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Specific T-Cell Response to a *Pneumocystis carinii* Surface Glycoprotein (gp120) after Immunization and Natural Infection

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T cells have been shown to be important in recovery from *Pneumocystis carinii* pneumonitis, although no specific antigen of *P. carinii* has been defined as containing T-cell epitopes. *P. carinii* has an abundant mannosylated surface glycoprotein of approximately 120 kDa (gp120) which induces a prominent host antibody response in experimental animals after exposure to *P. carinii* in the environment or after recovery from *P. carinii* pneumonitis. *P. carinii* gp120 was purified from infected lungs by lectin affinity chromatography. Standard in vitro lymphocyte stimulation assays using purified gp120 and control normal lung preparations were performed on isolated T cells obtained from BALB/c mice after immunization with *P. carinii*-infected crude lung homogenates or lectin-purified gp120. Lymphocytes from reconstituted severe combined immunodeficient mice which had recovered from naturally acquired *P. carinii* pneumonitis were also tested. A specific T-cell response was elicited by gp120 after immunization with *P. carinii* gp120 and after recovery from *P. carinii* pneumonitis. In addition, the mice developed a strong antibody response to gp120 as ascertained by Western blot (immunoblot). These data suggest that gp120 may be important in the recognition of *P. carinii* by T cells.

Pneumocystis carinii causes severe pneumonitis in immunocompromised hosts, such as patients undergoing chemotherapy for malignancy and patients with congenital or acquired immunodeficiencies (13). Despite the importance of this organism, the immunopathogenesis of *P. carinii* pneumonitis remains poorly defined.

Both clinical and experimental evidence support the involvement of T cells in the protection against or recovery from *P. carinii* pneumonitis. For example, it is well known that patients with low numbers of T cells or poorly functioning T cells are susceptible to *P. carinii* pneumonitis (17). Animal models have shown that T cells have a role in the ability of a host to recover from *P. carinii* pneumonitis. The adoptive transfer of normal spleen cells or isolated T cells to nude mice infected with *P. carinii* allows these animals to recover (2). Chronic $CD4^+$ T-cell depletion results in *P. carinii* pneumonitis in normal mice (22), implicating these cells in the host response to *P. carinii* pneumonitis. Most importantly, $CD4^+$ T cells must be present when reconstituting the immune system of severe combined immunodeficient (SCID) mice in order for them to recover from *P. carinii* pneumonitis (10). Although these reports clearly demonstrate the necessity of T-cell responses for recovery from *P. carinii* pneumonitis, neither the actual function of these cells nor the specific *P. carinii* antigens involved in this T-cell response have been defined.

We are studying the T-cell response to an abundant mannosylated glycoprotein (gp), which has a molecular mass of approximately 120 kDa, expressed by *P. carinii* obtained from animal models of infection (5, 15, 20, 23). The rationale for choosing to study the T-cell response to this antigen is that (i) this antigen is expressed on the surface of *P. carinii* (7), (ii) it has been shown to stimulate a prominent antibody response in animals after environmental exposure to *P. carinii* or after recovery from *P. carinii* pneumonitis (24), and (iii) passive administration of a monoclonal antibody to this antigen confers partial protection against *P. carinii* pneumonitis in animal models (6). These observations suggest that gp120 is a likely candidate antigen to be involved in the T-cell response to *P. carinii*. This report presents data which demonstrate a T-cell response in mice to gp120 after both immunization with the protein and recovery from natural infection with *P. carinii*.

MATERIALS AND METHODS

Animals. Six-to-eight-week-old female BALB/cByJ mice (Jackson Laboratories, Bar Harbor, Maine) were used for the immunization studies described below. They were housed in filter-top cages in the University of Rochester Vivarium. Female SCID mice were used as a source of mouse *P. carinii* and to study the lymphocyte response to *P. carinii* gp120 after reconstitution of their immune systems and recovery from *P. carinii* pneumonitis. These mice were bred and housed under barrier conditions at the Trudeau Institute. *P. carinii* was also obtained from female ferrets (Marshall Farms, North Rose, N.Y.) which were immunosuppressed with oral steroids to induce *P. carinii* pneumonitis as previously described (5). These animals were housed in open cages in the University of Rochester Vivarium.

