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Influence of Dose and Route of *Mycobacterium lepraemurium* Inoculation on the Production of Interleukin 1 and Interleukin 2 in C57BL/6 Mice

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Groups of C57BL/6 mice were infected either intravenously or subcutaneously with 10^5 or 10^8 *Mycobacterium lepraemurium* cells, and the ability of their splenic macrophages and T-cells to produce, respectively, interleukin 1 on lipopolysaccharide stimulation and interleukin 2 on concanavalin A stimulation was assessed during the course of infection. In all groups of infected mice, interleukin 1 production remained unaffected during the entire observation period, whereas interleukin 2 activity decreased as the infection progressed. Heavily infected mice (10^8 *M. lepraemurium* cells) showed an earlier and stronger deficiency interleukin 2 production by concanavalin A-stimulated spleen cells than did mice infected with a lower dose (10^5 bacilli), without detectable influence by the route of inoculation. In mice receiving 10^5 bacilli, minor differences were seen according to the route of infection, with a slight delay in interleukin 2 decrease in mice injected intravenously. In subcutaneously inoculated mice, the failure of spleen cells to produce interleukin 2 after concanavalin A stimulation did not correlate with the number of bacilli developing in the spleen, suggesting the existence of suppressor mechanisms acting at a distance from the site of inoculation.

Lepromatous leprosy is defined by an extensive dissemination of *Mycobacterium leprae* associated with an impairment of specific T-cell immune responses to the antigens of the bacilli both in vivo and in vitro (11, 33). The experimental model of murine infection by *Mycobacterium lepraemurium* provides a convenient model of human leprosy. Indeed, murine leprosy is also characterized by a severe deficit of specific and nonspecific T-cell-mediated immunity both in vivo and in vitro (2, 4, 27). Since *M. lepraemurium* is an intracellular pathogen, the induction of efficient cell-mediated immunity in this model was considered essential for the development of host resistance. (1, 5, 17, 21). Interleukins, such as interleukin 1 (IL1) and interleukin 2 (IL2), produced by macrophages and T-cells, respectively, have been shown to play a major role in the regulation of both cell-mediated immunity and humoral T-cell-dependent immune responses (8, 9, 24, 35). We have previously reported that an early in vitro decrease in IL2 production was observed in C57BL/6 mice during the course of *M. lepraemurium* infection after intravenous (i.v.) inoculation. This in vitro depression of IL2 activity was seen before the alteration of antigen- or mitogen-induced T-cell proliferative responses (15).

The route of administration and the dose of particulate antigens seemed to be important for the acquisition of T-cell-mediated immunity, with the dermal and subcutaneous (s.c.) routes being more effective than the i.v. route (19). In mycobacterial infections, similar observations have been reported (20, 30, 32). C57BL/6 mice were able to resist an s.c. inoculation of *M. lepraemurium* by mounting effective cell-mediated immunity, but they did not control an infection caused by i.v. inoculation (5, 6).

To investigate further the role of interleukins in the pathophysiology of experimental leprosy, IL1 and IL2 production was studied during the course of *M. lepraemurium* infection in C57BL/6 mice inoculated either s.c. or i.v. with a low or high dose of bacilli, e.g., 10^5 or 10^8, respectively. Our results suggested that the date of onset of the decrease of IL2 production was dependent on the dose of bacilli injected and to a lesser extent on the route of inoculation. However, after several months of infection, the deficiency in IL2 activity occurred regardless of the dose and the route used to infect the animals. On the other hand, IL1 production was never affected, even when IL2 production was low.

MATERIALS AND METHODS

Mice. Female C57BL/6 and C3H/HeJ (lipopolysaccharide negative) mice were obtained from Institut Pasteur, Paris, France. Mice were 7 to 8 weeks old at the time of infection.

M. lepraemurium infection. The *M. lepraemurium* strain was maintained as previously described (15). Mice were infected either s.c. or i.v. with 10^5 or 10^8 bacilli. Age-matched noninfected mice were used as controls. Every month, the progression of the infection was evaluated by counting the number of acid-fast bacilli (AFB) within the spleens and the footpads of infected animals by the auramin-staining method of Shepard and McRae (29).

