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A T Cell-Independent Protective Host Response against *Cryptococcus neoformans* Expressed at the Primary Site of Infection in the Lung

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T cell-independent host resistance expressed against a primary lung infection with *Cryptococcus neoformans* was investigated. Following intratracheal inoculation of the yeast, BALB/cBy *scid/scid* mice or $CD4^+$ plus $CD8^+$ T cell-depleted BALB/cBy mice developed a primary lung infection that remained stable for several weeks before progressing and disseminating to kill the host. By contrast, normal BALB/cBy hosts resolved the infection after 4 to 8 weeks. Thy^+ $CD4^-$ $CD8^-$ cells were found to accumulate in the pulmonary alveoli of infected *scid/scid* or normal mice. Depletion of these cells caused the infection to progress more rapidly and resulted 4 weeks later in a 30- to 70-fold increase in yeast numbers in the lungs and dissemination to extrapulmonary sites. Cytofluorometric studies revealed that the Thy^+ $CD4^-$ $CD8^-$ cells responsible were negative for the CD3 T cell marker. A small percentage of these Thy^+ $CD3^-$ cells expressed asialo-Gm_1, but treatment with asialo-Gm_1 antibody did not have the same infection-enhancing effect as Thy-1 monoclonal antibody treatment. Further experiments revealed that Thy-1 monoclonal antibody treatment had no effect on the establishment of infectious foci in the brain or liver following intravenous inoculation of the yeast. The data point to the existence of an early resistance mechanism for which Thy^+ $CD3^-$ $CD4^-$ $CD8^-$ cells are essential. This mechanism of host defense, while insufficient for complete protection, may be capable of delaying the development of cryptococcal meningoencephalitis by restricting the growth of the yeast at primary sites of infection in the lungs, even in immunodeficient mice.

While meningoencephalitis caused by *Cryptococcus neoformans* is a formidable disease in AIDS and other immunocompromised patient populations (13), the pneumonia that occurs at the initial site of infection is almost always mild or asymptomatic (1). On the basis of this common clinical observation, we have hypothesized (9) that some protective responses against this opportunistic pathogen in the lungs are spared by human immunodeficiency virus infection. One such response could be that mediated by $CD8^+$ T cells, which has been shown (9, 10) to be a component of anti-*Cryptococcus* immunity. There is also evidence that the *Cryptococcus*-infected host focuses T lymphocyte-independent mechanisms at the site of infection. For example, mice depleted of $CD4^+$ plus $CD8^+$ cells (9, 10), nude mice (11), and even mice with severe combined immunodeficiency (SCID) (8, 11) that are virtually devoid of functional T and B cells, are able to restrain the progressive multiplication of the yeast population in the lungs for at least 4 weeks.

The purpose of this study was to investigate whether this early stasis of an opportunistic cryptococcal lung infection is the result of an acquired cell-mediated mechanism of resistance. The results show that this is the case and that the resistance is due in part to the actions of Thy^+ $CD3^-$ cells, which accumulate in the pulmonary alveoli in response to infection. The results show, in addition, that this early resistance appears not to function against the yeast in the brain but acts within the lung alveoli, serving to delay the progression of disease until specific anti-*Cryptococcus* immunity is generated.

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MATERIALS AND METHODS

Mice. BALB/cBy and *Pneumocystis*-free BALB/cBy *scid/scid* (SCID) mice were bred at the Trudeau Institute and maintained as previously described (8).

***Cryptococcus* infection.** *C. neoformans* 184 (serotype A) was grown in Sabouraud broth and washed and suspended in phosphate-buffered saline (PBS). Mice were infected intratracheally with 10^6 yeast cells in 0.1 ml of PBS or intravenously via the lateral tail vein with 2×10^4 yeast cells in 0.2 ml of PBS. At various times of infection, four or five mice from each group were killed and alveolar exudate cells were sampled by tracheobronchial lavage (9). Viable yeast cells in the lung lavage fluid, lungs, liver, and brain were enumerated by plating aliquots of homogenized tissue on Sabouraud agar plates. The numbers ($\log_{10}$) of viable *C. neoformans* are expressed as the mean ± standard deviation. Data were statistically analyzed with Student's *t* test.

