number was tested in triplicate. Then, half of the supernatant (i.e., 100 μl) was collected from each well, and the radioactivity of each sample was counted in a gamma counter (Kontron). Controls consisted of 0.2 ml of medium alone (spontaneous release) and of 0.2 ml of a detergent solution (RBS 25, 2%; Verrefer, Chatenay-Malabry, France) (maximal release).

The percentage of specific lysis was calculated for each effector/target cell ratio as follows: percent specific lysis = 100 × [(experimental cpm release − spontaneous cpm release)/(maximal cpm release − spontaneous cpm release)]. Maximal release averaged 85 to 100% of the total radioactivity introduced, and the spontaneous release never exceeded 20% of the maximal release. Results for each mixed culture were expressed in 50% lytic units per culture as described by others (7).

ConA-induced IL2 production. A total of 10 × 10^6 spleen cells were cultured in 2 ml of medium supple-
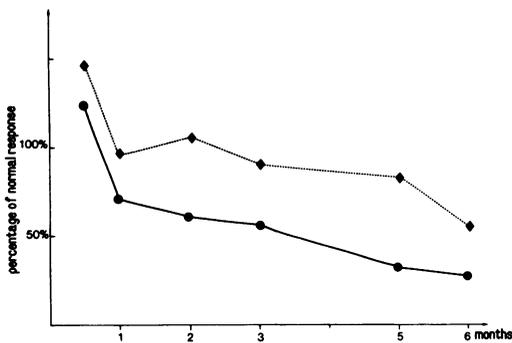
FIG. 2. Proliferative response to ConA (2.5 μg/ml) of spleen cells from MLM-infected mice. The proliferative response to ConA was assessed by [³H]TdR incorporation by a pool of five spleens from infected C57BL/6 (●) or BALB/c (◆) mice and age-matched controls. Results obtained in infected mice are expressed as the percentages of the response of the normal controls and represent the mean of three or more experiments. [³H]TdR incorporation averaged 114 ± 14 kcpm in normal C57BL/6 spleen cells cultured with ConA (4,107 ± 902 cpm without ConA) and 165 ± 19 kcpm in ConA-stimulated normal BALB/c spleen cells (4,307 ± 941 cpm without ConA) (mean ± the standard error of the mean of 16 experiments).

mented with 2% FCS in tissue culture plates (multidish nunclon delta, 24 wells; Nunc, Roskilde, Denmark) with 2.5 μg of ConA per ml. The supernatants were harvested 24 h later and supplemented with 20 mg of α-methyl-D-mannoside (αMM; Sigma Chemical Co., St. Louis, Mo.) per ml. These supernatants were then filtered through 0.2-μm filters (Schleicher & Schull, Dassel, Germany) and stored at −20°C until used.

IL2 activity in these supernatants were measured as described by Andersson et al. (3), using ConA blasts as target cells. ConA blasts were prepared by culturing 2 × 10⁶ spleen cells in 2 ml of culture medium containing 5% FCS and 2.5 μg of ConA per ml. After 72 h of culture, the cells were washed twice in RPMI 1640 supplemented with 20 mg of αMM per ml. These blasts (3 × 10⁴) were then recultured for 72 h in flat-bottomed wells of microtiter plates in 0.2 ml of medium containing 10% FCS and serial (1/2 to 1/16) dilutions of supernatants to be tested for their IL2 activities. IL2-induced blast proliferation was assessed by measuring the incorporation of tritiated thymidine as described above, after a 4-h pulse with 1 μCi [³H]TdR per well.

Proliferation induced by individual supernatants tested was compared with that obtained the same day with a supernatant from normal BALB/c spleen cells stimulated with ConA, harvested as described above, and used as a reference for IL2 activity. The same batch stored in samples at −20°C was used for all experiments.

Expression of IL2 activity. IL2 activity was evaluated and expressed as described by Dauphinee and al. (11). The stimulation index (SI) [(mean cpm from ConA blasts cultured with supernatants)/(mean cpm from ConA blasts cultured with medium)] was calculated for each dilution of the experimental supernatants and of the reference supernatant. SIs were

plotted against log 2 dilutions, and linear regression curves were calculated by the least-squares method. The intercept of each curve with the y axis, representing the theoretical SI obtained with undiluted supernatant, was determined. The value obtained for the reference supernatant was arbitrarily defined as 10² U of IL2 activity, and the number of units for each experimental supernatant was then calculated by the following formula: units of IL2 = 100 × (SI with undiluted experimental supernatant/SI with undiluted reference supernatant).