Culture medium and cell preparation. At different times after infection, a minimum of nine mice from each group of infected and control animals were sacrificed. The spleen cells were dissociated with a Potter homogenizer, washed twice in Hanks balanced salt solution and resuspended in RPMI 1640 medium (Flow Laboratories, Hamden, Conn.) supplemented with 2 mM glutamine, 100 IU of penicillin per ml, 100 μg of streptomycin per ml, 5×10^{-5} M 2-mercaptoethanol, and the appropriate concentration of fetal calf serum (FCS) (GIBCO, Glasgow, Scotland).

ConA-induced mitogenic responses. Mouse spleen cells (2.5×10^5) from infected and control animals were cultured for 48 h in 96-well flat-bottomed microtest culture plates (Corning Glassworks, Corning, N.Y.) in culture medium supplemented with 5% FCS and 2.5 μg of concanavalin A (ConA) per ml as previously described (15). At 24 h before cell harvesting, 1 μCi of tritiated thymidine ([^{3}H]TdR) (specific

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TABLE 1. Proliferative response to ConA of spleen cells from *M. lepraemurium*-infected C57BL/6 mice

Time after infection (mo)	[^{3}H]TdR incorporation (net cpm) by route and dose of *M. lepraemurium* infection:[a]			
	10^8 i.v.	10^5 i.v.	10^8 s.c.	10^5 s.c.
1	168,607 ± 20,615	126,791 ± 21,298	124,777 ± 22,830	135,670 ± 23,357
2–3	98,642 ± 13,709	132,240 ± 14,495	93,981 ± 16,088	90,177 ± 15,550
6–7	57,548 ± 15,453	76,266 ± 19,119	82,172 ± 18,529	91,399 ± 20,256
Uninfected control mice[b]			140,254 ± 9,971	

[a] Results are expressed as the mean of net counts per minute ± the standard error of the mean (mean counts per minute of ConA-stimulated triplicate cultures minus mean counts per minute of nonstimulated cultures) obtained from at least three experiments including groups of containing three mice. cpm, Counts per minute.

[b] Pooled results from 34 normal mice.

activity, 2 Ci/mol; Amersham Corp., Arlington Heights, Ill.) was added to each well. Cultures were then harvested onto glass fiber filters, and [^{3}H]TdR incorporation was measured. Each culture was tested in triplicate.

IL1 production. Spleen cells (10^7) from infected and uninfected mice were incubated for 2 h in 2 ml of culture medium supplemented with 5% FCS in 35-mm plastic petri dishes (Corning). Nonadherent cells were removed by five repeat washings with Hanks balanced salt solution. Adherent spleen cells were then cultured for 48 h in 2 ml of culture medium supplemented with 5% FCS in the presence of 50 μg of *Salmonella typhimurium* lipopolysaccharide (LPS; Sigma Chemical Co., St. Louis, Mo.) per ml. Supernatants were then collected, centrifuged, filtered through 0.22-μ filters, and stored at −20°C until used.

IL1 bioassay. IL1 activity was assayed by using the technique previously described by Mizel et al. (23). Briefly, thymocytes from young C3H/HeJ mice (1.5×10^6 cells per well) were cultured for 72 h in 96-well flat-bottomed microtest plates in 0.2 ml of culture medium supplemented with 5% FCS and one-fourth-diluted IL1-containing supernatant in the presence or absence of 1 μg of phytohemagglutinin (PHA; Welcome, Paris, France) per ml. Preliminary studies showed that a one-fourth dilution of IL1-containing supernatant sustained optimal PHA-induced proliferative response of thymocytes. For each supernatant, results were expressed by substracting the counts per minute of thymocytes cultured with supernatant in the absence of PHA from the counts per minute of thymocytes cultured with IL1-containing supernatant and PHA.

IL2 production. Spleen cells from infected or uninfected mice were adjusted to 5×10^6 cells per ml and were cultured in 24-well flat-bottomed culture plates (Linbro; Flow Laboratories) in 2 ml of culture medium supplemented with 2% FCS and 2.5 μg of ConA per ml as previously reported (15). After 22 h of culture, these supernatants were collected, supplemented with 10 mg of α-methylmannoside per ml (Sigma), filtered on 0.22-μm filters, and frozen at −20°C until they were tested.