Antibodies. Hybridomas secreting rat immunoglobulin G2b (IgG2b) monoclonal antibodies (MAbs) against mouse Thy-1.2 (hereafter referred to as Thy-1) (30-H12), CD4 (GK1.5), and CD8 (2.43) were obtained from the American Type Culture Collection (Rockville, Md.). Hybridoma 145-2C11 secreting a hamster anti-mouse CD3 MAb was obtained from J. Bluestone at the University of Chicago, Chicago, Ill. MAbs were purified from ascites, and their yield and purity were assessed by an enzyme-linked immunosorbent assay and high-pressure liquid chromatography, as previously described (8). To deplete either $CD4^+$ plus $CD8^+$ T cells or all Thy^+ cells, mice were injected intraperi-

toneally with 1 mg of the appropriate MAb(s) diluted in 0.5 ml of saline 1 week before yeast inoculation and with an additional 500 μg every week thereafter. For natural killer (NK) cell depletion, a commercially available rabbit asialo-Gm_1 polyclonal antibody (Wako BioProducts, Richmond, Va.) was administered to mice twice weekly at a dose (40 μl intraperitoneally) documented to deplete all poly(I · C)-inducible NK cell activity against YAC cell targets. Control mice in these experiments received an equivalent amount of normal rabbit IgG, generously provided by E. Havell (Trudeau Institute).

Cytofluorometry. Spleen cells and alveolar exudate cells were prepared as previously described (9) and stained for cytofluorometric analysis with fluorescein isothiocyanate (FITC; Sigma Chemical Co., St. Louis, Mo.)- or biotin (biotinamidocaproate *N*-hydroxysuccinimide ester; Sigma)-labelled antibodies. Staining of T cells was carried out with $F(ab')_2$ fragments of MAbs, which were prepared by pepsin digestion of purified IgG and then separated from small peptides on a Sephadex G-200 gel before conjugation to FITC or biotin. Cell-bound biotinylated MAbs were detected after incubation of samples with streptavidin-phycoerythrin (Becton Dickinson, Mountain View, Calif.). Asialo-Gm_1-positive cells in the Thy^+ alveolar exudate population were detected with rabbit asialo-Gm_1 IgG that had been purified on a protein A affinity column and conjugated directly to FITC. Asialo-Gm_1-positive spleen cells were detected by indirect staining with asialo-Gm_1 antibody and FITC–$F(ab')_2$–goat anti-rabbit IgG (Tago Immunochemicals, Burlingame, Calif.).

Single- or dual-color analysis of stained cells was performed with a FACScan cytofluorograph (Becton Dickinson), and 4,000 to 7,000 events per sample were analyzed with Lysis II software. For alveolar exudate cells, data were collected through an active gate with forward and side light scatter set to include lymphocytes but to exclude large, granular, autofluorescent alveolar macrophages. The gated cells were mainly lymphocytes and accounted for between 2 and 20% of the total exudate population. The highest percentage of lymphocytes was found in exudates from infected control BALBc/By mice (17.4%), whereas lower percentages were found in control infected (5.3%) and antibody-treated infected (2.7%) SCID mice. Active gates were not used for spleen cell analyses.

RESULTS

T cell-deficient mice possess a Thy-dependent resistance mechanism that prevents *C. neoformans* from multiplying in the lungs for 4 weeks. Figure 1 shows that following the deposition of 10^6 *C. neoformans* cells into the respiratory tract of normal, $CD4^+$ plus $CD8^+$ T cell-depleted, and SCID mice, the numbers of viable yeast cells in the lungs remained virtually unchanged for 4 weeks. After that time, however, the numbers of yeast cells began to decline in normal mice. In contrast, yeast cell numbers increased in SCID and T cell-depleted mice, all of which died of disseminated disease by 10 weeks.

Cytofluorometric analysis of cells obtained by bronchopulmonary lavage revealed that, in addition to macrophages, Thy^+ cells accumulated in the alveoli of SCID mice during this fungistatic response. Table 1 shows that at 4 weeks of infection, more than 2×10^5 Thy^+ cells could be lavaged from the lungs of SCID mice. At this time, 10 to 25% of the total lung burden, approximately 6×10^4 viable yeast cells, was also retrieved from the lungs by lavage, giving a 4:1 ratio

