INFECTION AND IMMUNITY, July 1987, p. 1657–1662
0019-9567/87/071657-06$02.00/0

Vol. 55, No. 7

Characterization of a Third-Order Suppressor T Cell (Ts3) Induced by Cryptococcal Antigen(s)

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Received 14 January 1987/Accepted 1 April 1987

Previous studies from our laboratory have shown that a high dose of cryptococcal culture filtrate antigen (CneF) administered intravenously induces a complex suppressor cell cascade which down-regulates the cell-mediated immune response to *Cryptococcus neoformans* antigens. The primary objective of this investigation was to determine whether a suppressor cell induced by immunization is required for efferent suppression of the cryptococcal delayed-type hypersensitivity (DTH) response. Our approach to this problem was to immunize CBA/J mice with CneF emulsified in complete Freund adjuvant and then 6 days later to collect spleen cells from the immunized mice and adoptively transfer these cells along with *C. neoformans*-specific second-order suppressor T cells (Ts2) to naive syngeneic recipients at the time of footpad challenge of the recipients with CneF. To establish which populations of cells in the spleens of immunized mice play a suppressive role, mass cytolysis with specific antibodies and complement was performed before the spleen cells were transferred to naive animals. Since the phenotype of the cells responsible for the transfer of the cryptococcal DTH response had not been completely determined, we first demonstrated that the cells responsible for DTH were L3T4$^+$ Lyt-2$^-$ cells. Subsequently, we established that a Thy-1$^+$ L3T4$^-$ Lyt-2$^+$ I-J$^+$ cell population induced by immunization was required along with *C. neoformans*-specific Ts2 cells for efferent suppression of the cryptococcal DTH response. In addition, we demonstrated that the suppressor cells in the immune cell population were derived from cyclophosphamide-sensitive precursors. These data indicate that a third suppressor cell population is required for efferent suppression of the cryptococcal DTH response. As in the azobenzenearsonate and 4-hydroxy-3-nitrophenyl acetyl hapten suppressor models, the Ts2 cells in the circuit mediate their effects through this third suppressor component. Since the mode of induction and the phenotype of the third *C. neoformans*-specific suppressor cells are similar to those reported for Ts3 cells in other antigen-specific suppression models, we referred to this third suppressor cell in the *C. neoformans*-specific suppressor cell cascade as a Ts3 cell.

Cryptococcosis is a fungal disease caused by an encapsulated yeastlike organism, *Cryptococcus neoformans*. Depressed cell-mediated immunity and high levels of cryptococcal antigen in body fluids are common coexisting findings in patients with progressive or disseminated cryptococcosis (6, 11, 31). Using a murine model, we have demonstrated a direct correlation between high cryptococcal antigen titers and depressed delayed-type hypersensitivity (DTH) responses (16, 17). Intravenous (i.v.) injection of mice with 20 μg to 1 mg of cryptococcal culture filtrate antigen (CneF) triggers the induction of a cascade of *C. neoformans*-specific suppressor cells and factors (23, 24, 26–28). Briefly, the sequence of events is as follows. At 7 days after CneF administration, a Lyt-1$^+$ I-J$^+$ first-order suppressor T-cell (Ts1) population is induced in lymph nodes from a cyclophosphamide (Cy)-sensitive precursor (24, 26, 28). Ts1 cells or the soluble factor derived therefrom, i.e., TsF1, injected at the time of immunization specifically suppress the cryptococcal DTH response by inhibiting the production of cells responsible for the response (T_{DH} cells) (24, 26, 28). In addition, both Ts1 cells and TsF1 induce second-order T suppressor cells (Ts2) (27). Ts2 cells, which are found in the spleens of animals given Ts1 cells or TsF1, are induced from Cy-resistant precursors, are Lyt-1$^-$-2$^+$ I-J$^+$, and are efferent suppressors (27). Ts2 cells mediate suppression by means of a soluble factor (TsF2) (27). The features of the *C. neoformans*-suppressive pathway that have been studied resemble the suppressive circuits that modulate the DTH responses to the haptens azobenzenearsonate (ABA) and 4-hydroxy-3-nitrophenyl acetyl (NP) (1, 3, 8, 9, 12, 22, 29, 30, 33, 34, 38, 39). In these two hapten models, as well as in certain other antigen-specific suppressor cell circuits, a third-order suppressor cell (Ts3) population induced by an immunization protocol has been defined (1–3, 8, 12, 13, 21, 22, 29).

