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Influence of Route of *Mycobacterium lepraemurium* Injection on Susceptibility to Mouse Leprosy and on Lymphoblastic Transformation

RAYMOND TURCOTTE

Centre de Recherche en Immunologie, Institut Armand-Frappier, C.P. 100, Laval-des-Rapides, Ville de Laval, Québec, Canada H7N 4Z3

Groups of female C57BL/6 and C3H/St mice were inoculated intraperitoneally (i.p.) with 10^9, 10^7, and 10^5 bacilli and into the right hind footpad with 10^7 and 10^5 bacilli of *Mycobacterium lepraemurium*. The incidence of death from leprosy and the mean survival time of leprous mice were recorded. In addition, the blastogenic responses to the T-cell mitogens phytohemagglutinin and concanavalin A and to the B-cell mitogens lipopolysaccharide and dextran sulfate were evaluated at various times during the course of infection in the spleen and peripheral lymph nodes of mice infected with 10^7 bacilli. When *M. lepraemurium* was administered i.p., the two strains of mice succumbed to the disease at about the same time, except for the C57BL/6 mice infected with 10^9 bacilli, which died earlier than the C3H/St mice. Moreover, in both strains of mice, a progressive depression of blastogenesis, first to the T-cell mitogens and then to the B-cell mitogens in the spleen, and to the T-cell mitogens in the peripheral lymph nodes, occurred during the course of the infection, whereas the response to the B-cell mitogens in the nodes increased slowly during the advanced stage of the disease. When 10^7 and 10^5 bacilli were injected into the footpad, the C3H/St mice succumbed to the disease at 298 and 344 days, respectively, and the modifications of blastogenesis were similar to those observed in i.p.-infected C3H/St mice. In contrast, the C57BL/6 mice appeared resistant to footpad inoculation of *M. lepraemurium*, since they lived until the end of the observation period (466 days postinfection) and the depression of blastogenesis was not detectable until 1 year after the infection. Thus, it is concluded that for the C57BL/6 mice (but not for the C3H/St mice), the route of administration of *M. lepraemurium* can markedly influence the susceptibility or resistance to leprosy.

The development of experimental murine leprosy varies markedly in different inbred strains of mice. C57BL/6 mice were found able to resist a subcutaneous (s.c.) inoculation of *Mycobacterium lepraemurium*, presumably by developing a cell-mediated immune response, whereas no histopathological indication of such a response was detected in C3H mice which eventually died from disseminated infection. These observations, originally reported by Kawaguchi (10) and thereafter confirmed and extended by Closs and Haugen (5), were recently challenged by Lefford et al. (14). According to the latter workers, neither C57BL/6 nor C3H mice resisted an intravenous (i.v.) inoculation, and C57BL/6 mice were even more susceptible than C3H mice. Moreover, a highly susceptible strain of mice (e.g., the F_1 hybrid of BALB/c × C57BL/6) can mount a cell-mediated immune response to *M. lepraemurium* (14). Although many factors, such as the strain and dose of *M. lepraemurium* and the strains of mice maintained in different breeding laboratories, could explain these contradictory results, we feel that the route of infection, which differed in the above-mentioned studies, might greatly influence the susceptibility of a given strain of mice to *M. lepraemurium*.

We observed recently that the i.v. or intraperitoneal (i.p.) injection of 1 mg of *M. tuberculosis* strain BCG into C57BL/6 mice induced suppressor cells in the spleen, whereas no suppressive activity could be detected either in the spleen or in the draining lymph nodes when a corresponding dose was administered into the footpad (24). Moreover, i.p. (23) and i.v. (3) inoculations of *M. lepraemurium* into C3H mice induced suppressor cells, first in the spleen and later in the peripheral lymph nodes. These suppressor cells persisted until the death of the mice from disseminated infection. It is not known yet whether suppressor cells are involved in the depression of cellular immunity as observed in the lepromatous form of human leprosy (25). On the other hand, the induction of cell-mediated

immunity, which is believed to play a major role in host resistance against *M. lepraemurium* (5, 11), depends on the route of administration of the particulate antigens. For instance, the intradermal and s.c. routes are more effective than the i.p. and i.v. routes (13).

The purpose of the present investigation was to study the influence of the i.p. and s.c. routes of infection on the susceptibility and resistance to *M. lepraemurium* and on the blastogenic response to polyclonal mitogens of the spleen and lymph node cells in C3H and C57BL/6 mice.