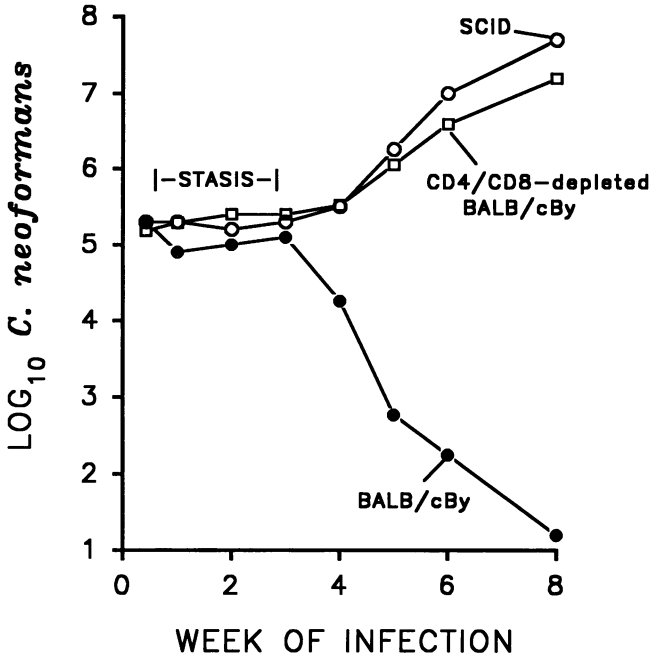

FIG. 1. Changes over time in the numbers of *C. neoformans* cells in the lungs of normal and T cell-deficient mice, showing that all mice are capable of restraining the multiplication of the yeast cells at the site of infection for 4 weeks. Datum points are means for four mice.

of Thy^+ cells to yeast cells in the alveoli. Given the high frequency of these Thy^+ cells among the nonmacrophage alveolar exudate cells of infected SCID mice, the possible role of these cells in resistance against *C. neoformans* during the static phase of the pulmonary infection was investigated. Their importance was revealed when Thy^+ cells were eliminated from the host before intratracheal inoculation with *C. neoformans*. Thus, Table 1 shows that treating SCID mice with a Thy-1 MAb but not CD4 plus CD8 MAbs rendered them more susceptible to *Cryptococcus* infection, as evidenced by a 45-fold higher lung burden of yeast cells at 4 weeks. An examination of lavage cells in stained cytocentrifuge preparations revealed that whereas yeast cells in control mice were predominantly single organisms surrounded by host cells, those recovered from Thy^+ cell-depleted mice were budding aggregates of three to six organisms. Despite an increased yeast cell burden, Thy^+ depletion caused no noticeable change in the total number of cells accumulating in the lungs of SCID mice. It is perhaps important to note, however, that Thy-1 MAb treatment resulted in a change in the forward and right-angle light-scattering properties of the cells, in that virtually all of them had characteristics of macrophages. Moreover, these macrophages were considerably larger and more granular than their counterparts in exudates of infected control or $CD4^+$ plus $CD8^+$ T cell-depleted SCID mice. In the latter groups of mice, cells with the light-scattering characteristics of lymphocytes comprised up to 20% of total cells, whereas they constituted less than 3% of total cells in exudates of Thy-1 MAb-treated mice.

Table 1 also shows that the alveolar exudate of control infected SCID mice contained small numbers of lymphocytes that were $CD4^+$ or $CD8^+$. However, these T lymphocytes were not obligatory participants in the early fungistatic

TABLE 1. Thy-1 MAb treatment abrogates early resistance expressed against *C. neoformans* in the lungs of BALB/cBy *scid/scid* (SCID) and normal BALB/cBy mice

Mice	MAb treatment[a]	No. of cells (10^4) in alveolar exudate at 4 wk					TLB[b]	P
		Viable yeast	Total	Thy^+	$CD4^+$	$CD8^+$		
SCID	None	6.3	120	22.8	2.0	0.3	5.39 ± 0.25	
	CD4 + CD8	4.6	220	5.3	<0.1	<0.1	5.32 ± 0.24	NS[c]
	Thy-1	156.0	170	<0.1	<0.1	<0.1	6.98 ± 0.54	≤0.005
BALB/cBy	None	14.8	375	14.5	11.3	3.5	5.74 ± 0.16	
	CD4 + CD8	30.8	240	3.3	<0.1	<0.1	5.82 ± 0.18	NS
	Thy-1	276.0	70	<0.1	<0.1	<0.1	7.35 ± 0.27	≤0.001

[a] Eight- to 10-week-old SCID and normal BALB/cBy mice were treated with MAbs to deplete Thy^+ or $CD4^+$ plus $CD8^+$ cells as outlined in Materials and Methods. They were infected intratracheally with 10^6 *C. neoformans* cells 1 week after MAb treatment was begun.
[b] TLB, total lung burden of viable yeast cells at 4 weeks of infection, reported as the mean $\log_{10}$ number ± standard deviation for four or five mice.
[c] NS, not significant.

response, because depleting them with a cocktail of CD4 plus CD8 MAbs did not result in an increase in yeast cell numbers in the lungs. The Thy-mediated mechanism of resistance was readily demonstrated in infected BALB/cBy mice, in that depletion of Thy^+ $CD4^-$ $CD8^-$ cells, but not $CD4^+$ plus $CD8^+$ T cells, resulted in a significant exacerbation of the lung infection similar to that found in SCID mice.