The primary purpose of this study was to determine whether a cell similar to the Ts3 cell in other models is required in the *C. neoformans*-specific suppressor cell pathway. To make this assessment, further characterization of the T_{DH} cells was necessary. T_{DH} cells can be detected by adoptive transfer of spleen cells from mice that have been immunized 6 days earlier with CneF in complete Freund adjuvant (CneF-CFA). Through adoptive transfer experiments, the phenotype of the T_{DH} cells was shown to be L3T4$^+$ Lyt-2$^-$. In similar transfer studies in which spleen cells from immunized mice were adoptively transferred to naive syngeneic mice along with *C. neoformans*-specific Ts2 cells, the transferred DTH was significantly suppressed. On the other hand, when Lyt-2$^+$ cells were removed by mass cytolysis with anti-Lyt-2.1 antibody and complement from the immune spleen cell pool and transferred along with Ts2 cells, efferent suppression was abrogated. These results indicate that Lyt-2$^+$ cells in the sensitized cell population are necessary for Ts2-mediated suppression of the cryptococcal DTH response. The suppressor cells in the immune population were further characterized as Thy-1$^+$ I-J$^+$ cells and were shown to be derived from Cy-sensitive precursors. Considering that the suppressor cells induced by immunization with

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CneF-CFA are phenotypically and functionally similar to Ts3 cells which have been described in other antigen-specific suppressor cell pathways (1, 3), the suppressor cells induced by immunization with CneF-CFA are referred to as *C. neoformans*-specific Ts3 cells.

MATERIALS AND METHODS

Mice. Male and female CBA/J mice (H-2^k Lyt-1.1 Lyt-2.1) purchased from Jackson Laboratory, Bar Harbor, Maine, were used at 7 to 10 weeks of age throughout these studies.

Antigen. CneF used for induction of suppression, immunization, and footpad challenge was prepared by the procedure described by Cauley and Murphy (4). Briefly, *C. neoformans* 184-A (25) was grown for 3 days in a neopeptone-dialysate broth supplemented with glucose and thiamine hydrochloride. After the cultures were treated with 2% Formalin for 18 h, the cells were removed by centrifugation and filtration, and the supernatant was then washed and concentrated on a membrane filter with a 50,000-molecular-weight exclusion limit (XM50 membrane; Amicon Corp., Lexington, Mass.). The CneF used in these studies had a protein concentration of 5.0 mg/ml, measured by the procedure of Lowry et al. (18) as modified by Miller (19), and a carbohydrate concentration of 2.4 mg/ml, based on results of the phenol-sulfuric acid assay (10).

Induction and elicitation of the DTH response and induction of Ts3 cells. Mice were immunized by injecting 0.1 ml of CneF-CFA emulsion (1:1) at each of two sites at the base of the tail. At 6 days after immunization, spleen cells were collected as the source of sensitized or T_{DH} cells and putative suppressor cells, i.e., Ts3 cells (26). To assay for the presence of the T_{DH} cells, 10^8 sensitized spleen cells were adoptively transferred to naive syngeneic recipients. Within 1 h after the transfer of cells, the recipient mice were footpad challenged with 30 μl of CneF, and 24 h later the footpads were measured to determine the DTH reactivity. Recipients of spleen cells obtained from immune animals were footpad challenged and served as positive DTH controls. Recipients of spleen cells obtained from naive animals were footpad challenged and were the negative DTH controls. Increases in footpad thickness were calculated as described by Cauley and Murphy (4).

