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Suppression of T-Lymphocyte Response by *Coccidioides immitis* Antigen

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Intravenous injection of BALB/c mice with coccidioidin or an alkali-soluble cell wall extract of *Coccidioides immitis* mycelia resulted in the induction of a splenic cell population(s) that suppressed delayed-type hypersensitivity response to coccidioidal antigen. To determine whether the levels of *C. immitis* antigen produced during the course of active coccidioidal disease might also cause suppression of T-lymphocyte response, BALB/c mice were infected by intranasal instillation of arthroconidia, and 2 weeks later, their sera were evaluated for suppression of T-lymphocyte response in syngeneic recipients. Intravenous transfer of sera, which were shown to contain high levels of coccidioidal antigen by an enzyme-linked immunoadsorbent assay, suppressed the delayed-type hypersensitivity response of recipients to immunization with coccidioidin. Solid-phase immunoadsorption of the sera with goat antibodies to *C. immitis* antigens removed the suppressive component(s). To determine whether the suppressive effect of circulating coccidioidal antigen(s) was associated with the activation of a splenic suppressor cell(s), as was observed in mice injected intravenously with coccidioidal antigen, spleen cell lysates were prepared from infected donors, and after filtration to remove viable fungi, the lysates were transferred to syngeneic mice. Recipients of lysates from infected but not noninfected donors were suppressed in their response to immunization with coccidioidin. Collectively, these results provide evidence that depressed T-cell responses observed in coccidioidomycosis are associated with, and may be attributable to, the activation of a suppressor cell or factor by circulating *C. immitis* antigens.

Coccidioidomycosis is a systemic mycotic disease caused by the dimorphic fungus *Coccidioides immitis*. Primary infection is acquired by inhalation of mycelial-phase arthroconidia, which convert to endosporulating spherules in host tissue. Manifestations of the disease range from a benign, self-limited pulmonary infection to a severe, progressive, and often fatal mycosis involving pulmonary and extrapulmonary tissues (14).

The profile of cell-mediated and humoral immune responses during the course of coccidioidal disease is well documented (6, 11, 12, 27, 28). Typically, persons with self-limited infection manifest strong T-lymphocyte responses and have low or nondetectable levels of serum antibodies to *C. immitis* antigens. Conversely, patients with progressive disease exhibit depressed T-lymphocyte reactivity and produce high levels of serum antibodies. The association of T-cell hyporesponsiveness or anergy with uncontrolled fungal growth is consistent with the crucial role of cell-mediated immunity in host defense against *C. immitis* (2–4).

In studies to elucidate the mechanistic basis of anergy in coccidioidal disease, we have previously reported that BALB/c mice are highly susceptible to pulmonary infection with *C. immitis* as compared to DBA/2 mice (9). The susceptibility of BALB/c mice was shown not to be associated with their inability to mount cell-mediated immune reactivity to the fungus; rather, BALB/c mice acquired a delayed-type hypersensitivity (DTH) response to coccidioidin (CDN) early during the course of disease, to a level comparable to the DTH response of DBA/2 mice. However, in contrast to the more resistant DBA/2 mouse strain, BALB/c mice developed anergy to CDN by day 15 postinfection. In the present report, we provide evidence that the acquired anergy is a result of active immunosuppression by *C. immitis* antigen.

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MATERIALS AND METHODS

Animals. Five- to 7-week-old female BALB/c mice were purchased from Jackson Laboratories (Bar Harbor, Maine). The mice were used after a 1- to 2-week quarantine period.

Infection. Mice were infected via a pulmonary route by intranasal instillation of viable arthroconidia of *C. immitis* Silveira (ATCC 28868), as described previously (9). In brief, arthroconidia were harvested from 6- to 8-week-old mycelial-phase cultures grown on 1% glucose–0.5% yeast extract agar. The cell suspension was passed over a sterile cotton column to remove hyphal elements, and the arthroconidia were enumerated by hemacytometer counts. Viability was confirmed by plate cultures.