MATERIALS AND METHODS

Experimental animals. Female mice of the inbred strains C57BL/6J and C3H/St (hereafter called B6 and C3, respectively) were obtained from Jackson Laboratories, Bar Harbor, Maine, and from Canadian Breeding Farm and Laboratory, Ltd., Laprairie, Quebec, Canada, respectively. They weighed 16 to 18 g at the beginning of the experiments. They were maintained under standard laboratory conditions and fed Purina chow and water ad libitum.

Infection of mice. The Hawaii strain of *M. lepraemurium*, obtained from O. K. Skinsnes, Honolulu, Hawaii, was maintained by successive passages in B6 and C3 female mice. At the time of infection, fresh bacilli were isolated from the spleen or liver of infected mice and counted according to the method of Shepard and McRae (21). Groups of B6 and C3 mice were injected i.p. with three doses (10^5, 10^7, and 10^9) of bacilli suspended in 0.5 ml of phosphate buffer (pH 7.4, 0.15 M) or s.c. via the right hind footpad with the same number of bacilli in 0.02 ml.

Experimental procedures. At various time intervals after infection, randomly selected C3 and B6 mice inoculated with 10^7 bacilli and control uninfected mice of the same age were killed by cervical dislocation. The blastogenic response to mitogens was evaluated in the spleen and the peripheral lymph nodes. For mice infected i.p., a pool of the right and left popliteal, inguinal, and axillary nodes was made; for mice infected via the right hind footpad, a pool of the right popliteal and inguinal nodes and a pool of the corresponding left nodes and axillary nodes were tested separately. The spleen and peripheral lymph nodes were removed under sterile conditions, and the lymphoid cells were isolated according to a method already described (24). The rest of the mice from these groups and those inoculated with 10^5 and 10^9 bacilli were observed two to three times a week until the time of death. The cause of death was established at autopsy by macroscopic examination of the pathological lesions in visceral organs and, in a few randomly selected mice, by histological examination of sections of spleen, liver, lymph nodes, and pelvic fatty pad, stained for acid-fast bacilli by the Ziehl-Neelsen method.

In vitro blast transformation. The blastogenic responses of spleen and lymph node cells from *M. lepraemurium*-infected mice were compared with those of age-matched, noninfected control mice. The responses to the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (ConA) and to the B-cell mitogens lipopolysaccharide (LPS) and dextran sulfate (DS) were determined by measuring the incorporation of [^{3}H]TdR into deoxyribonucleic acid. The method used for this assay has been described in detail elsewhere (24). Briefly, viable lymphoid cells (5×10^5) were cultured with optimal concentrations of mitogens in 0.1 ml of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) and antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml). Final concentrations were: PHA, 0.5 μl; ConA, 0.2 μg; LPS and DS, 5 μg. All cultures were set up in flat-bottomed tissue culture plates (Linbro Scientific Co., Inc, Hamden, Conn.) and incubated for 72 h at 37°C in a humidified atmosphere containing 5% CO_2. Cultures were pulsed with 1.0 μCi of [^{3}H]TdR 18 h before the end of the incubation period and harvested on glass fiber filters with a Titertek cell harvester (Flow Laboratories, Inc., Rockville, Md.). Radioactivity was counted in a Beckman DPM-100 scintillation spectrometer. In some experiments, the presence of suppressor cells was determined by evaluating the ability of lymphoid cells from infected mice to inhibit the incorporation of [^{3}H]TdR into normal splenic cells. All experiments were done in triplicate, and the results are expressed as net counts per minute (i.e., mean counts per minute of mitogen-stimulated triplicate cultures minus mean counts per minute of nonstimulated triplicate cultures).

RESULTS

Groups of B6 and C3 mice were infected i.p. and s.c. via the right hind footpad with decreasing doses of *M. lepraemurium*. The pooled results from two experiments, expressed as the incidence of death and the mean survival time of infected mice, are illustrated in Table 1. When *M. lepraemurium* was administered i.p., both strains of mice succumbed to the disease at the three infecting doses used. Thus, at a dose of 10^9 bacilli, the mean survival time of B6 mice was shorter than that of C3 mice ($P < 0.05$), but at the lower doses, no significant difference in the mean survival time was noted. As expected, the mean survival time of leprous mice varied inversely with the number of inoculated bacilli. When the mice were infected via the footpad with 10^7 and 10^5 bacilli, the C3 mice succumbed to leprosy after 298 and 344 days, respectively; in fact, they lived a little longer than those infected i.p. In contrast, the B6 mice lived with no clinical sign of illness, except for a footpad inflammatory reaction, until the end of the observation period (466 days after infection). Very similar results were obtained when these experiments were repeated; however, it is not yet known whether B6 mice would have survived for as long if infected s.c. with larger doses (e.g., 10^9 bacilli).