The Thy^+ cells of interest in the lungs of infected mice were not $CD4^+$ or $CD8^+$ T cells. Indeed, they are unlikely to be T cells at all, in that the results of cytofluorometric studies (Fig. 2) showed that ≥90% of Thy^+ cells in control infected SCID mice (Fig. 2b) and ≥98% of Thy^+ cells in those given CD4 plus CD8 MAbs did not express the CD3 T cell marker (Fig. 2c). In contrast, greater than 90% of Thy^+ cells in the exudate of infected BALB/cBy mice were Thy^+ $CD3^+$ (Fig. 2a), with the majority expressing either the CD4 or the CD8 T cell marker (data not shown). In these mice too, however, the Thy^+ cells of interest were not $CD4^+$ or $CD8^+$.

In vivo depletion of asialo-Gm_1-positive cells fails to block the early fungistatic response in the lungs of SCID mice. When tested, a small portion (6 to 8%) of the Thy^+ cells in the alveolar exudates of infected SCID mice also stained positively for the asialo-Gm_1 surface marker. This result suggested that Thy^+ NK cells may contribute to resistance expressed in the lungs of infected SCID mice. A role for NK cells in the early control of infection was therefore investigated by depleting mice of these cells by treatment with an asialo-Gm_1 antibody before intratracheal inoculation, and the infection was monitored over a 4-week period. Control groups of mice were left untreated or given an equivalent amount of rabbit control IgG. For comparison purposes, an additional group of mice was depleted of all Thy^+ cells, including Thy^+ asialo-Gm_1-positive cells, by treatment with a Thy-1 MAb. That asialo-Gm_1 antibody treatment was effective in depleting asialo-Gm_1-positive cells from the alveolar exudates and spleens of infected mice can be seen from the cytofluorometric data shown in Fig. 3. Nonetheless, this treatment did not render mice more susceptible to *C. neoformans* infection in the lungs (Table 2), unlike treatment with the Thy-1 MAb, which again caused exacerbated pulmonary disease. Although it was apparent from the cytofluorometric data (Fig. 3) that a portion of the remaining spleen cells in infected SCID mice expressed low-intensity staining for asialo-Gm_1, it seemed unlikely that these cells were NK cells. Their light-scattering properties were those of large activated macrophages, and it was possible that the low-intensity staining was due to nonspecific Fc-mediated binding of the asialo-Gm_1 antibody. All of the cells that were stained intensely with the asialo-Gm_1 antibody had the light-scattering properties of lymphoid cells and were characteristic of large granular lymphocytes reported by others (2) to have high NK cell activity.

Evidence that Thy^+ cells function locally in the lungs to delay the development of disease. Because it is known that SCID mice eventually succumb to cryptococcal infection that has disseminated to the brain (11), viable yeast cells in the brain were also enumerated in the above-described experiment. It was found (Table 2) that treatment with an asialo-Gm_1 antibody had no exacerbating effect on yeast growth at this extrapulmonary site. In fact, a small but noticeable decrease in yeast burden in the brain was observed for this group of mice. Treatment with a Thy-1 MAb, on the other hand, resulted in a significantly higher yeast burden in the brain. It thus appears that ablating the Thy^+ cell-mediated protective response not only causes progressive pulmonary disease but results in increased dissemination of yeast cells to the brain.

It is not clear whether the aforementioned Thy^+ cell-mediated mechanism is active systemically against *C. neoformans* infection, i.e., whether it protects the host against blood-borne yeast cells that escape the lungs to establish foci of infection in the extrapulmonary organs. To determine whether Thy^+ cells defend against yeast cells in the vasculature, the blood of intact, $CD4^+$ plus $CD8^+$ T cell-depleted, and Thy^+ cell-depleted SCID mice was seeded with a small number of yeast cells (2×10^4) via the tail vein, a route widely used to examine host responses controlling the dissemination of *C. neoformans* cells (15). Seven days later, the mice were killed, and viable yeast cells were enumerated in the lungs, liver, and brain. The results in the top half of Table 3 show that there was no difference in the organ yeast burden in the liver and brain of control and MAb-treated mice at 7 days. There was a small but statistically significant increase in the number of viable yeast cells in the lungs of Thy-1 MAb-treated mice at 7 days following the introduction of *C. neoformans* directly into the blood. This result suggests that SCID mice can express a Thy^+ cell-mediated protective response against yeast cells cleared from the vasculature in the lungs but not in the brain. At 12 to 14 days, in the presence of greater than 10^6 yeast cells in established foci in the brain, the remaining five mice in the control and MAb-treated groups died of meningoencephalitis.