Responsiveness to IL2 of ConA blast derived from normal and MLM-infected mice. At various times during MLM infection, ConA blasts were prepared as described above, from normal and infected mice, and their ability to proliferate in the presence of the reference IL2-containing supernatant was tested. Results are expressed as the mean of SIs with the undiluted reference supernatant as defined above.

Evaluation of spleen cell subsets in normal and MLM-infected mice. The percentages of immunoglobulin-containing (Ig⁺), Thy1⁺, Lyt1⁺, and Lyt2⁺ spleen cells were determined in an indirect immunofluorescence assay on the whole spleen, which includes both lymphoid and nonlymphoid spleen cells. A total of 10⁶ spleen cells were incubated at 4°C for 30 min with 5 μl of anti-Lyt1-2, anti-Lyt2-2, or anti-Thy1-2 (NEI 017, 006, 001; New England Nuclear Corp., Boston, Mass.) monoclonal antibodies (diluted 1/10) in a total volume of 50 μl. Cells were washed twice in HBSS supplemented with 0.2% sodium azide and 5% FCS. Purified fluorescein-conjugated goat anti-mouse IgG (Miles Laboratories, Inc., Elkhart, Ind.) antibodies were then added for 30 min. A sample was incubated with goat
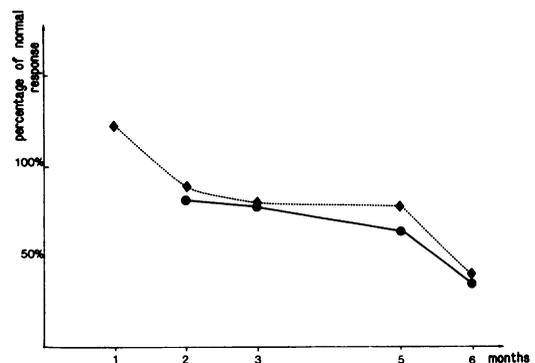

FIG. 3. Proliferative response to allogeneic cells of spleen cells from MLM-infected mice. The MLR was assessed by [³H]TdR incorporation by a pool of five spleens from MLM-infected C57BL/6 (●) or BALB/c (◆) mice and age-matched controls. Results obtained in infected mice are expressed as the percentages of the response of the normal controls and represent the mean of three or more experiments. [³H]TdR incorporation averaged 39.6 ± 4 kcpm in the MLR of normal C57BL/6 cells stimulated with allogeneic cells (5,040 ± 823 cpm with syngeneic cells) and 57 ± 8 kcpm in allogeneic-stimulated normal BALB/c cells (9,182 ± 1,766 cpm cultured with syngeneic cells) (mean ± the standard error of the mean of 19 experiments for C57BL/6 mice and 12 experiments for BALB/c mice).
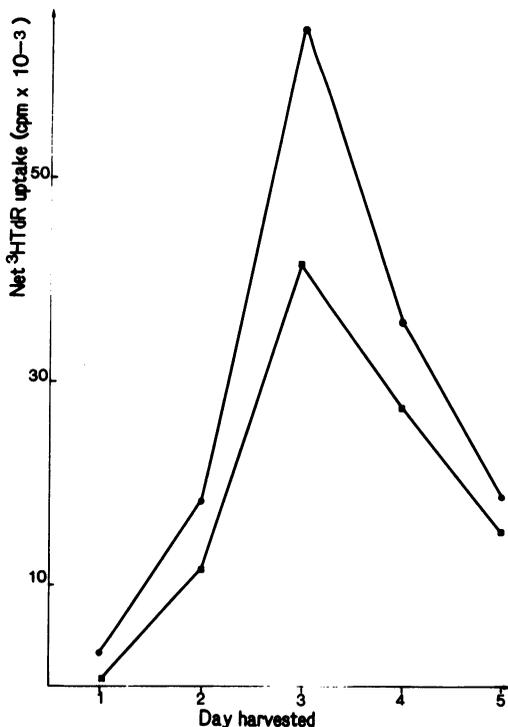
FIG. 4. Kinetics of MLR of spleen cells from MLM-infected BALB/c mice. A total of 2.5×10^5 responder cells from normal (●) or MLM-infected (■) mice (4 months after infection) were cultured with 5×10^5 irradiated (2,000 rads) syngeneic or allogeneic spleen cells and harvested on the days indicated, after a 24-h pulse with 1 μCi of tritiated thymidine.

