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Characterization of a Cell Population Which Amplifies the Anticryptococcal Delayed-Type Hypersensitivity Response

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Cell-mediated immunity to *Cryptococcus neoformans* can be detected by delayed-type hypersensitivity (DTH) to a culture filtrate antigen of *C. neoformans*. Recently, we have identified a population of cells in spleens of mice immunized with cryptococcal antigen that, when transferred to recipient mice at the time of immunization, amplifies the anticryptococcal DTH response. If the cell donor mice are treated with cyclosporin A during induction of the anticryptococcal DTH response, the amplifier cells are not induced, whereas the cells which transfer DTH (T_{DH} cells) are induced. The purpose of this study was to characterize the amplifier cells with respect to their surface and functional properties and, in so doing, determine whether or not the amplifier cells are analogous to long-lived memory cells. We demonstrated that the amplifier cells were nylon-wool-nonadherent, antigen-specific, CD4 ($L3T4^+$ $Lyt\text{-}2^-$) T lymphocytes which appear in the spleens of mice 5 days postimmunization with cryptococcal culture filtrate antigen in complete Freund adjuvant. The amplifier T (T_{amp}) cells are not considered to be memory cells because they are relatively short-lived, being present 14 but not 18 days after the stimulating immunization. Moreover, the amplified anticryptococcal DTH response does not fulfill the criteria of the typical secondary immune (anamnestic) response in that the amplified response does not appear early relative to the appearance of the primary anticryptococcal DTH response, and it does not persist longer than the primary DTH response. We speculate that T_{amp} cells are not long-lived memory cells but rather act in a T-helper cell capacity to amplify the anticryptococcal DTH response.

Cryptococcosis begins as a primary pulmonary infection and, in approximately 10% of the cases, progresses to a disseminated form (25). It is clear from studies with humans and laboratory animals that a functional cell-mediated immune response is the key to recovering from this disease (1, 2, 6, 12, 13). In previous investigations with the mouse model, we have demonstrated that there are various T-cell populations which regulate the anticryptococcal cell-mediated immune response as detected by the delayed-type hypersensitivity (DTH) response to cryptococcal antigen (4, 5, 9, 18, 19, 21–23). For instance, by injecting mice intravenously with soluble cryptococcal antigen to simulate the antigenemia observed in cryptococcosis patients, we induced a series of antigen-specific suppressor T cells that down regulate the anticryptococcal DTH response mediated by T_{DH} cells (9, 18, 21–23). Also, we have shown that intravenous injection of cryptococcal antigen specifically affects clearance of *C. neoformans* from mouse tissues (19).

More recently, we identified another regulatory cell population which, in contrast to suppressor cells, amplifies the anticryptococcal DTH response (5). The presence of the amplifier cells can be demonstrated by transferring spleen cells from immunized mice to recipient mice at the time of immunization of the recipient animals with the cryptococcal culture filtrate antigen CneF in complete Freund adjuvant (CFA). At 6 days postimmunization, the recipient animals respond to footpad challenge with DTH reactions which are significantly higher than those of the immune controls (5). If the recipients are not immunized following the transfer of immune spleen cells, their DTH reactions are equivalent to the levels of reactivity for passive transfer of DTH. Neither spleen cells from naive mice injected with saline in CFA nor sera from CneF-CFA-immunized mice induce an amplified DTH response in immunized recipient mice (5). Furthermore, mice given a population of cells containing T_{DH} cells without amplifier cells do not display amplified DTH responses 6 days postimmunization but instead show DTH reactivity equivalent to that of positive immune control mice (5). Thus, the amplified DTH response has been defined as a response that is significantly greater than the primary DTH response. Amplifier cells, which contribute to the amplified DTH response, can be distinguished from T_{DH} cells by the fact that amplifier cells are not induced in cyclosporin A-treated mice, whereas T_{DH} cells are (5).