Similar results were obtained with mice that were challenged intravenously after a Thy^+ cell-mediated response had been induced by a primary lung infection. In this experiment, SCID mice were first infected intratracheally

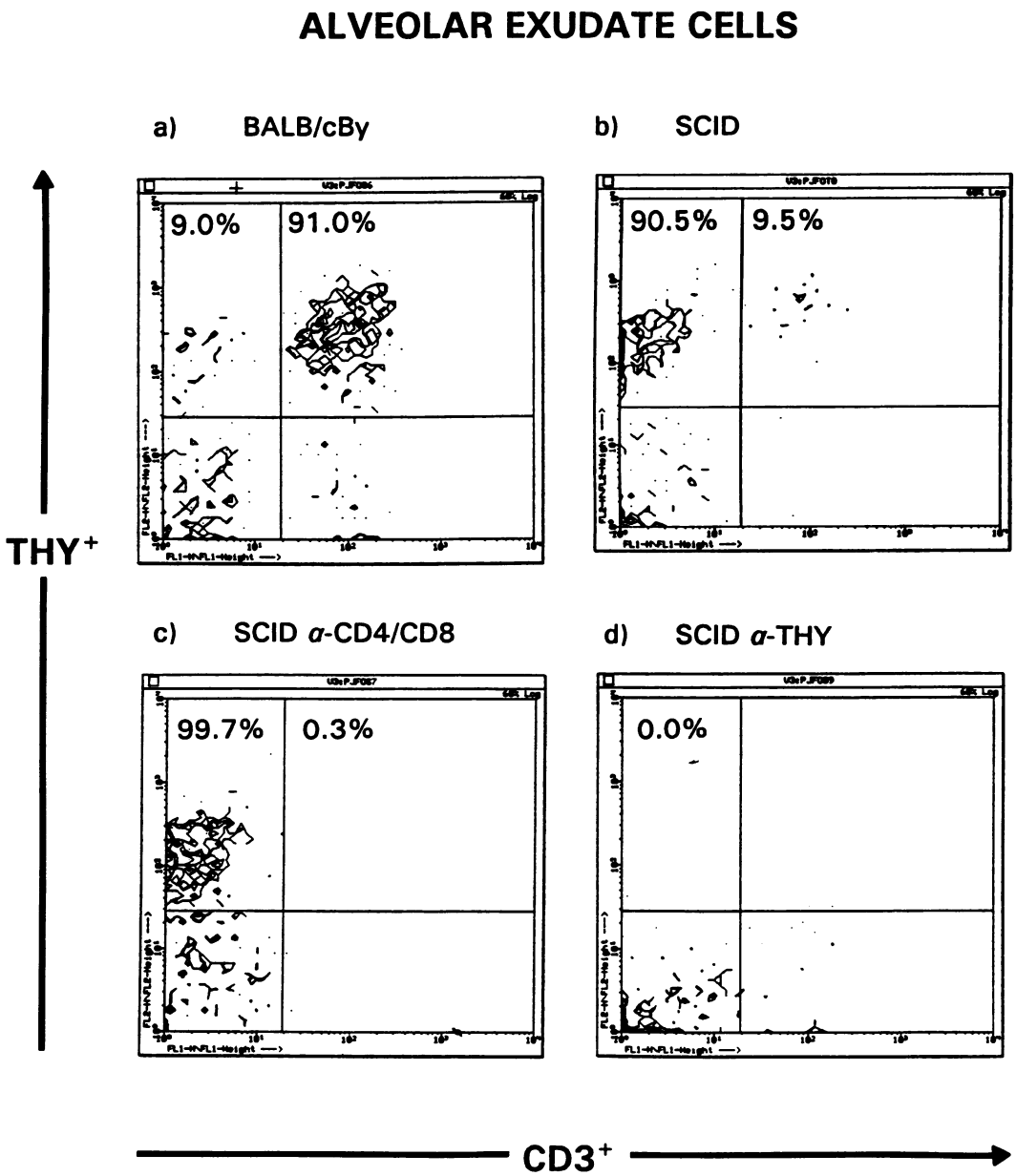

FIG. 2. Cytofluorometric analysis of alveolar exudate cells from 4-week-infected BALB/cBy (a), SCID (b), $CD4^+$ plus $CD8^+$ T cell-depleted SCID (c), or Thy^+ cell-depleted SCID (d) mice, showing that the majority of Thy^+ cells in the exudates of infected SCID but not infected BALB/cBy mice are Thy^+ $CD3^-$ $CD4^-$ $CD8^-$. Percentages refer to Thy^+ cells either negative or positive for CD3 expression. Data are representative of pooled exudate cells from four or five mice per group and are displayed as a contour diagram in which the x axis represents logarithmic increasing green fluorescence intensity (FITC) and the y axis represents increasing red fluorescence intensity (streptavidin-phycoerythrin).