anti-mouse immunoglobulin alone without previous incubation with monoclonal antibodies, to measure the percentage of Ig^+ positive spleen cells. Cells were counted under UV light by means of a Leitz fluorescent microscope. The percentages of $Thy1^+$, $Lyt1^+$, and $Lyt2^+$ cells were obtained by subtracting the percentage of directly stained Ig^+ cells from the percentages of cells labeled after successive incubations with the appropriate monoclonal anti-T-cell antibodies and goat anti-mouse IgG.

RESULTS

Course of the infection. The infection was evaluated by counting AFB in the spleens of five mice per group, every month after the intravenous injection of 10^7 MLM.

The progression of the infection is shown in Fig. 1. Bacillus growth progressed similarly in C57BL/6 and BALB/c mice, reaching the same level of 10^9 AFB per spleen at 6 months of infection.

Proliferative response to ConA. The proliferative response of spleen cells to ConA (from 1.25 to 2.5 μg/ml) tested at various times after infection is illustrated in Fig. 2. In both C57BL/6 and

BALB/c mice, a transient enhancement of the ConA-induced mitogenic response of spleens from infected mice was observed 15 days after MLM inoculation. In both infected mouse strains, a progressive decrease of ConA-induced proliferation was then observed. At any time during infection, the proliferative response to ConA, expressed as the percentage of the results obtained from normal controls, was lower in infected C57BL/6 mice than in infected BALB/c mice.

Proliferative response to allogeneic cells. The allogeneic response in vitro was also tested at various times after MLM injection in normal and infected BALB/c or C57BL/6 mice, (Fig. 3). In both C57BL/6 or BALB/c mice, a slow depression of MLR was observed during the first 5 months of infection, and a further decrease occurred after 6 months of infection. At any time during infection, MLR was similarly depressed in both strains. It should be stressed that in C57BL/6 mice, the allogeneic proliferative response declined less rapidly than did ConA-induced proliferation.

The deficient MLR manifested by infected spleen cells was not due to altered kinetics of the response. The experiment summarized in Fig. 4 demonstrates that the proliferative deficiency in
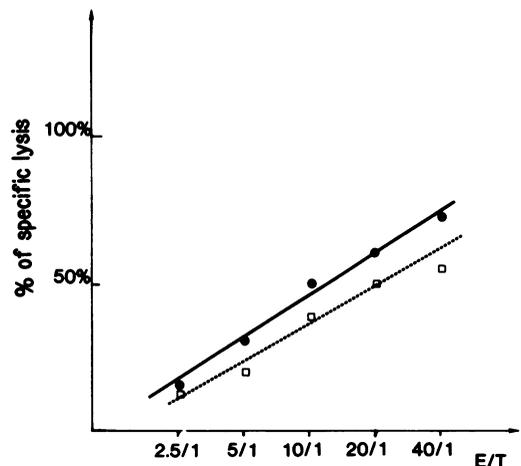