IL2 bioassay. IL2 activity contained in these supernatants was assessed as described by Gillis et al. (10). CTLL IL2-dependent cells provided by F. de Nizot (Centre d' Immunologie, Marseille-Lumigny, France) and grown in IL2-containing reference supernatant were washed and adjusted to 2×10^5 cells per ml of culture medium supplemented with 5% FCS, and 0.1 ml of the cell suspension was distributed into flat-bottomed microtiter plates. Each well also received 0.1 ml of a log 2 serial dilution of the material to be titrated, and cultures were incubated for 48 h. Then, cultures were harvested, and [^{3}H]TdR incorporation was measured as above. Each sample was tested in duplicate.

Results were expressed by using probit analysis, and one unit of IL2 activity was defined as the amount of IL2-containing supernatant that produced 50% of the maximal proliferative response obtained with the reference IL2 sample. Reference IL2 sample was obtained by stimulating normal rat spleen cells with ConA as described above and used as a positive control in the IL2 assay and also for routine maintenance of CTLL cells.

To investigate whether supernatants of ConA-stimulated spleen cells from infected mice contained factors able to inhibit IL2 activity, the following experiment was performed. IL2-dependent CTLL cell proliferation was assayed with serially diluted reference supernatant as described above and in the presence of a fixed concentration of supernatant from infected mouse spleen cells stimulated by ConA. Three different final concentrations (50, 25, and 12.5%) of these supernatants were tested. Results from experiments performed with supernatants from mice infected 6 months earlier were pooled, regardless of the route of *M. lepraemurium* inoculation, since in all cases the amount of IL2 contained in these supernatants was similarly low or undetectable.

Statistical analysis. Comparisons between experimental groups were performed according to Student's *t* test for unpaired data.

RESULTS

ConA-induced mitogenic responses. At various times after *M. lepraemurium* inoculation, the proliferative response to ConA of mouse spleen cells from infected mice and controls was evaluated. Since ConA-induced proliferative response of normal mouse spleen cells was not age dependent, data obtained from all control uninfected mice were pooled. A significant depression of the ConA-induced response of splenic cells was observed after 2 to 3 months of infection in all groups of infected mice except for those which received 10^5 *M. lepraemurium* cells i.v. (Table 1). Mice infected s.c. did show a significant decrease of ConA response, even when inoculated with 10^5 bacilli. After 6 months of infection, regardless of the route or the dose of *M. lepraemurium* used to infect the animals, there was a noticeable decrease in the proliferative response to ConA that accounted for 41 to 65% of the response of normal mouse spleen cells.

LPS-induced IL1 production. At various times after *M. lepraemurium* infection, adherent spleen cells from mice infected either s.c. or i.v. with 10^5 or 10^8 bacilli were studied for their ability to produce IL1 after stimulation with LPS

TABLE 2. LPS-induced IL1 production of plastic-adhering spleen cells from *M. lepraemurium*-infected mice

Time after infection (mo) and expt	[^{3}H]TdR incorporation (net cpm) by route and dose of *M. lepraemurium* infection:[a]				
	Age-matched uninfected controls	10^8 i.v.	10^5 i.v.	10^8 s.c.	10^5 s.c.
1					
1	3,301 ± 415	3,520 ± 1,651	3,827 ± 646	3,420 ± 1,562	2,051 ± 680
2	24,439 ± 8,487	28,800 ± 4,756	25,635 ± 8,104	ND[b]	ND
2–3					
1	12,925 ± 1,132	15,680 ± 96	13,570 ± 2,919	12,207 ± 247	11,921 ± 201
2	17,864 ± 2,455	14,213 ± 1,566	14,389 ± 2,736	22,636 ± 5,223	19,857 ± 3,499
6					
1	17,353 ± 2,203	17,144 ± 5,075	ND	ND	ND
2	3,697 ± 1,247	ND	7,726 ± 1,174	6,198 ± 1,414	10,607 ± 1,341
3	7,562 ± 2,380	ND	9,246 ± 1,513	6,427 ± 238	7,829 ± 1,937

[a] Results are expressed as the mean ± the standard deviation of net counts per minute (counts per minute of thymocyte cultured with PHA and one-fourth-diluted IL1-containing supernatant minus counts per minute of thymocytes cultured with one-fourth-diluted IL1-containing supernatant without PHA). Three mice were individually tested for each experiment. The net PHA-induced thymocytic proliferation without IL1-containing supernatant varied from 1,206 to 11,054 cpm. cpm, Counts per minute.