Mice were lightly anesthetized just before infection by an intraperitoneal injection of Nembutol (1 mg in distilled water). An inoculum of arthroconidia suspended in 20 μl of phosphate-buffered saline (PBS) was dispensed as drops into the nares. Control animals were treated in an identical manner with PBS alone.

Antigens. CDN was prepared as a toluene-induced lysate of mycelial-phase cells of *C. immitis* Silveira as described by Pappagianis et al. (24), except that the cells were grown in modified Converse medium as described by Levine et al. (21), but without Tamol. In brief, mycelia were collected after 5 days of growth at 33°C on a gyratory shaker. The mycelia were suspended to a slurry in sterile, pyrogen-free distilled water, and toluene was added to achieve a final concentration of 3%. The suspension was incubated at 33°C for 3 days on a gyratory shaker (120 rotations per min), and the soluble lysate fraction was collected by filtration, lyophilized to remove the toluene, and then dialyzed against distilled water. The nondialyzable CDN preparation was

filtered (0.45-μm pore size), lyophilized, and then stored at −20°C until used.

The alkali-soluble, water-soluble cell wall extract of *C. immitis* mycelia (designated C-ASWS) was isolated as described previously (7). Briefly, Formalin-killed mycelia were mechanically disrupted in a Braun homogenizer for 4 min at 2,000 strokes per min, using a CO_2-cooling device to minimize heat effects. Cell walls were collected by centrifugation at 27,000 × *g* for 10 min at 4°C, washed by centrifugation in distilled water, and then treated with trypsin (10 μg/ml) for 3 h at 25°C. The walls were extracted with 1 N NaOH for 3 h at room temperature, and the alkali-soluble supernatant was collected and dialyzed against distilled water until the pH of the dialysate was 7.0 to 7.2. The nondialyzable C-ASWS antigen was filtered (pore size, 0.45 μm), lyophilized, and stored at −20°C until used.

Mycobacterial purified protein derivative (PPD) was obtained from Connaught Laboratories (Ontario, Canada).

Immunization and footpad challenge. Immunization was performed by injecting mice subcutaneously in the nape of the neck with 1 mg of antigen (CDN, C-ASWS, or PPD) dissolved in 0.25 ml of PBS and admixed with an equal volume of complete Freund adjuvant (CFA). Control mice were given PBS alone in CFA.

Footpad challenge was performed by injecting mice in the right and left hind footpads with 100 μg of antigen diluted in 50 μl of PBS or, for a negative control, 50 μl of PBS alone. Footpad thicknesses were measured just before and 24 h after injection, using a Mitutoyo dial caliper (Mitutoyo Dial Calipers, Tokyo, Japan). All readings were performed in a double-blind manner. The results were calculated as difference in footpad thickness of antigen- and PBS-injected pads at 24 h minus the difference in footpad thickness of antigen- and PBS-injected pads before footpad challenge. The responses of the test and control groups were analyzed for statistical significance by using Student's unpaired *t* test.

Spleen cell transfers. Spleens were collected from mice 14 days after intravenous (i.v.) injection of CDN or C-ASWS. The spleens were gently teased through a 100-mesh stainless steel screen, and the cells were collected in cold Hanks balanced salt solution and treated with isotonic ammonium chloride to lyse erythrocytes (26). After two washes by centrifugation in Hanks balanced salt solution, the cells were assessed for viability by trypan blue dye exclusion and then suspended to 10^8 viable cells per ml of Hanks balanced salt solution. Recipients were injected in the tail vein with 0.5 ml of the cell suspension. Control mice were given an equivalent number of spleen cells obtained from normal syngeneic mice.

