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Effects of First-Order *Cryptococcus*-Specific T-Suppressor Cells on Induction of Cells Responsible for Delayed-Type Hypersensitivity

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Cell-mediated immunity is an important aspect of host resistance against *Cryptococcus neoformans*. Using a CBA/J murine model, we demonstrated that injection of cryptococcal antigen (CneF) at dosages sufficient to stimulate the antigenemia observed in cryptococcosis patients induces specific T-cell-mediated suppression of the cryptococcal delayed-type hypersensitivity response. The purpose of this study was to establish whether Lyt 1^+, first-order T-suppressor (Ts1) cells block the induction of T cells responsible for delayed-type hypersensitivity (T_{DH} cells) or whether they function by inducing Lyt 2^+, efferent suppressor (Ts2) cells. In one set of experiments, suppression was observed when Ts1 cells were adoptively transferred to recipient animals the day before, the day of, or the day after immunization; however, when Ts1 cells were transferred after T_{DH} cells were present, no suppression occurred. In other experiments, putative T_{DH} cells from lymph nodes (LN) or spleens were adoptively transferred from mice after immunization or after a suppressive dose of CneF or adoptive transfer of Ts1 cells and immunization. Delayed-type hypersensitivity could not be transferred with LN or spleen cells from mice receiving the suppressive dose of CneF or the Ts1 cells, even when the LN or spleen cells were treated with anti-Lyt 2.1 antibody and complement to remove any Ts2 cells. Delayed-type hypersensitivity was readily transferred with LN or spleen cells from immunized mice whether the cells were or were not treated with anti-Lyt 2 and complement. Furthermore, the cells in the tolerized LN cell pools responsible for suppression of T_{DH} cell induction were Lyt 1^+ 2^-, I-J$^+$ cells, which is the phenotype of the Ts1 cells. Taken together, these data indicate that Ts1 cells inhibit the induction of T_{DH} cells. This finding, coupled with the previous demonstration that Ts1 cells or a Ts1 cell-derived soluble factor (TsF1) induces Ts2 cells, establishes that the cryptococcal Ts1 cells are bifunctional in the suppressive pathway.

Cryptococcosis is a disease caused by an encapsulated yeastlike organism, *Cryptococcus neoformans*. It is well established that patients with disseminated cryptococcosis have detectable levels of cryptococcal antigen in their body fluids (4, 6, 9, 10, 12, 23, 25, 26), and high or increasing antigen titers are associated with progressive disease (4). Furthermore, it is not unusual to find individuals with active cryptococcosis displaying depressed cell-mediated responses to cryptococcal antigens (3, 11). Investigations with a murine cryptococcosis model demonstrated that mice with high numbers of cryptococci in their tissues have high cryptococcal antigen titers which in turn correlate with depressed delayed hypersensitivity (DTH) responses (14). Those results, together with the observations made in human cryptococcosis, stimulated us to propose that high levels of cryptococcal antigen might be down regulating the cryptococcal cell-mediated immune response, an important resistance mechanism against *C. neoformans* (13). In more recent studies in our laboratory it was demonstrated that cryptococcal antigen injected intravenously (i.v.) will trigger the induction of a series of suppressor cells and factors which specifically suppress cell-mediated immune responses to cryptococcal antigen (20–22). Briefly, the order of the induction of the suppressive elements and their functional and phenotypic characteristics are as follows. When cryptococcal antigen is injected i.v. into mice at levels simulating antigen titers in patients with systemic cryptococcosis, first-order suppressor (Ts1) cells are induced from cyclophosphamide-sensitive precursors and are found in the lymph nodes (LN) (20). Phenotypically, Ts1 cells are Lyt 1^+ 2^-, I-J$^+$ T cells (22). Functionally, they specifically suppress the cryptococcal DTH response if injected at the time of immunization, but they do not suppress if injected at the time the footpads are challenged (20). In addition, Ts1 cells induce a population of second-order T-suppressor (Ts2) cells from cyclophosphamide-resistant precursors in the spleens of animals receiving Ts1 cells (21, 22). Both afferent suppression and Ts2 induction can be achieved by treating mice with a Ts1-cell-derived soluble factor, referred to as TsF1. After injecting naive mice with Ts1 cells or TsF1, significant levels of Ts2 cell activity can be detected through adoptive transfer experiments by 6 days post-Ts1 or -TsF1 injection (21, 22). Because Ts1 cells can induce Ts2 cells, it is possible that the inhibition of DTH after Ts1 injection was not due to the suppression of the production of cells responsible for the DTH response (T_{DH} cells) but rather to the induction of Ts2 cells which in turn suppressed the expression of T_{DH} cells. The primary objective of this study was to determine whether Ts1 cells inhibited the production of T_{DH} cells. Using two different experimental approaches, we generated data which strongly support the concept that cryptococcal Ts1 cells inhibit the production of T_{DH} cells.