At autopsy, an enlargement of liver, spleen, and pelvic fatty pad was observed in both strains

of mice infected i.p. with the three doses of *M. lepraemurium*. However, splenomegaly was more marked in C3 mice (six to seven times normal size) than in B6 mice (two to three times normal size). Moreover, a whitish coloration of the spleen was visible in C3 mice only. In mice infected via the right hind footpad, a local inflammatory reaction occurred during the first few months after infection and persisted until the time of death (C3) or sacrifice (B6 mice). Measurements of footpad thickness showed no significant difference between the two strains of mice. However, staining of lesions revealed the presence of acid-fast bacilli in C3 mice, whereas no bacilli could be seen in two out of two footpads from B6 mice. The size of draining popliteal and inguinal lymph nodes was increased in both strains of mice. Internal organs were severely affected and to about the same extent in s.c.- and i.p.-infected mice; however, only a slight splenomegaly was observed throughout the observation period in s.c.-infected B6 mice. Histologically, a few intracellular bacilli could be detected in the draining lymph nodes and in the spleen of B6 mice at the time of sacrifice; in the organs of C3 mice, a larger number of mononuclear cells loaded with numerous bacilli were seen.

TABLE 1. *Effects of route of infection and dose of M. lepraemurium on the incidence of death from murine leprosy and on the survival time of female C57Bl/6J and C3H/St mice*[a]

Route of infection	Strain of mice	Dose of bacilli	Incidence of death (%)	Mean survival time (day ± SD)[b]
i.p.	C57Bl/6J	10^9	100[c]	104.4 ± 4.8
		10^7	100	255.5 ± 21.7
		10^5	100	304.3 ± 27.5
	C3H/St	10^9	100	159.4 ± 7.9
		10^7	100	250.4 ± 29.8
		10^5	100	291.9 ± 23.7
s.c. (footpad)	C57Bl/6J	10^7	0	466[d]
		10^5	0	466
	C3H/St	10^7	100	298.5 ± 22.4
		10^5	100	344.0 ± 24.6

[a] Pooled results from two experiments. An infecting dose of 10^7 *M. lepraemurium* was used in experiment 1, whereas doses of 10^5 and 10^9 were used in experiment 2.
[b] SD, Standard deviation.
[c] Each experimental group comprised 10 to 20 mice.
[d] Mice were sacrificed 466 days after the infection.

The next step was to compare the blastogenic response to polyclonal mitogens of spleen and lymph node cells in normal B6 and C3 mice. Due to large variations in the mitogenic responses in mice of similar age, no relationship could be established between the degree of lymphocyte transformation and the age of the mice; therefore, the results shown in Table 2 represent pooled data obtained from 2- to 14-month-old mice. For both strains of mice, the spontaneous (no mitogen added) [^{3}H]TdR incorporation was higher in the spleen than in the lymph node cells, and the spleen and lymph node cells from C3 mice incorporated significantly more thymidine than those from the B6 mice. The relatively high spontaneous incorporation by lymphoid cells from C3 mice could have been due to the fact that these cells may be more sensitive than those from B6 mice to the presence of fetal calf serum (or a substance contained therein). The proliferative response to PHA of spleen cells from C3 mice was quite low compared with the incorporation by unstimulated cells and the corresponding response obtained in B6 mice (Table 2). However, more comparable responses to ConA and LPS occurred in the spleen cells from the two strains of mice. Inversely, the proliferative responses to PHA and ConA of lymph node cells from C3 mice were markedly higher than those from B6 mice. Finally, the responses of lymph node cells to LPS were quite similar in the two strains.

