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Pharmacological Modulation of Suppressor Cell Activity in Mice with Disseminated Histoplasmosis

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Indomethacin and cyclophosphamide (CY) were used in an attempt to modify the suppressive effects of spleen cell populations from mice with disseminated histoplasmosis at 1 week of infection. In vitro addition of indomethacin did not alter the depressed plaque-forming cell response to sheep erythrocytes of normal spleen cells cocultured with unfractionated or nylon wool-fractionated spleen cells from infected mice. Likewise, indomethacin given intraperitoneally did not enhance the subnormal in vivo plaque-forming cell response of spleen cells from infected mice. Conversely, 20 mg of CY per kg given intraperitoneally 2 days before or 6 h after the inoculation with *Histoplasma capsulatum* partially reversed the suppression effected by splenic T cells (nylon wool passed) in vitro, whereas 50 mg of CY per kg given intraperitoneally 6 h after the injection of *H. capsulatum* ablated suppressor T cell activity in vitro; neither dosage of CY altered the suppression mediated by unseparated or nylon wool-adherent spleen cells. Furthermore, the administration of 50 mg of CY per kg failed to improve the depressed footpad responses of mice infected for 1 week to sheep erythrocytes in sheep erythrocyte-sensitized mice or to histoplasmin. These findings indicate that in experimental disseminated histoplasmosis, suppression effected by splenic T cells can be alleviated by CY; however, there is a persistent immunosuppressor mechanism(s) that cannot be counteracted by either indomethacin or CY.

Human infection with *Histoplasma capsulatum* is usually asymptomatic and recognized only by a positive histoplasmin skin test. Less commonly, a progressive intracellular infection may develop which, if untreated, is fatal in a majority of cases (36). Clinical studies have documented that patients with disseminated histoplasmosis frequently demonstrate abnormalities of cell-mediated immunity as evidenced by skin test anergy to histoplasmin and depressed blastogenic responses of peripheral blood mononuclear cells to mitogens and *Histoplasma* antigens (4, 28). Moreover, it has been reported that suppressor T cells may be, in part, responsible for the perturbations of immunoregulation observed in some anergic individuals with disseminated fungal infections (31).

To provide a better understanding of the immunoregulatory disturbances associated with *Histoplasma* infection, we have established in mice a highly reproducible model of disseminated histoplasmosis (2). During the first 4 weeks of infection, suppressor cells are activated within the spleens of mice; concomitantly, there is a severe depression of the in vitro and in vivo cellular immune responses of infected mice (3, 10). Two populations of spleen cells exert suppressor activity in this model. One population is composed of T cells, and the other is composed of macrophage-like cells that are poorly adherent to glass or plastic surfaces but adherent to nylon wool (24). Currently, however, the functional significance of suppressor cells in the pathogenesis of systemic infection in mice is not known.

Pharmacological modulation of immunosuppression represents one method by which the role of suppressor cells in immune responses can be assessed. In this regard, two agents that have been shown to abrogate suppressor cell activity, although by different means, are indomethacin, a prostaglandin synthetase inhibitor (15), and cyclophosphamide (CY), a DNA-alkylating agent (5). In this report, we describe studies designed to alter the immunosuppression associated with disseminated histoplasmosis and thereby restore an immunoregulatory balance in mice. The results demonstrated that indomethacin was unable to modify the in vitro suppression mediated by T cells or macrophage-like cells from the spleens of infected mice. In contrast, treatment with CY completely eliminated suppressor T cell activity in vitro, but did not reverse the suppression effected by unfraction-

ated spleen cells or by the macrophage-like cells that are nylon wool adherent. In vivo, CY failed to improve the depressed delayed-type hypersensitivity (DTH) responses of infected mice to sheep erythrocytes (SRBC) or histoplasmin.

MATERIALS AND METHODS

Mice. Male C57BL/6 mice were purchased from Jackson Laboratories, Bar Harbor, Maine, and male C3H/AnF mice were obtained from Cumberland View Farms, Clinton, Tenn. All animals were housed under identical conditions of humidity, temperature, and light. Food and acidified, chlorinated water were provided ad libitum.