[b] ND, Not determined.

(Table 2). Contrary to IL2 production, no age dependence was observed for IL1 production of the adherent spleen cells from age-matched controls. At no time during the infection was there a decrease in the LPS-induced IL1 production of the adhering cells recovered from 10^7 spleen cells, regardless of the dose or the route of inoculation, compared with the IL1 production of the adhering cells similarly recovered from 10^7 normal spleen cells. Moreover, a slight, though not significant, increase of IL1-activity was observed in both groups of mice inoculated i.v. or s.c. with 10^5 *M. lepraemurium* cells. A comparison of IL1 and IL2 production and ConA-induced proliferative response of the same mouse spleen cells showed that IL1 production was not affected even when a strong decrease of both ConA-induced blastogenesis and IL2 production was observed. In mice infected for 6 months, the IL2 production by T-cells recovered from 10^7 infected splenocytes strongly decreased, although the LPS-induced IL1 production by the adherent cells recovered from the same amount of splenocytes was unchanged.

IL2 production. At various times during the infection, IL2 production by ConA-stimulated spleen cells from the different groups of infected mice and age-matched controls was assessed. All of the different groups of infected mice exhibited a deficit of IL2 activity contained in the supernatants of ConA-stimulated spleen cells, the kinetics of which varied according to the dose and the route of *M. lepraemurium* inoculation. To exclude the possibility of a suppressive activity of mycobacterial products on ConA-induced IL2 production, normal mouse spleen cells were incubated with different amounts of *M. lepraemurium* (from 10^6 to 5×10^7 bacilli per culture), and the ability of spleen cells to produce IL2 on ConA stimulation was assessed. Results from two separate experiments showed that the addition of *M. lepraemurium* did not decrease IL2 production by normal mouse spleen cells (1.4 and 2 IL2 units per culture without *M. lepraemurium* versus 1.6 and 2.1, respectively, with 5×10^7 *M. lepraemurium*).

Absence of suppressive factors of IL2 activity in supernatants from *M. lepraemurium*-infected mice. The addition of ConA-stimulated spleen cell supernatants from mice infected for 6 months with either 10^5 or 10^8 *M. lepraemurium* cells to the IL2-containing reference supernatant did not affect IL2-dependent CTLL cell proliferation (Table 3). Since no inhibitory factors of IL2 activity could be found in any infected mouse spleen cell supernatants (even when a final concentration of 50% was used), the decrease of IL2 activity in these supernatants likely resulted from a defect of IL2 production.

Kinetics of IL2 production in the different groups of *M. lepraemurium*-infected mice. Results were expressed as the number of IL2 units per supernatant as described above (Fig. 1). IL2 activity in supernatant from ConA-stimulated normal mouse spleen cells was age dependent because a significant decrease was observed in 8 to 9-month-old age-matched controls. This age-related decline of IL2 production by spleen cells from normal C57BL/6 mice has been already described (7, 31). At 1 month after the *M. lepraemurium* injection, only highly infected mice (either i.v. or s.c. inoculated) showed a significant depression ($P < 0.01$) of IL2 activity in supernatants from ConA-stimulated spleen cells (57 and 50% of normal response, respectively).

After 2 to 3 months, spleen cells from mice s.c. infected

TABLE 3. Absence of suppressive factor of IL2 activity in supernatants of ConA-stimulated spleen cells from *M. lepraemurium*-infected mice

Concn of supernatant introduced (%)	ConA-simulated spleen cells (IL2 unit) from:[a]		
	10^8 *M. lepraemurium*-infected mice	10^5 *M. lepraemurium*-infected mice	Normal mice
0	1	1	1
12.5	1.05 ± 0.05	1.03 ± 0.07	1.55 ± 0.58
25	1 ± 0.07	1.065 ± 0.14	1.6 ± 0.64
50	0.95 ± 0.19	1.195 ± 0.37	1.985 ± 0.57
100	≤0.03	≤0.03	0.9 ± 0.24

[a] Proliferation of CTLL cells was assessed in the presence of serial dilutions of the reference IL2 sample with the addition of supernatants from ConA-stimulated spleen cells from either normal, highly, or weakly infected mice regardless of the route of inoculation. Results are expressed as the number of IL2 units contained in the reference supernatant after addition of supernatants from infected mice or aged-matched controls. Each value represents the mean ± the standard deviation of the results of four mice per group.