Induction of Ts2 cells. Ts2 cells were induced by a single i.v. injection of 5×10^7 Ts1 cells. Lymph node cells harvested 7 days after the injection of CBA/J mice i.v. with 0.4 ml of CneF served as the source of Ts1 cells (26). At 7 days after the treatment of naive mice with Ts1 cells, spleens were removed and served as the source of Ts2 cells (27, 28). Ts2-cell activity was assayed by adoptively transferring 10^8 sensitized spleen cells and 10^8 Ts2 cells i.v. to CBA/J mice and then footpad challenging the recipients within 1 h after the cell transfers. Footpads were measured 24 h after challenge, and the percent suppression was calculated as follow: % suppression = [(mean increase in footpad thickness of positive control − mean increase in footpad thickness of test group)/(mean increase in footpad thickness of positive control − mean increase in footpad thickness of negative control)] × 100. Positive controls were mice which had been given 10^8 sensitized spleen cells and footpad challenged with CneF. The negative controls were mice given the same number of naive spleen cells before footpad challenge with CneF.

Antibody treatment of spleen cells. The anti-Thy-1 antibody used in these studies has been previously described (26). Anti-I-J^k was obtained from Accurate Scientific, Hicksville, N.Y., and was used at a 1/5 dilution (28). Since anti-I-J^k was prepared in B10.A(3R) mice, serum from normal B10.A(3R) mice diluted 1/5 was used as a control in designated experiments. Monoclonal anti-Lyt-2.1 purchased from New England Nuclear Corp., Boston, Mass., was used at a 1/600 dilution (28). Anti-L3T4 monoclonal antibody was a culture supernatant produced from the GK 1.5 hybridoma obtained from the American Type Culture Collection, Rockville, Md., and was used at a 1/10 dilution. In all antibody treatments, 0.1 ml of antibody was added to 10^8 spleen cells. After the cells and antibody were incubated at room temperature for 30 min, the cells were washed once with Hanks balanced salt solution and suspended in the complement source which was 0.1 ml of 3% rabbit serum (28) made up with guinea pig serum diluted 1/16 (28). After 30 min of incubation at 37°C, the cells were extensively washed and suspended in 0.5 ml of Hanks balanced salt solution per 10^8 cells treated. Mice were injected i.v. with 0.5 ml of the treated cell suspension. To assure the efficacy of the antibody and complement treatments, prior to being used in these studies, the designated dilutions of each antibody along with the complement source described above were shown to abrogate specific immune functions known to be associated with specific cell phenotypes. For example, treatment of *C. neoformans*-specific Ts2 cells with anti-I-J^k or anti-Lyt-2.1 antibody and complement ablated their efferent suppressive activity, and the anti-L3T4 and complement abrogated *C. neoformans*-specific Ts1 cell-mediated suppression.

Adoptive transfers. For adoptive transfer experiments, five donors were used for five recipients. In certain experiments, 10^8 sensitized spleen cells either untreated or after treatment with the appropriate antibody and complement were adoptively transferred i.v. to naive syngeneic recipients. In other experiments, specified groups of mice were given 10^8 untreated or treated sensitized spleen cells and 10^8 Ts2 cells. Within 1 h after cell transfers, the footpads of recipient mice were challenged with 30 μl of CneF, and the footpads were measured 24 h later.

Cy treatment. In studies to investigate the Cy sensitivity of the Ts3 cell precursors, mice were injected intraperitoneally 2 days before immunization with Cy (100 mg/kg of body weight) obtained from Sigma Chemical Co., St. Louis, Mo. At 6 days after immunization, the spleen cells of the Cy-treated immunized mice were assayed for the presence of suppressor cells, i.e., Ts3 cells, by adoptively transferring spleen cells from Cy-treated immune mice along with Ts2 cells to naive syngeneic recipients before footpad challenge.

Statistical analysis. Calculations of means, standard errors of the mean, and unpaired Student's *t* tests were used to analyze the data.