Infection with *H. capsulatum*. Preparation and inoculation of *H. capsulatum* yeast cells of strain G 217-B have been described previously (2). Briefly, yeast cells were harvested after they were cultured for 36 h in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at a gyratory speed of 200 rpm and washed three times in Hanks balanced salt solution (HBSS). A final centrifugation was performed at 30 × *g* for 2 min to remove larger yeast aggregates. The yeast forms were enumerated in a hemacytometer. Mice, 6 to 8 weeks of age, were inoculated via the tail vein with 6×10^5 yeast cells in 0.2 ml of HBSS. In one set of experiments, normal mice were given 10^6 yeast cells that had been heat killed by immersion in a 60°C water bath for 1 h.

Antigens. SRBC in Alsever solution were obtained from Colorado Serum Co., Denver, Colo. The histoplasmin preparation, HKC-43, was a generous gift from Coy Smith, University of Kentucky College of Medicine, Lexington. It was prepared from seven mycelial strains of *H. capsulatum* grown in Smith asparagine medium. Before use, HKC-43 was dialyzed extensively against phosphate-buffered saline, pH 7.2. The protein content of the dialysate as determined by the method of Lowry et al. (19) was 875 μg/ml.

Drug treatments. For in vitro experiments, crystalline indomethacin (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 95% ethanol at a concentration of 10 mg/ml, filter sterilized, and serially diluted in fresh Mishell-Dutton medium (22). Indomethacin was added to spleen cell cultures in a volume of 0.15 ml, with final concentrations in cultures ranging from 0.05 to 10 μg/ml. Control spleen cells cultured without indomethacin contained 0.2% ethanol, which was the highest concentration of ethanol present in spleen cell cultures containing indomethacin.

For in vivo experiments, indomethacin was dissolved in 0.1 M Na_2CO_3, sterilized by filtration, and injected intraperitoneally (i.p.) into mice in a volume of 0.2 ml containing 0.1 mg. After the inoculation of *H. capsulatum*, i.p. injections of indomethacin were administered to groups of four mice (i) daily for 1 week, (ii) 8 and 24 h after the inoculation of organisms, or (iii) 24 and 2 h before the sacrifice of mice. Control mice were given indomethacin or 0.2 ml of 0.1 M Na_2CO_3 i.p.

CY powder (Sigma) was dissolved in physiological saline and sterilized by filtration before use. CY was given in a volume of 0.2 ml i.p. to groups of eight mice in dosages of either 20 or 50 mg/kg. Mice received 20 mg of CY per kg either 2 days before or 6 h after the inoculation of *H. capsulatum*; 50 mg of CY per kg was administered to mice either 6 h or 2 days after the injection of *H. capsulatum*. In all experiments, control mice were given CY or 0.2 ml of sterile saline i.p. at concomitant time intervals.

Measurement of DTH. To measure the DTH response to SRBC, groups of mice were sensitized by intravenous (i.v.) injection with 0.2 ml of a 0.01% SRBC suspension. After 5 days, mice were challenged intradermally (i.d.) with 0.05 ml of a 20% SRBC suspension into a hind footpad. Footpad swelling was measured 24 h later with a digital micrometer (Brown and Sharpe Co., North Kingston, R.I.). The DTH response was expressed as the percent increase in footpad size over that measured immediately before the SRBC challenge. Mean values were determined from groups of six mice. As an additional control, the degree of banal inflammatory response to the injection of the SRBC challenge dose only was measured for footpads of nonimmunized control mice from both the normal and the infected groups. In a similar fashion, the specific DTH responses to histoplasmin in paired control and infected mice also were measured as the percent increase in the footpad swelling 24 h after i.d. challenge with 0.05 ml of histoplasmin, HKC-43, at a dilution of 1:25. This dilution was chosen as optimal for i.d. challenge, based on preliminary dose-response experiments.

Organ culture for *H. capsulatum* yeast cells. Spleens from groups of six mice were removed aseptically and homogenized individually by a Teflon tissue grinder in 5 or 10 ml of sterile saline. Homogenates were diluted serially, and 0.1 ml of each dilution was plated in triplicate onto brain heart infusion agar (2% agar [wt/vol]), containing 1% (wt/vol) dextrose, 0.01% (wt/vol) cysteine hydrochloride, 10 μg of gentamicin per ml, and 5% (vol/vol) defibrinated SRBC. Cultures were incubated at 25°C in a closed cabinet for 2 weeks, and the number of mycelial colonies was counted on a colony counter. Verification of the organism was accomplished by random sampling of mycelial colonies for morphological identification.