MATERIALS AND METHODS

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

Cryptococcal antigen. A cryptococcal culture filtrate (CneF) antigen was used for induction of suppression, immunization, and footpad challenge. It was prepared by the method of Cauley and Murphy (2). The lot of CneF antigen used in these studies had a protein concentration of 4.1 mg/ml, determined by the procedure of Lowry et al. (15) as modified by Miller (16), and a carbohydrate concentration of 2.4 mg/ml, when assayed by the phenol-sulfuric method (5).

Antibody. Anti-Lyt 1.1, anti-Lyt 2.1, and anti-I-J^k antibod-

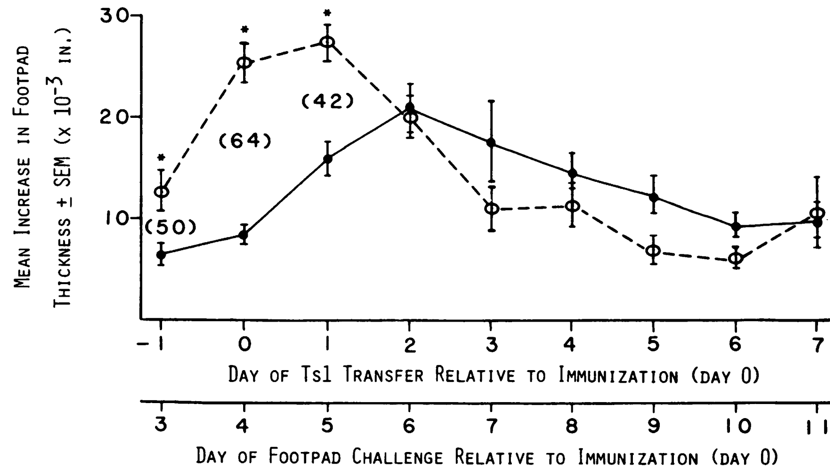

FIG. 1. Kinetics of suppression of the cryptococcal DTH response by Tsl cells. Symbols: ○, responses of mice immunized with CneF-CFA on day 0; ●, responses of mice immunized with CneF-CFA on day 0 and given Tsl cells on designated day; *, indicates P values of <0.025 when results of the immunized group were compared with those of the immunized, suppressed group. The numbers within parenthesis refer to percent suppression of the DTH response in the immunized, suppressed groups.

ies, complement, and cell treatment procedures used in these experiments were previously described (20, 22).

Induction and elicitation of DTH to *C. neoformans*. DTH was induced as described previously (20). Briefly, mice were inoculated at each of two sties at the base of the tail with 0.1 ml of an emulsion of CneF antigen in complete Freund adjuvant (CneF-CFA), and 6 days later the footpads of animals were challenged with 30 μl of CneF antigen as described by Cauley and Murphy (2). Final measurements of the footpads were made 24 h after challenge. Immune or positive and naive or negative DTH controls were included in each experiment. Previous data indicated that naive mice had similar levels of footpad responses to CneF antigen as did mice injected 6 days earlier with physiological saline-CFA (22); therefore, naive animals were used as negative controls in subsequent experiments.

Induction of cryptococcal Tsl cells. Mice were injected i.v. with 0.3 ml of CneF antigen 7 days before collecting peripheral and mesenteric LN cells. Single cell suspensions were made and served as the source of Tsl cells (20).

Induction of cryptococcal Ts2 cells. Tsl cells were injected i.v. into syngeneic mice, and 7 days later spleens of the recipient mice were collected as the source of Ts2 cells (22).

Kinetics of Tsl function. To determine when Tsl cells had to be adoptively transferred in relationship to the time of immunization to effectively suppress the DTH response, mice were injected i.v. with 5×10^7 Tsl cells the day before, the day of, or 1 to 7 days after immunization, and then their footpads were challenged with CneF antigen 4 days after the adoptive transfer of Tsl cells. Immunized mice whose footpads were challenged at each time period served as controls.