Partial purification of gp120. Ferrets or SCID mice with *P. carinii* pneumonitis were killed, and their lungs were harvested to provide *P. carinii* cysts. Lungs were homogenized in phosphate-buffered saline (PBS) and then digested for 30 to 60 min at 37°C with collagenase and hyaluronidase (Sigma, St. Louis, Mo.), each at a final concentration of 0.2% (wt/vol). Sodium deoxycholic acid (0.2%, wt/vol), DNase (10 μg/ml; Sigma), and RNase A (100 μg/ml; Sigma) were then added to the homogenate for an additional incubation of 30 to 60 min at 37°C. The homogenate was washed in PBS and centrifuged at 600 to 900 × *g* two times; the final pellet was

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suspended in 5 to 10 volumes of PBS. Homogenates of normal, uninfected ferret and SCID mouse lungs were prepared in a similar fashion. A 10-μl drop of each preparation was dried on a glass slide and stained with toluidine blue O to quantitate the number of cysts in the homogenates. The homogenates were stored at −70°C until needed.

Infected and normal lung homogenates were solubilized in a 1.0% sodium dodecyl sulfate (SDS)–PBS solution. The solutions were rocked at room temperature for 60 min and centrifuged for 10 min at 15,000 × *g* in a microcentrifuge, and the supernatants were filtered through a 0.45-μm-pore-size filter (Acrodisc; Gelman Sciences, Ann Arbor, Mich.). Samples were passed over a detergent binding affinity column (Extracti-Gel D; Pierce Immunotechnology, Rockford, Ill.) to remove the SDS. This material was then lectin affinity purified by using a modification of our previously published protocol (5).

Affinity chromatography was performed by using lentil lectin Sepharose (Sigma) followed by succinyl-concanavalin A (succinyl-ConA) agarose (Sigma) in disposable minicolumns (Isolab, Inc., Akron, Ohio). Both lectins are specific for mannose and glucose; however, we found that *P. carinii* gp120 binds poorly to lentil lectin. Thus, we used the lentil lectin as the first step in an attempt to reduce contamination by other mannose-containing gps in our final preparations. Briefly, a 3.0-ml volume of the soluble antigen preparation was passed over a 0.5-ml lentil lectin column. The column was washed twice with 0.5 ml of Tris-buffered saline (pH 7.4); the soluble antigen preparation and the two washes were then passed over a 1.0-ml succinyl-ConA agarose column, collected, and reapplied to the column four times. The column was washed with 80 column volumes of Tris-buffered saline and then eluted with 0.5 M NaCl–0.5 M α-D-methylmannopyranoside (α-MMP; Sigma). The elution buffer was prewarmed to 50°C, and 1.0 ml was passed over the column, collected, and immediately reapplied to the column with an additional 1.5 ml of the elution buffer after the column was stoppered. The column was incubated in a 50°C water bath for 60 min, which we found markedly enhanced the efficiency of displacing mouse or ferret *P. carinii* gp120. The eluting buffer was collected, and the columns were then washed with an additional 0.5 ml of prewarmed elution buffer, which was added to the final eluate. Uninfected lung homogenates were subjected to the same solubilization and affinity chromatography purification for use as control preparations.

The total protein concentration was checked on some of the lectin-purified *P. carinii* gp120 preparations by using a commercial kit (Bio-Rad, Richmond, Calif.). Protein concentrations were approximately 10 to 20 μg/ml. Each preparation was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) to monitor the purity of the 120-kDa gp and to check that each preparation contained a similar amount of antigen. The samples were mixed with sample buffer (final concentration: 1.0% SDS, 10% glycerol, 0.05 M Tris-HCl [pH 6.8], 5.0% 2-mercaptoethanol), boiled for 5 min, and subjected to electrophoresis at 30 mA through a 10% polyacrylamide gel as previously described (5). The gels were stained with a silver stain kit (Bio-Rad) to visualize the proteins. The presence of the gp120 antigen was also confirmed by Western blot (immunoblot) by using the monoclonal antibody 5E12 as previously described (7). Finally, the eluates were centrifuged in a microconcentrator (Centricon-30; Amicon, Danvers, Mass.) to decrease the concentrations of salt and sugar. The samples were resuspended back to the original precentrifugation volume in RPMI medium supplemented with 0.04 M α-MMP, filter sterilized, and frozen at −4°C until needed for the proliferation assays.