Cell preparation. Spleen cell suspensions were prepared from pools of at least four spleens by gently teasing the spleens between two ground glass slides in HBSS. The cell suspension was allowed to settle for 5 min to remove tissue debris. The cells were washed twice in HBSS and suspended in Eagle minimal essential medium supplemented with 10% fetal calf serum, 1% glutamine, 2% sodium pyruvate–nonessential amino acids, 10 μg of gentamicin per ml, 5×10^{-5} M 2-mercaptoethanol, and 100 U of nystatin (GIBCO Laboratories, Grand Island, N.Y.) per ml to inhibit the growth of *H. capsulatum*.

A population of spleen cells enriched for T lymphocytes was prepared by the nylon wool column method of Julius et al. (17). The nylon wool-adherent cells were obtained by compressing the nylon wool with a sterile syringe plunger containing HBSS, free of Ca^{2+} and Mg^{2+}, with 0.02% EDTA. Viabilities of cells recovered as nylon wool-fractionated cells were greater than 90% by trypan blue dye exclusion.

Cell culture and PFC assay. Spleen cells were cultured as described by Mishell and Dutton (22); 1.5×10^7 cells were dispensed into 35-mm tissue culture dishes (Falcon Plastics, Oxnard, Calif.) and immunized with 3.5×10^6 SRBC. The dishes were incubated at 37°C with gentle rocking in an atmosphere of 10%

CO_2–7% O_2–83% N_2 and fed daily with fresh medium. The immunoglobulin M antibody plaque-forming cells (PFC) were assessed by the Cunningham-Szenberg modification of the Jerne technique (9). Responses were expressed as PFC per 10^6 cells recovered, as calculated from triplicate or quadruplicate cultures. In all coculture experiments, 5×10^6 spleen cells from normal or infected mice were added to 1×10^7 syngeneic spleen cells from age-matched controls. The in vivo PFC responses to SRBC of spleen cells from normal and infected mice were determined 5 days after an i.p. inoculation of 2×10^8 SRBC.

Statistics. The two-tailed Mann-Whitney U test was used for statistical analysis (29).

RESULTS

Treatment with indomethacin. Studies were performed to examine the effects of indomethacin on the in vitro suppressor activity exerted by spleen cells from C3H/AnF and C57BL/6 mice at 1 week of infection. The addition of indomethacin to spleen cell cultures from normal mice in the maximum concentration employed (10 μg/ml) exerted no significant effect on the PFC response by normal control spleen cell cultures ($P > 0.05$) (Table 1). Unfractionated spleen cells from both C3H/AnF and C57BL/6 mice with *Histoplasma* infections markedly suppressed the PFC response of normal syngeneic spleen cells. However, indomethacin did not modify the suppressor capacity of whole spleen cells from either strain of infected mice. Likewise, indomethacin failed to reverse the respective suppressor function of either splenic T cells, prepared by nylon wool passage, or macrophage-like cells from infected C3H/AnF and C57BL/6 mice (Table 1).

In addition to these in vitro experiments, indomethacin was given i.p. to mice to evaluate whether in vivo administration could modulate the immunosuppression induced by *H. capsulatum*. After the i.v. injection of *H. capsulatum*, C3H/AnF mice were given 0.1 mg of indomethacin either daily, at 8 and 24 h after inoculation, or at 24 and 2 h before sacrifice of the animals. None of the above in vivo indomethacin regimens modified the depressed in vivo PFC responses of spleen cells from mice at 1 week of infection (data not shown). Thus, in both in vitro and in vivo experiments, indomethacin failed to alter the suppressor capacity of spleen cells from *H. capsulatum*-infected mice.