To investigate whether the low blastogenic response to PHA of spleen cells from normal C3

TABLE 2. *Proliferative responses to T- and B-cell mitogens of spleen and lymph node cells from normal C57BL/6J and C3H/St mice*[a]

Strain of mice	Origin of lymphoid cells	[^{3}H]TdR incorporation: Unstimulated cells	Cells stimulated with: PHA	ConA	LPS
C57BL/6	Spleen	12.1 ± 1.1	42.2 ± 9.2	90.4 ± 5.4	78.3 ± 12.9
	Lymph nodes	0.3 ± 0.1	69.8 ± 5.7	90.5 ± 5.6	6.1 ± 1.3
C3H/St	Spleen	22.7 ± 3.1	24.0 ± 4.4	86.7 ± 12.0	91.6 ± 5.2
	Lymph nodes	1.2 ± 0.2	142.4 ± 11.9	165.0 ± 15.7	7.3 ± 1.7

[a] Results of seven experiments expressed as the arithmetic mean ± standard error (× 10^{-3}) of counts per minute of [^{3}H]TdR incorporated per culture. The age of mice varied from 2 to 14 months.

mice was due to a relative lack or an absence of PHA-responding T lymphocytes or to an active suppression of blastogenesis, splenic macrophages were eliminated by treating of the whole spleen cell population with iron powder and magnet before stimulating the macrophage-depleted cells with mitogens. This treatment (Table 3) led to a significant increase of the blastogenic response to PHA, suggesting that splenic macrophages could actively depress the blastogenic response to this mitogen. Interestingly, the removal of macrophages also reduced the spontaneous incorporation of thymidine by unstimulated cells.

Since B6 and C3 mice were found to be about equally susceptible to leprosy after i.p. injection of 10^7 bacilli, the evolution of the lymphoproliferative response to T- and B-cell mitogens of the spleen and lymph node cells from infected mice was investigated next. For these experiments, mice were first infected with 10^7 bacilli and then tested at various times after infection. The results obtained with B6 mice, which were essentially the same as those already obtained with C3 mice (23), are illustrated in Fig. 1. In the spleen, no modification of the blastogenic response (by comparison with normal responses) occurred during the 1st month after infection. Thereafter, a marked depression of the response to ConA, to PHA and LPS, and finally to DS was observed at 2, 4, and 8 months, respectively. In the peripheral lymph nodes, a significant depression of the response to PHA and ConA was detectable only 4 months after infection. Thereafter, the responses to the T-cell mitogens were not totally suppressed, and an enhancement of the response to the B-cell mitogens, detectable at 4 months, increased till the terminal stage of the disease. These findings show that during the evolution of leprosy in mice infected i.p., the spleen cells become completely refractory to in vitro stimulation by the T- and B-cell mitogens, whereas the lymph node cells become partially refractory to stimulation by the T-cell mitogens and show an enhanced reactivity to the B-cell mitogens.

As found previously with *M. lepraemurium*-infected C3 mice (23), inhibition of the mitogen-induced transformation in the spleen was not due to a relative absence or a refractoriness of T and B lymphocytes, but to the presence of suppressor cells. Indeed, when spleen cells from infected B6 mice were added to normal spleen cells before their stimulation with the mitogens, a marked inhibition of the blastogenesis of the normal cells was observed. Moreover, splenic T lymphocytes from infected B6 mice obtained by passage through a nylon wool column still have

TABLE 3. *Deoxyribonucleic acid-synthetic response to PHA, ConA, and LPS of unfractionated and macrophage-depleted splenic cells from normal C3H/St mice*

Type of spleen cells	[^{3}H]TdR incorporation[a]			
	Unstimulated cells	Cells stimulated with:		
		PHA	ConA	LPS
Unfractionated	25.5 ± 5.8	16.5 ± 4.4	61.7 ± 22.6	81.2 ± 7.9
Macrophage depleted	15.8 ± 2.9	60.6 ± 18.0	74.7 ± 29.9	86.1 ± 13.3

[a] Results from three separate experiments, each done in triplicate, expressed as in Table 2.

the capacity to respond to mitogens (R. Turcotte, manuscript in preparation).