Adoptive transfer of DTH. Mice were immunized with CneF-CFA as described above, and 6 days later their spleens and LN were collected. Single cell suspensions were prepared, and then 10^8 spleen or LN cells were injected i.v. into naive syngeneic mice. In specified cases, the immune spleen or LN cells were mixed with 10^8 normal spleen cells or 10^8 Ts2 cells, and the mixtures of cells, either untreated or after treatment with monoclonal anti-Lyt 2.1 antibody plus complement as described previously (21, 22), were adoptively transferred to separate groups of mice. Within 1 h after adoptive transfer of cells, the footpads of mice were challenged with CneF antigen, and final measurements of the footpads were made 24 h later.

In experiments to assess effects of Tsl cells on T_{DH} cell induction, Tsl cells were either adoptively transferred i.v. into naive mice at the time of immunization or induced in mice by injecting i.v. CneF antigen 7 days before the animals were immunized; then spleen or LN cells of recipient mice were collected 6 days after immunization. The cells, either untreated or after treatment with anti-Lyt 2.1 antibody and complement to eliminate Ts2 cells, were adoptively transferred by i.v. injection into naive mice within 1 h before their footpads were challenged.

In studies designed to demonstrate that Tsl cells in the LN cell pools from tolerized mice were the cells responsible for inhibiting T_{DH} cell induction, the following protocol was used. LN cells from mice injected 7 days previously with CneF antigen were either untreated or treated with complement alone or with complement plus anti-Lyt 1.1, anti-Lyt 2.1, or anti-I-J^k antibody. Separate groups of naive syngeneic mice were injected i.v. with 5×10^7 cells from one of each of the treatment groups, and then the animals were immediately immunized with CneF-CFA. Immunized and unimmunized controls, as well as a control group of mice injected with normal LN cells and then immunized, were included. Six days after immunization, 10^8 spleen or LN cells from each group of mice were adoptively transferred to separate groups of naive mice whose footpads were challenged with CneF antigen. Final measurements of the footpads were made 24 h after challenge.

Statistical analyses. Calculations of means, standard errors of the means, and unpaired t tests were performed with an Apple II computer and statistics software.

RESULTS

Kinetics of cryptococcal Tsl cell function. The first approach in determining whether Tsl cells were inhibiting the induction of T_{DH} cells was to assess at what stages in the immune process Tsl cells suppressed the DTH response. The basic supposition was that if Tsl cells directly suppress the production of T_{DH} cells, then this cell population would have to be present soon after immunization, before the induction of T_{DH} cells. In contrast, if Tsl cells indirectly affect suppression by inducing Ts2 cells, then one would

	Treatment					Mean Increase in Footpad Thickness ± SEM (x 10^{-3} in.)	Comparison with Naive Control
	Donor		Recipient				
Group No.	CneF I.V.	Immunize with CneF:CFA S.C.	αLyt 2.1 + C' before Transfer	1 x 10^8 Donor LNC I.V.	1 x 10^8 Donor SPC I.V.	5 10	P <
1	0	0	0	0	0		-
2	+	+	-	+	-		0.4 (NS)
3	+	+	-	-	+		0.4 (NS)
4	+	+	+	+	-		0.25 (NS)
5	+	+	+	-	+		0.4 (NS)
6	-	+	-	+	-		0.0005
7	-	+	-	-	+		0.0005
8	-	+	+	+	-		0.0005
9	-	+	+	-	+		0.0005

FIG. 2. Adoptive transfer of DTH with LN cells (LNC) or spleen cells (SPC) from mice which were either immunized or immunized and suppressed with CneF antigen. Specified cell pools were treated with anti-Lyt 2.1 antiserum and complement (C') before transfer. S.C., Subcutaneously; NS, not significant.

expect to see suppression of the DTH response by Ts1 cells later in the induction phase and throughout the duration of the response. Earlier studies have demonstrated that DTH in this model could be detected as early as 3 days after immunization (unpublished data) and that Ts1 cells did not suppress the DTH response once it was stimulated (20). Therefore, the first experimental approach was as follows. Mice were immunized on day 0. Ts1 cells were adoptively transferred on days −1, 0, or 1 through 7. Four days after Ts1 cell transfer, the footpads of mice were tested, and footpad thicknesses were measured 24 h later. The results of one of three such experiments are shown in Fig. 1. Ts1 cells suppressed the DTH response only when they were transferred the day before, the day of, or the day after immunization. The percentages of suppression achieved at those time periods were 50, 67, and 42, respectively, whereas, no suppression was observed when Ts1 cells were transferred 2 or more days after immunization.