Effects of CY on in vitro suppressor cell activity. The results of two separate experiments designed to determine whether CY could alter the in vitro suppressor activity of spleen cells from infected C57BL/6 mice are summarized in Table 2. Mice initially were given 20 mg of CY per kg i.p. either 2 days before or 6 h after the i.v. injection of 6×10^5 yeast-phase organisms. Concomitantly, CY was administered to normal mice to assess the effect of CY on the PFC response by normal spleen cells. In these control experiments, the PFC response (± the standard error of the mean [SEM]) by normal spleen cells cocultured with spleen cells from normal mice given 20 mg of CY per kg 2 days before inocula-

TABLE 1. Indomethacin (10 μg/ml) added to cocultures of splenocytes from normal and *H. capsulatum*-infected C3H/Anf and C57BL/6 mice with normal syngeneic spleen cells

Splenocytes added to normal spleen cells[a]	Spleen cell donor	Indomethacin treatment	PFC/10^6 cells recovered ± SEM from cultures of:	
			C3H/Anf splenocytes	C57BL/6 splenocytes
Unfractionated	Normal	−	432 ± 42	4,777 ± 433
		+	442 ± 18	5,548 ± 369
	Infected	−	53 ± 9 (13)[b]	1,416 ± 186 (25)
		+	57 ± 5 (13)	1,135 ± 108 (20)
Nylon wool passed	Normal	−	500 ± 23	6,219 ± 825
		+	475 ± 45	5,343 ± 376
	Infected	−	244 ± 7 (49)	2,719 ± 193 (44)
		+	234 ± 17 (53)	2,271 ± 210 (43)
Nylon wool adherent	Normal	−	385 ± 37	4,896 ± 233
		+	392 ± 37	4,880 ± 372
	Infected	−	127 ± 13 (33)	1,392 ± 40 (28)
		+	114 ± 5 (29)	1,515 ± 86 (31)

[a] A total of 5×10^6 cells from normal or infected mice added to 1×10^7 normal syngeneic spleen cells.
[b] Numbers in parentheses indicate percentages of normal response.

tion with *H. capsulatum* (2,719 ± 64 PFC per 10^6 cells recovered) or at 6 h after inoculation (2,550 ± 119 PFC per 10^6 cells recovered) did not differ significantly ($P > 0.05$) from that of untreated normal mice (2,619 ± 40 PFC per 10^6 cells recovered). At 1 week of infection, 20 mg of CY per kg partially reversed ($P < 0.01$) the suppressor activity of splenic T cells (nylon wool passed) but did not alter the suppression mediated by unfractionated spleen cells or the macrophage-like cells that were nylon wool adherent (Table 2).

In subsequent studies, mice were treated with 50 mg of CY per kg either 6 h or 2 days after the inoculation with yeast-phase *H. capsulatum*, and the effect of these two regimes on splenic suppressor function in vitro was studied at 1 week of infection. Again, control experiments demonstrated that the PFC response by normal spleen cells cocultured with spleen cells from normal mice given 50 mg of CY per kg at 6 h (7,011 ± 544 PFC per 10^6 cells recovered) or at 2 days (7,009 ± 458 PFC per 10^6 cells recovered) after injection with *H. capsulatum* was not significantly different ($P > 0.05$) from that of untreated normal mice (6,764 ± 377 PFC per 10^6 cells recovered). CY given 6 h after i.v. injection of yeast organisms completely eliminated suppressor T cell activity (Table 2); however, when given on day 2 of infection, CY did not alter the suppressor capacity of T cells from infected mice (Table 2). Even at this higher dosage, there was no reversal of suppressor activity exerted by unfractionated or nylon wool-adherent spleen cells from infected mice (Table 2). Thus, despite abrogation by CY of T-cell-mediated suppression, the suppressor capacity of whole spleen cells and nylon wool-adherent cells remained intact.

CY therapy and DTH response. In a prior study, it was speculated that the poor DTH responses of mice to SRBC and histoplasmin resulted from a suppression of reactivity to these antigens (3). It was of interest, therefore, to determine whether the administration of 50 mg of CY per kg could improve the DTH responses of mice to SRBC and histoplasmin at 1 week of infection. At 6 h after the injection of organisms, mice were administered CY or saline, and normal control mice were treated identically. Subsequently, the DTH responses of these four groups of mice to an i.d. challenge with SRBC 5 days after immunization with SRBC were assessed. The mean increase in footpad thickness (± SEM) of CY-treated normal mice (52 ± 6.6%) was greater than, but not significantly different ($P > 0.05$) from that of untreated normal mice (45 ± 4.0%). These results are in accord with previous studies that examined the effects of CY pretreatment on the DTH response to SRBC in mice immunized with a dose of 0.01% SRBC (5, 26). In contrast, the footpad responses to SRBC of CY-treated and untreated infected mice increased by only 12 ± 0.9 and 13 ± 2.2%,

