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Immune Response to Atypical Mycobacteria: Immunocompetence of Heavily Infected Mice Measured In Vivo Fails To Substantiate Immunosuppression Data Obtained In Vitro

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The results of in vitro experiments designed to measure the immunocompetence of mice heavily infected with the atypical mycobacterial pathogens *Mycobacterium avium* and *Mycobacterium simiae* were compared with the results of experiments which used in vivo approaches. Blastogenic responsiveness in vitro both to mitogen and to alloantigen was severely depressed in the heavily infected mice; this responsiveness could be restored by removal of an inhibitory Thy-1.2$^-$, nylon wool-adherent cell population. No evidence was found to support the previous contention that suppressor T cells may play a role in the inhibition of this responsiveness. These results were then compared with experiments which measured the ability of the infected animal to elicit a delayed-type hypersensitivity response to sheep erythrocytes in vivo. However, although delayed-type hypersensitivity responses in vivo were also depressed, evidence was obtained which suggested that this unresponsiveness was due to inadequate sensitization of T cells, possibly due to catabolism of antigen, rather than due to the influence of an active, immunosuppressive mechanism. Finally, despite the severely depressed ability of cells from infected mice to respond to alloantigenic stimulation in vitro, infected animals were fully able to cause the regression of a tumor implant in vivo.

Acquired cell-mediated immunity to mycobacteria such as *Mycobacterium bovis* BCG and to *Mycobacterium tuberculosis* is established and is known to involve the generation within the host of protective T lymphocytes (12, 15, 18). In contrast, however, the nature of the host response to many of the so-called "atypical" mycobacteria remains to be defined, with particular regard to those pathogens such as *Mycobacterium avium* and *Mycobacterium simiae* which have been identified in the pathogenesis of chronic inflammatory lung disease in humans (25). This clinical profile is similar to that seen in experimental animal models (4, 22–24) in which the course of the infection is characterized by the persistence of the organism in target organs for the lifetime of the host. Results of experiments designed to investigate the cellular basis of this persistence initially led to the suggestion (22–24), based primarily on data obtained in vitro, that such mice may be actively immunosuppressed from mounting a cell-mediated immune response to the infection in vivo.

The present report is the first of a series which attempts to deal with the nature of the in vivo immune response to atypical mycobacteria in mice. It examines the validity of using in vitro measurements to determine the immunocompetence of mice which are heavily infected with *M. avium* or with *M. simiae* and compares data obtained in this way with comparative data obtained by in vivo approaches. The results of these studies show that the ability of spleen cells from heavily infected mice to respond in vitro to blastogenic stimulation by mitogen or alloantigen becomes gradually depressed as the infection progresses but that this responsiveness could be restored by passage of such cells through nylon wool columns. To test the hypothesis that these depressed responses in vitro were a reflection of suppressed responsiveness to antigen stimulation in vivo, heavily infected mice were tested for their capacity to mount a delayed-type hypersensitivity (DTH) reaction to sheep erythrocytes (SRBC). However, despite the observation that these response were also considerably depressed, this paper will present evidence that indicates that the lack of responsiveness in this in vivo assay was apparently a result of inadequate T cell sensitization, rather than a consequence of active immunosuppression. Furthermore, this report will show that despite the depression of allogeneic responsiveness in vitro, the intact animal was able to adequately cause the regression of an allogeneic tumor challenge in vivo.

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MATERIALS AND METHODS

Mice. Specific pathogen-free female B6D2 F_1 (C57BL/6xDBA/2) hybrids and A/Tru mice were supplied by the Trudeau Animal Breeding Facility, Saranac Lake, New York. They were given sterile food and acidified drinking water ad libitum and used when between the ages of 6 and 12 weeks.

Bacteria. *M. avium* (Trudeau Mycobacterial Culture Collection strain 724) and *M. simiae* (1226) were grown in modified Sauton's medium as previously described (4). Mice were infected via a lateral tail vein with appropriate numbers of bacteria suspended in an inoculum of 0.2 ml of phosphate-buffered saline (PBS). Growth curves for these infections in vivo are described in detail elsewhere (23, 24).