RESULTS

Further characterization of *C. neoformans*-specific T_{DH} cells. In previous studies, we have shown that *C. neoformans*-specific T_{DH} cells are not destroyed by treatment with anti-Lyt-2.1 antibody and complement (24). Considering that L3T4 antigen does not coexist with Lyt-2 antigen on mature T cells (5, 32), one might assume that the *C. neoformans*-specific T_{DH} cells are L3T4$^+$. To confirm this, spleen cells collected 6 days after immunization with CneF-CFA were treated with anti-L3T4 or anti-Lyt-2.1 antibody and complement and then adoptively transferred i.v. to naive recipients. The recipients were footpad challenged with CneF, and footpad swelling was measured 24 h later. The results of one such experiment are shown in Fig. 1. Treatment of the sensitized spleen cells with anti-L3T4 and complement vir-

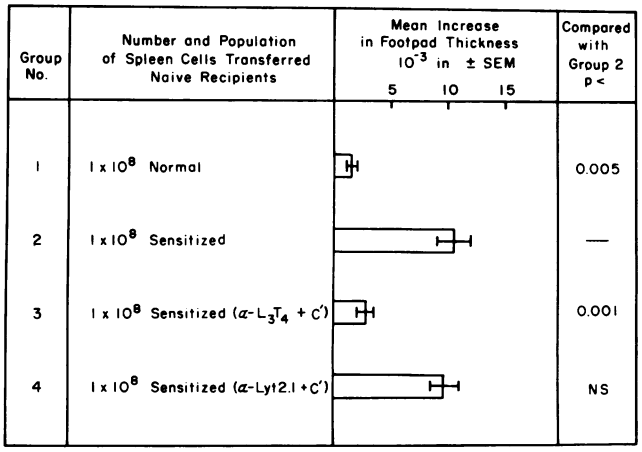

FIG. 1. Adoptive transfer of DTH with spleen cells from mice which were immunized with CneF-CFA (sensitized cells). The specified cell pools were treated before adoptive transfer with the antibody and complement (C') indicated in parentheses. Experiments were performed three times with five mice in each group. NS, Not significant.

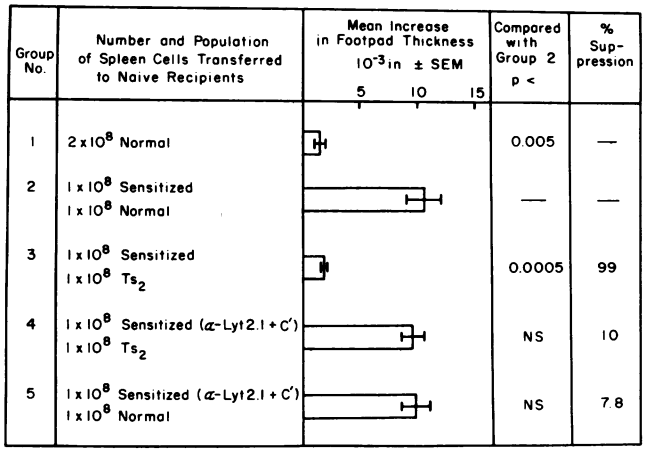

FIG. 2. Abrogation of Ts2-mediated suppression of cryptococcal DTH response by treating sensitized spleen cells with anti-Lyt-2.1 antibody and complement. The specified sensitized spleen cell pools were treated before adoptive transfer with the antibody and complement (C') indicated in parentheses. Experiments were performed three times with five recipients in each group. NS, Not significant.

tually eliminated the ability of the sensitized cells to adoptively transfer a DTH response (compare group 3 to the positive control group 2), as evidenced by the fact that the mean increase in footpad thickness in group 3 mice was not significantly different from the mean footpad reactions of mice given normal spleen cells (group 1). Recipient animals that received sensitized cells treated with anti-Lyt-2.1 antibody and complement (group 4) had DTH responses similar to those of the positive controls (group 2), indicating that Lyt-2^+ cells could be eliminated by using this mass cytolysis technique without affecting the transfer of DTH reactivity.

