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Immunoadsorption of *Cryptococcus*-Specific Suppressor T-Cell Factors

R. LEE MOSLEY,[1] JUNEANN W. MURPHY,[1]* AND REBECCA A. COX[2]

Department of Botany-Microbiology, University of Oklahoma, Norman, Oklahoma 73019,[1] and San Antonio State Chest Hospital, San Antonio, Texas 78223[2]

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In the murine cryptococcal suppressor cell circuit, two different T-cell suppressor factors, TsF1 and TsF2, have been identified which specifically suppress the delayed-type hypersensitivity (DTH) response to cryptococcal culture filtrate antigen (CneF). TsF1 is produced by a first-order T suppressor (Ts1) cell population and suppresses the afferent limb of the DTH response, whereas TsF2 is produced by a second-order T suppressor (Ts2) cell population and suppresses the efferent limb of the cryptococcal DTH response. The objective of this study was to ascertain whether TsF1 or TsF2 could bind to cryptococcal antigen. To assess this, adsorption of TsF1 and TsF2 was performed with heat-killed *Cryptococcus neoformans* cells and by solid-phase immunoadsorption (SPIA) on columns containing cryptococcal antigens, i.e., CneF covalently bound to Sepharose 4B. The suppressive effect of TsF1 was removed by adsorption with intact heat-killed cryptococci and by SPIA on CneF-Sepharose 4B. The binding of cryptococcal TsF1 to the cryptococcal SPIA column was shown to be specific since Sepharose 4B columns either coupled with *Saccharomyces cerevisiae* mannan or blocked with glycine did not adsorb the suppressor activity. In contrast, the suppressive component of TsF2 did not bind to heat-killed cryptococci, CneF-Sepharose 4B, *S. cerevisiae* mannan-Sepharose 4B, or glycine-Sepharose 4B columns. These results, together with the finding that cryptococcal antigen, anticryptococcal antibody, and C1q-binding immune complexes were not demonstrated in either TsF1 or TsF2, establish that TsF1 and TsF2 can be differentiated on the basis of their affinity for cryptococcal antigen.

Antigen-specific suppression of the cryptococcal delayed-type hypersensitivity (DTH) response can be induced in mice by intravenous (i.v.) injection of cryptococcal antigen to produce circulating antigen levels comparable to those found in patients with disseminated cryptococcosis (27). Within 6 days after antigen administration, a first-order T suppressor (Ts1) cell population can be isolated from lymph nodes of cryptococcal antigen-treated mice. These Ts1 cells suppress the afferent limb of the DTH response if adoptively transferred at the time of immunization (27). Phenotypically, Ts1 cells are Thy-1^+, Lyt-1^+2^-, and I-J^+ and are induced from cyclophosphamide-sensitive precursors (29). The suppressive effect of Ts1 cells is mediated through a soluble factor designated as TsF1 (29). In addition to inhibiting the induction of T cells responsible for DTH (T_{DH} cells), Ts1 cells and TsF1 induce a second-order T suppressor (Ts2) cell population in the spleens of Ts1- or TsF1-treated recipient mice (24, 29). Ts2 cells specifically suppress the cryptococcal DTH response when transferred to immunized mice at the time of footpad challenge with cryptococcal culture filtrate antigen (CneF) but do not suppress when given at the time of immunization (29). Therefore, Ts2 cells are referred to as efferent T suppressor cells. These second-order suppressor cells are Lyt-1^-2^+, I-J^+ T cells which are induced from cyclophosphamide-resistant precursors (28). As Ts1 cells do, Ts2 cells mediate their suppressive effects via a soluble suppressor factor designated as TsF2 (28).

Several groups of investigators have studied antigen-specific T suppressor cell circuits and suppressor cell factors induced by haptenic determinants such as azobenzenearsonate (ABA) (16), dinitrofluorobenzene (4), 4-hydroxy-3-nitrophenyl acetate (NP) (8), and picryl chloride (41). Suppressor factors have also been stimulated with several protein antigens, including keyhole limpet hemocyanin (35), glycoporphorin (10), and the synthetic polypeptides L-glutamic acid^{60}-L-alanine^{10}-L-tyrosine^{30} and L-glutamic acid^{50}-L-tyrosine^{50} (11, 38) and with particulate antigens such as sheep erythrocytes (19), alloantigens (31, 32), and tumor cells (14, 36). In fact, Webb et al. (38) concluded that in almost every immune response, suppressor cells are produced which release antigen- or determinant-specific T suppressor factors.