The primary purpose of this study was to define the surface antigen characteristics of the amplifier cells and to determine whether or not the amplifier cells are long-lived memory cells that contribute to a typical secondary immune response, i.e., a response that appears earlier and persists longer than a primary immune response. Our data indicate that the amplifier cells are nylon-wool-nonadherent $Thy\text{-}1^+$ $L3T4^+$ $Lyt\text{-}2^-$ Ia^- cells, are antigen specific, and are present within 5 days postimmunization with CneF-CFA. Furthermore, the amplified anticryptococcal DTH response mediated by amplifier T (T_{amp}) cells is not considered to be a typical secondary immune response. The basis of this conclusion is that (i) the amplified DTH response did not appear any earlier than a primary anticryptococcal DTH response, (ii) the amplified DTH response was of short duration, and (iii) the T_{amp} cells were not long-lived as would be expected for memory cells.

MATERIALS AND METHODS

Mice. Female CBA/J mice (*H-2^k Thy-1.2 Lyt-1.1 Lyt-2.1*) purchased from Jackson Laboratory, Bar Harbor, Maine, were used at 7 to 10 weeks of age for these studies.

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Antigen. The CneF used for immunization and footpad challenge was prepared by the procedure of Cauley and Murphy (1) from *C. neoformans* isolate 184-A (20). The lot of CneF used in these studies had a protein concentration of 0.27 mg/ml based on the procedure of Lowry et al. (14) as modified by Miller (15) and a carbohydrate concentration of 2.5 mg/ml as determined by the phenol-sulfuric acid assay (3).

Induction and elicitation of the amplified DTH response. Cells that amplify the anticryptococcal DTH responses were produced as previously described (5). Briefly, spleen cells from CBA/J mice that had been immunized subcutaneously 6 days earlier with CneF-CFA (21) served as the source of amplifier cells. To demonstrate the amplified DTH response, spleen cells from CneF-CFA-immunized mice (immune spleen cells) were transferred to naive syngeneic mice (10^8 cells per mouse) and the recipients were immunized with CneF-CFA immediately after cell transfer. At 6 days after immune spleen cell transfer and immunization, recipient mice were footpad challenged with 30 μl of CneF, and 24 h later, their footpads were measured to assess the level of DTH reactivity (21). To assess the level of DTH reactivity in immunized mice not treated with amplifier cells, we have previously used two different positive immune controls. In one case, mice were immunized with CneF-CFA and footpad tested 6 days later. In the other case, mice were immunized and given spleen cells from mice previously injected with physiological saline in complete Freund adjuvant before footpad testing (5). Since the mean DTH responses for these two control groups have proven to be similar, in this study we used immunized mice as the only positive control. Naive mice footpad challenged with CneF served as negative controls. The amplified DTH response is defined as a DTH response significantly greater than the response of positive immune controls.

Characterization and phenotyping of amplifier cells. To determine whether the amplifier cells had T-lymphocyte characteristics, we passed the immune spleen cells over nylon-wool columns as previously described (7) and collected the nonadherent and adherent populations. The nonadherent and adherent fractions were transferred into recipient mice (10^8 cells per mouse) at the time of immunization of the recipients. The DTH responses were assessed 6 days later.

For phenotyping, the amplifier cells were subjected to mass cytolysis with various surface receptor-specific antibodies and complement before adoptive transfer. The antibodies used were anti-Thy-1 (21), anti-immunoglobulin (Organon Teknika, Malvern, Pa.) (21), monoclonal anti-Lyt-2.1 (Dupont, NEN Research Products, Boston, Mass.) (23), anti-L3T4 (culture supernatant from the GK-1.5 hybridoma; American Type Culture Collection, Rockville, Md.) (9), and anti-Ia (Accurate Scientific, Hicksville, N.Y.) (23). The concentrations of anti-Thy-1, anti-Lyt-2, and anti-L3T4 antibodies used were as previously described (9, 21, 23), whereas, anti-immunoglobulin and anti-Ia were diluted 1:100 and 1:30, respectively. The complement source was Low-Tox complement (Accurate Scientific) diluted 1:10. All reagents and cell dilutions were made with Hanks balanced salt solution containing 1% fetal bovine serum. After the immune spleen cells were treated with the designated antibody and complement (9, 21, 23), the treated cells were transferred to mice which were immunized immediately and then assessed for DTH reactivity 6 days later.