Blastogenic assays. Spleens from normal or infected donors were removed asceptically, teased through stainless steel mesh, and washed in RPMI 1640 medium containing 1 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, antibiotics, and 5% heat-inactivated fetal calf serum. Spleen cells were enriched for T cells where indicated by passage through nylon wool columns (10). Cells (5×10^5) were seeded in triplicate into Linbro microtitration multiwell plates (Flow Laboratories, McLean, Va.) and cultured for 48 h at 37°C in the presence of 0.5 μg of phytohemagglutinin (Burroughs Wellcome, Triangle Park, N.C.) in 5% CO_2-enriched humidified air. This concentration of mitogen was determined to be optimum for both infected and normal cells by preliminary

titration. Cultures were pulsed with 0.5 μCi of tritiated thymidine (specific activity, 5 Ci/mmol; Radiochemical Center, Amersham Corp., Arlington Heights, Ill.) for another 20 h and then harvested on glass fiber filters with a Mash II sampler (Microbiological Associates, Bethesda, Md.). Uptake of radioactive label was measured with an LKB liquid scintillation counter.

Mixed lymphocyte cultures. Mixed lymphocyte cultures (MLC) were carried out by culturing spleen cells (5×10^5) from normal or infected B6D2 F_1 animals with 5×10^5 irradiated (1,000 rads) allogeneic (A/Tru, *H-2*^{a}) stimulator cells for 72 h at 30°C in 5% CO_2-enriched air, followed by a further 20 h in the presence of 0.5 μCi tritiated thymidine. At the end of this culture period, cells were harvested, and the incorporation of radiolabel was counted as above. For inhibition assays, 3×10^5 T cell-enriched normal responder spleen cells were cultured with stimulator cells in the presence of variously treated responder spleen cells from infected animals, and the effect of the presence of these additional cells on the blastogenic response of the normal cells was determined. In some experiments, spleen cells were treated with mitomycin C (Sigma Chemical Co., St. Louis, Mo.; 50 μg/ml) for 30 min at 37°C (5) or with antibody to Thy-1.2 plus complement as previously described (18).

Macrophage depletion. Spleen cell suspensions were depleted of macrophages by double passage through Sephadex G-10 columns by the method of Mishell et al. (14) or by adherence to plastic surfaces (2 h at 37°C). After this latter procedure, over 90% of the adhered cells exhibited morphology characteristic of macrophages by phase contrast microscopy.

DTH to SRBC. Mice were primed intravenously with a range of concentrations of SRBC and later tested at indicated times for their ability to mount a DTH response by challenge in a hind footpad with 10^8 SRBC in 40 μl of pyrogen-free saline. Footpad swelling was measured against time with dial calipers (Schnelltäster, Hessen, Germany) that are capable of measuring 0.05-mm increments in thickness.

Passive transfer of cells. Appropriate numbers of spleen cells were resuspended in cold PBS containing 1% fetal calf serum and infused via a lateral tail vein. In some experiments, cell suspensions were enriched for T cells by depletion of B cells by adherence to plastic dishes coated with antisera to mouse immunoglobulin (18). Plastic dishes (4030; Lab-Tek Products, Westmont, Ill.) were coated with goat anti-mouse immunoglobulin G (Cappel Laboratories, Cochranville, Pa.) by adding 20 ml of 0.05 M Tris-hydrochloride buffer (pH 9.5) containing 20 μg of antibody ml^{-1} and incubating dishes at 25°C for 25 min. Unbound antibody was washed away with cold PBS. Spleen cells were added in a volume of 20 ml of RPMI plus 2% fetal calf serum (2×10^7 cells ml^{-1}) and incubated at 4°C for 45 min. Unattached cells were harvested, washed, and resuspended as above.

Tumor regression in vivo. The SA-1 spindle cell sarcoma (*H-2*^{a}) was grown in ascites form in the peritoneal cavities of A/Tru mice. After 7 days, they were harvested in heparinized PBS, washed twice, and subjected to secondary passage for a further 7 days. After this period, cells were harvested, washed, and resuspended in PBS. Mice were inoculated in a hind footpad with 5×10^5 viable SA-1 tumor cells in 40 μl of PBS, and the growth of the tumor was followed against time by measuring the swelling of the tumor mass with dial calipers.