Immunization of BALB/c mice. Groups of three to six mice were immunized with lectin-purified ferret *P. carinii* gp120 eluate or infected ferret lung homogenates. Primary immunization with crude homogenate containing 2×10^6 cysts per mouse was given by direct intraperitoneal injection. No adjuvant was used. The animals were boosted 2 to 3 weeks later by subcutaneous tail injection of crude homogenate containing 5×10^5 to 7×10^5 cysts per mouse in incomplete Freund's adjuvant. Other mice received a primary intraperitoneal immunization of lectin-purified ferret *P. carinii* gp120 (3 to 5 μg per mouse) in a 1:1 emulsion with complete Freund's adjuvant. Booster immunization was done 2 to 3 weeks later by subcutaneous tail injection of approximately 3 μg of partially purified gp120 mixed 1:1 with incomplete Freund's adjuvant. A final group of mice were immunized and boosted with a protein concentration similar to that described above of bovine serum albumin (BSA) to act as a negative control. Mice were killed 8 to 10 days after booster immunization, and para-aortic and inguinal lymph nodes were harvested. Nodes were pressed through a fine mesh tissue screen, washed in RPMI medium, and passed over nylon wool columns (18) to isolate a T-cell-enriched population of cells.

Natural infection of SCID mice. To determine whether natural infection with *P. carinii* resulted in a specific T-cell response to *P. carinii* gp120, we utilized the SCID mouse model of *P. carinii* pneumonitis (10). For these studies, SCID mice were reconstituted with approximately 10^8 normal BALB/c spleen cells per mouse and the mice were killed 15 to 18 days later. The tracheobronchial lymph nodes (TBLN) were harvested and suspended in RPMI medium. Isolation of T cells was not performed because of low numbers of available cells.

Lymphocyte proliferation assays. Cell-mediated immunity to gp120 was measured by lymphocyte proliferation. T cells or SCID TBLN cells were cultured in 96-well, round-bottomed microtiter plates (Costar, Cambridge, Mass.) at a concentration of 10^5 cells per well in 0.1 ml of complete RPMI 1640 (2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 5×10^{-5} M 2-mercaptoethanol) supplemented with 10% heat-inactivated fetal bovine serum. The lymphocytes were cocultured with 10^5 irradiated, normal unimmunized BALB/c mouse spleen cells per well to serve as antigen-presenting cells. All cultures were performed in quadruplicate, except for one of the SCID cell assays (experiment 1), which was done in triplicate because of the low number of cells recovered. Cells were cultured in the presence of soluble gp120 or control normal lung eluates at a 1:1 mixture; the final volume was 0.2 ml per well. The final dilutions of soluble gp120 and control antigens were 1:4 and 1:40, with estimated protein concentrations of 0.5 to 1.0 and 0.05 to 0.1 μg, respectively. A 1:400 dilution of gp120 was also tried, but the results are not shown since it had no activity. The final culture medium contained 0.02 M α-MMP to block mitogenic activity from any succinyl-ConA which could have leached off the affinity columns. This concentration of α-MMP was shown to totally inhibit 20 μg of ConA per ml. ConA (5 μg/ml) was added to cells in medium without α-MMP to serve as a positive control. Plates were incubated in 6% CO_2 at 37°C for 3 or 5 days. On the third or fifth day, the cultures were pulsed with 1.0 μCi of [^{3}H]thymidine (2 μCi/mmol; Amersham, Arlington Heights, Ill.) for 18 h. The cells were harvested on glass fiber filter strips with an automatic multiple sample harvester (Cambridge Tech-

nology, Inc., Watertown, Mass.). The incorporation of thymidine was quantified by liquid beta-scintillography, and data were expressed as counts per minute or in terms of a stimulation index (SI; mean counts per minute in stimulated wells/mean counts per minute in background wells).