Specificity of amplifier cells. Spleen cells from mice immunized with CneF-CFA were transferred to four groups of recipient animals, and the recipient mice were immunized immediately with CneF-CFA, sterile physiological saline solution in complete Freund adjuvant, heat-killed *Listeria monocytogenes* (8) in complete Freund adjuvant, or 2,4-dinitrofluorobenzene (DNFB) (24). On the appropriate day after immunization, the mice were challenged with the homologous antigen in a footpad (CneF or *Listeria* intracellular product) (8) or on an ear (DNFB) (24). At 24 h postchallenge, the DTH reactivities were determined by measuring the thicknesses of the footpads or ears. Positive controls were mice immunized and challenged with each of the respective antigens, whereas the negative controls were naive mice footpad or ear challenged with the designated antigens.

In specific experiments, to determine whether or not cells equivalent to anticryptococcal amplifier cells could be detected in other antigen models, spleen cells from mice immunized with heat-killed *L. monocytogenes* in complete Freund adjuvant or sensitized with DNFB 6 days earlier were transferred to recipient mice (10^8 cells per mouse). Recipient animals were immunized with the homologous antigen immediately after cell transfer, and their DTH responses were assessed 6 days later. Positive and negative controls as described above were included for each antigen system.

Kinetics of induction of amplifier cells. On designated days after mice were immunized with CneF-CFA, their spleen cells were transferred to recipient mice. Immediately after cell transfer, the recipient mice were immunized, and their DTH reactions were assessed 6 days later.

Kinetics of expression of amplifier cells. Immune spleen cells were transferred to recipient mice at the time of immunization of the recipients, and then daily on days 3 to 7 after immune cell transfer and immunization, groups of five recipient mice were randomly selected and footpad challenged. Their footpads were measured 24 h postchallenge. Positive controls for these experiments consisted of (i) immunized mice footpad challenged with CneF on the designated days following immunization and (ii) nonimmunized mice footpad challenged with CneF on designated days after the transfer of immune spleen cells. Naive mice challenged with CneF were the negative controls.

Determination of the functional life span of amplifier cells. The length of time postimmunization for which amplifier cells were effective in amplifying the DTH response was determined by two different approaches. The first defined the length of time for which amplifier cells were functional after being transferred to recipient animals. For this, spleen cells from mice immunized 6 days earlier were transferred to recipient mice and then on designated days after the immune spleen cell transfer, the recipient mice were immunized. The footpads of the recipients were challenged 6 days postimmunization to determine their levels of DTH reactivity. The second approach defined the length of time postimmunization for which functional amplifying cells could be transferred from donor mice. For this, spleen cells from mice immunized 1 to 3 weeks earlier were transferred to recipient animals at the time of immunization of the recipients. The DTH responses of the recipients were assessed 6 days after cell transfer and immunization.

Statistical analysis. The unpaired Student *t* test was used to analyze the data.

RESULTS

Characterization of amplifier cells. Nylon-wool-nonadherent spleen cells from CneF-CFA-immunized mice were

TABLE 1. Nylon-wool adherence characteristics of amplifier cells[a]

Group	Treatment of Mice: Donor: Immunize with CneF-CFA day 0	Donor: Treatment of immune SPC day 6	Recipient: Transfer of immune SPC day 6	Recipient: Immunize with CneF-CFA day 6	Mean increase in footpad thickness ± SEM (10^{-3} in) day 13	Compared to Group 3 p<
1	-----	-----	-----	-----		0.0005
2	-----	-----	-----	+		0.001
3	+	None	+	+		-----
4	+	NWN	+	+		0.0025
5	+	NWAdh	+	+		0.025

[a] Experiments were repeated twice with five recipient mice per group. SPC, Spleen cells; NWN, nylon-wool nonadherent; NWAdh, nylon-wool adherent.

shown to be enriched for amplifier cells, as indicated by the fact that mice that received the nylon-wool-nonadherent cell fraction had significantly higher DTH responses (Table 1, group 4) than did animals given unfractionated cells (group 3) ($P < 0.0025$). Animals given the nylon-wool-adherent cell fraction (group 5) did not display amplified DTH responses, as their mean increase in footpad thicknesses postchallenge with CneF was not significantly different from that of the positive immune control group (group 2).