As mentioned above, when *M. lepraemurium* was inoculated into the footpad, the development of murine leprosy differed markedly in the two inbred strains of mice. In a subsequent series of experiments, we investigated whether there was a relationship between the presence of suppressor cells and the resistance of B6 and the susceptibility of C3 mice to *M. lepraemurium.* Table 4 shows the results obtained with the spleen cells of both strains of mice infected for various periods of time with 10^7 bacilli. In C3 mice, the blastogenic responses to the T-cell mitogens and to LPS started to decrease 3 months after infection, reaching a very low value at the 9th month, that is, at the time of death. On the other hand, the response to the B-cell mitogen DS increased from the 6th month until death. In B6 mice, a significant depression of the mitogenic responses to PHA, ConA, and LPS was observed as long as 13 months after infection, whereas for DS the depressed response was preceded by an enhancement of that response. It should be emphasized that no significant depression of the response to PHA, ConA, and LPS was detected in two infected B6 mice when tested at 12 months (data not shown). It is not yet known whether the blastogenic depression in B6 mice infected for more than 13 months would have been as strong as that of C3 mice at the time of death. Nevertheless, the reactivity of spleen cells differed markedly in these two strains of mice in that the depression of the blastogenic responses to PHA, ConA, and LPS in B6 mice occurred much later after infection.

The blastogenic responses of the draining (popliteal and inguinal) lymph node cells from C3 and B6 mice infected in the right hind footpad are illustrated in Table 5. In both strains of mice, the responses to PHA and ConA were not significantly modified during the first 6 to 8 months after infection but decreased drastically

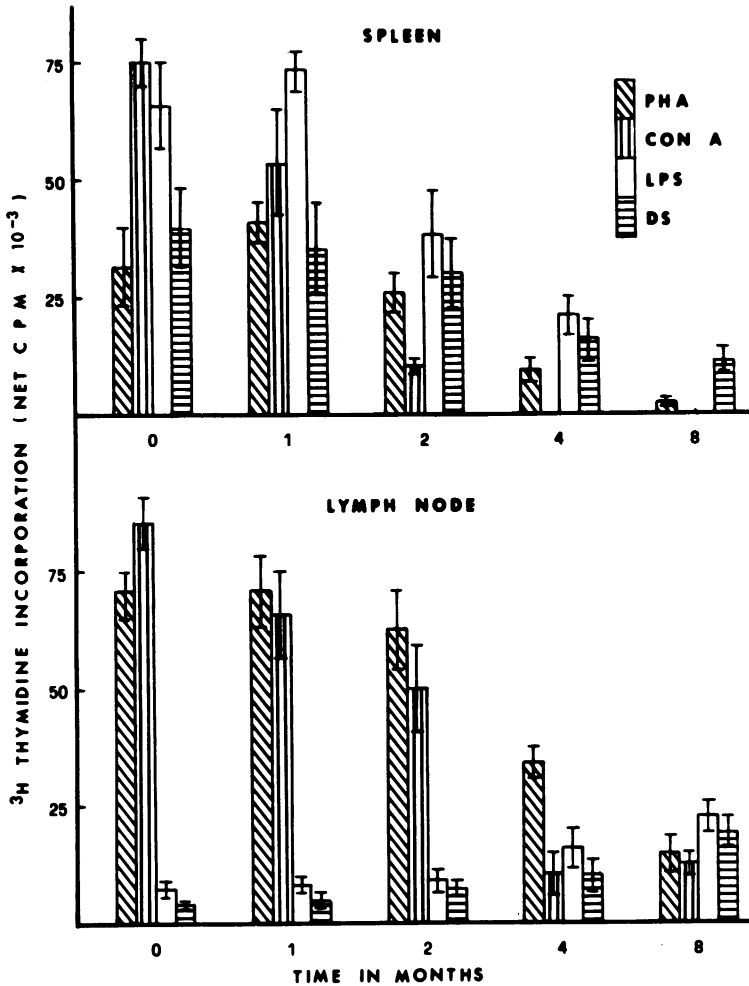

FIG. 1. *[^{3}H]thymidine incorporation in response to PHA, ConA, LPS, and DS in the spleen and lymph node cells of normal and M. lepraemurium-infected C57BL/6 mice, as a function of time after infection. For time zero, the data represent the mean net counts per minute × 10^{-3} ± standard error obtained from seven experiments; for each time after infection, the data represent mean net counts per minute obtained from two separate experiments. Each experiment was done in triplicate.*

especially in the C3 mice, during the following months. In contrast, the responses to LPS and DS were enhanced at 3 months and at 6 to 8 months, respectively, and persisted until death (C3 mice) or the end of the observation period (B6 mice). Thus, the evolution of the blastogenic response in the draining lymph node cells was essentially the same for the two strains of mice, except that the depression of the response to the T-cell mitogens was markedly delayed and the enhanced response to LPS reached higher values in the B6 mice.