with 10^6 *C. neoformans* cells and then challenged intravenously 3 weeks later with 2×10^4 organisms. They were killed 7 days later (i.e., 4 weeks after the initiation of the primary lung infection). The results in the bottom half of Table 3 show that Thy^+ cell-depleted mice again had higher lung burdens than CD4 plus CD8 MAb-treated or untreated control mice. However, none of the groups of mice was able to prevent the establishment and growth of *C. neoformans* in the brain. All mice died by 18 days after the intravenous challenge. Thus, there was no indication that mice could generate or express a protective Thy^+ $CD4^-$ $CD8^-$ response against yeast cells in the brain. It was concluded, therefore, that this response delays the development of systemic disease by preventing the progressive multiplication of yeast cells in the alveoli, probably denying *C. neoformans* access to the systemic circulation during the first month of the infection.

DISCUSSION

This study shows that Thy^+ $CD4^-$ $CD8^-$ cells that accumulate at the primary site of a *C. neoformans* infection in the

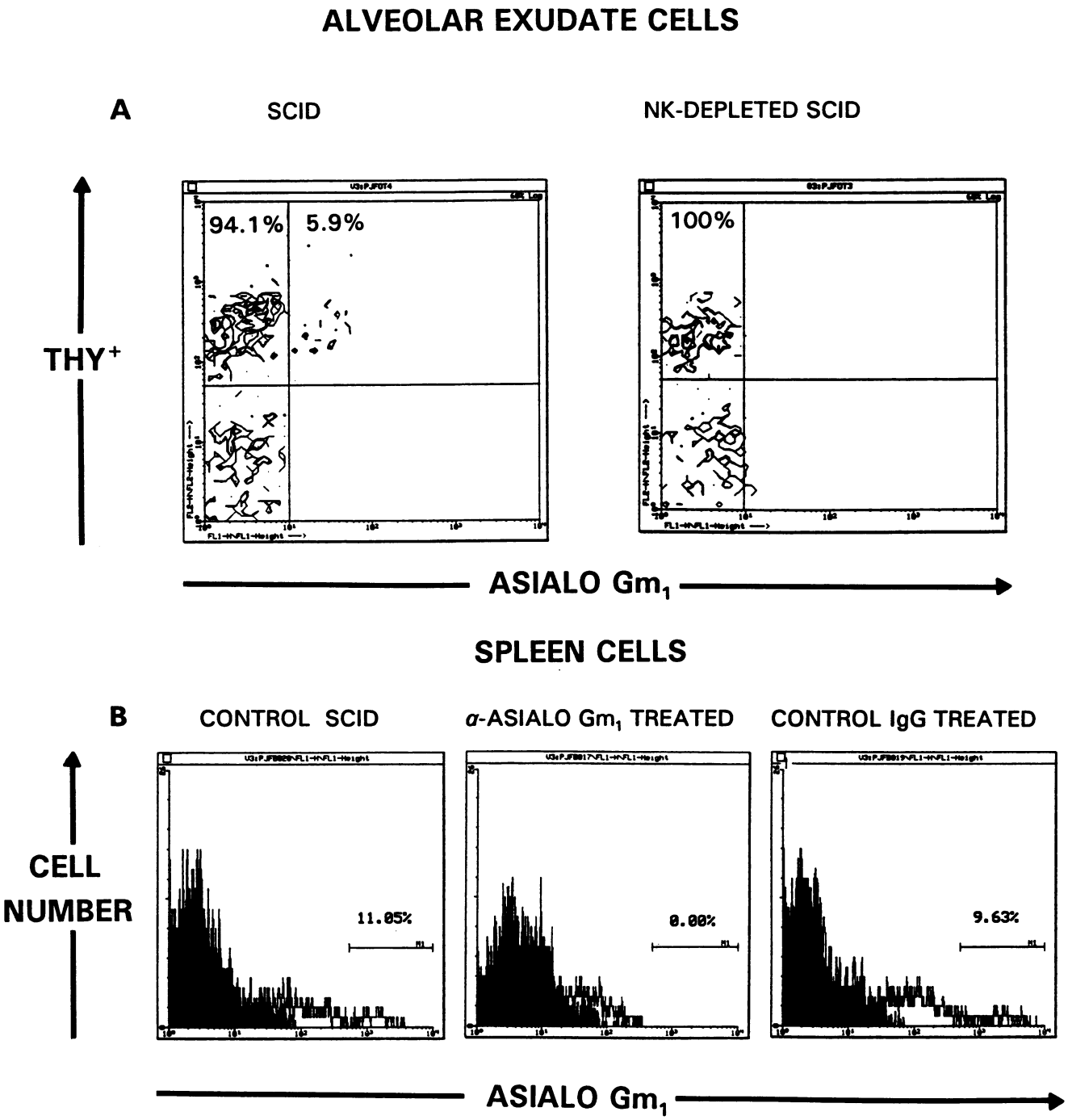

FIG. 3. Evidence (A) that a small percentage of Thy$^+$ cells in the alveolar exudates of infected SCID mice express asialo-Gm$_1$ and are depleted by in vivo treatment with an asialo-Gm$_1$ antibody and (B) that this treatment causes systemic depletion of other asialo-Gm$_1$-bearing cells, including splenic NK cells. Infected SCID mice were depleted of asialo-Gm$_1$-positive cells over 4 weeks, and pooled cells from alveolar exudates or spleens of four or five mice per group were examined. Dual-color staining was carried out on exudate cells; the percentage of positively stained cells is given on the contour plots in panel A. Spleen cells were analyzed by single-color indirect-fluorescence staining with asialo-Gm$_1$ antibody and then with FITC–F(ab')$_2$–goat anti-rabbit IgG. The data, presented as histograms in panel B, show the percentage of cells with high-intensity staining for asialo-Gm$_1$. The filled histogram represents the background staining obtained with the FITC-conjugated secondary antibody alone.