Demonstration and characterization of suppressor cells from spleens of immunized mice. *C. neoformans*-specific Ts2 cells have been shown to suppress the DTH response when transferred to immunized mice at the time of footpad challenge (27). It is possible that the observed suppression is due to Ts2 cells alone or that it results from the interaction of Ts2 cells with other cells in the immune mouse, as has been demonstrated in certain other suppressor models (1–3, 8, 9, 12, 21, 22, 29). To ascertain which situation exists in the *C. neoformans*-specific suppressor cell pathway, sensitized spleen cells treated with anti-Lyt-2.1 and complement were adoptively transferred to naive recipients along with Ts2 cells. Recipients were footpad challenged within 1 h after cell transfer with 30 μl of CneF, and 24 h later the footpad thicknesses were measured. As shown in Fig. 2, suppression of the DTH response was demonstrated in mice which received untreated sensitized spleen cells and Ts2 cells (group 3), whereas no significant suppression was detected in mice which received Ts2 cells and sensitized cells that had been treated with anti-Lyt-2.1 antibody and complement (group 4). Furthermore, as we showed in the previous experiment (Fig. 1), the anti-Lyt-2.1 antibody and complement treatment did not diminish the ability of the sensitized cell population to transfer DTH (group 5). Together these data indicate that Lyt-2^+ cells in the sensitized spleen were required for efferent suppression of the cryptococcal DTH response.

Although it may be assumed that the sensitized spleen cells needed for Ts2-mediated suppression are T cells, we confirmed this with the experimental data shown in Fig. 3. Groups 1 and 2 were negative and positive DTH controls, respectively. Animals in group 3 were given sensitized spleen cells that had been treated with normal mouse serum (NMS) and complement along with Ts2 cells. The DTH results from group 3 were similar to the DTH responses of mice given the same sensitized cells that had not been treated with NMS and complement (data not shown). The combined data from these two groups of mice confirm that the Ts2 cells used in these experiments were functional, in that they suppressed the DTH responses by 88% in group 3 and 86% in the group given the untreated sensitized cells. Animals in group 4 received Ts2 cells along with sensitized spleen cells which had been treated with anti-Lyt-2.1 antibody and complement. Significant suppression of the DTH response was not observed in group 4, confirming that

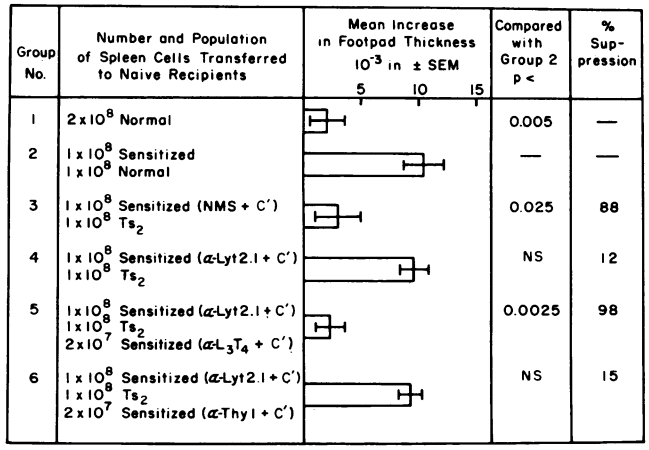

FIG. 3. Sensitivity of the suppressor cells in the sensitized spleen cell population to treatment with anti-Thy-1 antibody and complement. The specified cell pools were treated before adoptive transfer with the antibody and complement (C') indicated in parentheses. Experiments were performed three times with five recipient mice in each group. NS, Not significant.

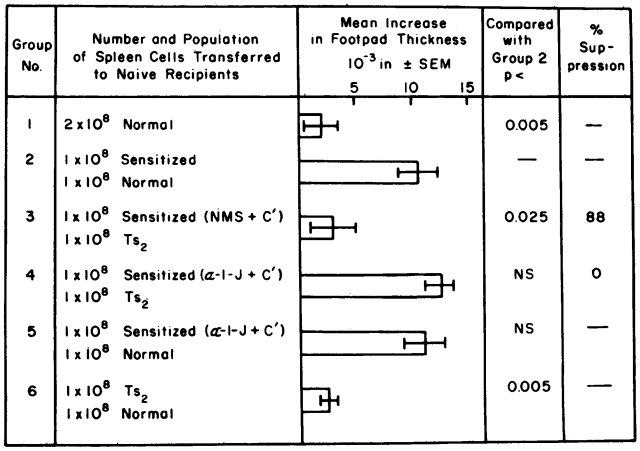

FIG. 4. Effects of anti-I-J antibody and complement treatment on the suppressor cells in spleens from CneF-CFA-immunized mice. The specified cell pools were treated before adoptive transfer with the antibody or complement (C') indicated in parentheses. Experiments were repeated three times with five mice in each group. NS, Not significant.