Western blot analysis. To determine the serum antibody response to gp120 after immunization or recovery from natural infection, a Western blot analysis was performed. For these studies, *P. carinii*-infected lung homogenates were solubilized in SDS-PAGE sample buffer and electrophoresed as noted above, and the separated antigens were transferred to nitrocellulose as previously described (7). Pooled serum samples corresponding to each group of mice used in the lymphocyte proliferation assays were serially diluted in PBS containing 5% fetal bovine serum and placed on nitrocellulose strips for 16 h at room temperature. After being washed, the strips were incubated in goat anti-mouse immunoglobulin G and immunoglobulin M conjugated to alkaline phosphatase (Pel-Freeze, Rogers, Ark.) diluted 1:1,000 in PBS–5% fetal bovine serum for 3 h. The strips were developed with Fast Red TR salt (2 mg/ml; Sigma) and naphthol AS-MX phosphate (1 mg/ml; Sigma) in 0.05 M Tris-HCl (pH 8.5) to detect any mouse antibody bound to the nitrocellulose strips.

Statistical analysis. A two-sample Wilcoxon rank-sum test was used to compare data from gp120-stimulated cultures with data from normal lung cultures.

RESULTS

Preparation of gp120. Sequential lectin affinity column chromatography was used to isolate gp120 from *P. carinii*-infected ferret or mouse lung homogenates and uninfected control lungs. This procedure worked especially well in the purification of ferret *P. carinii* gp120; our final preparation contained only trace contaminants of other proteins, as seen by SDS-PAGE separation and silver stain. (Fig. 1A, lane 2). Mouse *P. carinii* gp120 was also successfully purified in this fashion, although there was a somewhat higher rate of contamination by other proteins (Fig. 1A, lane 1). Normal, uninfected mouse and ferret lung preparations subjected to the same lectin affinity purification did not demonstrate any protein bands at these molecular masses. With longer exposures or by loading more protein, contaminants of other proteins are visible in the 40- to 60-kDa range (Fig. 1A, lanes 3 and 4). Western blot, by using antibody 5E12, confirmed that the 120-kDa molecule in our eluates was *P. carinii* gp120 (Fig. 1B).

Responses to gp120 after immunization. To determine whether gp120 could elicit a specific T-cell response after immunization, BALB/c mice were immunized with either crude *P. carinii* homogenates or lectin-purified *P. carinii* gp120 preparations. The first set of assays was designed as a pilot experiment to determine whether gp120 elicited the proliferation of immune T cells and to determine the optimal number of cells per culture and optimal duration of culture with antigen to obtain proliferative responses. Mice immunized with *P. carinii*-infected lung homogenates showed significant lymphocyte stimulation, with SIs of 13 and 11 times the background value when pulsed on day 3 of the culture. Similar results were also seen when the T cells were pulsed on day 5 and harvested and counted on day 6 (results not shown). There was no lymphocyte stimulation above the background level by a 1:4 dilution of *P. carinii* gp120 in the BSA-immunized BALB/c mice (SI, ≤1.3), nor did ferret *P. carinii* gp120 stimulate C57B/10 T cells sensitized to an equine myoglobin peptide (SI, ≤1.3; four experiments). These results with cells from two strains of mice indicate that the *P. carinii* gp120 preparations were not acting as a mitogen.