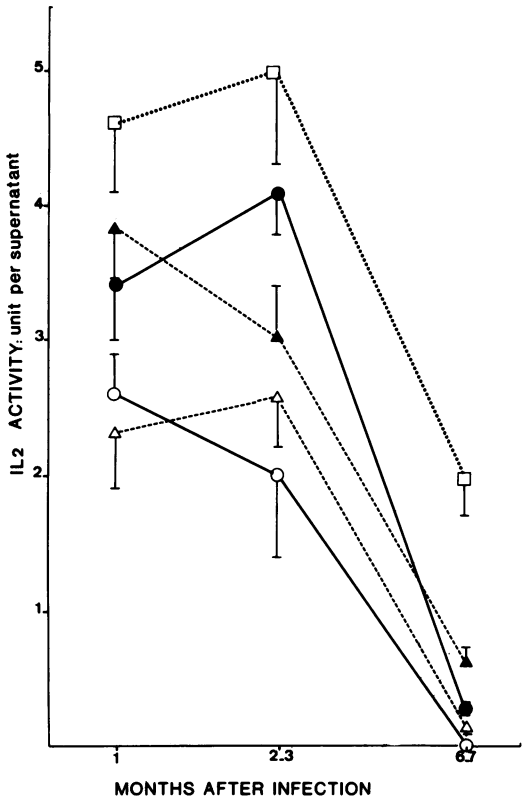

FIG. 1. IL2 production during the course of *M. lepraemurium* infection in C57BL/6 mice. IL2 activity was assessed in the supernatants of spleen cells stimulated with ConA. Results are expressed as the number of IL2 units per supernatant as described in the text. Each point represents the mean ± the standard error of the mean for three or more experiments, each including a minimum of three mice per group. Mice received 10^8 bacilli i.v. (○), 10^5 bacilli i.v. (●), 10^8 bacilli s.c. (△), 10^5 bacilli s.c. (▲), or no infection (□).

with 10^5 bacilli also exhibited a deficiency of ConA-triggered IL2 activity (60% of the normal response, $P < 0.05$), whereas spleen cells from mice infected i.v. with a similar *M. lepraemurium* inoculum did not. After 6 months of infection, the IL2 activity in spleen cell supernatants was strikingly depressed in all groups of mice. However, the defect of IL2 production was significantly more pronounced in highly infected mice (either i.v. or s.c. inoculated) than in mice infected with 10^5 bacilli (0 versus 15% of normal IL2 activity for the i.v.-inoculated mice and 7 versus 35% of normal IL2 activity [$P < 0.01$] for the s.c.-infected mice).

Correlation between bacilli growth and dissemination and spleen cell IL2 activity. (i) i.v. infected mice. IL2 activity in the spleen cell supernatants from mice infected i.v. decreased as the bacterial load increased (Fig. 2). Thus, no significant alteration of IL2 production was observed in the spleens of mice infected with 10^5 bacilli until the number of AFB recovered from the spleens reached 10^7. In mice infected with 10^8 bacilli, a strong decrease of IL2 production was already observed 1 month after infection, the time at which the number of AFB reached 10^7. A further drop of IL2 activity in the ConA-stimulated spleen cell supernatants was observed when the number of bacilli further increased in the spleen.

(ii) s.c.-infected mice. No correlation was observed between the defect of IL2 production and the number of AFB recovered from the spleens of mice infected s.c. with either 10^5 or 10^8 bacilli (Fig. 3A and B). Thus, although the amount of bacilli remained low and constant in the spleens of mice infected s.c. with either 10^5 or 10^8 *M. lepraemurium* cells, the IL2 deficiency in spleen cell supernatants progressed, suggesting the absence of a local influence of bacterial load on IL2 production. In mice infected with 10^5 bacilli, IL2 production by spleen cells seemed to decrease progressively, as the number of *M. lepraemurium* cells recovered from the footpad increased. In mice infected with 10^8 bacilli, a similar correlation was not found, since the number of AFB recovered from footpads was already high 1 month after the infection and remained unchanged thereafter, whereas the IL2 activity, measured in ConA-stimulated spleen cell supernatants, decreased steadily.