FIG. 5. Allogeneic CTL response of MLM-infected C57BL/6 mice. A total of 2.5×10^6 responder cells from normal (●) and MLM-infected C57BL/6 ($H-2^b$) (□) mice (4 months after infection) were cultured with 5×10^6 irradiated (2,000 rads) syngeneic or allogeneic DBA/2 ($H-2^d$) spleen cells. The cytotoxic activity was assessed by a microcytotoxicity assay employing ^{51}Cr-labeled P815 mastocytoma target cells as described in the text. Results are expressed as 50% lytic units per culture. Spontaneous ^{51}Cr release was 13% of the maximal release, which represents 90% of total radioactivity introduced. ^{51}Cr release in responder spleen cells cultured with syngeneic cells did not exceed the spontaneous release value.
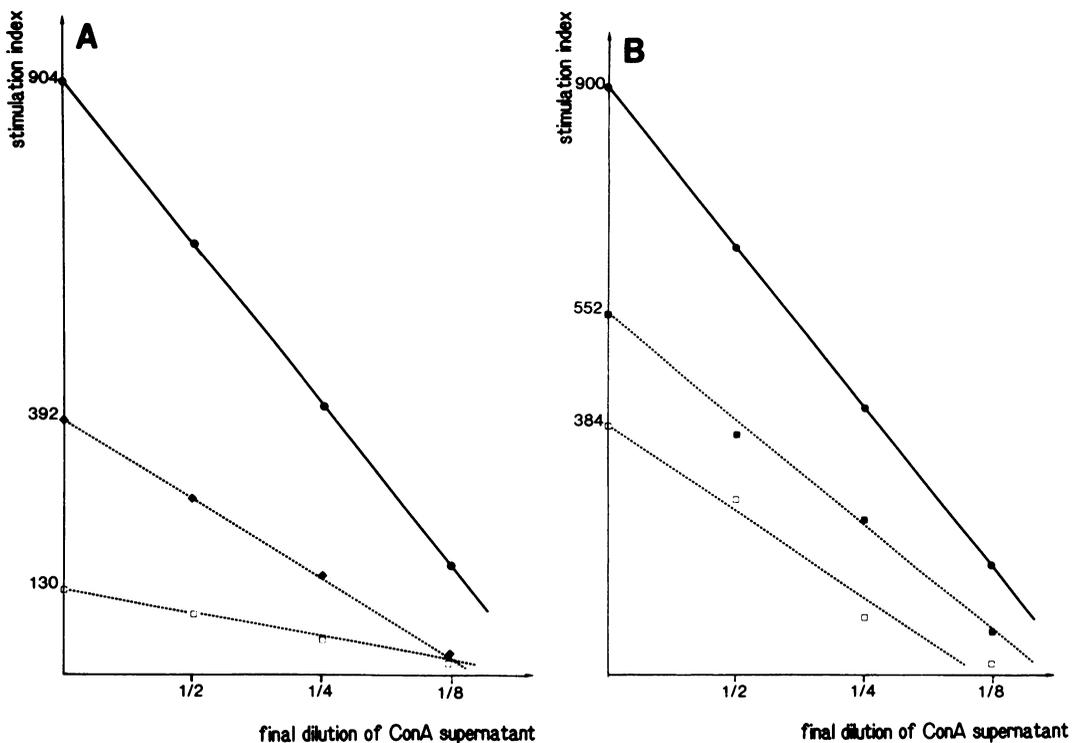
FIG. 6. Effect of MLM infection on IL2 production. IL2 production was assessed by measuring the proliferation of ConA blasts incubated with supernatants of ConA-stimulated spleen cells from 2-month-infected C57BL/6 (A) or BALB/c (B) mice. Symbols: □, MLM-infected mice; ■, age-matched controls; and ●, reference IL2-containing supernatants. Units of IL2 activity in ConA supernatants from infected C57BL/6 or BALB/c mice were 14 or 42, respectively, and 43 and 61 in supernatants from age-matched uninfected C57BL/6 or BALB/c mice.

the allogeneic MLR was consistent when cultures were harvested daily between days 1 and 4 after culture initiation.

CTL generation. No alteration of the generation of CTL among spleen cells from infected C57BL/6 mice stimulated with irradiated DBA/2 spleen cells was observed at any time during the infection, when compared with normal age-matched controls (Fig. 5). Spleen cells from infected C57BL/6 mice generated normal alloreactive CTL despite their marked deficit in alloantigen-induced proliferation.

IL2 production by spleen cells from MLM-infected mice. In both C57BL/6 and BALB/c strains, supernatants from the ConA-stimulated spleen cells of MLM-infected mice induced a much smaller proliferation of normal ConA blasts than did similar supernatants from normal spleen cells. A typical experiment is shown in Fig. 6 A and B, indicating that IL2 activity was decreased in supernatants from mice infected 2 months earlier. IL2 production was measured at different times during infection (Fig. 7). IL2 production declined early, from 1 month after infection in both strains, to reach its minimal level at 3 months in C57BL/6 mice and 5 months

in BALB/c mice (16 and 28% of the IL2 production of normal age-matched controls, respectively). Thus, IL2 production in both strains decreased more rapidly than did their proliferative responses to ConA or allogeneic cells.