TABLE 2. Effects of 20 and 50 mg of CY per kg on the splenic suppressor activity of mice infected with *H. capsulatum* for 1 week

Splenocytes added to normal spleen cells[a]	Spleen cell donor	CY treatment[b]	PFC/10^6 cells recovered ± SEM with:	
			20 mg of CY per kg	50 mg of CY per kg
Unfractionated	Normal	None	2,619 ± 40	6,764 ± 377
	Infected	None	710 ± 107 (27)[c]	1,099 ± 164 (16)
	Infected	−2 days	656 ± 74 (21)	ND[d]
	Infected	6 h	246 ± 47 (18)	948 ± 56 (14)
	Infected	2 days	ND	1,095 ± 230 (16)
Nylon wool passed	Normal	None	4,177 ± 81	8,799 ± 509
	Infected	None	1,323 ± 115 (32)	4,693 ± 546 (53)
	Infected	−2 days	2,210 ± 190 (53)	ND
	Infected	6 h	2,363 ± 195 (57)	9,074 ± 765 (103)
	Infected	2 days	ND	5,384 ± 524 (61)
Nylon wool adherent	Normal	None	4,034 ± 167	6,293 ± 339
	Infected	None	318 ± 45 (8)	1,559 ± 107 (25)
	Infected	−2 days	149 ± 42 (4)	ND
	Infected	6 h	91 ± 12 (2)	1,419 ± 124 (23)
	Infected	2 days	ND	1,713 ± 69 (27)

[a] A total of 5 × 10^6 spleen cells from normal or infected mice added to 1 × 10^7 normal spleen cells.
[b] CY given to mice 2 days before (−2 days), 6 h after (6 h), or 2 days after (2 days) inoculation with *H. capsulatum*.
[c] Numbers in parentheses indicate percentages of normal response.
[d] ND, Not done.

respectively. In fact, these responses did not differ from the banal inflammatory responses of both CY-treated (14 ± 1.3%) and untreated (11 ± 0.6%) infected mice.

Mice also were tested at 1 week of infection for their responsiveness to histoplasmin 24 h after an i.d. challenge to a 1:25 dilution of histoplasmin. The increase in footpad thickness of infected mice given CY (10 ± 1.5%) did not differ ($P > 0.05$) from that of untreated infected mice (6 ± 0.6%) or from that of CY-treated control mice (9 ± 1.7%). To ascertain whether the failure of CY to restore the DTH responses of infected mice to histoplasmin was merely a consequence of an inertial phase in the development of sensitivity to *Histoplasma* antigens, a heat-killed inoculum of 10^6 yeast-phase *H. capsulatum* cells was injected into normal mice and the DTH response to histoplasmin were measured 1 week later. Mice given a heat-killed inoculum demonstrated a 20 ± 1% increase in footpad thickness; this increase was significantly greater than the response of infected mice to histoplasmin ($P < 0.01$). Thus, the diminished footpad responses of infected mice to SRBC and histoplasmin appear to be caused by an immunosuppressor mechanism(s) that was unaffected by CY therapy.

Culture of spleens for *H. capsulatum*. Additional experiments were conducted to examine whether the inactivation of suppressor T cell activity in vitro effected by 50 mg of CY per kg given i.p. could be accompanied by a change in the number of *H. capsulatum* CFU recovered from the spleens of mice infected for 1 week. The mean number of CFU per gram of spleen (± SEM) from mice administered 50 mg of CY per kg at 6 h of infection ($25.0 \pm 9.3 \times 10^6$) was greater than, but did not significantly differ ($P > 0.05$) from, that of untreated infected mice ($7.6 \pm 2.3 \times 10^6$).

DISCUSSION

In recent years, numerous investigations have provided a much better understanding of the immunoregulatory activity of the prostaglandins, especially the E series (reviewed in reference 15). These substances are capable of suppressing immune responses and have been shown to inhibit lymphokine secretion (16), lymphocyte blastogenesis (34), and hemolytic plaque formation (20). In mice, populations of T lymphocytes (14, 33) and macrophages (33) have been characterized as prostaglandin-secreting suppressor cells. Since T cells and macrophage-like cells from the spleens of mice with disseminated histoplasmosis exert suppressor activity in this model, the present study was undertaken to determine whether prostaglandins are mediators of immunosuppression. Indomethacin, at concentrations known to block prostaglandin synthesis entirely (15), did not modify the in vitro suppressor capacity of whole spleen cells or nylon wool-fractionated spleen cells. Furthermore, indomethacin administered i.p. to mice could not enhance the depressed in vivo PFC response of spleen cells from mice to SRBC. Thus, the failure of indomethacin to reverse suppressor cell activity strongly suggests that prostaglandins are not a cause of immunosuppression in *Histoplasma* infection.