To determine the surface characteristics of the amplifier cells, immune spleen cells were subjected to the negative selection technique of mass cytolysis before the transfer of spleen cells to recipient mice. This experiment was repeated twice with similar results, and data from one of the two experiments are shown in Table 2. The results demonstrate that the amplified DTH response was present in mice given immune spleen cells and an immunization (Table 2, group 3 compared with group 2; $P < 0.001$). Complement treatment alone had no effect on the amplified DTH response (group 4 compared with group 3). Only treatments with anti-Thy-1 (group 5) and anti-L3T4 (group 7) antibodies and complement significantly reduced the amplified DTH response, whereas anti-immunoglobulin (group 6), anti-Lyt-2 (group 8), and anti-Ia (group 9) antibody and complement treatments of the immune spleen cell population had no effect on the amplified DTH responses.

TABLE 2. Phenotyping of amplifier cells by mass cytolysis[a]

Group	Treatment of Mice: Donor: Immunize with CneF-CFA day 0	Donor: Treatment of immune SPC day 6	Recipient: Transfer of immune SPC day 6	Recipient: Immunize with CneF-CFA day 6	Mean increase in footpad thickness ± SEM (10^{-3} in) day 13	Compared to Group 4 p<
1	-----	-----	-----	-----		0.0005
2	-----	-----	-----	+		0.001
3	+	None	+	+		NS
4	+	C	+	+		-----
5	+	anti-Thy 1 + C	+	+		0.02
6	+	anti-Ig + C	+	+		NS
7	+	anti-L3T4 + C	+	+		0.01
8	+	anti-Lyt 2.1 + C	+	+		NS
9	+	anti-Ia + C	+	+		NS

[a] Experiments were repeated twice with five recipient mice per group. SPC, Spleen cells; C, complement; NS, not significant when $P > 0.05$.

TABLE 3. Specificity of amplifier cells[a]

Group	Treatment of Mice: Donor: Immunizing antigen day 0	Donor: Transfer of immune SPC day 6	Recipient: Immunizing antigen day 6	Recipient: Challenge antigen day 12	Mean increase in footpad thickness ± SEM (10^{-3} in) day 13	Compared to immune control p<
1	-----	-----	-----	CneF		0.0005
2	-----	-----	CneF-CFA	CneF		-----
3	CneF-CFA	+	CneF-CFA	CneF		0.0005
4	CneF-CFA	+	SPSS-CFA	CneF		0.0005
5	-----	-----	-----	LIP		0.0005
6	-----	-----	HKL	LIP		-----
7	CneF-CFA	+	HKL	LIP		NS
8	HKL	+	HKL	LIP		NS
					Mean increase in ear thickness ± SEM (10^{-4} in)	
9	-----	-----	-----	DNFB		0.0005
10	-----	-----	DNFB	DNFB		-----
11	CneF-CFA	+	DNFB	DNFB		NS
12	DNFB	+	DNFB	DNFB		NS

[a] Two replica experiments were performed with five recipient mice per group. SPC, Spleen cells; SPSS, sterile physiological saline solution; HKL, heat-killed *L. monocytogenes*; LIP, *Listeria* intracellular product; NS, not significant when $P > 0.05$.

Specificity of amplifier cells. To determine the specificity of amplifier cells, immune spleen cells were transferred to recipient mice which were immunized immediately with CneF-CFA or one of the two heterologous antigens, i.e., heat-killed *L. monocytogenes* or DNFB. The DTH responses of the recipient mice were measured after footpad or ear challenge with the immunizing antigens. Spleen cells from CneF-CFA-immunized mice amplified the DTH responses of recipient mice immunized with CneF-CFA (Table 3, group 3 compared with the positive control for cryptococcal antigen, group 2) but had no effect on the DTH responses to *L. monocytogenes* (group 7 compared with group 6, the positive DTH control for *L. monocytogenes*) or DNFB (group 11 compared with group 10, the positive DTH control for DNFB). Also in this set of experiments, we demonstrated that recipient mice injected with complete Freund adjuvant in saline instead of CneF-CFA after immune cell transfer did not display amplified anticryptococcal DTH responses (group 4 compared with group 2). However, the immune spleen cells were able to transfer anticryptococcal DTH to the recipients (group 4 compared with group 1, the negative DTH control for cryptococcal antigen; $P < 0.0005$).