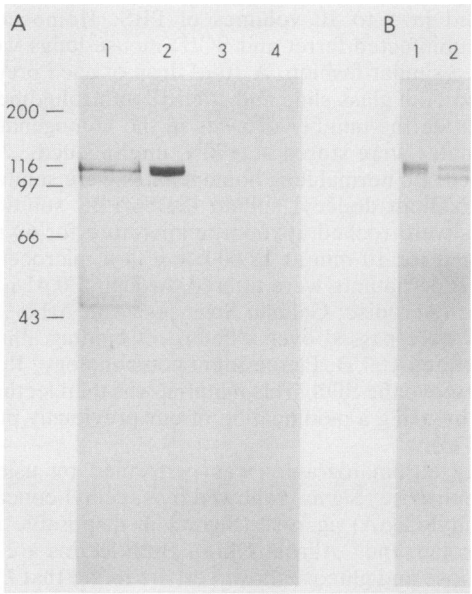

FIG. 1. SDS-PAGE (A) and Western blot analyses (B) of lectin-purified *P. carinii* gp120 preparations. (A) Lane 1, 150 μl of lectin-purified mouse *P. carinii* gp120; lane 2, 35 μl of lectin-purified ferret *P. carinii* gp120; lane 3, 150 μl of lectin-purified normal mouse lung; lane 4, 35 μl of lectin-purified normal ferret lung. Proteins were demonstrated by silver stain. (B) Western blot using a *P. carinii* gp120 specific monoclonal antibody to confirm that the lectin-purified preparations are ferret *P. carinii* gp120 (lane 1) and mouse *P. carinii* gp120 (lane 2).

Additional experiments were performed to confirm that the observed proliferative response was due to gp120 rather than to some other lung antigen which may have contaminated our preparations. Experiments using lymphocytes obtained after immunization with crude lung homogenates demonstrated a specific response to gp120 (Table 1). An SI of 4.9 (experiment 1) or 20.3 (experiment 2) was produced by a 1:4 dilution of ferret *P. carinii* gp120; similar dilutions of a normal lung preparation resulted in an SI of only 2.6 or 4.4, respectively. The 1:40 dilution of ferret *P. carinii* gp120 also resulted in significantly greater stimulation than seen with the corresponding dilution of normal lung preparations.

TABLE 1. Response to ferret *P. carinii* gp120 by BALB/c T cells obtained after immunization with homogenate of *P. carinii*-infected ferret lung

Stimulant (dilution or amt)	[^{3}H]thymidine incorporation, cpm (mean ± SD)[a]	
	Expt 1	Expt 2
None	4,167 ± 2,019	1,186 ± 318
gp120 (1:4)	20,549 ± 3,382 (4.9)[b]	24,107 ± 11,296 (20.3)[b]
Normal lung (1:4)	10,610 ± 1,790 (2.6)	5,194 ± 500 (4.4)
gp120 (1:40)	17,823 ± 3,816 (4.3)[b]	10,042 ± 2,304 (8.5)[b]
Normal lung (1:40)	10,546 ± 2,982 (2.5)	2,843 ± 1,643 (2.4)
ConA (5 μg/ml)	41,133 ± 13,858 (9.9)	22,089 ± 4,412 (18.6)

[a] Values in parentheses are SIs.
[b] $P < 0.05$, compared with the value for normal lung.

TABLE 2. Response to ferret *P. carinii* gp120 by BALB/c T cells obtained after immunization with lectin-purified ferret *P. carinii* gp120

Stimulant (dilution or amt)	[^{3}H]thymidine incorporation, cpm (mean ± SD)[a]	
	Expt 1	Expt 2
None	1,366 ± 373	1,693 ± 566
gp120 (1:4)	3,837 ± 1,603 (2.8)	9,321 ± 4,173 (5.5)[b]
Normal lung (1:4)	2,440 ± 687 (1.8)	2,393 ± 899 (1.4)
gp120 (1:40)	1,775 ± 774 (1.3)	2,131 ± 593 (1.3)
Normal lung (1:40)	1,862 ± 642 (1.4)	1,778 ± 474 (1.1)
ConA (5 μg/ml)	25,195 ± 11,369 (18.4)	22,480 ± 9,436 (13.3)

[a] Values in parentheses are SIs.
[b] $P < 0.05$, compared with the value for normal lung.