Transfer of T_{DH} cells from immunized or immunized and suppressed mice. The second approach in these studies was to determine whether T_{DH} cells were present in mice which had been tolerized with CneF antigen before immunization or which had been given 5×10^7 Ts1 cells at the time of immunization. To assess the presence of T_{DH} cells, spleen and LN cells were collected 6 days after immunization from the two groups of mice, i.e., immunized and tolerized-immunized groups, and adoptively transferred to separate groups of naive syngeneic recipients. The footpads of the recipient mice were immediately tested with CneF antigen, and 24 h later the final measurements of the footpads were made. Since there was a possibility that Ts2 cells could have been induced in the donors of T_{DH} cells by either the tolerizing dose of CneF antigen or Ts1 cells, portions of the spleen and LN cell populations collected from those animals were treated with anti-Lyt 2.1 antibody and complement to eliminate Ts2 cells before cell transfer. The results of adoptively transferring putative T_{DH} cells from either immunized mice or mice tolerized with cryptococcal antigen 7 days before immunization are shown (Fig. 2). Figure 3 presents the results obtained from similar adoptive transfer experiments in which suppression was achieved with cryptococcal Ts1 cells given at the time of immunization. It is apparent that DTH could be transferred with either LN or spleen cells from immunized mice (Fig. 2 and 3, groups 6 and 7, respectively). However, neither LN nor spleen cells from mice tolerized with cryptococcal antigen or Ts1 cells transferred significant responses (Fig. 2 and 3, groups 2 and 3, respectively). Furthermore, the responses in the suppressed groups (groups 2 and 3) could not be restored when any Ts2 cells that might be present were removed by treatment with anti-Lyt 2 antibody and complement (Fig. 2 and 3, groups 4 and 5, respectively). When the footpads of groups 8 and 9 (Fig. 2 and 3) were tested, the results were not significantly different from those obtained from the respective untreated immune groups (Fig. 2 and 3, groups 6 and 7), demonstrating that treatment with anti-Lyt 2.1 and complement did not affect the T_{DH} cells.

Since the possibility existed that treatment with anti-Lyt 2 and complement could be lysing Ts2 cells and releasing TsF2, a soluble factor which will suppress T_{DH} cells (21), an additional study was done to establish that under the conditions used, released TsF2 was not effective in suppressing the DTH response. T_{DH} and Ts2 cells were mixed together and then adoptively transferred to naive recipient mice before and after treatment with anti-Lyt 2 antibody and complement. Control animals were adoptively given a mixture of T_{DH} cells and normal spleen cells which were either untreated or treated with anti-Lyt 2 antibody and complement before transfer. The footpads of all groups of recipient mice plus a group of naive mice were challenged at the time of cell transfer. Footpad responses were measured 24 h later (Fig. 4). LN and spleens of immunized mice contained T_{DH} cells, and the addition of normal spleen cells did not abrogate the DTH response as indicated by the positive transfer of DTH (Fig. 4, groups 2 and 3, respectively). When Ts2 cells were mixed with the same immune cell pools, the transfer of DTH was significantly inhibited (Fig. 4, groups 4 and 5). Treatment of the immune and normal spleen cell mixtures with anti-Lyt 2 antibody and complement did not affect the transfer of DTH (Fig. 4, groups 6 and 7). However,

Group No.	Treatment: Donor: 5 x 10^7 LNC I.V.	Treatment: Donor: Immunize with CneF:CFA S.C.	Treatment: Recipient: αLyt 2.1 + C' before transfer	Treatment: Recipient: 1 x 10^8 Donor LNC I.V.	Treatment: Recipient: 1 x 10^8 Donor SPC I.V.	Mean increase in footpad thickness ± SEM (x 10^{-3} in.) (scale: 5, 10)	Comparison with naive control P<
1	0	0	0	0	0		-
2	Ts1-LNC	+	-	+	-		0.4 (NS)
3	Ts1-LNC	+	-	-	+		0.4 (NS)
4	Ts1-LNC	+	+	+	-		0.1 (NS)
5	Ts1-LNC	+	+	-	+		0.25 (NS)
6	NLNC	+	-	+	-		0.0005
7	NLNC	+	-	-	+		0.0005
8	NLNC	+	+	+	-		0.0005
9	NLNC	+	+	-	+		0.0005

FIG. 3. Adoptive transfer of DTH with LN cells (LNC) or spleen cells (SPC) from mice which were immunized and given normal LN cells (NLNC) or which were immunized and suppressed with Tsl cells. Specified cell pools were treated with anti-Lyt 2.1 antiserum and complement (C') before transfer. S.C., Subcutaneously; NS, not significant.

the suppressive effect of Ts2 cells in the immune and Ts2 cell mixtures was abolished by treatment with anti-Lyt 2 and complement, as indicated by the reversal of the negative transfers in groups 4 and 5 to positive transfers of DTH in groups 8 and 9, respectively (Fig. 4). These data demonstrate that Ts2 cell activity can be abrogated without affecting T_{DH} cells when mixtures of T_{DH} and Ts2 cells are treated with anti-Lyt 2 and complement; therefore, it can be assumed that DTH would have been effectively transferred in groups 4 and 5 (Fig. 2 and 3) if T_{DH} cells had been present.