We also assessed whether a similar amplifier cell was present postimmunization with *L. monocytogenes* or DNFB. To determine this, spleen cells from mice immunized with *L. monocytogenes* or DNFB were transferred to recipient mice and the recipients were immunized immediately with the homologous antigen. DTH responses to the respective antigens were determined in recipient mice 6 days after cell transfer and immunization. The data in Table 3 indicate that antigen-specific amplifier cells were not present in the spleens of *Listeria*- or DNFB-immunized mice, since the DTH responses of recipient animals (groups 8 and 12, respectively) were not significantly higher than the DTH responses of the respective positive controls (groups 6 and 10).

Kinetics of induction of amplifier cells. In our initial studies,

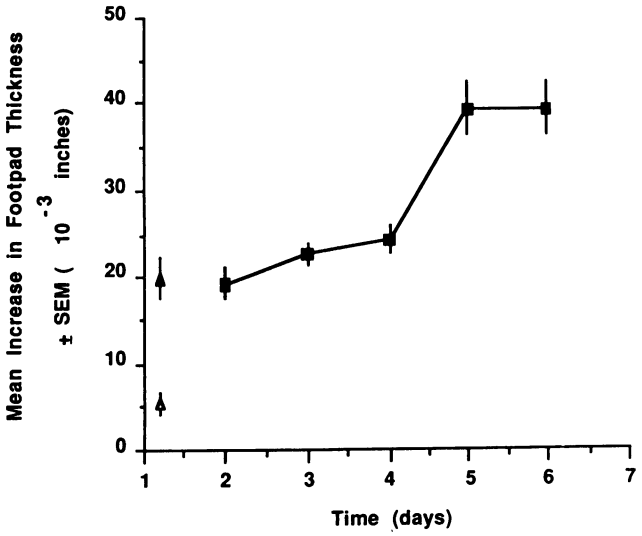

FIG. 1. Kinetics of induction of amplifier cells. On designated days postimmunization of mice with CneF-CFA, spleen cells from these mice were transferred to syngeneic recipient mice. Recipient animals were immunized immediately with CneF-CFA, and their DTH responses were elicited 6 days later (■). Positive controls (▲) were mice immunized 6 days before footpad challenge. Naive mice footpad challenged with CneF were the negative controls (△). Experiments were repeated twice with five recipient mice per group.

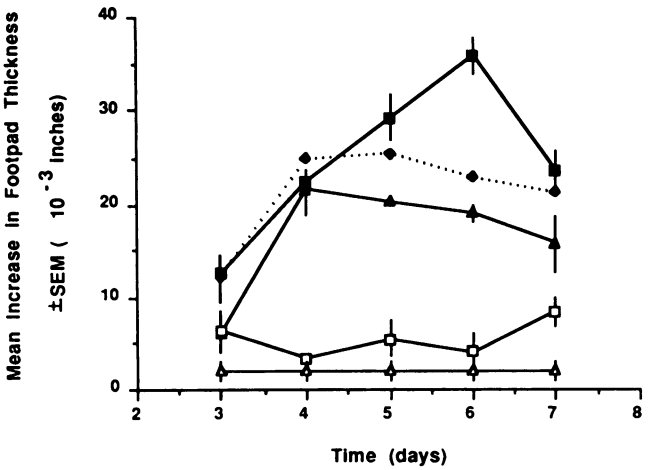

FIG. 2. Kinetics of expression of amplifier cells. On designated days posttransfer of immune spleen cells with (■) or without (□) immunization with CneF-CFA, recipient mice were footpad challenged with CneF and footpad swelling was measured 24 h later. The positive controls were mice footpad challenged on the designated days postimmunization (▲), whereas the negative controls were naive mice that were footpad challenged with CneF (△). Also shown are additive DTH responses of mice given immune spleen cells and immunized and of mice given immune spleen cells only (◆ ····· ◆). Experiments were repeated at least twice with five recipient mice per group.