IL2-induced proliferation of ConA blasts of MLM-infected mice. ConA blasts were prepared with spleen cells from infected and control C57BL/6 mice, and their capacity to proliferate when incubated with a reference IL2-containing supernatant was tested. ConA blasts prepared from infected C57BL/6 mice 6 months after infection proliferated less than did control ConA blasts in the presence of the reference IL2 ($P \leq$ 0.02). The arithmetic means ± the standard error of the mean of SIs calculated from six experiments were 352 ± 93 for infected mice and 552 ± 125 for normal mice.

B- and T-cell surface markers. In the two mouse strains, no variation in the lymphocyte subset percentages was observed during the first 4 months of infection, as illustrated in Table 1. However, a slight reduction of the number of Ig^+, $Thy1^+$, and $Lyt1^+$ spleen cells was observed in infected BALB/c mice ($P \leq 0.05$) after 6 months of infection, with a correlative increase
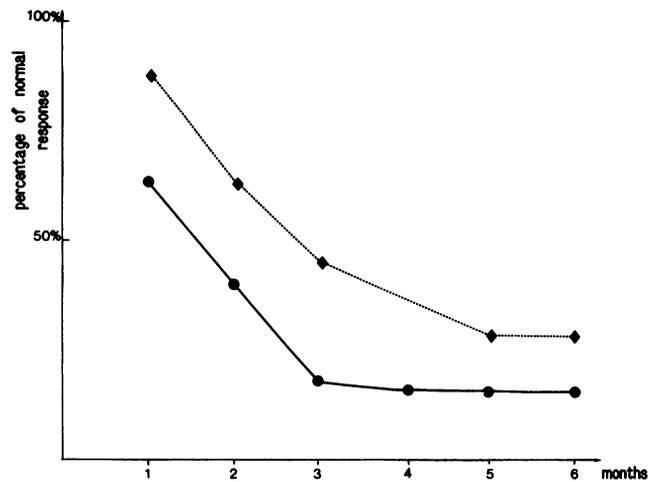
FIG. 7. Kinetics of IL2 production in MLM-infected C57BL/6 and BALB/c mice during infection. Results are expressed as the percentages of the number of IL2 activity units obtained in age-matched control mice in a T-cell growth assay described in the text. Units of IL2 activity are calculated from the mean of at least three experiments. In each experiment, data were obtained from a pool of five normal or MLM-infected C57BL/6 (●) or BALB/c (♦) mice.

in the percentage of null cells (Ig⁻, Thy1⁻). In C57BL/6 mice, a significant decrease of Ig⁺ spleen cells was also observed, as well as an augmentation of the relative number of null cells. A diminution of the percentage of Thy1⁺ and Lyt1⁺ also appeared, but did not reach statistical significance.

DISCUSSION

We have shown that proliferative responses to ConA and to allogeneic cells were depressed throughout MLM infection, whereas no significant modification in the generation of CTL could be detected. It has been well established that the inoculation of rats and mice with large doses of

MLM or other pathogenic mycobacteria induces deficits of in vitro immune functions, such as responses to polyclonal T-cell mitogens (32, 38) and specific antigens (21) and an impairment of in vivo T cell-mediated immunity, such as delayed skin graft rejection (6, 23), reduced delayed-type hypersensitivity response to sheep erythrocytes (6), and T-dependent antibody response to sheep erythrocytes (5, 25, 38).

The early decrease of IL2 production that we observed in MLM-infected mice may contribute to the alteration of T-cell functions, since the presence of IL2 is considered an absolute requirement for in vitro T-cell proliferation and maturation (34). It should be stressed that IL2 production in MLM-infected mice started to

TABLE 1. Percentage of splenic lymphocytes from normal and infected BALB/c and C57BL/6 mice bearing surface Ig, Thy1, and Lyt antigens during MLM infection[a]