Because spleen cell preparations from *C. immitis*-infected BALB/c mice contained viable spherules and endospores, transfers were performed with the soluble lysate fraction obtained from spleen cells which had been subjected to three successive freeze-thaw cycles at −20 and 37°C, respectively. The lysates were centrifuged, and the soluble supernatant was filtered through a 3-μm-pore-size filter to remove fungal cells. The lysate from 10^8 spleen cells from infected or, for a control, noninfected donors was transferred to syngeneic mice via injection in the tail vein.

Serum transfers. Mice were lightly anesthetized with diethyl ether and then bled via the retro-orbital sinus. The blood was allowed to clot at room temperature, and the sera were collected by centrifugation and then pooled before transfer. Recipient mice were each given 0.5 ml of the serum pool into the tail vein. Controls were given 0.5 ml of a serum pool obtained from noninfected BALB/c donors.

ELISA. The enzyme-linked immunosorbent assay (ELISA) was conducted using goat antiserum directed against CDN antigen 2 (Ag2) and the incomplete precipitating antigen (IPA). The preparation of this antiserum, hereafter designated as goat anti-Ag2/IPA, has been reported previously (8). The immunoglobulin G (IgG) fraction of the serum was isolated by precipitation with Rivanol (17), dialyzed against distilled water, and then lyophilized.

Goat IgG anti-Ag2/IPA was diluted in 0.1 M bicarbonate buffer (pH 9.6) to a concentration of 5 μg/ml. Samples of 0.2 ml (1 μg) were added to wells on a polystyrene microtiter plate (Immulon II, Dynatech), and the plates were incubated overnight at 4°C. Nonbound components were removed by washing in PBS containing 0.05% Tween 20 (PBS-TW), and the wells were blocked by the addition of 1% bovine serum albumin in PBS-TW. Samples of 0.2 ml of mouse sera, diluted 1:10 in PBS-TW and heated at 100°C for 30 min to dissociate immune complexes, were added to a series of six wells. After overnight incubation at 37°C, the plates were washed with PBS-TW, and then 0.2 ml of alkaline phosphatase-conjugated goat anti-Ag2/IPA or, for a control, alkaline phosphatase-conjugated normal goat IgG, both diluted 1:320 in PBS-TW, was added to triplicate wells. The plates were incubated for 4 h at 37°C and then washed with PBS-TW, and 0.2 ml of substrate (*p*-nitrophenyl phosphate diluted in 10% diethanolamine buffer) was added to each well. Enzymatic reactions were terminated after a 1-h incubation at 25°C by the addition of 50 μl of 3 M NaOH.

The results of the ELISA were calculated as the difference in the A_{410} of sera reacted with enzyme-conjugated goat anti-Ag2/IPA minus the absorbance of any nonspecific reactivity with enzyme-conjugated normal goat IgG. A standard curve was prepared by the addition of CDN to a serum pool obtained from noninfected mice. The absorbance values obtained with the various concentrations of CDN were analyzed using a least-squares method for a log-log relationship (30). The resulting linear curve was used to calculate unknown concentrations of antigen by converting the sample absorbance into nanograms per milliliter.

Solid-phase immunoadsorption. Solid-phase immunoadsorbents were prepared with goat anti-Ag2/IPA covalently coupled to cyanogen bromide-activated Sepharose 4B (Sigma Chemicals). Coupling was performed by adding 75 mg of ammonium sulfate-precipitated immunoglobulins (8), dissolved in 0.1 M bicarbonate buffer (pH 8.3), to 5 g of activated Sepharose 4B. The suspensions were tumbled overnight at 4°C, and the gels were then washed by centrifugation in bicarbonate buffer. Nonreactive groups were blocked with 0.2 M glycine, and the coupled gels were washed three times with alternating cycles of 0.1 M acetate buffer (pH 4.0) and 0.1 M borate buffer (pH 8.0), each containing 1 M NaCl. The immunoadsorbent was transferred to a column (2.5 by 10 cm) and equilibrated with 0.0067 M PBS (pH 7.2), containing 0.02% sodium azide as preservative.