it was clear that amplifier cells were present in immunized mice 6 days postimmunization. The objective here was to determine how early after immunization amplifier cells could be detected. For this, spleen cells were collected from mice on consecutive days after immunization with CneF-CFA and were transferred to syngeneic recipient animals. The recipient mice were immunized immediately after cell transfer and were footpad challenged 6 days later. Amplifier cells were not present until 5 days after immunization of the donor mice (Fig. 1). This was indicated by the lack of a significant increase in DTH responses of immunized recipient mice that received spleen cells from donor mice immunized 4 days or less before cell transfer compared with the positive controls. Spleen cells collected and transferred 5 or 6 days after immunization of donor mice mediated a significant increase in the DTH reactivity of recipient mice ($P < 0.0005$ compared with the positive controls).

Kinetics of expression of amplifier cells. To determine the optimal time after the transfer of immune spleen cells and immunization for maximal expression of the amplified DTH responses, footpad reactivity to CneF was monitored daily in recipient mice beginning 3 days posttransfer of immune spleen cells and immunization of the recipients. Significantly amplified DTH responses, relative to the positive controls, were not apparent until day 5 after immune cell transfer and immunization (Fig. 2). A linear increase in DTH reactivity was observed between days 3 and 6 after the immune spleen cell transfer and immunization; however, only on days 5 and 6 were the DTH responses clearly amplified over the positive immune control responses. In fact, the amplified DTH responses were also significantly greater than the more exaggerated figure of the additive DTH responses of the positive controls and the DTH responses of the unimmunized mice that had DTH passively transferred by immune spleen cells.

Functional life-span of amplifier cells. Two experimental approaches were taken to determine the life-span of amplifier cells. The first assessed the life-span of amplifier cells following transfer into recipient animals. For this, spleen cells from mice immunized 6 days earlier were transferred to recipient mice, and on the designated day after immune cell transfer, groups of five randomly selected recipient mice were immunized with CneF-CFA. The DTH responses of the recipients were assessed 6 days postimmunization. The results (Fig. 3) illustrate that amplifier cells were functional

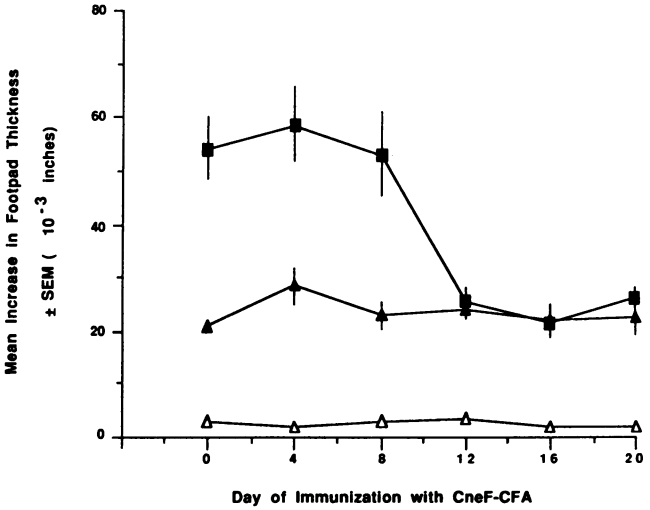

FIG. 3. Life-span of amplifier cells in recipient animals. Immune spleen cells were transferred on day zero, and on designated days thereafter, recipient mice were immunized with CneF-CFA (■). At 6 days postimmunization of the recipients, their DTH responses were assessed. Positive controls were mice footpad challenged with CneF 6 days postimmunization (▲), whereas naive mice footpad challenged with CneF served as negative controls (△). Experiments were performed twice with five recipient mice per group.

for at least 8 days after transfer to recipient mice. This was shown by the fact that the DTH responses of recipient mice immunized at any time through 8 days after the immune spleen cell transfer were significantly amplified over positive control mice, whereas the DTH responses of recipient mice immunized 12 or more days after immune spleen cell transfer (18 days postimmunization of immune spleen cell donor mice) were similar to positive control levels.

