

Biological Properties of Factors Secreted by Antigen-Reactive Suppressor Cells in Mice Infected with *Mycobacterium lepraemurium*

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Antigen-reactive cells were isolated from the spleens of *Mycobacterium lepraemurium*-infected C57BL/6 mice on petri dishes coated with mycobacterial antigens. When adoptively transferred to syngeneic mice, the mycobacterial antigen-reactive cells were found to depress the induction and expression of the delayed-type hypersensitivity (DTH) reaction to *M. lepraemurium* antigens. The adoptive transfer of soluble suppressor factors (SF) secreted by these cells inhibited only the expression of DTH. The cells depressing the induction of DTH mainly belonged to the L3T4⁺ (CD4⁺) T-lymphocyte subset, whereas those depressing its expression differed from the L3T4⁺ and Lyt-2⁺ (CD8⁺) subsets. Treatment of *M. lepraemurium*-infected mice with SF reduced their mean survival time and enhanced the multiplication of bacilli at the site of infection and their dissemination to the spleen and liver. In vitro at least, SF appeared to interfere at the level of mycobacterial antigen recognition by T lymphocytes rather than at the levels of antigen processing and presentation by macrophages.

Suppressor cells have been found to develop in the spleens of various strains of mice during the course of infection with *Mycobacterium lepraemurium* (6, 30). These suppressor cells can depress several in vitro immune functions, such as the T-cell proliferative response to polyclonal mitogens (30), to alloantigens (12), and to specific antigens (4) and the antibody response to sheep erythrocytes (6). There is also evidence that these suppressor cells are involved in the depressed production of interleukin-2 (11, 31) and in the depressed expression of high-affinity interleukin-2 receptors in concanavalin A-activated spleen cells from *M. lepraemurium*-infected mice (31). In general, the *M. lepraemurium*-induced suppressor cells have been identified as T lymphocytes and/or macrophages (6, 30). However, the exact phenotype displayed by the suppressor T cells is still a matter of controversy. In most of the above-mentioned studies, cellular depletion techniques were used for the purification and characterization of *M. lepraemurium*-induced suppressor cells and for the study of their immune properties. Recent results from our laboratory (24) have shown that *M. lepraemurium*-induced suppressor cells, at least those involved in the inhibition of T-cell proliferative responses to mitogens and antigens, can be isolated on petri dishes coated with mycobacterial antigens and that these cells have the ability to release suppressor factors (SF) in culture supernatants. The purpose of the present study was to investigate by means of adoptive-transfer experiments some of the biological properties of these antigen-reactive suppressor cells and of SF derived from them.

Suppressor cells were found able to depress both the induction and the expression of the delayed-type hypersensitivity (DTH) response to mycobacterial antigens, whereas SF inhibited only the expression of this cutaneous reaction. In addition, SF-containing culture supernatants interfered

with the mechanisms of acquired resistance to *M. lepraemurium* infection, as demonstrated by the reduction of the mean survival time of infected mice and the enhancement of acid-fast bacterial growth at the site of infection and the dissemination of acid-fast bacteria to the spleen and liver.

MATERIALS AND METHODS

Microorganisms. The Hawaiian strain of *M. lepraemurium* was maintained by serial passages in C57BL/6 female mice as previously described (30). When needed, fresh bacilli were isolated from the livers or spleens of infected mice and counted by the slide technique of Shepard and McRae (27). *Mycobacterium avium* (TMC 706; obtained from the Trudeau Mycobacterial Culture Collection, Saranac Lake, N.Y.) was also used because of its very high DNA relatedness (>85%) to *M. lepraemurium* (2) and its ability to induce a proliferative response in spleen cell cultures from *M. lepraemurium*-sensitized mice (unpublished observations). It was cultured as a pellicle on the surface of Sauton medium, and the bacilli were harvested by centrifugation (1,000 × g for 30 min) at the end of the exponential growth phase. *Corynebacterium* sp. (ATCC 15927) was used as a control. It was grown on dextrose proteose no. 3 agar for 2 days at 37°C; the bacilli were harvested by scraping the surface of the solid medium and were washed twice in Hanks balanced salt solution.