In the nondraining lymph nodes of both mouse strains, the blastogenic response to the T-cell mitogens was not depressed throughout the whole observation period, whereas an enhancement of the response to LPS and DS was detected at months 9 and 13, respectively, in the C3 and B6 mice (data not shown).

A comparison of the evolution of the blastogenic responses of the spleen and lymph node cells (Table 4 versus Table 5), as a function of time after infection, revealed that the suppression of the T-cell responses occurred first in the spleen. Moreover, a marked stimulation of the response to the B-cell mitogens (LPS and DS) was observed in the draining and the nondraining lymph nodes in comparison with a depressed

response to LPS in the spleen. Finally, in the nodes, the response to LPS was stimulated before the response to DS.

DISCUSSION

The present study shows that, depending on the route of administration of *M. lepraemurium*, the development of murine leprosy as determined by the mean survival time can differ markedly in two inbred strains of mice. Thus, at a dose of 10^7 bacilli, C3 mice were susceptible to the disease when infected both i.p. and s.c., whereas B6 mice were susceptible to an i.p. inoculation (to about the same extent as the C3 mice) but were highly resistant to an s.c. (footpad) inoculation. As expected, for a given route of infection, susceptibility to the disease could be influenced by the dose of *M. lepraemurium*. Indeed, when both strains of mice were infected i.p. (Table 1) or i.v. (14) with 10^9 bacilli, the B6 mice were more susceptible than the C3 mice. These findings can explain the divergent results obtained by some workers concerning the susceptibility of B6 mice to experimental leprosy; Kawaguchi (10) and Closs and Haugen (4) had infected their mice s.c., whereas Lefford et al. (14) used the i.v. route of infection. These results suggest that, for B6 mice (but not for C3 mice), the route of inoculation of *M. lepraemurium* is an important factor in determining susceptibility to the disease. The reason for the opposite behavior of these two strains of mice is presently unknown. However, the induction of cell-mediated immunity, which is believed to play an important role in the defense against murine leprosy (6, 11), might be favored by an s.c. inoculation of *M. lepraemurium*, as already shown with unrelated particulate antigens (13) and with BCG (Turcotte, submitted for publication), and could therefore be responsible for the resist-

TABLE 4. *Mitogenic responses of spleen cells from C3H/St and C57BL/6 mice infected in the right hind footpad with 10^7 M. lepraemurium for various periods of time*

Time after infection (mo)	[^{3}H]TdR incorporation (net cpm)[a] induced with:			
	PHA	ConA	LPS	DS
C3H/St mice				
0 (controls)	1.3 ± 4.4	64.0 ± 12.0	68.9 ± 5.2	24.1 ± 4.5
3	0	50.2 ± 2.9	52.3 ± 4.5	23.2 ± 11.4
6	0	5.7 ± 5.7	24.6 ± 3.2	50.9 ± 9.6
9	0	0	11.2 ± 9.7	51.0 ± 7.8
C57BL/6 mice				
0 (controls)	30.1 ± 9.2	78.3 ± 5.6	66.2 ± 10.3	38.4 ± 13.4
3	34.9 ± 1.9	66.7 ± 7.2	65.3 ± 9.5	60.6 ± 13.4
8	54.9 ± 17.5	70.5 ± 9.2	79.2 ± 3.7	69.7 ± 3.8
13	8.2 ± 2.4	8.0 ± 1.7	41.5 ± 5.8	25.9 ± 1.4

[a] Mean counts per minute of stimulated triplicate cultures minus mean counts per minute of nonstimulated triplicate cultures. Results are expressed as counts per minute $\times 10^{-3}$ ± standard error. For control mice, the data represent the mean net counts obtained from seven experiments; for each time after infection, the data represent mean net counts obtained from two separate experiments. Each experiment was done in triplicate.