RESULTS

Depression of in vitro blastogenic responses. Whole spleen cells taken from infected donors were depressed in their ability to respond to blastogenic stimulation in vitro either by mitogen (Table 1) or by alloantigen (Table 2). This depression, which was first evident by about day 30, persisted for several weeks in both groups of infected animals before showing some evidence of recovery. In contrast, however, passage of cells from both groups of infected donors over nylon wool columns restored blastogenic responsiveness to close to, or in some cases above, that of control values. Only on day 30 of these infections was there a significant depression in responsiveness to phytohemagglutinin stimulation (Table 1). These results, therefore, were taken to indicate that the observed depression of blastogenic responses was mediated principally by a nylon wool-adherent cell population.

Characterization of the inhibitory adherent cell population. To further characterize the nylon wool-adherent cell population identified by the above results, spleen cells were harvested from infected donors at the height of the depression in vitro (day 30) and subjected to various depletion procedures. After these procedures, cells were then assayed for their ability to enhance or inhibit the MLC response when cocultured with a similar number of normal (indicator) spleen cells. The results obtained (Table 3) demonstrate that the ability of infected cells to inhibit the MLC response of cocultures was mediated by a population of strongly adherent macrophage-like spleen cells, as evidenced by the failure to elute these cells from the nylon wool column by the addition of ice-cold medium and by their adherence to plastic surfaces. To further determine whether these adherent cells possessed characteristics typical of macrophages, spleen cells were fractionated by double passage over Sephadex G-10 columns; this procedure completely removed the inhibitory effect on the MLC response. Moreover, the data also shows that the inhibitory cell population was resistant to treatment with mitomycin C and to treatment with antibody to Thy-1.2 plus complement. These results are, therefore,

TABLE 1. Blastogenic response to phytohemagglutinin stimulation

Day of infection	Uptake of tritiated thymidine (cpm)[a]					
	Unfractionated spleen cells from:			Nylon wool-passed spleen cells from:		
	Normal	*M. avium* infected	*M. simiae* infected	Normal	*M. avium* infected	*M. simiae* infected
5	96,102 ± 6,537	92,703 ± 8,278	90,138 ± 7,328	108,230 ± 9,440	91,239 ± 8,394	91,237 ± 7,321
15	72,936 ± 2,922	73,914 ± 9,219	81,345 ± 6,233	97,127 ± 8,133	97,929 ± 7,212	90,139 ± 7,992
30	88,187 ± 6,990	46,129 ± 3,888[b]	29,138 ± 4,757[b]	83,103 ± 6,123	63,123 ± 4,122[b]	72,127 ± 1,301[b]
70	90,189 ± 7,991	31,280 ± 6,931[b]	65,127 ± 4,001[b]	104,555 ± 7,328	89,123 ± 10,127	99,128 ± 10,979
120	102,550 ± 8,948	67,998 ± 5,344[b]	83,323 ± 7,442[b]	118,989 ± 7,383	121,191 ± 9,202	109,335 ± 11,002

[a] Mean ± standard error of the means ($n = 3$). Background responses of all cultures were <5,000 cpm.
[b] Significantly lower than resposne of normal control ($P < 0.01$).

TABLE 2. Blastogenic response of mixed lymphocyte cultures

Day of infection	Uptake of tritiated thymidine (cpm)[a]					
	Unfractionated spleen cells from:			Nylon wool-passed spleen cells from:		
	Normal	*M. avium* infected	*M. simiae* infected	Normal	*M. avium* infected	*M. simiae* infected
5	32,321 ± 1,290	28,198 ± 2,737	28,120 ± 2,327	40,124 ± 3,017	37,179 ± 5,329	39,138 ± 5,353
15	21,938 ± 1,393	24,325 ± 3,828	22,127 ± 2,007	29,297 ± 3,127	36,937 ± 4,327	33,440 ± 3,071
30	44,027 ± 3,881	31,230 ± 4,404[b]	32,676 ± 4,320[b]	45,721 ± 3,089	40,129 ± 4,345	46,012 ± 6,019
60	37,175 ± 3,306	33,126 ± 1,293	28,741 ± 2,002[b]	45,149 ± 5,979	53,152 ± 5,678	46,074 ± 4,608
90	40,005 ± 5,341	33,910 ± 3,405	31,999 ± 4,990	46,176 ± 5,790	45,159 ± 3,677	54,987 ± 4,028

[a] Means ± standard error of the means ($n = 3$). Background responses (no stimulator cells added) were <3500 cpm.
[b] Significantly lower than response of normal control ($P < 0.01$).

consistent with the hypothesis that the inhibitory cell population was of the macrophage-monocyte cell type. Similar data was obtained with PHA-stimulated blastogenesis as the indicator response (data not shown).