Surface markers	BALB/c			C57BL/6		
	2- to 4-month infected[b]	6-month infected[c]	Normal[d]	2- to 4-month infected[e]	6-month infected[c]	Normal[f]
Ig	46 ± 2	44 ± 1	48 ± 2	50 ± 2	45 ± 2	51 ± 1
Thy1	39 ± 3	28 ± 2	40 ± 2	27 ± 2	21 ± 1	26 ± 2
Lyt1	39 ± 3	27 ± 1	38 ± 3	31 ± 3	22 ± 3	27 ± 3
Lyt2	20 ± 3	13 ± 3	16 ± 2	16 ± 4	13 ± 2	16 ± 3

[a] Spleen cells from infected and uninfected mice were labeled with monoclonal antibodies and fluorescein-conjugated purified goat anti-mouse IgG antibodies. Stained cells were counted by fluorescent microscopy. Since no modification of lymphocyte subset distribution occurred with age, results from all age-matched controls were pooled.

[b] Pooled results from 10 experiments are expressed as the arithmetic mean ± the standard error of the mean.

[c] Pooled results from three experiments.

[d] Pooled results from 12 experiments.

[e] Pooled results from six experiments.

[f] Pooled results from eight experiments.

decline before the alteration of proliferative responses to ConA and allogeneic cells. Surprisingly, allogeneic CTL were normally generated by spleen cells from infected mice, even after 6 months of infection, a time when both IL2 production and T-cell proliferation in MLR were strongly reduced. Such a discrepancy had already been noted by Altman et al. (2) in mice developing lupus-like syndrome. It might be suggested that residual IL2 levels are sufficient to support CTL cell differentiation but not T-cell proliferation.

The decline of IL2 production might be due to a primary numerical or functional defect of the Lyt1-bearing T cell secreting IL2. The cellular composition of spleens of both strains was not disturbed during the first 4 months of infection. The relative proportions of the different subsets of splenic lymphocytes were similar in infected mice and in age-matched controls, and no increase of Ig^- Thy^- null cells was observed. Thus, these results demonstrated that the dilution of Lyt1 IL2-secreting cells did not account for the early defect of IL2 production at this stage of infection. However, in spleen cells from 6 month-infected C57BL/6 and BALB/c mice, the relative number of Lyt1 cells and also Ig^+ cells tended to diminish. At this stage of infection, macrophages and other cells which participated in granulomatosis inflammation likely diluted out lymphoid cells; this dilution of Lyt1 T cells could contribute to the deficiency of proliferative responses and of IL2 production.

Since IL2 production is IL1 dependent (20–29), it could be hypothesized that the lack of IL1 secretion by infected macrophages could play a role in the failure of IL2 production. Indeed, mouse strains susceptible to subcutaneous MLM inoculation display macrophage abnormalities, such as a diminished efficacity in controlling mycobacterial multiplication in vivo as compared with resistant mice (22) or an enhancement of superoxide dismutase activity in splenic macrophages of MLM receptive mice (30).

In addition to the decrease of IL2 production, other mechanisms could likely participate in the depressed proliferative responses to ConA and allogeneic cells, especially during the advanced stage of infection. One of these mechanisms could be a defective sensitivity of ConA blasts to IL2 from infected mice since these splenic blast cells from 6 month-infected mice failed to respond in a normal manner to the reference IL2-containing supernatant. Moreover, at this late stage of infection, some suppressive material (factor or cell or both) might appear which would inhibit the proliferation of T helper cells and would be associated with the defect in IL2 production. It has previously been shown that

mice infected with various species of mycobacteria have splenic suppressor cells, either T cells (10, 33) or macrophages (16, 33), which can inhibit in vitro T cell-mediated responses (15, 39).

There is no major difference between C57BL/6 and BALB/c mice, since the levels of IL2 activity and of in vitro proliferative responses are very similar in both strains. Previous reports showed that neither C57BL/6 nor BALB/c mice resisted an intravenous injection of MLM, and both strains were found to be about equally susceptible to an inoculum of 10^7 bacilli (32), which is in agreement with our present findings of a minor difference in the course of infection between C57BL/6 and BALB/c mice when infected intravenously.

In conclusion, the data presented here suggest that the altered synthesis, recognition, or utilization (or all three) of T cell-secreted lymphokines, such as IL2, may contribute to the impaired T immune functions observed in MLM-infected mice. On the basis of these in vitro observations, the question is raised as to the possibility of restoring specific and nonspecific T cell-mediated immunity by injecting exogenous IL2 into mycobacteria-infected mice.