In the cryptococcal suppressor circuit, the soluble factors derived from first- and second-order T suppressor cells specifically suppress the afferent and efferent limbs of the DTH responses, respectively, in a manner similar to that described for the ABA and NP suppressor factors. First-order T suppressor cell factors in other systems recognize or bind to the specific antigen that induces them; whereas, suppressor factors from second-order T suppressor cells do not (1, 2, 15, 17, 19, 30, 35–40). The purpose of this study was to determine whether cryptococcal TsF1 and TsF2 could be differentiated on the basis of their affinity for cryptococcal antigen.

MATERIALS AND METHODS

Mice. Inbred CBA/J female mice, 7 to 10 weeks of age, purchased from the Jackson Laboratory, Bar Harbor, Maine, were used throughout these studies.

Reagents. Unless otherwise specified, chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

Organism. *Cryptococcus neoformans*, isolate 184, serotype A, which has been previously described (25, 26), was used for the preparation of antigen and as an immunoadsorbent in adsorption experiments.

Antigen preparation and analysis. CneF, which was used

* Corresponding author.

for induction of suppression, immunization, and footpad challenge, was prepared by the method of Cauley and Murphy (3). The lot of CneF used in these studies had a protein concentration of 3.1 mg/ml, as determined by the procedure of Lowry et al. (20) with modifications of Miller (21), and a reducing carbohydrate concentration of 3.0 mg/ml, based on results of the phenol-sulfuric acid method of Dubois et al. (9).

Induction and elicitation of DTH. DTH was induced as described previously (27). Briefly, mice were immunized by inoculating 0.1 ml of an emulsion of CneF in complete Freund adjuvant (CFA; CneF-CFA) at each of two sites at the base of the tail. Six days later, mice were footpad challenged with 30 μl of CneF, and DTH was measured as the increase in footpad thickness 24 h after antigen challenge as described by Cauley and Murphy (3). Positive (i.e., immune) and negative DTH controls were included in each set of experiments. DTH-negative controls consisted of either untreated mice or mice inoculated with sterile physiological saline solution in CFA 6 days before footpad challenge. Both types of negative controls gave similar footpad responses to CneF. DTH was calculated from the individual measurements of five mice per group and expressed as the mean increase in footpad thickness plus or minus the standard error of the mean (SEM).

Preparation and assay of suppressor factors. TsF1 or TsF2 was prepared as previously described (28, 29, 34). Briefly, 5×10^8 Ts1 or Ts2 cells per ml of Hanks balanced salt solution (HBSS) was subjected to four cycles of alternate snap freezing at −70°C and thawing at 37°C. Cellular debris was removed by centrifugation and the supernatant was centrifuged at 100,000 × *g* for 1 h. The soluble supernatant was collected and maintained at −20°C until used.

TsF1 was assayed for suppressor activity (28, 29) by administering 10^7 cell equivalents of TsF1 to mice intravenously (i.v.) on each of 5 consecutive days beginning at the time of immunization. Six days later, the mice were footpad challenged with CneF to assess the level of DTH. The activity of TsF2 preparations was assayed by giving 10^8 cell equivalents of TsF2 i.v. to mice immunized 6 days previously and footpad challenged at the time of TsF2 administration (28).

Percent suppression of the cryptococcal DTH response was calculated from the mean increase in footpad thickness by the following formula: Percent suppression = [(footpad thickness of immune control group − footpad thickness of experimental group)/(footpad thickness of immune control group − footpad thickness of naive control group)] × 100.

Adsorption of TsF1 and TsF2 with cryptococcal cells. To assess whether TsF1 or TsF2 would bind to cryptococci, heat-killed *C. neoformans* cells were washed and suspended in (i) 2×10^8 cell equivalents of TsF1 per ml of HBSS, (ii) 2×10^8 cell equivalents of TsF2 per ml of HBSS, or (iii) an equivalent volume of HBSS to make 8.3% (vol/vol) cell suspensions. The mixtures were incubated at 4°C for 90 min. The suspensions were centrifuged, and the supernatants were collected and then stored at −20°C until assayed.