lungs are a component of the protective host response against this opportunistic pathogen. Studies with immunocompetent mice have shown that the targets of a protective host response to *C. neoformans* include yeast cells that have been enclosed by histiocyte rings, which eventually fuse to form multinucleated giant cells under the influence of CD4$^+$ T lymphocytes (8). The Thy$^+$ cells responsible for the early resistance described here are CD3$^-$ and therefore are not T

TABLE 2. Lack of effect of systemic asialo-Gm_1 antibody treatment on the fungistatic response against *C. neoformans* in the lungs of BALB/cBy *scid/scid* (SCID) mice

Antibody treatment[a]	No. of viable *C. neoformans* cells (mean $\log_{10}$ ± SD) at wk 4 of infection in:	
	Lungs	Brain
None	5.35 ± 0.65	3.16 ± 0.10
Thy-1	6.63 ± 0.84[b]	4.95 ± 1.00[b]
Asialo-Gm_1	5.30 ± 0.20	1.77 ± 0.85
Rabbit serum	5.08 ± 0.53	2.98 ± 0.19

[a] Eight- to 10-week-old SCID mice were treated with a Thy-1 MAb or an asialo-Gm_1 antibody. These mice, along with rabbit serum-treated control mice, were infected intratracheally with 10^6 *C. neoformans* cells. Mice were retreated with additional doses of antibodies as described in Materials and Methods.

[b] $P \leq 0.005$ versus controls.

cells. However, it is not yet known whether these Thy^+ cells are constituents of the host cell-yeast cell aggregates present in the alveoli. It is possible that Thy^+ cells do not need to come into direct contact with the yeast capsule or cell wall, but mediate their effects through the secretion of cytokines. In this regard, Dunn and North (3) recently described Thy^+ $CD4^-$ $CD8^-$ cells that function in resistance against a systemic bacterial infection by secreting gamma interferon (4). The cytokine-producing properties of Thy^+ $CD4^-$ $CD8^-$ cells in the lungs of *Cryptococcus*-infected mice warrant study, as perhaps they are a source of the gamma interferon that mediates the macrophage fungistasis described by Flesch et al. (5).