Bacterial extract preparation. Suspensions containing 5 × 10⁹ *M. lepraemurium* bacilli and ca. 1.0 g (wet weight) of *M. avium* or *Corynebacterium* sp. per ml of phosphate-buffered saline were sonicated for 20 min under cooling on ice with a Braunsonic 1510 sonicator (B. Braun Melsungen AG) set at 80 W. The bacterial debris was eliminated by centrifugation at 40,000 × g for 30 min. The proteins in the supernatants were precipitated at 4°C overnight with 50% ammonium sulfate, dissolved in phosphate-buffered saline, dialyzed against distilled water until free of ammonium ions, and lyophilized. The protein content of the lyophilized prepara-

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tions was estimated by a protein assay (Bio-Rad Laboratories, Mississauga, Ontario, Canada) with bovine serum albumin as a standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in slab gels (11%) under reducing conditions as described by Laemmli (15). The gels were loaded with sonic extracts of bacteria at a protein concentration of 2.5 µg per gel. Electrophoresis was done overnight at a current of 5.0 mA, and the gels were stained with the Bio-Rad silver-staining kit.

Infection of mice. Female mice of the inbred strain C57BL/6, purchased from Charles River Breeding Laboratories, Wilmington, Mass., were maintained under standard laboratory conditions and fed Purina chow and water ad libitum. They were infected intravenously (i.v.) with 10^7 *M. lepraemurium* bacilli. Nine weeks after infection, that is, at the time suppressor cells were detected in vitro (24), spleens from infected and age-matched control mice were removed under sterile conditions and the lymphoid cells were isolated by a previously described method (30).

Spleen cell separation. Spleen cells (10 ml; 10^7 cells per ml) were layered on Teflon-coated petri dishes (150 mm) and incubated in a humidified atmosphere of 5% CO₂ at 37°C for 2 h to remove adherent cells (14). Nonadherent cells were further separated into surface immunoglobulin-positive and immunoglobulin-negative cells by a panning technique (33). In brief, nonadherent cells (3 ml; 10^7 cells per ml) were layered on plastic petri dishes (100 mm) coated with affinity-purified goat anti-mouse immunoglobulins G and M (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.). The surface immunoglobulin-negative cells, hereafter referred to as the T-cell-enriched population, were further separated into mycobacterial antigen-reactive and -nonreactive cells on antigen-coated plastic petri dishes as follows. A sonic extract of *M. avium* (10 ml; 75 µg of lyophilized extract per ml of phosphate-buffered saline) was poured into 100-mm-diameter dishes (catalog no. 8-757-13; Fisher Scientific Co.). The dishes were incubated for 24 h at 4°C and washed four times with 10 ml of Hanks balanced salt solution. The T-cell-enriched spleen cell population (5 ml; 10^7 cells per ml) from *M. lepraemurium*-infected mice was layered on the dishes. After incubation for 1 h at 37°C in 5% CO₂ (the dishes being occasionally swirled during that period), the nonadherent (antigen-nonreactive) cells were removed by pouring off the contents of the dishes and washing the dishes gently four times with 10 ml of warm (37°C) RPMI 1640 culture medium. Finally, the adherent (antigen-reactive) cells were harvested by three successive flushings of the dishes with 10 ml of chilled (4°C) culture medium and scraping of the dish surfaces with a rubber policeman.

Monoclonal anti-L3T4 (GK 1.5) and anti-Lyt2 (53-6.7) antibodies (Becton Dickinson, Mountain View, Calif.) were used for selective cell depletion and for determination of the phenotypes of mycobacterial antigen-reactive cells. The antibodies were used in the presence of Low Tox rabbit complement (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) as recommended by the supplier. Treatments with anti-L3T4-complement and with anti-Lyt2-complement were found to reduce the number of antigen-reactive cells by about 52 and 24%, respectively. The effectiveness of T-cell depletion was determined by indirect immunofluorescence in an Epic-C flow cytometer (Coulter, Hialeah, Fla.) with fluorescein-labeled goat anti-rat immunoglobulin (heavy plus light chains) (Kirkegaard & Perry). Depletion was usually 100% with anti-Lyt2-complement, but it was 90 to 95% with anti-L3T4-complement.