SPIA of TsF1 and TsF2. In preparation for solid-phase immunoadsorption (SPIA) of TsF1 and TsF2, CneF and *Saccharomyces cerevisiae* mannan were covalently coupled to Sepharose 4B through bifunctional oxirane reactive groups as described by Sundberg and Porath (33). Briefly, 1.0 g of washed and suction-dried Sepharose 4B was suspended in 1.0 ml of 1,4-diglycidyl ether and 1.0 ml of 0.6 N NaOH containing 2 mg of $NaBH_4$ per ml and reacted for 8 h at 25°C. The reaction was stopped by washing with water over a sintered-glass filter, and the resulting retentate served as the epoxy-activated Sepharose 4B. Coupling of the ligands was achieved by suspending 5.0 g of suction-dried epoxy-activated Sepharose 4B with 10 ml of CneF, 10 ml of *S. cerevisiae* mannan (3.0 mg/ml), or 10 ml of 1.0 M glycine (Gly) in 0.15 M NaCl-NaOH (coupling buffer; pH 11.0). Each reaction mixture was incubated at 37°C with slow gyratory motion. The reactions were terminated after 16 h by washing the ligand-coupled Sepharose 4B with coupling buffer. The unreacted oxirane groups were then blocked by incubating in 1.0 M Gly in coupling buffer overnight at room temperature. The ligand-coupled Sepharose 4B was washed and stored in 0.5 M NaCl–0.01% thimerosol at 4°C until used.

SPIA columns were prepared by packing 5-ml syringe barrels with 3.0-ml bed volumes of each ligand-coupled Sepharose 4B. The columns were washed extensively with phosphate-buffered saline (PBS; pH 7.2). A 1-ml portion containing 5×10^8 cell equivalents of TsF1 or 5×10^8 cell equivalents of TsF2 was applied to each of the columns and allowed to move into the column matrix. Adsorption was performed at 4°C for 90 min. Each column was washed with 5 ml of PBS, which was five times the void volume. The unbound fractions were collected and designated as the column effluents. The material adsorbed to the column was eluted with 0.1 M Gly/HCl buffer (0.1 M Gly adjusted to pH 2.8 with 0.2 M HCl). The eluates were collected and immediately neutralized to pH 7.2 with 0.1 N NaOH. All fractions were adjusted to their original volumes with PBS and washed with PBS by ultrafiltration with a PM10 membrane (Amicon Crop., Lexington, Mass.) at 4°C. The effluent and eluate fractions were frozen at −20°C until they were assayed for suppressor activity as described above.

Assays for cryptococcal antigen. Latex agglutination assays for cryptococcal antigen were run on the TsF1 and TsF2 preparations and on each supernatant from adsorbed cryptococcal cells with a commercial kit purchased from Immuno-Mycologics, Inc., Norman, Okla.

Assays for anticryptococcal antibody. Whole yeast cell agglutination, as described by Murphy and Cozad (25), was used to assess the presence of anticryptococcal antibody in the TsF1 and TsF2 preparations. Hyperimmune rabbit anticryptococcal antiserum or mouse anticryptococcal antiserum were included as positive controls. Mouse anticryptococcal antiserum was prepared by intraperitoneally injecting mice with 10^5 heat-killed, dimethyl sulfoxide-treated cryptococci (12) 7 days before serum collection. Samples of undiluted TsF1, TsF2, and 1:10 dilutions of rabbit and mouse anticryptococcal antisera were serially diluted twofold to a final volume of 25 μl in sterile physiological saline solution in lucite microtiter plates with V-shaped wells. To each well was added 25 μl of a suspension of 10^7 washed, heat-killed *C. neoformans* per ml. The plates were sealed, incubated at room temperature for 2 h, and refrigerated overnight. Each well was scored for agglutination the following day, and the titer was taken as the reciprocal of the highest dilution in which there was not a smooth button of cryptococci in the well apex.

Anticryptococcal antibody activity was also measured by a solid-phase enzyme-linked immunosorbent assay (ELISA). Briefly, this assay consisted of coupling CneF to poly-L-lysine with cyanuric chloride as a coupling agent by the method of Gray (13). The coupled antigen was diluted to 1 μg/ml, based on carbohydrate concentration. Wells of polystyrene plates (Immunoplate II; Nunc, Roskilde, Denmark) were coated with poly-L-lysine-coupled cryptococcal

antigen by dispensing 100 μl of CneF–poly-L-lysine per well. The plates were incubated overnight at room temperature and then refrigerated for 3 days. Before use, each well was blocked with 100 μl of 0.1% bovine serum albumin in PBS. Rabbit or mouse anticryptococcal antiserum, diluted 1:10 in PBS as described above, was used as a positive control. TsF1, TsF2, and the anticryptococcal antisera were serially diluted twofold and added to cryptococcal antigen-coated wells. The plates were incubated for 3 h at room temperature. After incubation, the wells were washed with 0.5% Tween 20–PBS before adding 100 μl of a 1:5,000 dilution of either horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin or horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Cappel Laboratories, West Chester, Pa.) depending on the primary antibody used in the assay. After another 3 h of incubation at room temperature and three washes with Tween 20-PBS, 100 μl of substrate solution composed of 3% H_2O_2 and 40 mg of *O*-phenylendiamine per ml of 0.1 M citric acid-phosphate buffer (pH 5.3) was added to each well. The reaction was allowed to proceed for 15 min and then terminated by the addition of 50 μl of 4.0 N H_2SO_4. Spectrophotometric readings were obtained at 490 nm on a Microelisa reader MR-580 (Dynatech Laboratories, Inc., Alexandria, Va.). Titers were taken as the reciprocal of the highest dilution that gave an absorbance reading greater than 0.2.