TABLE 5. *Mitogenic responses of draining (popliteal and inguinal) lymph node cells from C3H/St and C57BL/6 mice infected in the right hind footpad with 10^7 M. lepraemurium for various periods of time*

Time after infection (mo)	[^{3}H]TdR incorporation (net cpm)[a] induced with:			
	PHA	ConA	LPS	DS
C3H/St mice				
0 (controls)	141.2 ± 11.9	163.8 ± 15.7	6.1 ± 1.7	1.4 ± 0.2
3	141.8 ± 10.2	146.2 ± 12.5	16.0 ± 2.3	1.2 ± 0.3
6	137.4 ± 9.0	158.3 ± 54.7	25.7 ± 9.9	15.4 ± 13.7
9	0	12.5 ± 1.2	18.7 ± 5.6	47.3 ± 10.7
C57BL/6 mice				
0 (controls)	69.5 ± 5.7	90.2 ± 5.6	5.8 ± 1.3	5.0 ± 1.4
3	74.4 ± 4.8	82.9 ± 4.4	29.5 ± 5.1	9.5 ± 1.3
8	85.0 ± 11.3	67.5 ± 9.7	38.4 ± 3.6	20.3 ± 3.4
13	39.6 ± 12.4	41.4 ± 14.8	61.6 ± 1.1	27.3 ± 5.7

[a] See Table 4.

ance of B6 mice. On the other hand, since s.c.-infected C3 mice did not seem to be resistant, there must be a deficiency or a blockage in the expression of resistance to murine leprosy in this strain. Recently, the C3H/He mouse strain (derived from the C3H/St strain) was examined for delayed-type hypersensitivity to BCG and found to be a low responder to this bacillus (15).

To determine the mechanism(s) involved in the difference in susceptibility to murine leprosy between C3 and B6 mice, a comparison of mitogen-induced lymphoproliferation was undertaken in normal and *M. lepraemurium*-infected mice. In normal C3 mice, the response to PHA of unfractionated spleen cells was practically absent as compared with that of B6 mice (Table 2), thus confirming the recent observation of Nakamura and Tokunaga (15). This "natural" unresponsiveness could be partially due to the inhibiting activity of splenic phagocytic cells (Table 3). Our observations strongly suggest that suppressor cells are normally present in the spleen of normal C3 mice, as was also found by Folch and Waskman (8), who used spleen cells from normal rats. Furthermore, the unresponsiveness to PHA might be inherent to the spleen, since lymph node cells responded quite well to this mitogen (and to ConA), even more so than lymph node cells from B6 mice, suggesting that C3 mice possess PHA-responding T cells. It is not known whether the suppressor cells, as detected in vitro in the spleen cells of normal C3 mice, are involved in susceptibility to murine leprosy after infection via the footpad. The variations in the mitogenic responses, as found in this study, between the spleen and lymph node cells from a given mouse, and probably between the two strains, could be due to the different ratios of T and B lymphocytes (17) and of macrophages present in these lymphoid organs (see below).

Several factors might be responsible for the gradual depression of the mitogen-induced blastogenesis in the spleen and lymph node cells of *M. lepraemurium*-infected C3 and B6 mice. First, there could be a lack of lymphocytes in the lymphoid organs or a loss of their viability in tissue culture or a refractoriness to mitogenic stimulation. It is well known that granulomas are formed, lymphocytes are replaced by histiocytes (4), and the movement of T cells is perturbed (2) in the spleen and lymph nodes of *M. lepraemurium*-infected mice and rats. Our preliminary experiments had shown that, in fact, more cells could be isolated from infected spleens and lymph nodes (due to their enlargement), but that the relative number of nylon wool-nonadherent T lymphocytes and of anti-Thy 1-sensitive lymphocytes was significantly lower than in control mice. On the other hand, the ability of these T lymphocytes to be stimulated in vitro by PHA and ConA was preserved provided that 2-mercaptoethanol was supplied to the culture medium in order to maintain cell viability or compensate for the lack of macrophages (1).

A second reason for the lack of responsiveness concerns the accessory role of macrophages in mitogen-induced lymphoproliferation. Although it is still a matter of controversy, macrophages would play an important role. For instance, at low concentration they enhance, whereas at high concentration they inhibit, the blastogenic response (19, 20), and the extent of inhibition is increased by macrophage activation (12). Moreover, it appears that various mitogens require different lymphocyte-to-macrophage ratios for optimal stimulation (16). On the other hand, it is not known yet whether the overloading of macrophages with *M. lepraemurium* would lead to a defect in presenting the mitogens to the lymphocytes. This seems not to be the case, since the addition of 2-mercaptoethanol to the cultures of infected spleen cells did not restore the blastogenic response (Turcotte, unpublished data). The possibility remains, however, that the depression of blastogenesis, as observed in this study, was due to the inhibitory effect of an increase in the percentage of macrophages in the cultures.