In previous studies, we demonstrated that the suppressor (Tsl) cells induced in the LN by i.v. injecting mice with CneF antigen are Lyt 1^+ 2^-, I-J^+ T cells (22). Since the LN cell pools used to suppress T_{DH} cell induction (Fig. 3) were not purified Tsl cells, the objective of this experiment was to show that the cells in the tolerized LN cell pools, which were responsible for suppression of T_{DH} cells, were in fact cells with phenotypic characteristics of Tsl cells. For this study, Tsl cells were induced in the usual manner by i.v. injecting CneF antigen into normal mice. Seven days later, the LN cells from the tolerized mice, either untreated or after treatment with complement alone or complement and anti-Lyt 1.1, anti-Lyt 2.1, or anti-I-J^k antibody, were transferred to naive, syngeneic mice which were immediately immunized. Six days after adoptive transfer and immunization, spleen and LN cells from each of the treatment groups

Group No.	Treatment of recipient: αLyt 2.1 + C' before transfer	Treatment of recipient: 1 x 10^8 Immune LNC I.V.	Treatment of recipient: 1 x 10^8 Immune SPC I.V.	Treatment of recipient: 1 x 10^8 NSPC I.V.	Treatment of recipient: 1 x 10^8 Ts2-SPC I.V.	Mean increase in footpad thickness ± SEM (x 10^{-3} in.) (scale: 5, 10)	Comparison with naive control P<
1	0	0	0	0	0		-
2	-	+	-	+	-		0.0005
3	-	-	+	+	-		0.0005
4	-	+	-	-	+		0.4 (NS)
5	-	-	+	-	+		0.25 (NS)
6	+	+	-	+	-		0.0005
7	+	-	+	+	-		0.0005
8	+	+	-	-	+		0.0005
9	+	-	+	-	+		0.0005

FIG. 4. Adoptive transfer of DTH with mixtures of immune LN cells (LNC) or spleen cells (SPC) and normal spleen cells (NSPC) or Ts2-containing spleen cells (Ts2-SPC). Specified cell pools were treated with anti-Lyt 2.1 antibody and complement (C') before transfer. NS, not significant.

Group No.	Treatment: Donor: 5×10^7 LNC i.v.: Source of LNC	Treatment of LNC before transfer	Immunize with CneF:CFA s.c.	Recipient: 1×10^8 Donor SPC i.v.	Mean increase in footpad thickness ± SEM (x 10^{-3} in.) 5 10	Comparison with naive control P <
1	0	0	0	0		-
2	0	0	+	+		0.0005
3	Normal	Untreated	+	+		0.0005
4	Ts1	Untreated	+	+		0.4 (NS)
5	Ts1	C' alone	+	+		0.4 (NS)
6	Ts1	αLyt 1.1 + C'	+	+		0.0005
7	Ts1	αLyt 2.1 + C'	+	+		0.25 (NS)
8	Ts1	αI-J^k + C'	+	+		0.0005

FIG. 5. Adoptive transfer of DTH with spleen cells (SPC) from mice which were immunized and given LN cells (LNC) from normal mice or which were immunized and given LNC from tolerized mice (Ts1). Specified LNC pools were treated with complement (C') alone or anti-Lyt 1.1, anti-Lyt 2.1, or anti-I-J^k antibody and C' before injection into donor mice. S.C., Subcutaneously; NS, not significant.