Production of SF. Mycobacterial antigen-reactive cells

were plated in tissue culture flasks (no. 25100; Corning, Corning N.Y.) at a density of 5×10^5 cells per ml of RPMI 1640 medium in the absence of fetal calf serum, antibiotics, and mitogen or antigen. After 15 h of incubation at 37°C in a humidified atmosphere of 5% CO₂, supernatants were harvested by centrifugation ($500 \times g$ for 10 min), filtered through a 0.22-µm-pore-size membrane (Acrodisc no. 4192; Gelman Sciences Inc., Ann Arbor, Mich.), and stored at -20°C until testing for suppressive activity in adoptive-transfer experiments.

Adoptive transfer of suppressor spleen cells and SF. Mycobacterial antigen-reactive cells (1 ml; 5×10^5 cells per ml) from infected mice (or from normal mice, as controls) and supernatant (1 ml) derived from the same number of cells were inoculated i.v. into syngeneic mice just prior to or 5 weeks after footpad infection with 10^7 *M. lepraemurium* bacilli. In some experiments, antigen-reactive cells (1 ml; 5×10^5 cells per ml) were first treated with anti-L3T4-complement or anti-Lyt2-complement, and then the residual cells, suspended in 1 ml of culture medium, were inoculated into each mouse. Normal recipient mice were challenged 5 weeks later in the opposite rear footpad with 20 µl of a sonicated preparation of *M. lepraemurium* (equivalent to 10^8 bacilli) to study the effects of suppressor cells or SF on the induction of DTH, whereas infected recipient mice were challenged 24 h after the transfers to study the effects of suppressor cells or SF on the expression of DTH. The footpad thickness was measured with a dial-gauge caliper prior to and 24 and 48 h after the challenge and was expressed in 0.1-mm units.

Measurement of bacterial growth. *M. lepraemurium* bacilli isolated from the footpads, spleens, and livers of infected mice were counted by the slide technique of Shepard and McRae (27).

Priming of macrophages. Resting peritoneal macrophages from normal mice were obtained by peritoneal washings with 15 ml of cold RPMI 1640 medium containing 0.5% heparin (Linson, Montreal, Quebec, Canada), followed by 2 h of incubation of 3 ml of peritoneal cells (10^7 cells per ml) on plastic petri dishes coated with Teflon. The adherent cells were collected by scraping the dishes with a rubber policeman in the presence of 0.02% EDTA (J. T. Baker Chemical Co., Phillipsburg, N.J.), adjusted to 10^6 cells per ml, and incubated at 37°C in a humidified atmosphere of 5% CO₂ with *M. avium* antigens (75 µg/ml) in the presence of SF-containing supernatants at a dilution of 50% (vol/vol). After 4 h of incubation in siliconized test tubes, primed macrophages were washed four times with RPMI 1640 medium and adjusted to a concentration of 5×10^5 cells per ml.

Sensitization of T lymphocytes. The T-cell-enriched population from normal mice (5×10^6 cells per ml) was incubated with the antigen-primed macrophages in plastic petri dishes at a ratio of 10:1 in the presence of SF-containing supernatants (50% [vol/vol]). After 4 h of incubation at 37°C in a humidified atmosphere of 5% CO₂, the plastic-nonadherent, sensitized T cells were washed three times in RPMI 1640 medium and 5×10^6 cells in 40 µl were injected subcutaneously in an admixture with a sonicated preparation of *M. lepraemurium* (equivalent to 10^8 bacilli) into the left hind footpad of normal syngeneic mice. The reading of the DTH reaction was done at 24 and 48 h after local transfers as described above.

Statistical analysis. Statistical significance was determined with the Student *t* test; $P < 0.05$ was considered significant.