Lyt-2^+ cells in the spleens of immune mice were involved in Ts2-mediated efferent suppression of the DTH response. Recipient mice in groups 5 and 6 were given three sets of cells. Group 5 mice received (i) 10^8 sensitized cells treated with anti-Lyt-2.1 and complement as a source of T_{DH} cells, (ii) 10^8 Ts2 cells, and (iii) 2×10^7 sensitized spleen cells treated with anti-L3T4 and complement as a source of putative Ts3 cells. As expected, when Lyt-2^+ cells from sensitized mice were added back, the DTH responses in the recipients (group 5) were suppressed when compared with the positive controls (group 2). However, if the sensitized spleen cell population providing the putative Ts3 cells was treated with anti-Thy-1 and complement prior to transfer (group 6), the suppression was abrogated. These data clearly demonstrate that L3T4$^-$ Lyt-2^+ T cells in the sensitized cell population are needed for Ts2-mediated suppression.

In general, suppressor T cells of antigen-specific suppressor cell circuits have been shown to express I-J determinants on their surface (1, 3, 8, 12, 21, 36). To determine whether the T cells in the sensitized spleen cell population required for Ts2-mediated suppression of the cryptococcal DTH response were I-J-bearing cells, we treated sensitized spleen cells with anti-I-J antibody and complement before adoptively transferring the cells to naive recipients. As can be seen by the results from one such experiment (Fig. 4), the DTH response was suppressed in group 3 mice which received Ts2 cells and sensitized spleen cells pretreated with NMS and complement. In contrast, animals in group 4 which received Ts2 cells along with sensitized spleen cells that had been treated with anti-I-J antibody and complement did not have suppressed DTH responses, demonstrating that the suppressor cells in the sensitized cell population were I-J^+. The anti-I-J antibody and complement treatment did not affect T_{DH} cells, because the DTH responses in recipients of sensitized spleen cells treated with anti-I-J antibody and complement (group 5) were comparable to the positive controls (group 2). Results from group 6 demonstrate that the spleen cell population used as the source of Ts2 cells did not contain significant numbers of T_{DH} cells. The data from these latter two control groups 5 and 6 help confirm that the cells in spleens of CneF-CFA-immunized mice functioning in conjunction with Ts2 cells to suppress the DTH response are I-J^+.

Cy sensitivity of the precursor of the suppressor cells in the sensitized cell population. To assess whether the precursors of the suppressor cells in the sensitized spleen cell population were sensitive to Cy, mice were treated with Cy (100 mg/kg of body weight) 2 days before immunization. At 6 days after immunization, sensitized spleen cells from the Cy-treated immunized mice were transferred to naive recipients. Within 1 h after the adoptive transfers, recipients were footpad challenged with CneF, and 24 h later the footpads were measured. In these experiments, animals that received spleen cells from Cy-treated nonimmune animals (Fig. 5, group 3) served as the negative controls, and mice that received spleen cells from Cy-treated immune mice (group 4) served as the positive DTH controls. These special controls for the Cy experiments were not significantly different from the routine negative (group 1) and positive (group 2) controls. Animals in group 5, given (i) 10^8 spleen cells from Cy-treated immune animals, (ii) 10^8 Ts2 cells, and (iii) 2×10^7 sensitized cells treated with anti-L3T4 antibody and complement as the source of Ts3 cells, showed suppression of the DTH response. On the other hand, animals in group 6 that received (i) 10^8 sensitized spleen cells from Cy-treated animals, (ii) 10^8 Ts2 cells, and (iii) 2×10^7 normal spleen cells treated with anti-L3T4 antibody and complement in place of the putative Ts3 cells did not have significantly suppressed DTH responses. Furthermore, suppression of the DTH response was not observed in animals that received 2×10^7 sensitized cells treated with anti-L3T4 antibody and complement as the source of Ts3 cells, 10^8 normal spleen cells in place of Ts2 cells, and 10^8 sensitized spleen cells from Cy-treated animals (group 7), indicating that normal spleen cells could not replace the *C. neoformans*-specific Ts1-induced Ts2 cells in efferent suppression of the cryptococcal DTH response. These combined data suggest that the precursors of the immune T cells required for Ts2-mediated suppression are sensitive to Cy.