Depressed responsiveness in vivo. The above results, taken alone, could be interpreted as evidence that mice heavily infected with *M. avium* or *M. simiae* acquire a population of macrophages which possess the capacity to suppress the blastogenic or alloreactive responsiveness of resident T cells. To examine this possibility further, the generation of two types of cell-mediated immune responses, believed to require the presence of macrophages as accessory or antigen-presenting cells was measured in these heavily infected animals in vivo. Table 4 shows the elicitation of DTH responses after the sensitization of animals with a standard intravenous inoculum (10^5) of SRBC at various times in mice heavily infected with *M. avium* or *M. simiae*. It shows that mice with either infection become heavily depressed in their capacity to mount a 24-h footpad swelling response to SRBC; this depression was evident by day 30 of the infection and remained low for the duration of the experiment. The data shown in Table 5 indicates, furthermore, that this depression was not a consequence of a shift in the kinetics of the sensitization process.

To examine the underlying nature of this depression, we first tested the possibility that responsiveness could be restored by increasing the sensitizing dose of antigen; this was based on the premise that earlier observations (13) had indicated that infection with mycobacteria can change the dose requirements for optimal sensitization with SRBC. The results shown in Fig. 1 confirm this possibility by demonstrating that the DTH response to SRBC (heavily depressed on day 30) could be restored by increasing the numbers of sensitizing SRBC to 10^8 cells per inoculum.

To test for the possibility that effector T cells had been sensitized to the lower dose of antigen but were immunosuppressed by a mechanism which in turn could be overcome by increasing the sensitizing dose, 8×10^7 T cell-enriched spleen cells from donor animals were passively transferred to normal recipients, which were then immediately challenged with SRBC. The results (Fig. 1) demonstrate that the amount of DTH which could be transferred was proportional to that expressed by donor animals and that the capacity of cells to transfer DTH was ablated by prior treatment with antibody to Thy-1.2 plus complement, thus showing that the transferred response to this model was mediated by T cells.

The above results, however, did not rule out the possibility that T cell effector mechanisms were suppressed after sensitization with the smaller doses of SRBC and that this suppression was subsequently also transferred to the recipients in the above assay. To test this possibility, spleen cells from infected mice inoculated with 10^5 SRBC were transferred to normal mice either immediately after sensitization of these recipients with 10^5 SRBC or just before their challenge in the footpad. Neither procedure (Table 6) affected the capacity of the recipient to mount a DTH response.

Taken together, therefore, these results are consistent with the hypothesis that suppressed responses to SRBC antigens in the infected mice were consequences of inadequate sensitization of T cells in these animals and that sensitization could be restored by increasing the antigen dose; no evidence could be provided to support the possibility that the sensitization process was actively immunosuppressed at either the induction or the effector level.

Tumor regression in vivo. In a second example of cell-mediated immunity in vivo, the ability of infected mice to cause the regression of an allogeneic tumor challenge was determined. These experiments followed from the results shown in Table 2 in which it was shown that spleen cells from heavily infected mice were depressed in their ability to respond to allogeneic stimulation by *H-2*^{a}-bearing stimulator cells. To test the relevance of this finding in the intact

TABLE 3. Characterization of inhibitor cells

Cells added to indicator cells[a]	Blastogenic response (cpm)[b]	% Suppression
Unfractionated test cells		
Normal cells	22,112 ± 1,890	
724 cells	11,927 ± 1,005	46[c]
1226 cells	10,829 ± 1,220	51[c]
Treated cells		
NWP (724)[d]	21,929 ± 2,322	1
NWP (1226)	25,444 ± 1,421	None
G10 (724)[e]	24,491 ± 2,021	None
G10 (1226)	24,527 ± 1,992	None
PNAD (724)[f]	19,931 ± 1,083	10
PNAD (1226)	26,142 ± 1,361	None
Mitomycin treated (724)	13,421 ± 955	39[c]
Mitomycin treated (1226)	10,129 ± 895	54[c]
Anti-Thy-1.2 + complement (724)	9,229 ± 1,207	58[c]
Anti-Thy-1.2 + complement (1266)	10,555 ± 977	52[c]