A 4-ml volume of serum from *C. immitis*-infected mice was applied to the column and allowed to slowly enter the gel matrix. Nonbound serum components were eluted in PBS at a descending flow rate of 12 ml/h until the A_{280} of the effluent returned to base line. The column effluent fraction was dialyzed against distilled water, lyophilized, and then reconstituted in 4 ml of sterile physiologic saline.

RESULTS

Suppression of DTH by intravenously administered antigen. The suppressive effect of CDN was evaluated by injecting

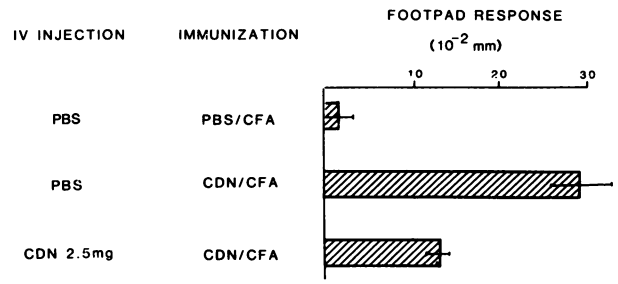

FIG. 1. CDN-induced suppression of response to immunization in BALB/c mice. Normal BALB/c mice were injected i.v. with PBS or 2.5 mg of CDN and then immunized with PBS or CDN in CFA 1 week later. Seven days after immunization, the mice were footpad challenged with CDN. Bars depict mean ± standard error obtained in groups of 15 mice.

BALB/c mice i.v. with a high dose of this antigen and, 1 week later, immunizing the mice with CDN in adjuvant. Antigen-treated and, for a control, PBS-injected mice were footpad challenged 7 days after immunization. Injection of BALB/c mice with 2.5 mg of CDN suppressed their DTH response to immunization with CDN by 54% ($P < 0.0001$; Fig. 1).

The specificity of the suppression induced by CDN was assessed by comparing the DTH response of CDN-treated with that of nontreated mice to immunization with PPD. CDN-suppressed mice responded to immunization with PPD, to a level comparable to the response of PBS-injected controls (Fig. 2); hence, suppression is antigen specific.

Suppression of DTH response to coccidioidal antigen was also induced, and in a dose-dependent manner, by i.v. injection of C-ASWS (Fig. 3). The degree of suppression induced by C-ASWS ranged from 11% in mice given 0.01 mg ($P > 0.05$) to 64% in mice given 1 mg of this antigen ($P < 0.025$).

Adoptive transfer of antigen-induced suppression. To determine whether antigen-induced suppression of T-lymphocyte reactivity could be attributed to the activation of a suppressor cell population(s), spleen cells from C-ASWS-treated mice and, for a comparison, nontreated donors were adoptively transferred to syngeneic mice. The recipients were immunized with CDN in adjuvant on the day of adoptive transfer and then footpad challenged 7 days later. Antigen-induced suppression was transferable to normal, syngeneic mice by i.v. injection of 5×10^7 viable spleen cells from

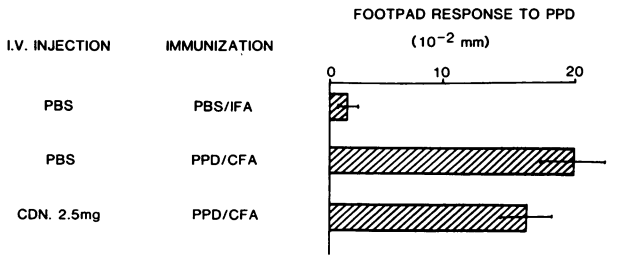

FIG. 2. Antigen specificity of suppression induced by i.v. injection of CDN. Normal BALB/c mice were injected i.v. with PBS or 2.5 mg of CDN. The mice were immunized 7 days later with PPD in CFA or, for a negative control, PBS in incomplete Freund adjuvant (IFA). The mice were footpad challenged with PPD 1 week after immunization. Bars depict mean ± standard error obtained in groups of seven mice.