C1q binding assay for immune complexes. The C1q binding assay was performed by the method of Zubler et al. (42). Human C1q (DNW Biologics, San Antonio, Tex.) was radiolabeled by the lactoperoxidase method (18) to a specific activity of 1.25 to 2.0 μCi of ^{125}I per μg and stored at −70°C until used. Ninety-five percent or more of the radioactivity was precipitable by trichloroacetic acid. Samples (50 μl) to be tested and 100 μl of 0.2 M EDTA (pH 7.4) were added to polypropylene tubes. After incubation for 30 min at 37°C, the tubes were transferred to an ice bath. Radiolabeled C1q (50 μl at 0.05 μg/ml) and 1.0 ml of 3% (wt/vol) polyethylene glycol (6,000 daltons) were then added. After 1 h of incubation in the ice bath, the percent [^{125}I]C1q precipitated was measured. The total trichloroacetic acid-precipitable [^{125}I]C1q (50 μl in HBSS) served as 100% binding. Heat-aggregated human gamma globulin (2.5 to 250.0 μg in HBSS), prepared by heating Cohn fraction II at 63°C for 20 min, served as a positive control.

Statistics. Means, standard error of the means, and the unpaired Student's *t* test were used to analyze the data.

RESULTS

Adsorption of TsF1 and TsF2 by cryptococcal cells. The results obtained in the adsorption of TsF1 and TsF2 by cryptococcal cells are shown in Table 1. Unadsorbed TsF1 (experiment 1, group 3) significantly suppressed the induction of DTH response to cryptococcal antigen. Adsorption of TsF1 with cryptococcal yeast cells abrogated the suppressor activity of TsF1 compared with the activity of unadsorbed TsF1. Contrasting results were obtained with TsF2 (experiment 2). TsF2 adsorbed with cryptococcal yeast cells (group 4) suppressed the expression of DTH to a level comparable to that of unadsorbed TsF2 (group 3), indicating that TsF2 had no affinity for cryptococcal antigen(s). As a control for these experiments, cryptococcal yeast cells were incubated in HBSS, and the supernatants were transferred either at the time of immunization (experiment 1) or at the time of footpad challenge (experiment 2). In both instances, the HBSS control was without suppressor activity, thereby establishing that any cryptococcal antigen(s) released from the yeast cells during the adsorption procedure did not contribute to suppressor activity.

TABLE 1. Adsorption of TsF1 and TsF2 with heat-killed cryptococci

Expt and group no.	i.v. treatment[a]	Adsorption with *C. neoformans*	Immunization with CneF-CFA s.c.	Mean increase in footpad thickness ± SEM (10^{-3} in.)[b]	% Suppression of DTH
Expt 1					
1		−	−	1.0 ± 0.3	
2		−	+	22.6 ± 1.5	
3	TsF1	−	+	14.6 ± 2.6[c]	37
4	TsF1	+	+	21.8 ± 1.5[d]	4
5	HBSS	+	+	24.4 ± 1.7[d]	−8
Expt 2					
1		−	−	1.6 ± 0.9	
2		−	+	18.0 ± 1.7	
3	TsF2	−	+	9.4 ± 1.7[e]	52
4	TsF2	+	+	9.4 ± 1.2[e]	52
5	HBSS	+	+	19.0 ± 1.0[d]	−6

[a] TsF1, 5 × 10^7 putative cell equivalents i.v.; TsF2, 10^8 putative cell equivalents i.v.
[b] Expressed as mean ± SEM of five animals per group (1 in. = 2.54 cm).
[c] $P < 0.01$ when compared with group 2.
[d] $P < 0.01$ when compared with group 3 and > 0.05 when compared with group 2.
[e] $P < 0.01$ when compared with group 2 and > 0.05 when compared with group 3.