[a] Test cells (3×10^5) cocultured with 3×10^5 indicator (normal) cells. Background responses of unstimulated spleen cells: normal cells, 891 ± 23; *M. avium* 724 cells, 1,021 ± 120; *M. simiae* 1226 cells, 523 ± 91.
[b] Blastogenic response to 5×10^5 irradiated A/Tru (*H-2*^{a}) spleen cells; mean ± standard error of the mean ($n = 3$).
[c] Significantly lower than response of normal controls ($P < 0.01$).
[d] Mixture of cells recovered from nylon wool columns plus cells removed by agitation of the nylon wood in ice-cold medium.
[e] Cells recovered from G10 column fractionation.
[f] Plastic nonadherent cells.

TABLE 4. Depression of footpad responsiveness to SRBC in infected mice

Day of infection	Increase in footpad size (×0.1 mm)[a]		
	Normal	*M. avium* infected	*M. simiae* infected
5	9.7 ± 1.7	9.6 ± 1.4	10.7 ± 2.2
15	12.1 ± 2.2	13.4 ± 1.2	17.1 ± 3.0
30	9.0 ± 1.5	1.9 ± 0.6[b]	2.3 ± 0.7[b]
60	10.7 ± 2.3	4.6 ± 1.0[b]	2.3 ± 0.9[b]
90	11.7 ± 2.1	6.7 ± 1.4[b]	2.9 ± 1.2[b]

[a] Means ± standard error of the means (n = 5). Mice primed with 10^5 SRBC intravenously 5 days earlier.
[b] Significantly lower than response of normal controls ($P < 0.01$).

animal, mice at the height of depression in vitro (day 30, MLC) were challenged in the footpad with an immunogenic *H-2*[a]-bearing tumor, the SA-1 sarcoma. In contrast to the results obtained in vitro, however, the growth and regression of the tumor in vivo (Fig. 2) was similar in both normal and infected mice. These results thus demonstrate that, whatever the mechanism of depressed alloreactivity in vitro, it did not apparently have any influence on the alloreactivity of the animal in vivo.

DISCUSSION

The purpose of the present study was to examine the validity of using in vitro measurements of T cell reactivity to assess the immunological status of mice infected with the mycobacterial pathogens *M. avium* and *M. simiae*. This examination was prompted by preliminary evidence (22–24), based primarily on in vitro observations, which was interpreted as indicating that heavily infected mice were undergoing active nonspecific immunosuppression in vivo. This interpretation, in turn, was put forward as an explanation for the basis of the persistence of certain atypical mycobacterial infections in experimental animal models.

The results of the present study show first that the depression of responsiveness observed in vitro with phytohemagglutinin responses or MLC responses as indicator assays was a consequence of an adherent macrophage-like cell population; no consistent evidence was obtained to support the original suggestion (22–24) that this depression was a result of the preferential generation of a population of long-lived or continuously generated suppressor T cells. Since the present data was obtained under carefully controlled conditions with only minimal technical changes from the previous reports, it must be considered as a retraction of these earlier claims. However, it is not the purpose of this report to attempt to explain the inconsistency of these in vitro observations; its purpose, instead, is to demonstrate that results obtained in vivo in the present report are not consistent with the earlier interpretation of active T cell-mediated nonspecific immunosuppression which has led from previous in vitro data. For example, this report demonstrates that depressed DTH responses to SRBC in infected mice may be a consequence of the failure of the inoculum of SRBC to sensitize adequate numbers of DTH effector cells by showing that responsiveness could be restored by increasing the dose of antigen. On the contrary, however, this study has failed to show, by means of the passive transfer of cells, that infected mice acquire in the spleen a cell population capable of inhibiting the SRBC-sensitization process in vivo. It demonstrates, furthermore, that the depression of MLC responses in vitro, which alone could be taken to indicate that the alloreactivity of the host was in some way compromised, was not supported by the demonstration that the ability of the animal to cause the regression of an allogeneic tumor challenge in vivo was completely unimpaired.