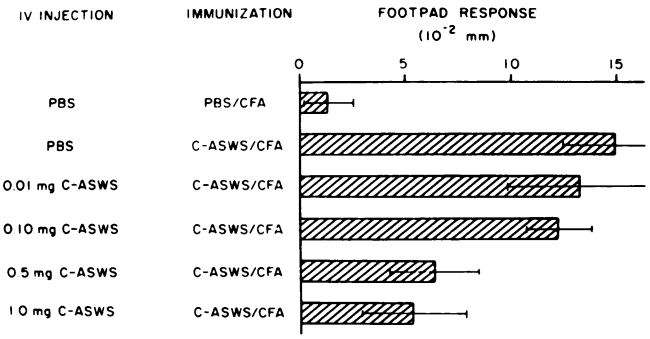

FIG. 3. C-ASWS-induced suppression of response to immunization in BALB/c mice. Normal BALB/c mice were injected i.v. with PBS or gradient doses of C-ASWS in PBS. Seven days later, the mice were immunized with C-ASWS in CFA and then footpad challenged with C-ASWS 1 week after immunization. Bars depict mean ± standard error in groups of 10 mice.

donors treated 2 weeks earlier with C-ASWS (Fig. 4). The footpad hypersensitivity response of mice given spleen cells from donors injected with 0.01 mg of C-ASWS was reduced by 32% ($P < 0.05$); recipients of spleen cells from mice treated with 0.5 mg of C-ASWS exhibited a 73% reduction in their footpad hypersensitivity response ($P < 0.0001$).

In evaluating the kinetics of suppression by spleen cell transfer, we observed that suppression was optimally demonstrable when recipients were immunized on the day of transfer (data not shown). In contrast, suppression by i.v.-administered antigen was optimally demonstrable when recipients were immunized 1 week after antigen treatment. These differences in kinetics would be consistent with an inductive phase for the activation of a splenic suppressor cell(s) by coccidioidal antigen.

Detection of circulating antigen in sera of infected mice. The suppressogenic effects of both CDN and C-ASWS suggested that a suppressor-inducer component(s) may be common to these two antigen preparations. In previous studies (7), we have shown that C-ASWS is comprised of two antigens which, by tandem two-dimensional immunoelectrophoresis, are of antigenic identity to Ag2 and the IPA in CDN. The isolation of Ag2 and the IPA from CDN and the production of goat antisera reactive with these components (8) enabled the development of an ELISA to determine whether these

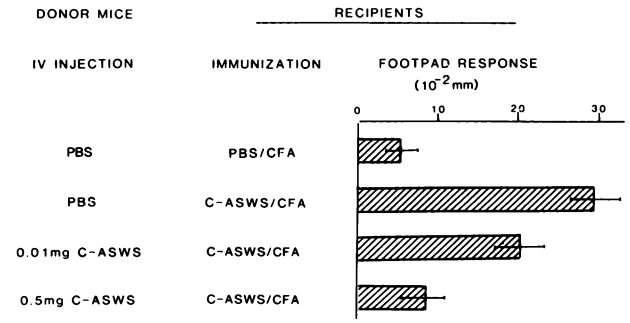

FIG. 4. Adoptive transfer of suppression with spleen cells from C-ASWS-treated BALB/c mice. Normal, syngeneic mice were injected in the tail vein with 5×10^7 spleen cells from donors treated with PBS or C-ASWS 14 days earlier. The recipients were immunized with PBS or C-ASWS in CFA on the day of transfer and then footpad challenged 7 days later. Bars depict the mean ± standard error obtained in groups of 12 mice.

antigens might be detectable in sera of infected BALB/c mice.