DISCUSSION

In a previous paper, we reported an early decrease of IL2 activity in supernatants of ConA-activated spleen cells from C57BL/6 mice infected i.v. with 10^7 *M. lepraemurium* cells which occurred before any detectable decrease of spleen cell proliferative response to ConA (15). In the present work, we extended our analysis of spleen cell-secreted interleukins in *M. lepraemurium*-infected C57BL/6 mice. We confirmed, with two different doses of bacilli (10^5 and 10^8), that *M. lepraemurium* infection depressed IL2 activity in supernatants of ConA-triggered spleen cells, a deficit which manifested, at any dose or route of inoculation, before the decrease of ConA-induced proliferation. Whether such a

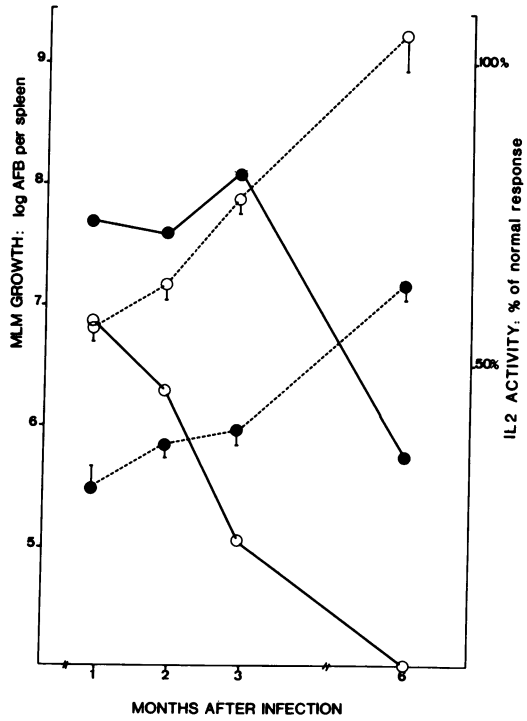

FIG. 2. *M. lepraemurium* growth and IL2 production in the spleens of C57BL/6 mice infected i.v. Results of the bacilli growth are expressed as the mean ± the standard deviation of the bacilli counts from a minimum of 10 spleens (dotted lines). Results of IL2 activity of infected mouse spleen cells are expressed as the percentage of IL2 activity of spleen cells from age-matched controls. Each value represents the mean of the results of 10 mice. Mice received 10^8 (○) or 10^5 (●) bacilli. MLM, *M. lepraemurium*.