Adsorption of TsF1 and TsF2 by SPIA. To confirm that TsF1 is specifically adsorbed by cryptococcal antigen, SPIA was performed on columns containing CneF-Sepharose 4B and, for comparison, *S. cerevisiae* mannan-Sepharose 4B and Gly-blocked Sepharose 4B (Gly-Sepharose 4B). Initial experiments were performed to establish that CneF was coupled to Sepharose 4B by adding rabbit anticryptococcal antibody-sensitized latex beads in Gly-buffered saline containing 0.1% bovine serum albumin and 0.1% sodium azide (pH 8.6) (Immuno-Mycologics, Inc.) to each of the three ligand-coupled Sepharose 4B matrices. The anticryptococcal antibody-sensitized latex beads attached to the surface of the CneF-Sepharose 4B (Fig. 1A) but did not associate with *S. cerevisiae* mannan-Sepharose 4B (Fig. 1B) or Gly-Sepharose 4B (not shown). These results confirmed that CneF was coupled to Sepharose 4B and the matrix could be used as a solid-phase immunoadsorbent. Since the *S. cerevisiae* mannan- and Gly-Sepharose 4B matrices did not bind components that were reactive with cryptococcal antigen, they were regarded as adequate controls.

The results obtained in two separate experiments with TsF1 are presented in Table 2. SPIA of TsF1 on CneF-Sepharose 4B completely adsorbed suppressor factor activity, as evidenced by the lack of suppression by the column effluent ($P < 0.005$ when compared with group 3). The component(s) responsible for afferent suppression was desorbed from the column in Gly/HCl buffer; i.e., suppressor activity was demonstrated in the column eluate fraction (group 5). Passage of TsF1 on *S. cerevisiae* mannan-Sepharose 4B or Gly-Sepharose 4B had no effect on TsF1 suppressor activity. This was evident by the suppressor activity of the column effluents, which was comparable to that of unfractionated TsF1, and the absence of suppressor activity in the eluates.

In contrast to the results obtained with TsF1, SPIA of

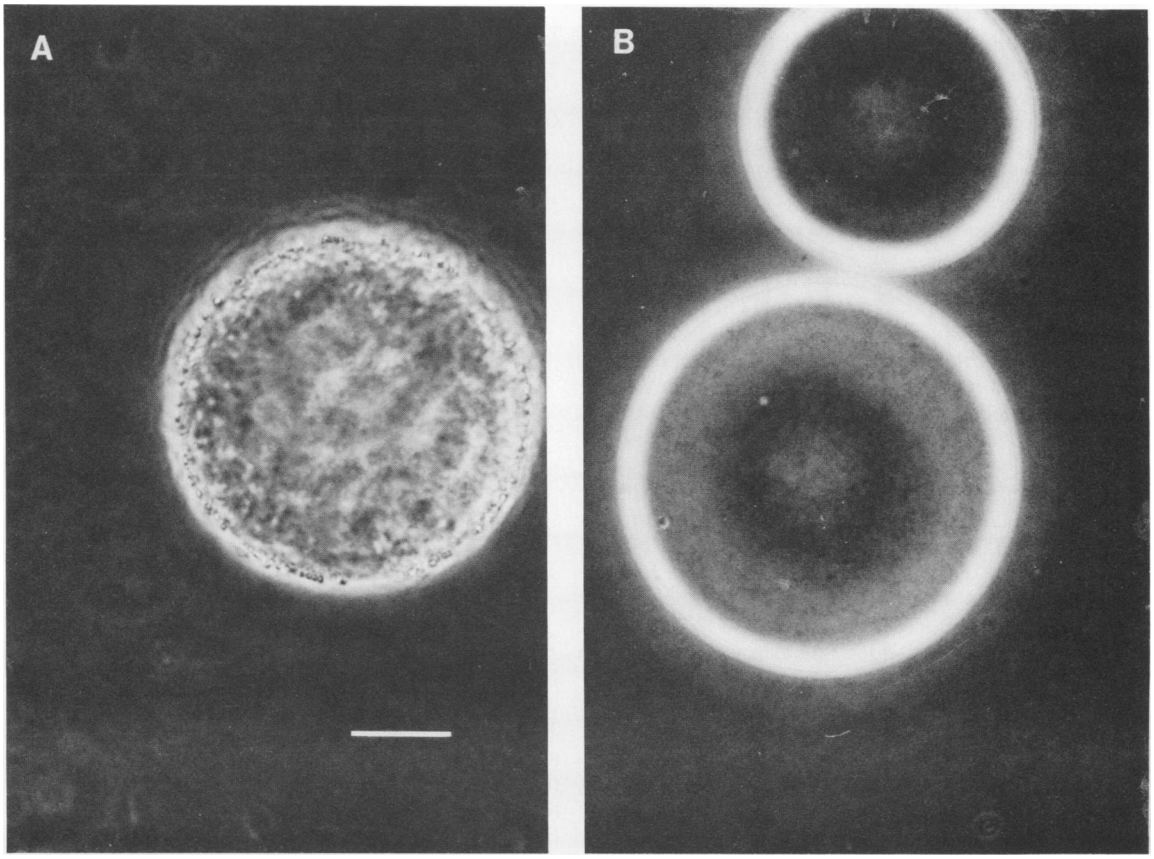

FIG. 1. Photomicrographs showing reactivity of rabbit anticryptococcal antibody-sensitized latex beads with CneF-Sepharose 4B (A) but not with *S. cerevisiae* mannan Sepharose 4B (B). Bar, 10 μm.