Information obtained in these experiments indicated that the inhibitory effect in vitro was mediated at the level of the macrophage, and it is possible that the basis of the perturba-

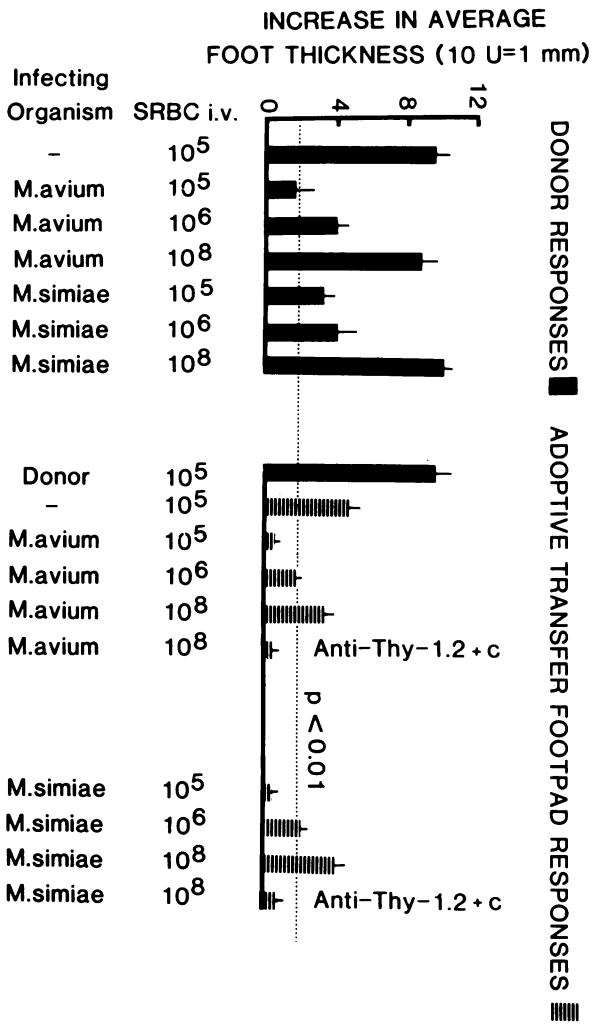

FIG. 1. Demonstration that DTH responsiveness could be restored by increasing the inoculum size of SRBC. Note that the capacity of spleen cells to adoptively transfer DTH to SRBC was proportional to that expressed by donor animals. Data are expressed as mean ± standard error of the mean (n = 5).

TABLE 5. Kinetics of emergence of footpad response to SRBC

Day of challenge[b]	Increase in footpad size (×0.1 mm)[a]		
	Normal	*M. avium* infected	*M. simiae* infected
2	3.5 ± 0.7	1.0 ± 0.5	0.5 ± 0.5
4	10.1 ± 1.1	2.6 ± 0.4	2.4 ± 0.6
5	10.4 ± 1.4	3.3 ± 1.0	2.5 ± 0.7
6	9.6 ± 0.4	1.3 ± 0.7	2.7 ± 0.7
8	3.1 ± 0.5	0.4 ± 0.4	1.1 ± 0.3

[a] Means ± standard error of the means (n = 5).
[b] Mice primed intravenously with 10^5 SRBC on day 0.

tion of both the in vitro and in vivo assays described in this report may well lie with this cell population. For instance, it is known that macrophages and other mononuclear phagocytes during infection with mycobacteria undergo adaptive physiological changes which allow them to express considerably enhanced phagocytic and digestive properties (16). It is quite possible therefore that a standard immunizing dose of SRBC which will normally allow the animal to mount a DTH response is rapidly catabolized or inadequately presented by cells expressing enhanced phagocytic activity in the infected host. Evidence in support of this hypothesis was provided by the observation that adequate sensitization to SRBC was obtained by increasing the immunizing dose, indicating that this unresponsiveness could be overcome by increasing the available antigen. As for the data obtained in vitro, it is possible that the presence of activated macrophages harvested from infected mice may inhibit the responsiveness of cultured cells in a number of ways; for instance, it is known that activated macrophages cultured in vitro can express considerably enhanced levels of arginase activity (6). The possibility that such phenomena may play a role in the present model is currently under examination in this laboratory.