Immunization with lectin-purified ferret *P. carinii* gp120 also resulted in specific recognition by T cells. Lymphocytes obtained from these animals showed SIs of 2.8 (experiment 1) and 5.5 (experiment 2) in response to the culture with lectin-purified ferret *P. carinii* gp120 (Table 2). The normal lung preparations at 1:4 and 1:40, as well as the lectin-purified ferret *P. carinii* gp120 preparation at 1:40, produced an SI of less than twice the background value.

Responses to gp120 after natural infection and recovery. SCID mice with *P. carinii* pneumonitis were reconstituted with normal BALB/c spleen cells and allowed to recover. TBLN cells were used in culture to determine their proliferative response to gp120 (Table 3). In the first of these experiments, the response to mouse *P. carinii* gp120 at 1:4 resulted in an SI of 6.1, while the SI in response to the normal lung control at 1:4 was 2.5. An even greater response to mouse *P. carinii* gp120 was seen in the second experiment, in which the SI in response to mouse *P. carinii* gp120 was 7.6 compared with an SI of 1.5 in response to the normal control. Interestingly, if ferret *P. carinii* gp120 was substituted for mouse *P. carinii* gp120 in the lymphocyte proliferation assays, there was no significant stimulation above the background level (data not shown).

Antibody response to gp120 after immunization or infection. Analyses of mouse sera after immunization with gp120 or crude homogenates and of the SCID mouse sera after recovery from *P. carinii* pneumonitis were done by Western blot. Mice immunized and boosted with lectin-purified ferret *P. carinii* gp120 developed a prominent antibody response, with titers of >1:156,250. The reconstituted SCID mice developed an antibody response to mouse *P. carinii* gp120 at titers of 1:1,250 to 1:6,250. Consistent with the finding that T cells from recovering SCID mice failed to proliferate in response to ferret *P. carinii* gp120, there also was no antibody response to ferret *P. carinii* gp120 detected in the SCID mouse sera (data not shown).

TABLE 3. Proliferation of reconstituted SCID mouse TBLN cells in response to mouse *P. carinii* gp120 after recovery from *P. carinii* pneumonitis

Stimulant (dilution or amt)	[^{3}H]thymidine incorporation, cpm (mean ± SD)[a]	
	Expt 1	Expt 2
None	522 ± 33	1,772 ± 914
gp120 (1:4)	3,206 ± 2,035 (6.1)[b]	13,396 ± 3,650 (7.6)[b]
Normal lung (1:4)	1,320 ± 231 (2.5)	2,890 ± 662 (1.6)
gp120 (1:40)	774 ± 190 (1.5)	5,751 ± 2,192 (3.3)[b]
Normal lung (1:40)	1,277 ± 939 (2.4)	2,596 ± 498 (1.5)
ConA (5 μg/ml)	26,315 ± 5,951 (50.4)	23,149 ± 4,205 (13.1)

[a] Values in parentheses are SIs.
[b] $P < 0.05$, compared with the value for normal lung.

DISCUSSION

The purpose of these studies was to begin to define the host cellular immune response to a *P. carinii* surface gp designated gp120. We have demonstrated that gp120 can elicit a specific in vitro proliferative response from T cells obtained from mice after immunization with *P. carinii* gp120 or from reconstituted SCID mice after recovery from natural infection with *P. carinii*. This indicates that gp120 is involved in the host immune responses to infection with *P. carinii*, possibly as a target in a coordinated cellular and humoral response. Our findings are consistent with the observation of Walzer et al. showing a prompt serum antibody response to *P. carinii* gp120 among rats after recovery from *P. carinii* pneumonitis (24).

A cellular immune response to *P. carinii* has been implicated in previous studies of in vitro proliferative responses to the organism (9, 12). Crude *P. carinii* cyst preparations were used to demonstrate the in vitro proliferation of lymphocytes obtained from normal human adults (12) and from patients with human immunodeficiency virus infection (9); the specific antigen or antigens responsible for this response were not defined. Utilizing lectin-purified gp120 from *P. carinii* of ferret and mouse origins, our studies now demonstrate a T-cell immune response to a specific *P. carinii* surface antigen. It has been recently stated that gp95, the human homolog of ferret and mouse *P. carinii* gp120, did not stimulate proliferation of peripheral blood mononuclear cells from normal adult humans (16). Although no details were mentioned as to how these studies were done, it is possible that the inability to demonstrate stimulation of human T cells by this antigen reflects low numbers of circulating T cells. If exposure to and infection with *P. carinii* occurs at an early age, as suggested by seroepidemologic data (13), then it would not be surprising that a healthy adult would have low circulating numbers of *P. carinii*-specific T cells. In contrast, our studies were done in a fashion which would optimize our ability to detect a *P. carinii* gp120-specific response.