Previous studies have demonstrated that treatment with CY before antigen injection potentiates antibody formation and cell-mediated immunity through the elimination or inactivation of suppressor T cells (5, 7, 23). In addition, CY therapy reduces the mortality of mice infected with *Plasmodium bergheii* subsp. *yoelii* (12) or the PR8 influenza virus (27). On the other hand, CY, given in dosages of 200 to 400 mg/kg, promotes the susceptibility of mice to infection with *H. capsulatum* and increases the mortality rate of infected mice (8). Based on these observations, we investigated the possibility that lower dosages of CY could abolish the in vitro and in vivo immunosuppression associated with *Histoplasma* infection and perhaps confer protection against infection upon mice. Clearly, 20 mg of CY per kg partially reversed suppressor T cell activity in vitro when given 2 days before or 6 h after the injection of yeast-phase *H. capsulatum*, whereas treatment with 50 mg of CY per kg completely abrogated the in vitro suppression expressed by T cells. It should be noted, however, that 50 mg of CY per kg eliminated the expression of suppressor T cell activity only when it was administered 6 h after the inoculation of *H. capsulatum* and not on day 2 of infection. Nonetheless, ablation of suppressor T cell activity did not induce an alteration in the suppressor capacity of whole spleen cells or nylon wool-adherent cells, nor did it restore the diminished DTH responses of infected mice. Moreover, the number of *H. capsulatum* CFU cultured from the spleens of mice that received CY therapy was increased.

The immunopotentiating property of CY was demonstrated only when it was administered before or very soon after the inoculation of yeast-phase *H. capsulatum*. These results are consistent with the observations that precursors of suppressor T cells are more susceptible to the action of CY than are antigen-activated suppressor T cells (18, 26). During the course of disseminated histoplasmosis in mice, suppressor cells are generated as early as day 1 of infection, and the onset of peak suppressor activity occurs as early as day 4 (10, 24). Therefore, the inability of CY given on day 2 to ablate suppressor T cell function probably is related to the existence at

this time of a more differentiated population of suppressor T cells that is CY resistant.

The persistence of splenic suppressor cell function and anergy despite CY therapy suggests that CY-resistant macrophage-like cells are important mediators of immunosuppression in *H. capsulatum*-infected mice. Indeed, suppressor macrophages have been described for mice experimentally infected with *Trypanosoma rhodesiense* (35) or *Toxoplasma gondii* (32), as well as for anergic individuals with pulmonary tuberculosis (11) or diffuse cutaneous leishmaniasis (25). In addition, macrophages from some anergic patients with systemic fungal diseases produce a factor that inhibits the blastogenic responses of lymphocytes from normal individuals to mitogens and antigens (30). Thus, it will be of interest to determine whether inactivation of the suppressor activity exerted by macrophage-like cells during systemic *Histoplasma* infection can effect a reversal of the anergic status, augment host defenses against this intracellular pathogen, or both.

At present, there are very few pharmacological agents that are known to be capable of modifying the suppressor activity of macrophages. Substances toxic to macrophages, such as carrageenan (6) or silica (1), may be able to do so; however, if given in vivo, they are likely to be deleterious to the phagocytic mechanism of macrophages. Consequently, susceptibility to *Histoplasma* infection might be increased. Nevertheless, despite the potential adverse effects of these agents, further studies on their immunomodulatory effects in vivo, especially in low concentrations, seem to be indicated. An alternative approach to the reversal of suppressor activity by macrophage-like cells from *H. capsulatum*-infected mice is suggested by recent evidence that hydrogen peroxide contributes to the cytostatic and immunosuppressive effects of macrophages in some systems (13, 21). Therefore, by the addition of catalase to cultures containing macrophage-like suppressor cells from infected mice, it may be possible to diminish suppressor activity through the reduction of oxygen intermediates, particularly hydrogen peroxide, derived from these cells.

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