TsF2 on CneF-Sepharose 4B did not alter the suppressor activity of TsF2 (Table 3). This was evident because the column effluent from the CneF-Sepharose 4B column, as well as the effluents from the *S. cerevisiae* mannan- and Gly-Sepharose 4B columns, suppressed expression of the DTH response at a level comparable to that achieved by unfractionated TsF2.

Assessment of TsF1 and TsF2 for anticryptococcal antibody, cryptococcal antigen, and immune complexes. The preceding results establish that TsF1 specifically binds cryptococcal antigen, whereas TsF2 does not. Studies were undertaken, therefore, to determine whether binding or lack of binding was attributed to the presence of anticryptococcal antibody, antigen, or immune complexes.

Anticryptococcal antibody was not demonstrable in TsF1 or TsF2 by the whole-cell agglutination assay or by the ELISA. Both assay procedures were capable, however, of detecting antibody in rabbit and mouse antisera to cryptococcal antigen. The titers obtained with rabbit anticryptococcal antiserum were 640 and 20,480 in the whole-cell agglutination assay and ELISA, respectively, and those obtained with mouse antisera were 64 in the agglutination assay and 160 in the ELISA. Hence, the negative responses obtained with TsF1 and TsF2 are not attributed to a lack of sensitivity of the assays for antibody. Both TsF1 and TsF2 were also negative for cryptococcal antigen, as measured by the latex agglutination assay, a procedure that can detect as little as 5 ng of cryptococcal antigen (Immuno-Mycologics, Inc.).

In the C1q binding assay, TsF1 showed 18.5% binding, and TsF2 showed 18.2% binding. These values are not significantly different from the 15.7% binding exhibited by the HBSS medium control. For comparison, the C1q assay was performed on sera from noninfected BALB/c mice or from BALB/c mice infected with a single intraperitoneal injection of 10^4 *C. neoformans* cells given 28 days before collecting sera (3, 27). Sera from infected mice yielded a mean binding of 82.5 ± 1.2% (SEM); whereas, sera of noninfected mice showed a binding of 30.9 ± 7.9%. These results establish that the C1q assay, as performed, is capable of specifically detecting immune complexes or immune complex-like substances.

DISCUSSION

In earlier work, we have shown that specific suppression of the cryptococcal DTH response is mediated by at least two phenotypically and functionally different T suppressor cell populations, Ts1 cells and Ts2 cells (24, 27–29). We have also shown that suppression is mediated through soluble suppressor factors, TsF1 and TsF2, derived from the Ts1 and Ts2 cell populations, respectively (28, 29). These factors are functionally different since they suppress at different stages during the cryptococcal DTH response (28, 29). In this study, we have demonstrated that TsF1 and TsF2 can be differentiated further on the basis of their ability to bind cryptococcal antigen.

In the initial phases of this work, the suppressive activity of TsF1 was shown to be removed by adsorption with

heat-killed cryptococci, whereas the suppressive effect of TsF2 was not. The ability of TsF1 to bind to cryptococcal components was confirmed by immunoaffinity chromatography. SPIA of TsF1 on CneF-Sepharose 4B resulted in adsorption of the suppressive factor, which, when desorbed from the column, was shown to suppress the afferent limb of the DTH response. Passage of cryptococcal TsF1 over a column matrix to which an unrelated polysaccharide was attached, i.e., *S. cerevisiae* mannan, or a column which had no carbohydrate attached, i.e., Gly-Sepharose 4B, did not alter TsF1 activity, thereby establishing that the binding of the TsF1 was to the cryptococcal antigen(s). On the other hand, TsF2 was not bound by CneF-Sepharose 4B, as demonstrated by the fact that TsF2 activity was detected in the column effluent and not the eluate fraction. These data establish that TsF1 specifically binds to cryptococcal antigen(s), whereas TsF2 has no affinity for cryptococcal antigen.