Because it was possible that transfer of amplifier cells to recipient mice affected their life-span, we determined the longevity of amplifier cells in donor animals. Mice were immunized with CneF-CFA, and on day 6, 18, or 22 post-immunization, the spleen cells were harvested and transferred to recipient mice that were immunized immediately. The DTH responses of the recipients were assessed 6 days after the cell transfer and immunization. As usual, when spleen cells were collected and transferred 6 days postimmunization of the donor mice, the recipient mouse DTH responses were clearly amplified ([36.4 ± 2.9] × 10^{-3} in. [1 in. = 2.54 cm.]) compared with those of the positive controls ([15 ± 0.84] × 10^{-3} in.; $P < 0.0005$). However, if 18 days elapsed between the immunization of donor mice and the collection of spleen cells, there was no evidence of an amplified DTH response in recipient mice ([17 ± 1.76] × 10^{-3} in.). Similarly, amplified DTH responses were not seen in recipient mice given donor spleen cells that were collected and transferred 22 days postimmunization of donor mice ([19.6 ± 2.33] × 10^{-3} in.). These results confirmed that the amplifier cells were relatively short-lived.

DISCUSSION

Immunization with cryptococcal antigen in complete Freund adjuvant induces, in addition to T_{DH} cells (9, 18) and third-order suppressor (Ts3) cells (9), a third population of cells which upon transfer significantly amplify the anticryptococcal DTH response of immunized recipient mice (5). The amplifier cells are not produced in mice treated with cyclosporin A and immunized, whereas T_{DH} and Ts3 cells are produced (5). The purpose of the present study was to elucidate the properties of the amplifier cells and, in so doing, gain a better understanding of the role of the amplifier cells in the anticryptococcal cell-mediated immune response.

Our initial experiments focused on defining the surface characteristics of the cells responsible for the amplified DTH response. We found that the nylon-wool-nonadherent fraction of the immune spleen cell population enhanced the amplifying effect, whereas adherent cells had no effect (Table 1). Furthermore, treatment of immune spleen cells with anti-Thy-1 antibody and complement abrogated the transfer of the amplifier cell population (Table 2). These data indicate that the cells responsible for the amplified DTH response are T cells; therefore, we refer to them as T_{amp} cells. T_{amp} cells, like T_{DH} cells in the cryptococcal model (9), are CD4 ($L3T4^+$ $Lyt\text{-}2^-$) cells, as evidenced by abrogation of the amplified response after treatment of immune spleen cells with anti-L3T4 antibody and complement and by the lack of effect on the amplified DTH response when immune spleen cells were treated with anti-Lyt-2 antibody and complement (Table 2). Anti-Ia antibody and complement treatment did not ablate the amplification of the DTH response, indicating that T_{amp} cells either lack or have very reduced amounts of major histocompatibility complex class II antigens (I-A and/or I-E) on their surfaces. Thus, T_{amp} cells are also similar to anticryptococcal T_{DH} cells with regard to lack of expression of significant quantities of class II antigens (9).

T_{amp} cells were shown to be specific for cryptococcal antigen, as indicated by the fact that cryptococcal antigen-induced T_{amp} cells were not able to amplify DTH responses to *L. monocytogenes* or DNFB (Table 3). These results are consistent with the antigen-specific property of cryptococcal T_{DH} and Ts cells (9, 18, 21–23).

The induction kinetics of T_{amp} cells were similar to those of anticryptococcal T_{DH} cells. In both cases, the earliest time when we could demonstrate functional cells was 5 days after immunization with cryptococcal antigen (18; Fig. 1). Although these findings, along with the phenotyping results, indicate that anticryptococcal T_{DH} and T_{amp} cells have several features in common, the two cell populations can be distinguished by their functions and by their differential sensitivities to cyclosporin A (5).