Regardless of whether the cells need to contact yeast cells directly to mediate their effects, it seems likely that a major contribution of accumulating Thy^+ cells is to restrict the multiplication of yeast cells at an early stage of infection until specific anti-*Cryptococcus* immunity is fully generated, an idea that has already been put forward (19). There is accumulating evidence in the literature in support of the view that murine (7, 21) and human (14, 17, 19) NK cells can bind directly to and inhibit the growth of *C. neoformans* in vitro. Given the knowledge that SCID mice have a normal complement of NK cells (8) and that at least some NK cells express Thy-1 (16), it seems logical to suggest that the Thy^+ $CD3^-$ cells in the lungs of *Cryptococcus*-infected SCID mice are indeed NK cells. Our results, however, would argue against that hypothesis, in that while a portion of the Thy^+ cells in the alveolar exudates and spleens of infected SCID mice expressed the asialo-Gm_1 surface marker, their elimination by systemic antibody treatment did not exacerbate pulmonary or extrapulmonary disease. There is also a study (15) showing that in immunocompetent mice, NK cells play a minor role in immunity against a virulent strain of *C. neoformans* introduced into the lungs, in that the depletion of $NK1.1^+$ cells from mice failed to cause a difference in the numbers of yeast cells recovered from primary and secondary sites of infection after the first 24 h. Similarly, asialo-Gm_1 antibody treatment has failed to affect innate systemic resistance against *Candida albicans* in SCID mice (6, 12). The effects of Thy-1 MAb treatment were not examined in these latter studies. However, it is not possible to preclude a role for NK cells in anticryptococcal resistance in SCID mice because $Thy\text{-}1^+$ $CD4^-$ $CD8^-$, asialo-Gm_1-negative, NK-like cytotoxic cells have been described elsewhere (22). Moreover, studies of the interaction between yeast cells and selected populations of human lymphoid cells have implicated $CD4^-$ $CD8^-$ non-NK cells in fungistasis (14). Whether these are similar in other ways to the cells active in the lungs of mice remains to be determined.

It was disappointing to find that the response mediated by Thy^+ $CD4^-$ $CD8^-$ cells is apparently not expressed at therapeutic levels in the brain. SCID mice, regardless of whether they have generated a Thy-dependent resistance mechanism, cannot prevent the establishment or growth of yeasts in that organ. The delay in the development of systemic disease in Thy^+ cell-competent mice can thus be attributed to the success of these mice in confining the yeast cells to the lung parenchyma during the first month of the pulmonary infection. Overall, the findings of this study and others (6, 12) leave no doubt that SCID mice can resist a *C. neoformans* infection for several weeks. The observations in this study argue against an exclusive role for $CD4^+$ and $CD8^+$ lymphocytes in anti-*Cryptococcus* immunity. Recent findings showing early in vivo protection against the progressive growth of several other pathogens in SCID mice, including bacteria (3), viruses (18), and protozoa (20), lend support to the notion that powerful T and B cell-independent resistance mechanisms can be generated by severely immunodeficient hosts. Therefore, there might be protective mechanisms in AIDS patients that function in the lungs independently of $CD4^+$ cells. It would be worthwhile to examine AIDS patients for the presence of Thy^+ non-T cells that are fungistatic against *C. neoformans*. Knowledge of $CD4^+$ T cell-independent responses against this and other opportunistic pathogens could be important in the develop-

TABLE 3. Effect of Thy-1 MAb treatment on resistance against *C. neoformans* introduced into the vasculature of naive SCID mice and SCID mice with a 3-week pulmonary infection

Mice	MAb treatment[a]	No. of viable *C. neoformans* cells (mean $\log_{10}$ ± SD)[b] in:		
		Lungs	Brain	Liver
Naive	None	1.61 ± 0.90	6.32 ± 0.28	3.76 ± 0.34
	CD4 + CD8	1.68 ± 0.31	6.32 ± 0.27	3.58 ± 0.35
	Thy-1	2.49 ± 0.77[c]	6.48 ± 0.24	3.60 ± 0.15
3-wk pulmonary infection	None	5.24 ± 0.39	6.16 ± 0.35	3.46 ± 0.26
	CD4 + CD8	4.39 ± 0.60	6.38 ± 0.26	3.61 ± 0.19
	Thy-1	6.26 ± 0.57[c]	5.81 ± 0.37	3.97 ± 0.54

[a] Eight- to 10-week-old SCID mice were treated with a Thy-1 MAb or CD4 plus CD8 MAbs. Half of the mice were infected intratracheally with 10^6 *C. neoformans* cells. Three weeks later, all of the mice were challenged intravenously with 2×10^4 yeast cells. After 7 more days, all of the mice were sacrificed, and viable yeast cells in the lungs, liver, and brain were enumerated.

[b] Four or five mice per group.

[c] $P \leq 0.05$ versus $CD4^+$ plus $CD8^+$ T cell-depleted mice and versus untreated control mice.

ment of therapies for AIDS-related infections, as the success of a therapy may be based on its capacity to stimulate a host defense mechanism spared by human immunodeficiency virus.

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