Antigen-specific suppression of the cryptococcal DTH response by TsF1 and TsF2 is not attributed to the presence of cryptococcal antigen, anticryptococcal antibody, or C1q-binding immune complexes. In previous studies, we have shown that concentrations of 50 to 2,500 μg of cryptococcal antigen suppress the cryptococcal DTH response (27). Concentrations below 50 μg did not induce measurable levels of suppression. Therefore, the lack of reactivity of TsF1 and TsF2 in the latex agglutination assay, a procedure that detects as little as 5 ng of cryptococcal antigen (Immuno-Mycologics, Inc.) argues against the possibility that these factors contain suppressive levels of antigen. The results obtained in the whole-cell agglutination assay and the ELISA provide evidence that neither TsF1 nor TsF2 contain detectable levels of anticryptococcal antibody. Even though TsF1 would be expected to bind cryptococcal antigen in the agglutination and ELISA procedures by virtue of its affinity for antigen, agglutination would not be expected due to the monovalent nature of antigen-specific suppressor factors (38), and in the ELISA, TsF1 would not be detected by antisera specific for immunoglobulin heavy and light chains. The insignificant level of binding of TsF1 and TsF2 in the C1q-binding assay provides evidence that these suppressor factors are void of immune complexes, although we cannot rule out the presence of immune complexes which do not bind complement.

TABLE 2. Afferent suppression of cryptococcal DTH by fractions of TsF1 from SPIA columns

Expt and group no.	TsF1 treatment[a]	Immunization with CneF-CFA s.c.	Mean increase in footpad thickness ± SEM (10^{-3} in.)[b]	% Suppression of DTH
Expt 1				
1	None	−	2.4 ± 0.5	
2	None	+	32.0 ± 3.3	
3	Unfractionated	+	19.8 ± 2.4[c]	41
4	CneF-Sepharose 4B effluent	+	29.6 ± 2.4[d]	8
5	CneF-Sepharose 4B eluate	+	19.2 ± 0.9[c]	43
6	SM-Sepharose 4B effluent	+	16.6 ± 2.8[c]	52
7	SM-Sepharose 4B eluate	+	30.6 ± 1.7[d]	5
8	Gly-Sepharose 4B effluent	+	18.2 ± 2.1[c]	47
9	Gly-Sepharose 4B eluate	+	31.6 ± 2.0[d]	1
Expt 2				
1	None	−	1.6 ± 0.2	
2	None	+	23.8 ± 2.5	
3	Unfractionated	+	12.4 ± 2.8[c]	51
4	CneF-Sepharose 4B effluent	+	25.8 ± 2.7[d]	−9
5	CneF-Sepharose 4B eluate	+	13.6 ± 0.9[c]	46
6	SM-Sepharose 4B effluent	+	14.4 ± 1.7[c]	42
7	SM-Sepharose 4B eluate	+	24.2 ± 1.5[d]	−6
8	Gly-Sepharose 4B effluent	+	15.8 ± 1.5[e]	36
9	Gly-Sepharose 4B eluate	+	23.6 ± 3.5[d]	1

[a] 5×10^7 putative cell equivalents of TsF1. SM, *S. cerevisiae* mannan.
[b] Expressed as mean ± SEM of five animals per group (1 in. = 2.54 cm).
[c] $P < 0.01$ when compared with group 2.
[d] $P < 0.01$ when compared with group 3 and > 0.05 when compared with group 2.
[e] $P < 0.01$ when compared with group 2 and > 0.05 when compared with group 3.

TABLE 3. Efferent suppression of cryptococcal DTH by fractions of TsF2 from SPIA columns

Group no.	TsF2 treatment[a]	Immunization with CneF-CFA s.c.	Mean increase in footpad thickness ± SEM (10^{-3} in.)[b]	% Suppression of DTH
1	None	−	0.8 ± 0.7	
2	None	+	21.3 ± 1.9	
3	Unfractionated	+	7.0 ± 0.9[c]	70
4	CneF-Sepharose 4B effluent	+	7.6 ± 1.6[d]	67
5	CneF-Sepharose 4B eluate	+	19.8 ± 3.1[e]	7
6	SM-Sepharose 4B effluent	+	6.2 ± 1.2[d]	74
7	SM-Sepharose 4B eluate	+	19.2 ± 1.1[e]	10
8	Gly-Sepharose 4B effluent	+	6.4 ± 0.7[d]	73
9	Gly-Sepharose 4B eluate	+	20.0 ± 1.4[e]	6

[a] 10^8 putative cell equivalents of TsF2. SM, *S. cerevisiae* mannan.
[b] Expressed as mean ± SEM of five animals per group (1 in. = 2.54 cm).
[c] $P < 0.0005$ when compared with group 2.
[d] $P < 0.0005$ when compared with group 2 and > 0.05 when compared with group 3.
[e] $P < 0.0005$ when compared with group 3 and > 0.05 when compared with group 2.