The results of the ELISA on sera obtained from mice at various time intervals after intranasal instillation of 22 arthroconidia are depicted in Fig. 5. Whereas none of 5 mice were positive at day 6 postinfection, antigenemia was detected in 1 of 7 (14%) BALB/c mice at day 9 postinfection, 6 of 10 (60%) mice at day 12, and 8 of 8 mice at day 15. Serum antigen levels directly correlated with the number of CFU of *C. immitis* recovered from the lungs, livers, and spleens of infected mice ($P < 0.001$; data not shown).

Transfer of suppressogenic activity with sera of infected mice. To determine whether antigenemia might be associated with suppressor activity, sera from BALB/c mice, infected 12 days earlier by intranasal instillation of 25 arthroconidia, were transferred to syngeneic recipients. The recipients were immunized with CDN 1 week later and then footpad challenged 7 days after immunization. Recipients of serum from infected BALB/c donors were significantly suppressed in their DTH response to immunization as compared with the response of mice given serum from normal donors ($P < 0.005$, Fig. 6). The kinetics of suppression by sera were similar to those of i.v.-injected antigen. Namely, suppression was optimally demonstrable when the mice were immunized 7 days after transfer, as opposed to immunization on the day of transfer (data not shown).

To directly examine the possibility that the suppressive effect of sera from *C. immitis*-infected mice was attributable to coccidioidal antigen, a serum pool was applied to a solid-phase immunoadsorbent containing goat anti-Ag2/IPA. The column effluent was collected and assayed for suppressor activity by i.v. transfer to syngeneic mice (Table 1). Whereas nonadsorbed serum significantly suppressed the DTH response of recipients to immunization as compared with the response of recipients of normal mouse serum ($P < 0.025$), serum adsorbed with goat anti-Ag2/IPA was without suppressor activity. The loss of suppressive activity was accompanied by an eightfold reduction in antigen content, i.e., from 261 ng/ml of unadsorbed serum to 33 ng of antigen per ml of the column effluent fraction.

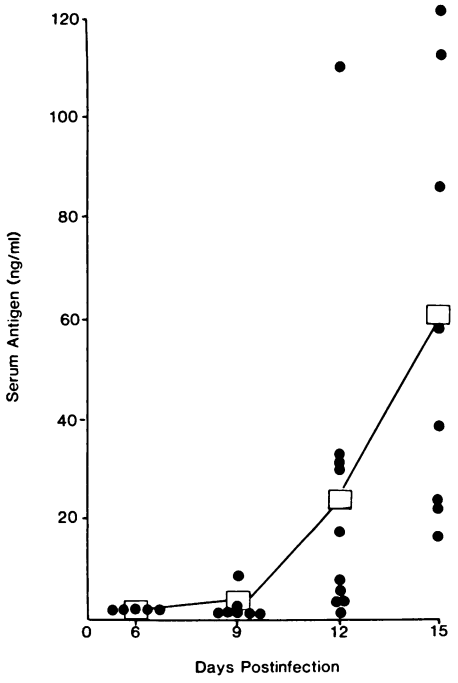

FIG. 5. Circulating antigen levels in *C. immitis*-infected BALB/c mice at days 6, 9, 12, and 15 after pulmonary instillation of 22 arthroconidia. Antigen levels were quantitated by using an ELISA with goat anti-Ag2/IPA as described in Materials and Methods.

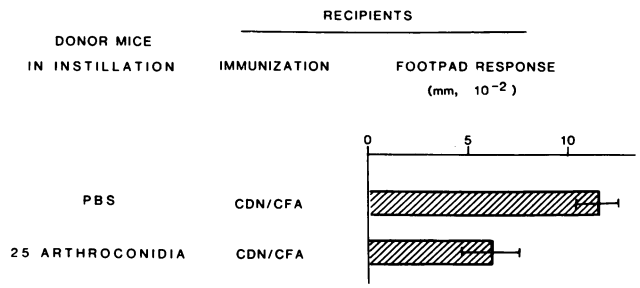

FIG. 6. Transfer of suppression with sera from *C. immitis*-infected BALB/c mice. Samples of 0.5 ml of sera from donors infected 12 days earlier with 25 arthroconidia or, for a control, sera from noninfected donors were injected into the tail vein of normal syngeneic mice. The recipients were immunized 7 days later with CDN in CFA and then footpad challenged 1 week after immunization. Bars depict mean ± standard error obtained in groups of eight mice.