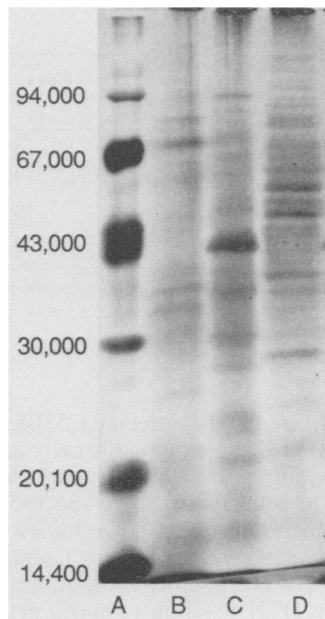


FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of bacterial sonic extracts. Samples (5.0 μ g of protein from sonic preparations) were electrophoresed on 11% polyacrylamide gels and stained with silver. Lanes: A, protein standards; B, C, and D, *M. lepraemurium*, *M. avium*, and *Corynebacterium* sp. extracts, respectively. Numbers at left are masses in daltons.

RESULTS

T-cell-enriched populations from the spleens of *M. lepraemurium*-infected mice and from age-matched control mice were incubated in plastic dishes coated with a sonic extract of *M. avium* to isolate the mycobacterial antigen-reactive cells. The results (means from eight experiments \pm standard deviations) revealed that $12.5\% \pm 2.5\%$ of splenic T cells from infected mice and $3.6\% \pm 1.4\%$ of those from normal mice had the capacity to adhere to mycobacterial antigen-coated dishes. Dishes coated with a sonic extract of *Corynebacterium* sp. had also the capacity to retain splenic T cells. However, the relative numbers of retained cells were about the same (ca. 4.0%) for the T-cell-enriched populations originating from *M. lepraemurium*-infected or normal mice.

The three bacterial extracts were analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide after having been boiled and reduced. The *M. lepraemurium* and *M. avium* sonic extracts were composed of 20 to 25 protein species ranging in molecular masses from about 100 kDa to about 15 kDa (Fig. 1, lanes B and C). Major qualitative and quantitative differences existed between these two mycobacterial preparations. Many more (at least 40) protein species were observed in the *Corynebacterium* sp. extract (Fig. 1, lane D).

Effects of adoptive transfer of mycobacterial antigen-reactive cells and culture supernatants derived from them on the DTH response to mycobacterial antigens. Mycobacterial antigen-reactive cells isolated from the spleens of mice infected i.v. 9 weeks earlier with *M. lepraemurium* and known to possess suppressor activity in vitro (24) were adoptively transferred i.v. to mice prior to and 5 weeks after subcutaneous infection with *M. lepraemurium* to study their effects on the induction and expression of the DTH reaction to *M.*

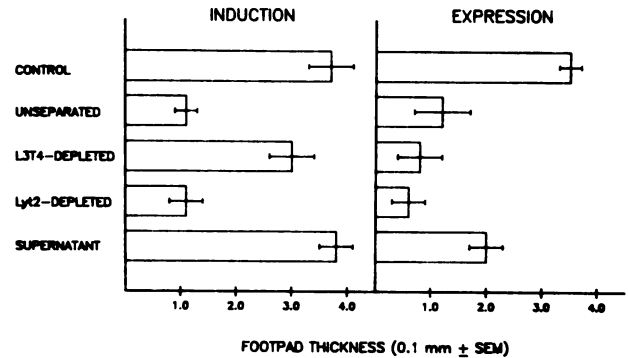


FIG. 2. Effects of transferred antigen-reactive spleen cells (5×10^5 per mouse) and their culture supernatants on the induction and expression of DTH to *M. lepraemurium* antigens. CONTROL, Footpad reaction detected in mice which received adoptive transfers of either the same number of normal spleen cells that adhered to mycobacterial antigen-coated petri dishes or the culture supernatants derived from these cells. Mice were challenged in one rear footpad with *M. lepraemurium* antigens 5 weeks (induction) or 24 h (expression) after the transfers. Footpad thickness was measured 48 h later and expressed as the mean thickness for five or six mice \pm the standard error of the mean (SEM).

lepraemurium antigens. The unseparated population of antigen-reactive cells had the ability to depress the induction ($P < 0.001$) and the expression ($P < 0.01$) of DTH to *M. lepraemurium* antigens (Fig. 2). The antigen-nonreactive spleen cells, that is, those which did not adhere to antigen-coated dishes, were devoid of such properties (data not shown). Surprisingly, the culture supernatant derived from the unseparated population of mycobacterial antigen-reactive cells had the capacity to depress the expression ($P < 0.01$) but not the induction of DTH, results which were in good agreement with our previous results obtained when culture supernatants derived from the whole spleen cell population were used (25).