The specificity of suppression in the *Cryptococcus* model has been evaluated in previous studies which showed that responses to heterologous antigens such as purified protein derivative and the hapten, dinitrofluorobenzene, were unaffected by cryptococcal Ts1 (27) and Ts2 (28) cells. In the present study, TsF1 was adsorbed by cryptococcal cells and CneF but not by *S. cerevisiae* mannan. While these results establish that TsF1 does not bind *S. cerevisiae* they do not address the potential cross-reactivity of the cryptococcal TsF1 with other fungal antigens such as those derived from the yeast-phase cells of *Candida*, *Blastomyces*, and *Histoplasma* spp.

Investigators using quite different antigens to stimulate T suppressor cells and T suppressor factors have shown that first-order T suppressor factors specifically bind to the inducing antigen, whereas second-order T suppressor factors do not bind. For instance, in the ABA suppressor cell system, ABA TsF1 binds to columns of Sepharose 4B coupled with ABA-fowl gamma globulin (FGG) and is recovered in the acid eluate from the columns, but does not bind to fowl gamma globulin-coupled columns (15). Unlike TsF1, ABA TsF2 permeates through ABA-fowl gamma globulin-Sepharose 4B columns and is recovered in the effluent, thus indicating ABA TsF2 does not bind the ABA haptenic group (7). Similarly, in the NP system, when a T cell hybridoma-derived NP TsF1 is subjected to SPIA over an NP-keyhole limpet hemocyanin-Sepharose 4B column, the suppressive activity is recovered in the acid eluate of the column,

whereas the suppressive activity of a hybridoma-derived NP TsF2 is not retained by the column but is recovered in the effluent (22). Also, the Lyt-1^+2^- T suppressor cell-derived factor which suppresses the DTH response to sheep erythrocytes can be adsorbed by sheep erythrocytes but not by chicken erythrocytes (18). The results obtained in this study clearly demonstrate that cryptococcal TsF1 binds cryptococcal antigen, whereas cryptococcal TsF2 does not, and are therefore in complete accordance with the results obtained in the aforementioned studies.

The fact that TsF1 binds to cryptococcal antigen(s) suggests that the cryptococcal TsF1 may possess idiotypic characteristics, a feature that has been demonstrated for the antigen-binding suppressor factors in the NP and ABA models (30, 34). The lack of antigen binding by TsF2 suggests it may be anti-idiotypic, a characteristic of TsF2 in other suppressor circuits (7, 22). If this is the case, then one might postulate that the idiotype–anti-idiotype characteristics could function in a network fashion to specifically regulate the cryptococcal immune response in a manner similar to the one proposed for other suppressor pathways (8, 16, 22).

Other suppressor cell factors in fungal infection models have recently been reported. Deepe et al. (5, 6) described a suppressor factor derived from the spleens of mice infected with *Histoplasma capsulatum*. The suppressor factor is a protein or glycoprotein having a low molecular weight which nonspecifically suppresses primary in vitro immunization to sheep erythrocytes. Additionally, this factor is produced from a Lyt-2^+, I-J^+ T cell and requires the participation of macrophages for its production (6). Morgan et al. (23) have also described a suppressor factor derived from the spleens of mice infected with *C. neoformans*. This factor suppresses the ability of unstimulated peritoneal macrophages to phagocytize either *C. neoformans* or *S. cerevisiae*. Production of the factor by T cells from infected mice must be specifically triggered by the addition of a cryptococcal cell wall extract. The T suppressor cell factors which we have previously described (28, 29) and used in this study appear to be different from those of Deepe et al. (5, 6) and Morgan et al. (23). Cryptococcal TsF1 and TsF2 specifically suppress the cryptococcal cell-mediated immune response; whereas the suppressor factor of Deepe et al. is nonspecific. Also, our TsF1 and TsF2 are prepared as lysates of cryptococcal Ts1 and Ts2 cells, respectively, without restimulating with cryptococcal antigen, whereas the aforementioned suppressor factors require either additional antigen (23) or macrophages (5, 6) to be liberated. Since different experimental protocols were used, further studies will be required to ascertain the similarities among the various fungal-antigen-induced suppressor factors. By demonstrating differential cryptococcal antigen-binding abilities in this study, we have begun to identify characteristics other than functional differences of the two T suppressor cell factors in the cryptococcal circuit which may be useful in delineating similarities and differences among suppressor cell factors.

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