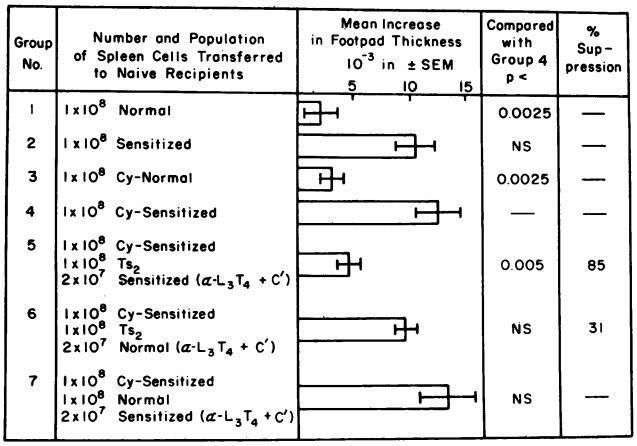

FIG. 5. Effects of Cy on precursors of the putative Ts3 cells. Mice injected with Cy 2 days before immunization with CneF-CFA were used as the source of Cy-sensitized cells. See Materials and Methods for a description of the preparation of sensitized and Ts2 cell populations. The specified cell pools were treated before adoptive transfer with anti-L3T4 antibody and complement (C'). Experiments were performed three times with five recipients in each group. NS, Not significant.

DISCUSSION

Antigen-specific suppressor cell circuits which regulate cell-mediated immune responses have been relatively well characterized for a number of hapten models (1, 3, 8, 9, 12, 13, 20, 35, 37, 39). Although there are many similarities among these various complicated circuits, there also appear to be some differences. The *C. neoformans*-specific suppressor pathway that affects the cell-mediated immune response to cryptococcal antigen and thus has the potential to play a role in an infectious disease process, as defined thus far, resembles the NP- and ABA-specific suppressor circuits regulating the hapten cell-mediated immune responses (1, 8). For example, in all three of these models, antigen-specific Ts1 cells are induced by i.v. administration of hapten-coupled spleen cells or antigen (8, 24, 26, 28, 29, 33, 38). The Ts1 cells or TsF1 bind to the specific antigens and specifically suppress the DTH response when given to recipients at the time of immunization (1, 8, 9, 24, 28, 38). Another similarity in these models is that Ts1 cells or TsF1 induce Ts2 cells without a second antigen stimulation which seems to be required in certain other models (1, 3, 8, 9, 27, 28, 30, 37, 39). Ts2 cells or TsF2 in the NP and ABA systems are anti-idiotypic and thus do not bind the specific antigen but do mediate specific suppression at the efferent phase of the DTH response (8, 9, 29, 30, 33, 37, 39). The anti-idiotypic characteristic of the *C. neoformans*-specific Ts2 cells has not been determined; however, we have demonstrated that *C. neoformans*-specific TsF2 does not bind cryptococcal antigens (23) but Ts2 cells do specifically suppress the cryptococcal DTH response (27), leading us to predict that the Ts2 cells in the cryptococcal model may be anti-idiotypic also. The Ts2 cells or TsF2 in the NP or ABA models do not directly interact with the T_{DH} cells to suppress the NP or ABA DTH responses but require a Ts3 cell that is found in mice immunized with a subcutaneous injection of the respective antigen-conjugated spleen cells (3, 8, 21, 29, 33). The need for a suppressor cell induced by an immunizing protocol is not unique to the NP and ABA suppressor cell pathways. Sy et al. (35) showed that contact sensitivity to 1-fluoro-2,4-dinitrobenzene (DNFB) in recipients of lymphoid cells from Cy-treated DNFB-immunized mice cannot be suppressed by a population of DNFB suppressor cells, whereas the contact sensitivity response in recipients of DNFB-immune lymphoid cells is readily suppressed by the same DNFB suppressor cells. These investigators referred to these Cy-sensitive suppressor cells stimulated by the standard immunization protocol as T auxiliary cells (35). The T auxiliary cells and Ts3 cells described for the NP and ABA models appear to be similar (1, 8). The phenotype of the NP and ABA Ts3 cells has been reported to be Lyt-2^+ I-J^+ (1, 8).