In an attempt to characterize the phenotypes of these suppressor cells, we treated the unseparated population with anti-L3T4-complement and anti-Lyt2-complement prior to adoptive transfer. The depression of the induction of DTH was maintained after the anti-Lyt2 treatment, whereas a significant loss ($P < 0.01$) of the suppressive activity was observed after the anti-L3T4 treatment (Fig. 2). These results were interpreted to indicate that the depression of the induction of DTH was mediated by antigen-reactive L3T4⁺ suppressor T cells. In the expression model, however, the treatment of antigen-reactive cells with both antisera did not significantly reduce their suppressive activity, indicating that the depressed expression of DTH could be mediated by a cell population different from these two T-cell subsets. The possibility that these suppressor cells belong to the monocyte-macrophage lineage appears unlikely, since plastic-adherent cells were eliminated prior to the isolation of antigen-reactive suppressor cells. Moreover, natural killer cells, another potential candidate (29), were not found in the antigen-reactive cell population (data not shown). The suppressive activity of mycobacterial antigen-reactive cells appeared to be specific in the sense that upon adoptive transfer, these cells affected neither the induction nor the expression of DTH to sheep erythrocytes in mice (data not shown).

Effects of culture supernatants derived from mycobacterial antigen-reactive cells on the evolution of *M. lepraemurium* infection. The effects of SF-containing supernatants on the

TABLE 1. Effects of culture supernatants derived from mycobacterial antigen-reactive cells on the evolution of *M. lepraemurium* infection

DTH model	Donor mice	No. of bacilli (log ₁₀ /organ ± SEM in ^a :			Mean survival time of mice (days ± SEM) ^b
		Footpad	Spleen	Liver	
Induction	Normal	8.5 ± 0.4	7.8 ± 0.1	7.1 ± 0.1	536.5 ± 21.6
	Infected	9.3 ± 0.5	7.6 ± 0.2	7.1 ± 0.1	491.5 ± 29.9
Expression	Normal	8.4 ± 0.12	7.5 ± 0.0	7.8 ± 0.4	544.8 ± 22.3
	Infected	9.9 ± 0.4 ^c	10.1 ± 0.3 ^c	10.3 ± 0.4 ^c	374.8 ± 22.3 ^c

^a Bacterial growth was estimated at 40 weeks postinfection in mice receiving culture supernatants from normal and *M. lepraemurium*-infected mice. Each group consisted of four mice.

^b Each group consisted of five or six mice.

^c Statistically different at $P < 0.05$, at least, when compared with the corresponding group of mice receiving normal supernatants.

mean survival time of infected mice and on the multiplication of *M. lepraemurium* bacilli at the inoculation site and their dissemination to the spleen and liver were next investigated. In the group of mice receiving culture supernatants just prior to infection (induction model), none of the investigated parameters differed significantly from those observed in infected mice treated with culture supernatants from normal spleen cells (Table 1). In contrast, in mice treated with culture supernatants at 5 weeks postinfection (expression model), a significant increase in the number of acid-fast bacilli at the infection site and in the spleen and liver was observed. In addition, the mean survival time of SF-treated mice was shorter than that of control mice. These results were not due to *M. lepraemurium* bacilli contaminating the SF-containing supernatants, since these supernatants were filtered through a 0.22- μ m-pore-size membrane prior to adoptive transfers to eliminate free bacilli. On the other hand, the possibility of desensitization of recipient mice by soluble *M. lepraemurium* antigens cannot be completely excluded.