of mice were transferred i.v. to naive syngeneic recipient groups of mice. The footpads of the recipient animals were challenged within 1 h of cell transfer. Final footpad measurements were made 24 h later (Fig. 5). Similar results were obtained from the various groups of mice which had LN cells rather than spleen cells adoptively transferred (data not shown). As observed in earlier experiments, mice given normal LN cells at the time of immunization had spleen and LN cells which could adoptively transfer DTH responsiveness (Fig. 5, group 3), whereas, mice given LN cells from tolerized mice did not produce sufficient numbers of T_{DH} cells in either organ to adoptively transfer the response (Fig. 5, group 4). When the Ts1-containing cell pools were treated with complement alone or with anti-Lyt 2.1 antibody and complement, they maintained the ability to suppress the induction of T_{DH} cells (Fig. 5, groups 5 and 7, respectively). However, when the Ts1 cell pools were treated with anti-Lyt 1.1 or anti-I-J^k antibody plus complement, they no longer suppressed the induction of T_{DH} cells (Fig. 5, groups 6 and 8, respectively). These data show that the suppressor cells inhibiting T_{DH} induction had the Lyt 1^+ 2^-, I-J$^+$ phenotype of the cryptococcal Ts1 cells.

DISCUSSION

From data generated in earlier studies, specific suppression of the cryptococcal DTH response appeared to be mediated by two different sets of suppressor cells, Ts1 and Ts2 cells, functioning at two different stages in the response, i.e., the induction and expression phases, respectively (20–22). The suppressor cell populations, as defined thus far for the cryptococcal model, are Ts1 cells which are induced from cyclophosphamide-sensitive precursors by administering cryptococcal antigen i.v. and have an Lyt 1^+ 2^-, I-J$^+$ phenotype and Ts2 cells which are induced by Ts1 cells from cyclophosphamide-resistant precursors and have an Lyt 1^- 2^+, I-J$^+$ phenotype. The Ts1 cell pools have been shown to suppress the DTH response when injected at the time of immunization and induce the Ts2 or efferent suppressor cells (20, 22). Furthermore, it was established that the cells in the Ts1 cell pools which were responsible for the apparent afferent suppression of the DTH response and induction of Ts2 cells were not only induced under the same conditions but were phenotypically the same with regard to the parameters assayed, i.e., Thy 1^+, Lyt 1^+ 2^-, I-J$^+$, and had cyclophosphamide-sensitive precursors (22). It is possible that two different cell populations within the Ts1 pools were mediating the two different observed effects; however, the available evidence does not support this idea but rather indicates that one cell type was responsible for the apparent afferent suppression and Ts2 cell induction (22). Even if only one cell population is responsible for the observed effects, it is feasible that the two effects are the result of only one event not two. For example, Ts1 cells may in fact not be suppressing the induction or afferent phase of the DTH response but rather inducing Ts2 cells which suppress the expression or efferent phase of the DTH response. The data presented in this paper, together with earlier studies (22), support the contention that the cryptococcal Ts1 cells play a dual role in the suppressive circuit, i.e., suppression of T_{DH} cell induction and induction of Ts2 cells, rather than a single role, i.e., induction of Ts2 cells. Two different experimental approaches were taken to answer this question, and the data from both indicate that Ts1 cells inhibit T_{DH} cell induction.

First, the kinetic studies concerned with the effects of Ts1 cells on induction of the DTH response suggested that Ts1 cells inhibit T_{DH} cell production (Fig. 1). This conclusion was drawn because Ts1 cells were only effective in suppression of DTH when given at the time of immunization or within 1 day of immunization. Our previous unpublished studies indicated, under the conditions of immunization used in these experiments, that 3 days after immunization was the earliest time that significant levels of DTH could be detected by footpad challenge. Therefore, to effectively suppress, Ts1 cells had to be in the system before or during the time T_{DH} precursor cells were being stimulated by the immunizing dose of antigen. When Ts1 cells were introduced into the system after T_{DH} cells were generated (2 or more days postimmunization), suppression was not achieved (Fig. 1). The possibility that Ts2 cells were present in the mice 4 days after Ts1 cell injection, which was the time of footpad challenge, is slight, since it was demonstrated that Ts2 cells cannot be detected until 6 days after Ts1 injection (21).

Furthermore, if Ts2 cells had been induced within 4 days after injection of Ts1 cells and were responsible for the suppression, suppression should have been observed for each of the times that the function of Ts1 cells was assessed. However, that was not the case, indicating that Ts1 cells were not indirectly affecting the DTH response by inducing Ts2 cells but rather were suppressing the induction of T_{DH} cells.