Transfer of suppressor activity with spleen cell lysates of infected mice. The preceding results provide evidence for an immunosuppressive role by *C. immitis* Ag2 or the IPA or both at levels produced during the course of experimental disease in BALB/c mice. To determine whether suppression by in vivo-produced antigen was associated with the activation of a splenic suppressor cell, normal BALB/c mice were injected i.v. with the soluble lysate fraction obtained from 10^8 spleen cells from *C. immitis*-infected mice. The recipients were immunized with CDN in adjuvant on the day of transfer and then footpad challenged 1 week later.

Recipients of lysates from infected BALB/c mice were suppressed in their response to immunization with CDN by 59% as compared with the response of mice given spleen cell lysates from noninfected mice ($P < 0.0001$, Fig. 7). The transfer of suppression by spleen cell lysates was similar in kinetics to that of viable spleen cells from antigen-treated mice; i.e., suppression was optimally demonstrable when recipients were immunized on the day of transfer (data not shown).

DISCUSSION

Numerous studies have established that cell-mediated immunity is a crucial component of host defense against *C. immitis* (2–4). Elucidation of mechanisms that might suppress cell-mediated immune response in coccidioidal disease is, therefore, of primary importance. In a recent report (9),

TABLE 1. Adsorption of the suppressive activity of infected mouse sera by immunoaffinity chromatography with goat anti-Ag2/IPA

Serum source	Antigen level (ng/ml)	Footpad response of recipients[a] (10^{-2} mm)
Noninfected mice	0	31.5 ± 3.7
Infected mice		
Nonadsorbed serum	261	18.5 ± 3.2
Adsorbed serum	33	37.4 ± 3.3

[a] Mean ± standard error of results obtained in groups of seven mice.

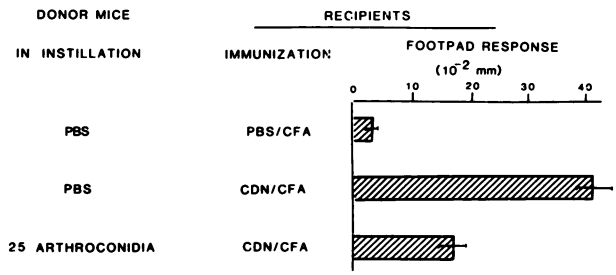

FIG. 7. Transfer of suppression with spleen cell lysates from *C. immitis*-infected BALB/c mice. Samples of 0.5 ml of the soluble lysate fraction obtained from spleen cells of BALB/c mice infected 12 days earlier with 25 arthroconidia or, for a control, noninfected mice, were injected i.v. into normal syngeneic mice. The recipients were immunized with CDN in CFA on the day of transfer and then footpad challenged 1 week later. Bars depict mean ± standard error obtained in groups of seven mice.

we showed that BALB/c mice are highly susceptible to pulmonary infection with *C. immitis* and that the susceptibility of this mouse strain is associated with the development of anergy during the course of infection. The results of this investigation provide evidence that the acquired anergy is associated with, and perhaps attributable to, the activation of a splenic suppressor cell by circulating *C. immitis* antigen.