Although the specific type of T cells responding to gp120 cannot be determined from our experiments, it is possible that these cells are $CD4^+$ helper cells involved in signaling B-cell production of specific antibody to gp120. After the transfer of splenic T cells to infected nude mice and rats, a prompt serum antibody response to *P. carinii* was noted as these animals recovered from *P. carinii* pneumonitis (2, 3). $CD4^+$ T cells are necessary for SCID mice to recover from naturally acquired *P. carinii* pneumonitis (10). There is also more direct evidence for the involvement of antibody as a host defense against *P. carinii*. Recent experiments have demonstrated that B cells, along with $CD4^+$ cells, are necessary for the recovery from *P. carinii* pneumonitis in the SCID mouse (11). Clearly, a strong antibody response to gp120 is mounted after infection with *P. carinii* (19, 24), and our studies confirm this production of antibody after both infection and immunization with either crude infected preparations or the lectin-purified antigen. Finally, passive immunoprophylaxis with a specific monoclonal antibody to gp120 confers partial protection against *P. carinii* infection of immunosuppressed animals (6). The importance of a humoral response in the protection or recovery from *P. carinii* infection is also implied in the many reports of

patients who develop *P. carinii* pneumonitis with only hypogammaglobulinemia as their defined immunodeficiency (1, 21). Therefore, it is our hypothesis that a humoral response to *P. carinii* gp120 is an important host defense mechanism against *P. carinii* and that T cells are necessary to provide a helper function for the production of specific antibody.

An interesting observation from our proliferation assays was that T cells from mice immunized with ferret *P. carinii* gp120 failed to respond to *P. carinii* gp120 of mouse origin. In a similar fashion, lymph node cells from reconstituted SCID mice infected with mouse *P. carinii* failed to respond to gp120 of ferret origin. Monoclonal antibodies to *P. carinii* antigens, especially gp120, have been developed, and most show host species specificity (7, 8, 14). The cross-reactive monoclonal antibody 5E12 is the only antibody described in the literature that recognizes gp120 antigens from many different species (7). This antibody is specific for the mannose-containing portions of the molecule (4). Therefore, it is possible that slight differences within T-cell epitopes of *P. carinii* gp120 from different host species account for the specific host cell-mediated responses seen here. It is also possible that the differences seen in our experiments reflect experimental artifacts secondary to our purification procedures. For example, the size of ferret *P. carinii* gp120 was unaffected by treatment with collagenase, whereas when collagenase treatment was eliminated from the isolation procedure of mouse *P. carinii* gp120, the size of the purified molecule was approximately 150 kDa (4). A limitation of our studies was that we were unable to increase the concentration of *P. carinii* gp120 in our lectin-purified preparations. This prevented us from doing formal antigen concentration-response curves and probably accounted for the lower SIs seen in the mice immunized with lectin-purified *P. carinii* gp120 compared with those immunized with the crude, infected lung homogenates. As the recombinant protein portion of *P. carinii* gp120 becomes available, it will be possible to optimize immunization and assay conditions as well as to definitively map and compare T-cell epitopes on this molecule obtained from various hosts.

In summary, we have demonstrated a specific T-cell response to a major surface gp of *P. carinii*, both after immunization and natural infection in an animal model. After either immunization or natural infection with recovery, mice developed a prominent antibody response to this protein. This suggests that gp120 is important in the immunopathogenesis of *P. carinii* pneumonitis and that further studies of the host response to this molecule may provide important information about the prophylaxis and treatment of the disease caused by *P. carinii*.

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