In the present study, we investigated whether Ts3 cells are functional components in the *C. neoformans*-specific suppressor cell circuit. Considering that in the other suppressor cell models Ts3 cells are induced by an immunizing dose of antigen, our approach was to determine by adoptive transfer whether an immunizing regimen with CneF would induce a similar suppressor cell population. However, before it was possible to use this technique, we had to ascertain the phenotype of cryptococcal T_{DH} cells so that the T_{DH} cells could be distinguished from the suppressor cells induced by immunization with CneF. The cryptococcal T_{DH} cells were shown to be sensitive to anti-L3T4 antibody and complement but not to anti-Lyt-2.1 antibody and complement treatment, indicating that their phenotype is L3T4$^+$ Lyt-2^- (Fig. 1). The phenotype of the cryptococcal T_{DH} cells is the same as that reported for cells which transfer DTH responses to tumor antigens (7) and *Mycobacterium tuberculosis* (14). However, our results are in contrast to the findings with *Listeria monocytogenes* in which a mixture of L3T4$^+$ and Lyt-2^+ cells from *L. monocytogenes*-sensitized mice are required to transfer DTH (15).

Ts2-mediated suppression of the cryptococcal DTH response was evident in recipients which received *C. neoformans*-specific Ts2 cells and untreated spleen cells from *C. neoformans*-immunized mice. However, the suppression was abrogated when Ts2 cells were transferred to naive recipients with sensitized cells which had been treated with anti-Lyt-2.1 and complement (Fig. 2) but not when Ts2 cells were transferred to naive recipients with sensitized cells which had been treated with anti-L3T4 antibody and complement (Fig. 3, group 5), indicating that L3T4$^-$ Lyt-2^+ cells in the sensitized cell population were required for the *C. neoformans*-specific Ts2-mediated suppression. Furthermore, mice which received Ts2 cells in conjunction with sensitized cells treated with anti-I-J antibody and complement showed no suppression of the cryptococcal DTH response (Fig. 4), demonstrating that sensitized cells from the spleens of immune mice needed for efferent suppression were I-J^+. Additional experiments were conducted to confirm that the cells in the immune population involved in efferent suppression of the DTH response were T cells (Fig. 3). Suppression was only achieved when recipients were supplied with T_{DH} cells, Ts2 cells, and a sensitized cell population containing Lyt-2^+ T cells (Fig. 2 and 3).

In studies in which mice were treated with Cy 2 days before immunization, it was clearly demonstrated that Cy treatment did not affect the development of the T_{DH}-cell population but it inhibited the induction of the suppressor cells (Fig. 5). Therefore, the *C. neoformans*-specific suppressor cells induced by immunization with CneF were similar to the T auxiliary suppressor cells described by Sy et al. (35).

Based on the above data, it was apparent that Ts2 cells and a population of T cells induced as the consequence of immunization were needed for the Ts2-mediated suppression of the cryptococcal DTH response. Furthermore, the L3T4$^-$ Lyt 2^+ I-J^+ suppressor T-cell population induced by immunization with CneF and derived from Cy-sensitive precursors appears to be comparable to the Ts3 cells in the NP and ABA suppressor cell cascades (1, 8, 9, 12) and the T auxiliary cells in the DNFB model (35).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-15716 from the National Institute of Allergy and Infectious Disease.

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