An excess of stimulating agents caused by the simultaneous presence of *M. lepraemurium* (as specific antigen) and of mitogens in cultures of infected spleen cells could also depress blastogenesis. However, no significant inhibition was observed when noninfected spleen cells were cultured with amounts of bacilli similar to those usually detected in cultures of infected spleen cells. A 10-fold increase in the number of bacilli did result in a significant inhibition which appeared to be caused by competition between the normal spleen cells and the bacilli for the mitogens (S. Lemieux et al., manuscript in preparation).

Finally, evidence was presented recently by ourselves (23) and other workers (3) that suppressor cells are induced in the spleen of mice after *M. lepraemurium* infection; these cells are currently believed to be responsible for the suppression of blastogenesis. The fact that the depression of the response to T-cell mitogens precedes depression of the response to B-cell mitogens in the spleen could be due either to a higher sensitivity of T lymphocytes to the action of suppressor cells or to the presence of two types of suppressor cells, one appearing earlier during

the infection and affecting the T lymphocytes, and the other appearing later and affecting the B lymphocytes. Recently, Bullock et al. (3) reported that two types of suppressor cells appearing at different times during the course of infection were present in the spleen of *M. lepraemurium*-infected C3H/Anf mice.

On the basis of the evolution of mitogenic responses, it is likely that suppressor cells originate first in the spleen and that some then migrate into the peripheral lymph nodes. The depression of the response to T-cell mitogens at the lymph node level could be due, as mentioned above, to the higher sensitivity of T lymphocytes to suppressor cells or to the migration into the lymph nodes of only the type of suppressor cells that affect T lymphocytes. However, the enhanced response of lymph node cells to the B-cell mitogens should be studied further in order to determine whether this enhancement is related to the increase in the percentage of B cells in the draining lymph nodes of mice stimulated with mycobacteria (9).

In contrast to C3 mice, B6 mice inoculated in the hind footpad did not present any clinical sign of murine leprosy, except for a local inflammatory reaction at the end of the observation period (466 days after infection). However, the occurrence of the depression of the T-cell response in the spleen, even though delayed compared with that of C3 mice, suggests that the B6 mice might have eventually succumbed to the disease if they had been kept for a longer period of time. This suggestion is also supported by the fact that acid-fast bacilli were detected histologically in the draining lymph nodes and in the spleen at the time of sacrifice. Obviously, nothing is known about the viability and other biological properties of these bacilli. In the experiments of Closs (4), in which 2.7×10^6 *M. lepraemurium* were inoculated s.c., the C57BL/6 mice were observed for 225 days, and after an early phase of bacillary proliferation the number of bacilli in the footpad and in the draining lymph nodes remained constant until the end of the observation period.

During the early stage of leprous infection in mice infected i.p., the depression of the blastogenic response to ConA occurred earlier than that to PHA (Fig. 1). This indicates that these two types of mitogens do not stimulate the same subpopulation of T lymphocytes (22) and also suggests that the ConA-responding T lymphocytes are more sensitive to the action of suppressor cells. With the B-cell mitogens, no parallelism in the modification of the blastogenesis was found; for instance, in the spleen of mice infected in the footpad, the response to DS increased whereas that to LPS decreased (Table 4); in the lymph nodes, the increase of the response to LPS preceded the increase of the response to DS (Table 5). It has been reported that DS can activate immature B lymphocytes to proliferate, whereas LPS acts on a more differentiated population of B cells (7). Therefore, in advanced murine leprosy, a deficiency in the maturation of B lymphocytes would be present and could be responsible for the depression of antibody syntheses, as already reported by other workers (18, 26).

No definitive conclusion about the presence of suppressor cells in mouse leprosy and their role in the susceptibility of various strains of mice to the disease can be reached until the exact number of macrophages has been evaluated in *M. lepraemurium*-infected lymphoid organs and detailed dose-response studies have been done. Moreover, it should be noted that macrophages may not be the only type of regulatory cells responsible for the suppressive activity observed in murine leprosy (3, 23). Further experiments should reveal whether the depression of in vitro blastogenesis can be related to the loss of cutaneous delayed-type hypersensitivity to antigens of *M. lepraemurium* in both strains of mice according to the route of infection and to the generalized state of anergy that characterizes the disseminated infection to *M. lepraemurium* (2).

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