Effects of SF on the priming of macrophages and the sensitization of T cells. To study the mechanism of action of SF on the depressed expression of DTH to mycobacterial antigens, we first primed normal macrophages and T-cell-enriched populations in vitro in the presence or absence of SF. Then, the in vitro sensitized nonadherent cells were adoptively transferred in an admixture with *M. lepraemurium* antigens to the footpads of normal mice. When normal macrophages were primed with mycobacterial antigens and then placed in contact with normal T lymphocytes, the latter cells became sensitized, since they acquired the capacity to transfer a positive DTH-like reaction (Table 2, group 1). In the absence of primed macrophages (group 2) or when in vitro sensitized T lymphocytes were injected without mycobacterial antigens (data not shown), no transfer of a positive DTH-like reaction was observed. The presence of SF-containing supernatants in the culture medium did not interfere with the priming of macrophages, since SF-treated macrophages induced T-cell sensitization which, upon adoptive transfer, induced a DTH-like response (group 3). On the other hand, the presence of SF at the time of T-cell sensitization with the antigen-presenting cells markedly reduced the DTH-like response (group 4). It would appear, therefore, that SF can interfere at the level of T-cell sensitization rather than at the level of macrophage priming.

DISCUSSION

The present study clearly demonstrates that cells depressing both the induction and the expression of the DTH response to mycobacterial antigens are present in the

spleens of *M. lepraemurium*-infected C57BL/6 mice and that many (if not all) of these suppressor cells can be positively selected on petri dishes coated with mycobacterial antigens. These suppressor cells appear to be specific for mycobacterial antigens, since they were not retained on petri dishes coated with antigens prepared from *Corynebacterium* sp. and since they did not inhibit, upon adoptive transfer, the induction of the DTH reaction to sheep erythrocytes (data not shown). The ability of *M. lepraemurium*-induced suppressor cells to react with mycobacterial antigens would suggest the presence of antigen-specific T-cell receptors and/or idiotypes on their surfaces. In fact, T-cell receptors have recently been shown to be present on the surfaces of some suppressor T-cell clones (10, 28). Mycobacterial antigen-reactive suppressor cells have also been found by Campa et al. (7) in the spleens of C57BL/6 mice infected i.v. with a massive dose of *Mycobacterium bovis* BCG, an organism which is nonpathogenic for mice. The ability of suppressor cells to specifically react with antigens would represent a general phenomenon, since antigen-reactive and idio-type-positive suppressor T cells down-regulating both humoral and cell-mediated immune responses have repeatedly been observed in mice rendered tolerant of a variety of nonreplicating antigens and haptens (review in reference 1).

The exact nature of suppressor T cells down-regulating the afferent and efferent arms of DTH to mycobacterial antigens in mice is still a matter of controversy. For example, radiosensitive T lymphocytes displaying the Lyt1⁺ and Lyt2⁺ phenotypes depressed the induction of DTH to *M. lepraemurium* antigens in C3H mice, whereas radioresistant T lymphocytes expressing the Lyt1⁺ and Lyt2⁻ phenotypes depressed the expression of this DTH (25). Moreover,

TABLE 2. Effects of SF on the priming of macrophages and the sensitization of T cells

Group	Primed macrophages (SF) ^a	Sensitized T cells (SF) ^b	Footpad thickness (0.1 mm ± SEM) ^c
1	+ (-)	+ (-)	2.7 ± 0.2
2	- (-)	+ (-)	0.7 ± 0.3 ^d
3	+ (+)	+ (-)	2.5 ± 0.2
4	+ (-)	+ (+)	0.7 ± 0.2 ^d

^a Resting normal peritoneal macrophages were primed in vitro with mycobacterial antigens in the presence or absence of SF-containing supernatants (see Materials and Methods).

^b Primed macrophages were added to normal T cells at a ratio of 1:10, and the mixture was incubated for 4 h in the presence or absence of SF, after which the nonadherent cells were adoptively transferred in an admixture with *M. lepraemurium* antigens to the footpads of normal syngeneic mice.

^c The footpad thickness was measured 48 h after local transfer. Each value represents the mean thickness ± standard error of the mean for six mice.