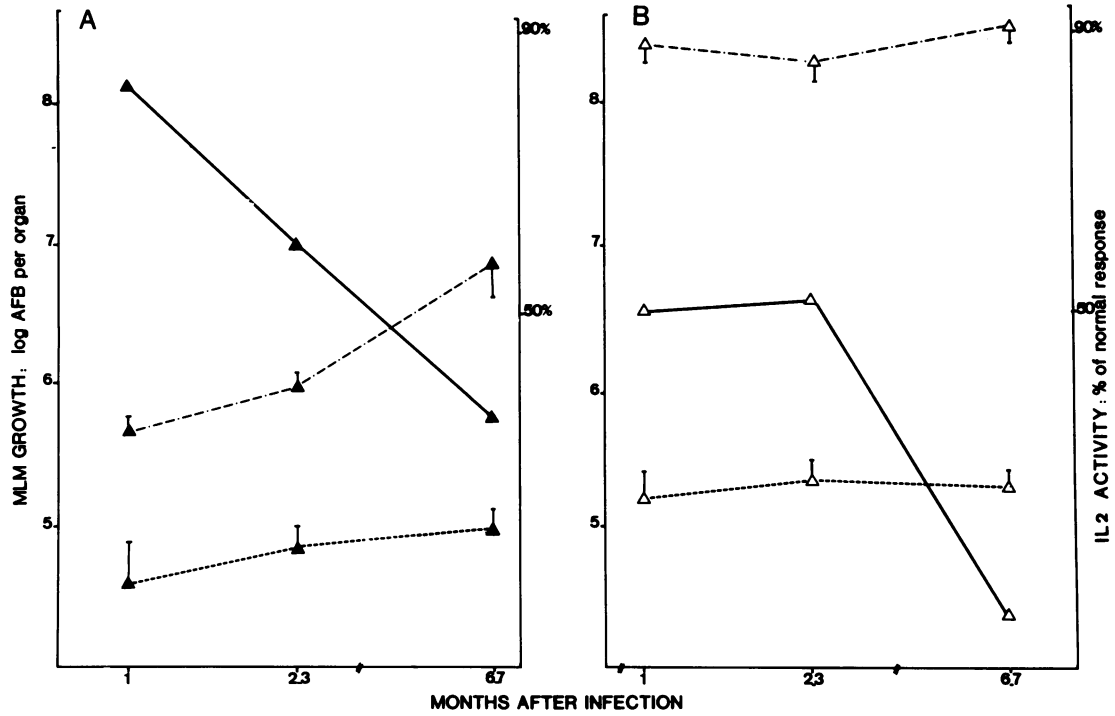

FIG. 3. Correlation between bacilli growth in spleens and footpads of mice infected s.c. and IL2 production by spleen cells stimulated with ConA. Results are expressed as shown in the legend to Fig. 2. Mice received either (A) 10^5 (▲) or (B) 10^8 (△) bacilli. Bacilli counts in the spleen (– – –) and ——— in the foot (. – – .). MLM, *M. lepraemurium*.

decrease of ConA-induced lymphocyte blastogenesis was a consequence of IL2 defect could not be ascertained, since assays for IL2 activity and ConA-induced proliferation might differ in their sensitivity. Moreover other factors, such as accessory cell activity, could influence the ConA-induced proliferative response.

Decrease of IL2 activity could be attributed to a decrease of IL2 secretion itself, rather than to the concomitant secretion of inhibitory factors counteracting the T-cell growth-inductive activity of IL2, since supernatants of ConA-activated spleen cells from infected mice did not alter the IL2 activity of a reference supernatant. IL2 production defect was not associated with a similar decrease of IL1 production by splenic adherent cells in any group of infected mice. LPS-induced IL1 activity was found in infected mice that was quite similar to that of normal controls, in most cases and tended in a few cases to increase. Spontaneous IL1 activity (not LPS induced) of infected mouse adherent spleen cells was also assessed (data not shown) and was found to be equal to that of normal adherent spleen cells. These findings suggested that IL2 deficiency was not due to an impaired production of IL1 by the infected macrophage population. However, since the results were not expressed as IL1 activity units per equal number of macrophages in infected mice versus normal mice, we could not totally exclude that an actual decrease in IL1 production per adherent cell unit might exist and be overlooked owing to the increased proportion of macrophages within infected spleens, especially after 6 months of infection. Identical data have been reported in *Trypanosoma cruzi*-infected mice, which developed a deficit in IL2 production with normal or enhanced levels of IL1 (14).

Similarly, Haregewoin et al. (13) showed that *M. leprae* antigens failed to trigger IL2 secretion and to elicit proliferation in lymphocytes from lepromatous leprosy patients and that IL1 did not restore lymphocyte blastogenesis to *M. leprae*. Taken together, these data would suggest that it might be a general feature of intracellularly developing infectious agents to induce a deficit of IL2 production (14, 15, 28). This deficit would not result from a failure of infected macrophages to secrete IL1 but rather from a defect of the IL2-secreting T-cell subset itself. Such a defect would be primary or secondary to active suppression (12, 22).

We compared ConA-induced IL2 production in the spleens of mice infected with a low (10^5) or high (10^8) dose of *M. lepraemurium* cells, either i.v. or s.c., since previous work showed that both dose and route of inoculation could modulate the consequences of mycobacterial infection on cell-mediated immunity. Two comments can be made on our results. (i) Kinetics and intensity of the depression of IL2 production in the spleen were merely dependent on the dose of bacilli injected and less or not at all on the route of inoculation. Heavily infected mice, whether s.c.- or i.v.-inoculated, exhibited a similar early and marked IL2 deficiency. Conversely, groups of mice infected with the low dose of 10^5 *M. lepraemurium* cells, either i.v. or s.c., showed a delay in the decrease of IL2 production. In these mice the delay was slightly longer for i.v.-infected compared with s.c.-infected animals. (ii) Although the defect of IL2 production by ConA-stimulated spleen cells increased with time after the infection, there was no direct relation between the number of bacilli recovered from the spleen and the level of depression of IL2 production. Thus, mice inoculated s.c. developed an IL2 defect with kinetics similar to those of

mice inoculated i.v. with the same dose, although very few bacilli disseminated to the spleen after s.c. infection, even in heavily infected animals.