The amplified DTH response observed after transfer of T_{amp} cells was reminiscent of a typical secondary immune response in that the response was significantly higher than the primary immune response. If we were observing a typical secondary immune response in T_{amp}-cell-recipient mice, then we would expect the amplified DTH response to appear earlier and be maintained over a longer time period than the primary immune response. However, our data show that this was not the case. The amplified DTH response mediated by T_{amp} cells was not detectable any earlier than the primary DTH response and declined as rapidly (Fig. 2). These findings make the possibility of a secondary immune response in T_{amp}-cell recipients less attractive. Another feature of a secondary immune response is the presence of long-lived memory cells. If we were observing a typical secondary immune response, we would expect our system to display long-lived memory cells. However, when we determined the functional life-span of T_{amp} cells, we found that the T_{amp} cells were not functional 12 days after transfer to recipient mice, which is analogous to 18 days after being induced (Fig. 3). Using another approach, we confirmed that T_{amp} cells were relatively short-lived by showing that T_{amp} cells were not present in immunized mice 18 days after immunization. One would expect typical immune memory cells capable of transferring a secondary response to be functional for more than 18 days after immunization; therefore, on the basis of this criterion, it seems unlikely that T_{amp} cells are typical immune memory cells. Our combined data dispel the notion that we observed a typical secondary immune response. It is more likely that T_{amp} cells function by providing help in the form of lymphokines or other accessory molecules which boost the primary immune response in recipient mice. The possibility that T_{amp} cells are helper T cells distinct from T_{DH} cells is reasonable, since T_{amp} cells have several characteristics in common with helper T cells induced by other antigens (10, 26). For example, the helper T cells in other systems and anticryptococcal T_{amp} cells are CD4 cells that are sensitive to cyclosporin A (10, 26). Helper T cells have been subdivided into two subsets, (i) TH1 cells, which are characterized by production of gamma interferon, interleukin 2, and lymphotoxin and which transfer DTH, and (ii) TH2 cells, which are characterized by production of interleukin 4, interleukin 3, and interleukin 6 (17). One might speculate that anticryptococcal T_{DH} cells are TH1 cells; however, without data on lymphokine production, it is difficult to speculate as to the subset of T_{amp} cells. Further investigations will be necessary to establish the TH subset of T_{amp} cells and to define their

mode of action in amplifying the anticryptococcal DTH response.

Our attempts to reproduce the amplified DTH response with other antigens, such as *L. monocytogenes* and DNFB, by using the same protocol used for anticryptococcal amplifier cells were unsuccessful (Table 3). This does not eliminate the possibility that similar amplifier cells exist in the *L. monocytogenes* and DNFB-immune cells circuits. It is possible that the protocol used for the cryptococcal system does not satisfy the conditions for induction and detection of amplifier cells in the other two antigen systems. In fact, amplifier or augmenting cells are not unique to the cryptococcal system. Cells which augment the anti-trinitrophenol (16), anti-erythrocyte (27, 28), and anti-influenza (11) DTH responses have been reported. However, in these other models (11, 16, 27, 28), different methods of detecting amplified DTH responses were used. For example, to demonstrate augmentation of the DTH response to trinitrophenol, recipient mice were immunized with a suboptimal antigen dose (a dose of antigen that does not stimulate detectable DTH levels) at the time of transfer of immune spleen cells (16). In studies in which erythrocytes (27) and influenza virus (11) were used as antigens, in vivo-induced T cells were shown to induce or amplify induction of T_{DH} cells in vitro. In the latter situations, the cells responsible for augmenting the DTH response were referred to as helper T cells (11, 27). The fact that our T_{amp} cells are similar in many ways to the helper T cells in these previously reported models provides another reason for suggesting that our T_{amp} cells are helper T cells.

In summary, we have further characterized and begun to study the role of the cells that amplify the anticryptococcal DTH response. Although our initial findings suggested that the amplified DTH response was a secondary immune response produced by immune T cells, the results of the present study indicate that T_{amp} cells do not mediate a typical secondary immune response and are not long-lived memory cells. On the basis of the sensitivity of T_{amp} cells to cyclosporin A (5), their phenotype (Table 2), specificity (Table 3), kinetics of induction (Fig. 1), the kinetics of the response mediated by T_{amp} cells (Fig. 2), and the similarity to helper T cells in other antigen-cell circuits, we speculate that our T_{amp} cells are helper T cells.

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