LITERATURE CITED

1. **Aarden, L. A., et al.** 1979. Revised nomenclature for antigen nonspecific T cell proliferation and helper factors. J. Immunol. 123:2928–2929.
2. **Altman, A., A. N. Theofilopoulos, R. Weiner, D. H. Katz, and F. J. Dixon.** 1981. Analysis of T cell function in autoimmune murine strains. J. Exp. Med. 154:791–808.
3. **Andersson, J., K. O. Gronvik, E. L. Larsson, and A. Coutinho.** 1979. Studies of T lymphocyte activation. I. Requirement for the mitogen dependent production of T cell growth factors. Eur. J. Immunol. 9:581–587.
4. **Bullock, W. E.** 1978. Leprosy: a model of immunological perturbation in chronic infection. J. Infect. Dis. 137:341–354.
5. **Bullock, W. E., E. M. Carlson, and R. K. Gershon.** 1978. The evolution of immuno-suppressive cell populations in experimental mycobacterial infection. J. Immunol. 120:1709–1716.
6. **Bullock, W. E., P. E. Evans, and A. R. Filomeno.** 1977. Impairment of cell mediated immune responses by infection with MLM. Infect. Immun. 18:157–164.
7. **Cerottini, J. C., M. D. Euger, H. R. MacDonald, and K. T. Brunner.** 1974. Generation of cytotoxic T lymphocytes in vitro. I. Response of normal and immune mouse spleen cells in mixed leucocyte culture. J. Exp. Med. 140:703–717.
8. **Closs, O.** 1975. Experimental murine leprosy: growth of Mycobacterium lepraemurium in C3H and C57BL/6 mice after footpad inoculation. Infect. Immun. 12:480–489.
9. **Closs, O.** 1975. Experimental murine leprosy: induction of immunity and immune paralysis to Mycobacterium lepraemurium in C57BL/6 mice. Infect. Immun. 12:706–713. 713.
10. **Collins, F. M., and D. S. Cunningham.** 1981. Systemic

Mycobacterium kansaii infection and regulation of the alloantigenic response. Infect. Immun. **32:**614–624.

11. **Dauphinee, M. J., S. B. Kipper, D. Wofsy, and N. Talal.** 1981. Interleukin 2 deficiency is a common feature of autoimmune mice. J. Immunol. **127:**2483–2487.

12. **Farrar, J. J., W. R. Benjamin, M. L. Hilfiker, M. Howard, W. L. Farrar, and J. Fuller-Farrar.** 1982. The biochemistry, biology and role of interleukin 2 in the induction of cytotoxic T cell and antibody forming B cell responses. Immunol. Rev. **63:**129–166.

13. **Gillis, S., and K. A. Smith.** 1977. Long term culture of tumor specific cytotoxic T cells. Nature (London) **268:**154–156.

14. **Godal, T.** 1978. Immunological aspects of leprosy. Present status. Prog. Allergy **25:**211–242.

15. **Klimpel, G. R., and C. S. Henney.** 1978. BCG-induced suppressor cells. I. Demonstration of a macrophage like suppressor cell that inhibits cytotoxic T cell generation *in vitro.* J. Immunol. **120:**563–569.

16. **Klimpel, G. R., M. Okada, and C. S. Henney.** 1979. Inhibition of *in vitro* cytotoxic responses by BCG-induced macrophage like suppressor cells. II. Suppression occurs at the level of a "helper" T cell. J. Immunol. **123:**350–357.

17. **Lagrange, P. H., and B. Hurtrel.** 1979. Local immune response to MLM in C3H and C57BL/6 mice. Clin. Exp. Immunol. **38:**461–474.

18. **Larsson, E. L.** 1981. Mechanisms of T cell activation. II. Antigen- and lectin dependent acquisition of responsiveness to TCGF is a non-mitogenic active response of resting T cells. J. Immunol. **126:**1323–1326.

19. **Lefford, M. J., P. J. Patel, L. W. Poulter, and C. B. Mackaness.** 1977. Induction of cell mediated immunity to *Mycobacterium lepraemurium* in susceptible mice. Infect. Immun. **18:**654–659.