Suppression of T-cell reactivity by coccidioidal antigens was demonstrated in an earlier study by Ibrahim and Pappagianis (18). Daily intraperitoneal administration of CDN into guinea pigs previously sensitized with killed hyphae in CFA suppressed the skin test reactivity and production of the lymphokine macrophage migration inhibitory factor to CDN. Suppression was antigen specific in that DTH responses to PPD were not impaired. In evaluating the effect of different routes of administering a killed spherule vaccine, Levine et al. (22) reported that intramuscular or subcutaneous routes were highly effective in inducing protection against challenge, whereas the i.v. route was ineffective. Rather, i.v. injection of killed spherules before, concomitant with, or within 35 days after intramuscular vaccination with the same antigen diminished the protective effects of the vaccine in mice. In the present study, antigen-specific suppression of T-lymphocyte reactivity was induced in mice injected i.v. with soluble *C. immitis* antigen. The suppression occurred via the activation of a splenic suppressor cell; specifically, adoptive transfer of spleen cells from antigen-treated mice suppressed the response of recipients to immunization. These results are in accord with the activation of suppressor cells in other biologic systems when antigen is administered by an i.v. route (1, 5, 13, 23, 25).

The possibility that the levels of coccidioidal antigen(s) produced in vivo during the course of active disease activate a splenic suppressor cell(s) is supported by the following evidence: (i) the anergy that is acquired in *C. immitis*-infected BALB/c mice (9) is temporally associated with the detection of circulating Ag2/IPA; (ii) the transfer of antigen-containing sera from infected mice suppresses the DTH response of recipients, while adsorption of serum antigen with goat anti-Ag2/IPA abrogates this suppressive activity; and (iii) the transfer of spleen cell lysates from infected mice suppresses T-lymphocyte reactivity in recipients. Although we cannot exclude the possibility that the suppressive effect of spleen cell lysates is attributable to coccidioidal antigen which may have been solubilized during the preparation of lysates by successive freeze-thaw cycles, the kinetics of suppression by the lysates differs from that of i.v.-administered antigen. That is, suppression by coccidioidal antigen (or serum containing antigen) is optimally demonstrable when recipients are immunized 1 week after transfer, whereas suppression by spleen cell lysates is optimally demonstrable when recipients are immunized on the day of transfer. These differences in kinetics would be consistent with the presence of a suppressor-inducer component in CDN and C-ASWS (and in serum) and a suppressor-effector component in the spleen cell lysates, i.e., a suppressor factor (13). Definitive support for this interpretation awaits the isolation and characterization of the splenic suppressor cell and the soluble factor(s) therefrom.

The adsorption of the serum suppressor-inducer component on columns containing goat anti-Ag2/IPA is consistent with suppression by Ag2, the IPA, or both. However, the possibility exists that other suppressor components, such as immune complexes or anti-idiotypic antibody, might also bind goat anti-Ag2/IPA. Circulating immune complexes comprised of anti-*C. immitis* IgG, *C. immitis* antigen, and C1q have been documented in coccidioidomycosis and commonly occur in patients who have multifocal disease (10, 31). Anti-idiotypic antibodies have not yet been reported in experimental or naturally acquired coccidioidomycosis, but they may be produced, and in other biologic models (15, 16, 29) such antibodies have been shown to suppress T-cell function. In studies to explore these possibilities, we plan to subject sera with suppressive activity to immunoaffinity chromatography using ligands which differ in their binding affinities for *C. immitis* antigen, immune complexes, and anti-idiotypes.

The temporal sequence between the induction of suppressor activity, the development of anergy, and the progressive disease that occurs in BALB/c mice suggests that suppressor cells may adversely modulate the course of coccidioidomycosis by down-regulating T-lymphocyte function. If this supposition is correct, then suppressor activity should be absent or reduced in DBA/2 mice, since this mouse strain is significantly more resistant to pulmonary (9) or intraperitoneal (19, 20) infection with *C. immitis*. In preliminary studies, we have demonstrated that spleen cell lysates from infected DBA/2 mice do not transfer suppressor activity (unpublished data). Paradoxically, sera of infected DBA/2 mice do transfer suppressor activity, to a level comparable to that obtained with sera from BALB/c mice. Further experiments are in progress to elucidate the basis for these differences and their possible effects on the course of coccidioidal disease.

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