Whatever the reasons for the inhibitory activity of macrophages in vitro, however, it was clear that inhibitory activity in vivo was dependent on the site of antigen inoculation. For example, inoculation with SRBC via the intravenous route subsequently led to a depression of the DTH response, presumably due to the destruction of antigen by activated macrophages present in the infected liver and spleen. In contrast however, the ability of the animal to cause the regression of an allogeneic tumor challenge in the footpad was unimpaired. It can therefore be assumed that the generation of cytotoxic T cells in the draining popliteal lymph node, upon which rejection of the tumor presumably ultimately depends (3), proceeded normally and was unaffected by the presence of the atypical mycobacterial infections (indeed, very few mycobacteria could be detected in peripheral lymph nodes after intravenous infection with these organisms [I. M. Orme, unpublished data]).

Although reports of immunosuppression in mice infected with *M. avium* or *M. simiae* are essentially restricted to those cited above (22–24), there are a number of reports in the literature which have dealt with the induction of suppressor T cells (5, 8), suppressor macrophages (1, 7, 9, 11), or both (2, 17, 20, 21) after mycobacterial infection both in mice and in humans. However, most observations of immunosuppression have followed exclusively from in vitro approaches, and it is now questionable whether such observations may be relevant to the intact animal. As an illustration, the observation that peak suppressor T cell activity in vitro (5) in mice infected with 10^8 BCG occurs at the same time at which the animal acquires large numbers of protective T cells in vivo, as measured by their ability to adoptively protect against *M. tuberculosis* (18), disputes the relevance of the in vitro data. As for suppressor macrophages, it is argued above that macrophage-mediated inhibition may be an irrelevant byproduct of activation of this cell population by the mycobacterial infections; a similar conclusion has been reached by Playfair (19) elsewhere.

To summarize, therefore, data obtained in in vitro assays which suggested the presence of nonspecific immunosuppression in mice heavily infected with two atypical mycobacterial strains was not supported by data obtained by in vivo approaches. These results strongly suggest that data obtained in vitro should be interpreted with extreme care and, where possible, as in the case of the depressed MLC responses, should be directly tested in the relevant in vivo model.

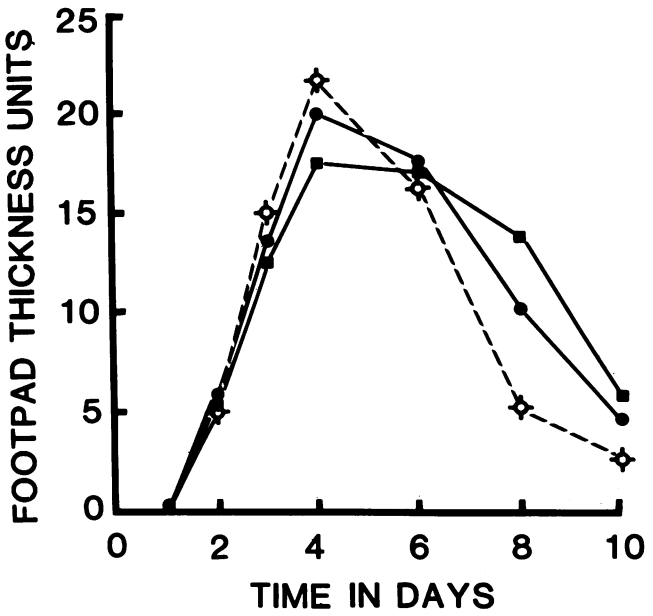

FIG. 2. Ability of normal mice (◈) and mice infected with *M. avium* (●) or *M. simiae* (■) to respond to and cause the regression of an SA-1 tumor implant in the footpad. Footpad swelling is expressed as dial gauge units (0.1 mm); each point represents the mean value of five determinations (standard error of the mean ranged from 0.4 to 1.6).

TABLE 6. Effect of passive transfer of spleen cells from infected mice on DTH to SRBC

Passive transfer of cells[a]	Day of transfer	Footpad swelling response to SRBC[b] ($\times 10^{-1}$ mm)
None		16.4 ± 2.1
Normal cells	0[c]	14.2 ± 1.8
724 cells	0	12.9 ± 2.2
1226 cells	0	16.1 ± 1.5
Normal cells	5[d]	13.8 ± 1.8
724 cells	5	14.2 ± 3.0
1226 cells	5	17.9 ± 2.5

[a] Spleen cells (8×10^7) infused intravenously.
[b] Day 5 response in normal recipients primed intravenously on day 0 with 10^5 SRBC; mean ± standard error of the mean ($n = 4$).
[c] At 1 h after recipient was primed with SRBC.
[d] At 1 h before recipient was challenged in the footpad with SRBC.

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