It is commonly assumed that mice mount a more efficient cell-mediated immunity when sensitized s.c. than when sensitized i.v. (19). This statement also applies to specific cell-mediated immunity developed after mycobacterial infections, at least in C57BL/6 mice, which were shown to survive to s.c. infection better than some other strains (18, 25). Conversely, the i.v. route of infection has been shown to induce in several strains, including the C57BL/6 strain, a strong impairment of both mycobacterial specific or nonspecific T-cell immune response in the spleen, often found associated with suppressor cells (4, 16, 36). Very little, however, is known about the distant consequences of an s.c. infection on T-cell responsiveness in the spleen. Turcotte (32) compared PHA- and ConA-induced proliferative responses of spleen cells from C57BL/6 mice infected with 10^7 *M. lepraemurium* by route of inoculation. They reported that spleen cells from s.c.-infected mice were not affected in their response to T-cell mitogen until month 13 of infection in contrast to i.v.-infected mice, which exhibited an early impairment of T-cell mitogen-induced proliferation in the spleen. These results partly contradict our own data since we found an equal depression of both ConA-induced proliferation and IL2 secretion in s.c.- or i.v.-infected mice. This discrepancy could be due to minor strain differences, different environmental conditions, or the use of different doses of bacilli. Moreover our period of study only extended to 6 to 7 months of infection instead of 13 months as in the study of Turcotte (32), and we cannot exclude that a possible influence by route of inoculation on ConA-induced spleen cell proliferation could have appeared later.

Finally, IL2 production and T-cell proliferation are related but are not identical phenomena, as exemplified by our own results showing that IL2 production started to decrease much earlier than proliferation. One could hypothesize that T-cells from i.v.-infected mice developed a resistance to IL2 activity as the infection progressed more easily than T-cells from s.c.-infected mice owing to the high amount of bacilli or bacilli products in the spleen. Such a difference in sensitivity to IL2 would explain the lower mitogen-induced proliferative responses observed by Turcotte in i.v.-infected mice later in the infection. Indeed, we previously reported that spleen cells from C57BL/6 mice infected i.v. with 10^7 bacilli exhibited, after 6 months of infection, a decreased sensitivity to IL2 in addition to their failure to produce IL2 (15).

In this paper, we showed that the deficiency of IL2 activity seemed to be linked to a defect of IL2 production. This defect could be secondary to several different suppressive mechanisms. Many previous reports have indicated the appearance of specific and nonspecific suppressor cells in the spleens of mice i.v. inoculated with large amounts of mycobacteria (4, 16, 36). To explain the IL2 defect in spleens from s.c.-infected mice, which is not directly related to the number of AFB since the amount of bacilli remains constant in the spleen, it could be suggested that certain suppressive mechanisms act at a distance from the initial site of infection. Suppressor cells originating in the inoculated footpad could circulate and be trapped in the spleens. Abnormalities of cell recirculation have been reported in rats infected with *M. lepraemurium* (3).

Circulating factors could also participate in the suppressive mechanisms. Soluble suppressor factors have been described which inhibit the in vivo anti-mycobacterial activity of macrophages in *M. lepraemurium*-infected BALB/c mice but not in C57BL/6 mice (26). Moreover, addition of sera from heavily infected C57BL/6 mice to normal mouse spleen cells did not depress their ConA-induced proliferation and IL2 production (data not shown). Finally, soluble mycobacterial products could induce a suppressive activity in the spleen of infected animals. It has recently been reported that phospholipids released from *Mycobacterium tuberculosis*-infected macrophages could activate OKT8-enriched human lymphocytes to suppress mitogen- or antigen-induced proliferation of normal lymphocytes (34). However, our experiments did not show any modification of IL2 production by incubating normal mouse spleen cells with *M. lepraemurium*, suggesting that the whole bacillus and the antigens released after in vitro macrophage processing could not directly induce a suppressive activity on IL2 secretion.

In an attempt to better understand the events leading to the immunodepression observed in murine leprosy, similar to that observed in lepromatous leprosy, suppressor mechanisms of IL2 production in spleen cells from *M. lepraemurium*-infected mice are currently under investigation in our laboratory.

LITERATURE CITED

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