The second experimental approach which involved demonstrating by adoptive transfer the presence or absence of T_{DH} cells in mice suppressed by injection of either CneF antigen or Ts1 cells confirmed that T_{DH} cells were few to absent when Ts1 cells were present at the time of immunization. The inability to transfer DTH from mice in which Ts1 cells had been present at the time of immunization, along with the inability to reverse the negative transfers to positive transfers by eliminating any Ts2 cells which could have been present, supports the contention that T_{DH} cell induction was inhibited by Ts1 cells. However, there were two complicating factors which had to be eliminated before the previous conclusion could be considered valid. First, Ts2 cells mediate their effect via a soluble factor, TsF2 (21), which could have been released by treatment with anti-Lyt 2 antibody and complement. If TsF2 had bound to its target cells before the washings of the treated cells took place and if it bound tightly enough to resist removal by the washes, then reversal of negative transfers to positive transfers of DTH would not have occurred even when T_{DH} cells were present. We demonstrated that such a scenario was not taking place by showing that mixtures of T_{DH} and Ts2 cells did not tranfer DTH before treatment with anti-Lyt 2 and complement but did so after treatment (Fig. 4). Second, cells other than Ts1 cells in the LN cell populations from tolerized mice could have been responsible for suppression of T_{DH} cell induction. This possibility was eliminated by demonstrating that suppression of T_{DH} cell induction was abrogated when Ts1 cells, which are Lyt 1^+ 2^-, I-J^+, were removed from the suppressor cell-containing LN cell population (Fig. 5). Taken together, the data presented (Fig. 1 to 5) provide strong evidence which supports the hypothesis that Ts1 cells suppress cryptococcal T_{DH} cell induction. In addition, considering earlier data demonstrating that Ts1 cells induce Ts2 cells (21, 22), we favor the concept of a dual functional role for Ts1 cells in the cryptococcal suppressor pathway.

Inhibition of induction of T_{DH} cells is not unique to cryptococcal afferent suppressor cells. In the 2,4-dinitro-1-fluorobenzene suppressor cell model, it has been demonstrated that afferent Ts cells were effective in suppressing the DTH response only when they were present during early stages of the afferent phase of sensitization (18, 19). Furthermore, the afferent suppressor cells specific to 2,4-dinitro-1-fluorobenzene were shown to inhibit antigen-induced cell proliferation (18, 19), suggesting that T_{DH} cell induction, including the proliferative aspect, was inhibited by the afferent Ts cells. However, in contrast to the cryptococcal suppressor cell pathway, as well as several other antigen-specific suppressor cell circuits (1, 7, 8, 24), the suppressor cell sequence specific to 2,4-dinitro-1-fluorobenzene induced by 2,4-dinitrophenyl-coupled syngeneic LN cells has as its first-appearing suppressor cell an efferent suppressor that in turn induces a population of afferent suppressor cells (17).

In addition to more specifically defining the role of the first-order suppressor cells in the cryptococcal model, this study demonstrated that the cryptococcal T_{DH} cells were Lyt 2^-, inferring that they were Lyt 1^+ cells. Although it would be expected that cryptococcal T_{DH} cells would be Lyt 1^+ because that is the phenotype of other T_{DH} cells, this is the first experimental documentation of the Lyt characteristics of the cryptococcal T_{DH} cells.