^d $P < 0.01$.

suppressor cells expressing the Lyt2⁺ phenotype depressed both the induction and the expression of DTH to *M. intracellulare* Mino antigens in C57BL/6 mice (20). Finally, in tuberculin-anegetic BCG-infected mice (7), idiotype-positive suppressor T cells (the phenotype of which remains to be established) depressed the expression of DTH to tuberculin purified protein derivative, whereas in the present study, the antigen-reactive L3T4⁺ subset depressed the induction of DTH to *M. lepraemurium* antigens. The reasons for these divergent results are presently unknown. These results may be related to differences in the susceptibilities of various strains of mice to mycobacteria (4), the time at which suppressor cells are isolated after infection (7), and/or the method used for isolating and purifying suppressor cells. Together, these studies suggest that, as already demonstrated in many other experimental systems (1), the *M. lepraemurium*-induced suppressive activity depends on complex immunosuppressive circuits involving different cell types and suppressive mediators.

The reason why SF-containing supernatants can depress the expression but not the induction of DTH to *M. lepraemurium* antigens (Fig. 2) is presently unknown. A simple explanation would be that a direct cell-cell contact is required for activating the cellular events leading to the depressed induction of DTH, whereas those leading to the depressed expression of DTH can be triggered by soluble mediators. Experiments are currently in progress to study the physicochemical nature of SF to determine whether they are related to T-cell receptors (9, 22) or to other DTH- and granuloma-depressing cytokines, such as those described in other infection models (3, 8, 18).

It is well established that mice infected with intracellularly growing microorganisms, including *M. lepraemurium*, develop a state of immune resistance against these organisms (17, 21). This acquired resistance largely depends on the recruitment of macrophages at the site of infection and on their activation by the macrophage-activating lymphokines produced by the antigen-activated T lymphocytes (5), the activated macrophages possessing an increased bactericidal and/or bacteriostatic activity. As shown in this study, a marked enhancement of mycobacterial growth at the inoculation site and in the spleen and liver was observed when *M. lepraemurium*-infected mice were treated with SF-containing supernatants at 5 weeks postinfection. The most simple explanation for these results would be that *M. lepraemurium*-induced SF have the ability to interfere in vivo with the molecular events leading to macrophage activation by depressing, for example, the production of macrophage-activating lymphokines, including gamma interferon. It is well known that interleukin-2 and gamma interferon play a major role in the development of cell-mediated immunity to mycobacteria by enhancing or promoting the bactericidal activity of the macrophages (13, 26). The possibility that SF can compete for the specific gamma interferon receptors and thus depress the expression of Ia markers on the surfaces of activated macrophages (32) appears unlikely, since the results of in vitro sensitization experiments (Table 2) suggested that SF-treated normal macrophages maintained their ability to process and present mycobacterial antigens (an Ia-dependent mechanism) to the T lymphocytes.

On the other hand, SF-treated normal lymphocytes did not acquire the ability to induce a positive DTH-like response to mycobacterial antigens when adoptively transferred to normal recipient mice. The absence of in vitro sensitization could result from a failure of mycobacterial antigen-reactive T cells to recognize specific antigens and/or to proliferate

and differentiate into the DTH-mediating T-cell subset. This interpretation would agree with recent results from this laboratory showing that SF-containing supernatants, at least those which were active in the suppression of the T-cell proliferative response to mitogens, depressed the production of interleukin-2, a lymphokine that plays a major role in the clonal expansion of antigen-reactive T lymphocytes and their differentiation into effector cells (16). In addition, Ptak et al. (23) demonstrated that the SF induced in mice immunodepressed with dinitrofluorobenzene had the capacity to inhibit the secretion of vasoactive mediators needed for the recruitment of T lymphocytes involved in the development of DTH at the site of the cutaneous reaction. Using the same experimental model, Miller and Jenkins (19) found that dinitrophenol-specific SF could abolish the secretion of the migration inhibitory factor by T lymphocytes involved in the development of DTH. Further experiments will be needed to determine whether SF act either directly at the level of T-cell receptors (e.g., by competitive inhibition) or at the level of cellular proliferation.

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