20. **Mizel, S. B.** 1982. Interleukin 1 and T cell activation. Immunol. Rev. **63:**51–72.

21. **Navalkar, R. G., P. J. Patel, and M. V. Kanchana.** 1980. Studies on immune response to *Mycobacterium lepraemurium.* Int. Arch. Allergy Appl. Immunol. **62:**423–432.

22. **Preston, P. M.** 1982. Macrophages and protective immunity in *Mycobacterium lepraemurium* infections on a "resistant" (C57BL) and a "susceptible" (BALB/c) mouse strain. Clin. Exp. Immunol. **47:**243–252.

23. **Ptak, W., J. W. Gaugas, R. J. W. Rees, and E. C. Allison.** 1970. Immune responses in mice with murine leprosy. Clin. Exp. Immunol. **6:**117–122.

24. **Robb, R. J., A. Munck, and K. A. Smith.** 1981. T cell growth factor receptors. Quantification, specificity and biological relevance. J. Exp. Med. **154:**1455–1474.

25. **Rojas-Espinosa, O., M. Casoluengo-Mendez, and G. Vil-**

lanueva-Diaz. 1976. Antibody mediated immunity in CFW mice infected with *Mycobacterium lepraemurium.* Clin. Exp. Immunol. **25:**381–387.

26. **Shaw, J., V. Manticone, G. Mills, and V. Paetkau.** 1978. Effects of costimulator on immune response *in vitro.* J. Immunol. **120:**1974–1980.

27. **Shepard, C. C., and D. H. McRae.** 1978. A method for counting acid fast bacteria. Int. J. Lepr. **36:**78–82.

28. **Smith, K. A.** 1980. T cell growth factor. Immunol. Rev. **51:**337–357.

29. **Smith, K. A., L. B. Lachman, J. J. Oppenheim, and M. F. Favata.** 1980. The functional relationship of the interleukins. J. Exp. Med. **151:**1551–1556.

30. **Stach, J. L., F. Fumoux, M. Strobel, A. Baret, and M. Michelson.** 1981. Augmentation de l'activité dismutasique dans les leucocytes spléniques de souris sensibles au BCG et à *Mycobacterium lepraemurium.* C. R. Acad. Sci. **293:**575–578.

31. **Thorn, R. M., J. C. Palmer, and L. A. Manson.** 1974. A simplified ^{51}Cr release assay for killer cells. J. Immunol. Methods **4:**301–315.

32. **Turcotte, R.** 1980. Influence of route of MLM infection on susceptibility to mouse leprosy and on lymphoblastic transformation. Infect. Immun. **28:**660–668.

33. **Turcotte, R.** 1981. Evidence for two distinct populations of suppressor cells in the spleens of *Mycobacterium bovis* BCG sensitized mice. Infect. Immun. **34:**315–322.

34. **Wagner, H., and M. Rollinghoff.** 1978. T-T cell interactions during *in vitro* cytotoxic allograft responses. I. Soluble products from activated Ly 1+ T cells trigger autonomously antigen primed Ly 23+ T cells to cell proliferation and cytolytic activity. J. Exp. Med. **148:**1523–1538.

35. **Watson, J., S. Gillis, J. Marbrook, D. Mochizuki, and K. A. Smith.** 1979. Biochemical and biological characterization of lymphocyte regulatory molecules. I. Purification of a class of murine lymphokines. J. Exp. Med. **150:**849–861.

36. **Watson, J., and D. Mochizuki.** 1980. Interleukin 2: a class of T cell growth factors. Immunol. Rev. **51:**257–278.

37. **Watson, S. R., V. S. Sljivic, and I. N. Brown.** 1975. Defect of macrophage function in the antibody response to sheep erythrocytes in systemic *Mycobacterium lepraemurium* infection. Nature (London) **26:**206–208.

38. **Watson, S. R., and F. M. Collins.** 1980. Development of suppressor T cells in mice heavily infected with mycobacteria. Immunology **39:**367–373.

39. **Watson, S. R., and F. M. Collins.** 1981. The specificity of suppressor T cells induced by chronic *Mycobacterium avium* infection in mice. Clin. Exp. Immunol. **43:**10–19.