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LITERATURE CITED

1. **Adorini, L., V. Colizzi, G. Doria, and P. Ricciardi-Castagnoli.** 1984. Immunoregulation of lysozyme-specific suppression. II. Hen egg-white lysozyme-specific monoclonal suppressor T cell factor suppresses the afferent phase of delayed-type hypersensitivity and induces second-order suppressor T cells. Eur. J. Immunol. **14**:826–830.
2. **Cauley, L. K., and J. W. Murphy.** 1979. Response of congenitally athymic (nude) and phenotypically normal mice to *Cryptococcus neoformans* infection. Infect. Immun. **23**:644–651.
3. **Diamond, R. D., and J. E. Bennett.** 1973. Disseminated cryptococcosis in man: decreased lymphocyte transformation in response to *Cryptococcus neoformans*. J. Infect. Dis. **127**:694–697.
4. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis. Ann. Intern. Med. **80**:176–184.
5. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
6. **Eng, R., H. Chmel, M. Corrado, and S. M. Smith.** 1983. The course of cryptococcal capsular polysaccharide antigenemia/human cryptococcal polysaccharide elimination kinetics. Infection **11**:132–136.
7. **Germain, R. N., and G. Benacerraf.** 1978. Antigen-specific T cell-mediated suppression. III. Induction of antigen-specific suppressor T cells (Ts2) in L-glutamic acid[60]-L-alanine[30]-L-tyrosine[10] (GAT) responder mice by nonresponder-derived GAT-suppressor factor (GAT-TsF). J. Immunol. **121**:608–612.
8. **Germain, R. N., J. Theze, C. Waltenbaugh, M. E. Dorf, and B. Benacerraf.** 1978. Antigen-specific T cell-mediated suppression. II. In vitro induction by I-J-encoded L-glutamic acid[50]-L-tyrosine[50] (GT)-specific T cell suppressor factor (GT-TsF) of suppressor T cells (Ts2) bearing distinct I-J determinants. J. Immunol. **121**:602–607.
9. **Goodman, J. S., L. Kaufman, and M. G. Koenig.** 1971. Diagnosis of cryptococcal meningitis. Value of immunologic detection of cryptococcal antigen. N. Engl. J. Med. **285**:434–436.
10. **Gordon, M. A., and D. K. Vedder.** 1966. Serologic tests in diagnosis and prognosis of cryptococcosis. J. Am. Med. Assoc. **197**:961–967.
11. **Graybill, J. R., and R. H. Alford.** 1974. Cell-mediated immunity in cryptococcosis. Cell. Immunol. **14**:12–21.
12. **Kauffman, C. A., A. G. Bergman, P. J. Severance, and K. D. McClatchey.** 1981. Detection of cryptococcal antigen. Am. J. Clin. Pathol. **75**:106–109.
13. **Lim, T. S., and J. W. Murphy.** 1980. Transfer of immunity to cryptococcosis by T-enriched splenic lymphocytes from *Cryptococcus neoformans*-sensitized mice. Infect. Immun. **30**:5–11.
14. **Lim, T. S., J. W. Murphy, and L. K. Cauley.** 1980. Host-etiological agent interactions in intranasally and intraperitoneally induced cryptococcosis in mice. Infect. Immun. **29**:633–641.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Miller, G. L.** 1959. Protein determination for large numbers of samples. Anal. Chem. **31**:964–966.
17. **Miller, S. D., L. D. Butler, and H. N. Claman.** 1982. Suppressor T cell circuits in contact sensitivity. I. Two mechanistically distinct waves of suppressor T cells occur in mice tolerized with syngeneic DNP-modified lymphoid cells. J. Immunol. **129**:461–468.
18. **Miller, S. D., M. S. Sy, and H. N. Claman.** 1978. Suppressor T cell mechanisms in contact sensitivity. II. Afferent blockade by alloinduced suppressor T cells. J. Immunol. **121**:274–280.

19. **Moorhead, J. W.** 1976. Tolerance and contact sensitivity to DNFB in mice. VI. Inhibition of afferent sensitivity by suppressor T cells in adoptive tolerance. J. Immunol. **117**:802–806.
20. **Murphy, J. W., and J. W. Moorhead.** 1982. Regulation of cell-mediated immunity in cryptococcosis. I. Induction of specific afferent T suppressor cells by cryptococcal antigen. J. Immunol. **128**:176–283.
21. **Murphy, J. W., and R. L. Mosley.** 1985. Regulation of cell-mediated immunity in cryptococcosis. III. Characterization of second-order T suppressor cells (Ts2). J. Immunol. **134**:577–584.
22. **Murphy, J. W., R. L. Mosley, and J. W. Moorhead.** 1983. Regulation of cell-mediated immunity in cryptococcosis. II. Characterization of first-order T suppressor cells (Ts1) and induction of second-order suppressor cells. J. Immunol. **130**: 2876–2881.
23. **Neill, J. M., J. Y. Suggs, and D. W. McCauley.** 1951. Serologically reactive material in spinal fluid, blood and urine from a human case of cryptococcosis (torulosis). Proc. Soc. Exp. Biol. Med. **77**:775–778.
24. **Sy, M. S., M. H. Dietz, R. N. Germain, B. Benacerraf, and M. I. Greene.** 1980. Antigen and receptor driven regulatory mechanisms. IV. Idiotype-bearing I-J^+ suppressor T cell factors induce second order suppressor T cells which express anti-idiotypic receptors. J. Exp. Med. **151**:1183–1195.
25. **Wold, L. E., G. D. Roberts, N. S. Brewer, D. J. Zeller, L. D. Fineman, C. L. Paschall, and R. C. Rosenlof.** 1980. Massive antigenemia during disseminated cryptococcosis. Mayo Clin. Proc. **55**:513–515.
26. **Young, E. J., D. D. Hirsh, V. Fornstein, and T. W. Williams.** 1980. Pleural effusions due to *Cryptococcus neoformans*: a review of the literature and report of two cases with cryptococcal antigen determinations. Am. Rev. Respir. Dis. **121**:743–747.