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Proliferative and Cytokine Responses to a Major Surface Glycoprotein of *Pneumocystis carinii*

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Naturally derived T-cell responses by rats to a 120-kDa major surface glycoprotein (MSG) of rat-derived *Pneumocystis carinii* were analyzed in vitro. Specific cytokines elicited by the T-cell response to the MSG were also identified. MSG was purified from rat-derived *P. carinii* by three different techniques: lectin affinity chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by electroelution, and size-exclusion high-performance liquid chromatography. The cell-mediated immunity of spleen cells isolated from Lewis rats with and without natural exposure to *P. carinii* to the purified MSG was studied. Exposure to *P. carinii* was monitored by the presence or absence of serum antibodies to *P. carinii* antigens by Western blotting (immunoblotting). A T-cell proliferative response to the MSG was identified only with spleen cells isolated from rats exposed to *P. carinii* and peaked at 4 days. Flow cytometric analysis revealed that the percentage of CD4 cells was significantly increased during the proliferative response to MSG. MSG also elicited secretion of tumor necrosis factor alpha, interleukin-1, and interleukin-2, with peak activity of these cytokines occurring after 12, 24, and 48 h, respectively, of culture. These findings suggest that MSG is important in host T-cell recognition of and immune response to *P. carinii* by recruitment of inflammatory cells and cytokine production.

Pneumocystis carinii is an important opportunistic pulmonary pathogen in persons with AIDS and other immunosuppressed patients. Immunocompetent humans and rodents exposed to *P. carinii* develop vigorous humoral and cellular immune responses, as evidenced by antibody production, delayed hypersensitivity, and blastogenic assays (9, 13, 15, 31, 38, 43). This exposure is also accompanied by production of cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6 (4–6, 21, 39). Since most of these studies were performed with crude, whole-organism preparations, it has been difficult to determine the specific *P. carinii* antigen(s) eliciting these responses.

P. carinii contains a prominent surface glycoprotein called by names such as gp120, gpA, or major surface glycoprotein (MSG) which has been the subject of considerable investigative interest (10, 23, 34). Much of the confusion concerning the nomenclature and molecular weight of MSG can be attributed to differences in the *P. carinii* host of origin and method of purification. This antigen complex has the following characteristics: it is encoded by a family of genes; it has a molecular mass under reducing conditions of 95 to 140 kDa; it has a pK of 5.0 to 6.9; it has shared (carbohydrate) as well as species-specific epitopes; and about 10% of the molecular mass is composed of N-linked carbohydrates rich in glucose, mannose, and *N*-acetylglucosamine residues (10, 11, 14, 16, 22, 23, 26–28, 34, 42). One important function of MSG is to mediate the attachment of *P. carinii* to host cells (33). The administration of monoclonal antibodies directed against MSG has been of therapeutic value (12).

Previous reports have demonstrated that MSG can elicit cell-mediated immune responses (9, 13). In these studies, mice were naturally exposed to *P. carinii* or had been injected with MSG prepared from rat or ferret *P. carinii*. In the present study, we have examined the cellular immune responses and cytokine production elicited by different preparations of rat *P. carinii* MSG in rats with naturally acquired *P. carinii* infection.

MATERIALS AND METHODS

Animals. Two groups of adult Lewis rats, of either sex, were obtained from Charles River Laboratories (Wilmington, Mass.). The first group of animals were retired breeders that had been raised in a protected environment free from exposure to common viruses and *P. carinii*. Upon arrival, this group was placed in a Bioclean Porta-Room to prevent any exposure to *P. carinii*. The second group of animals were also retired breeders but had been raised in a conventional colony providing ample environmental exposure to *P. carinii*. In all cases, the presence or absence of antibodies to *P. carinii* was ensured by Western blot (immunoblot) analysis performed as described previously (37).

Microorganisms. *P. carinii* organisms were isolated from the lungs of corticosteroid-treated Sprague-Dawley rats as described previously (37). Briefly, infected lungs from immunosuppressed animals were removed, minced, and ground through a 60-mesh screen in phosphate-buffered saline (PBS). The homogenate was centrifuged at 1,000 × *g* for 10 min at 4°C, and the resulting pellet was treated with 0.85% ammonium chloride to lyse erythrocytes. The pellet was washed twice and resuspended in PBS. Aliquots of the homogenate were examined by Diff-Quick stain (American Scientific Products, McGaw Park, Ill.) for quantitation of organisms. Samples of the homogenate were also streaked on Mueller-Hinton and Sabouraud dextrose agar plates for detection of any bacterial or fungal contaminants. Specimens with no detectable contamination were pooled and digested with Zymolyase 100 T (ICN Biomedicals, Costa Mesa, Calif.).

Antigen purification. (i) ConA affinity chromatography. A soluble species of MSG was purified by Zymolyase digestion and concanavalin A (ConA) affinity chromatography as described previously (27). Briefly, rat-derived *P. carinii*

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organisms were digested with Zymolyase (ICN) for 30 min at room temperature (7). The treated organisms were then fractionated by differential centrifugation at 1,000 × *g*, 10,000 × *g*, and 100,000 × *g*. The 100,000 × *g* supernatant was applied to a ConA-Sepharose 4B (Sigma Chemical Co. St. Louis, Mo.) column. To prevent leaching of the ConA during the purification, the ConA-Sepharose had been further stabilized by glutaraldehyde cross-linking (24). The column was washed extensively, and bound antigen was eluted with a gradient of 0 to 500 mM methyl-D-mannopyranoside. Fractions containing MSG were identified by protein assay, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and Western blotting. The MSG-containing fractions were pooled, dialyzed overnight against 0.1 M ammonium bicarbonate, and lyophilized. Purified MSG preparations were not contaminated with ConA as demonstrated by Western blotting with a ConA-specific polyclonal antibody (E*Y Labs, San Mateo, Calif.; data not shown).

(ii) Electroelution. Whole, rat-derived *P. carinii* organisms were run under reducing conditions on 10% SDS-polyacrylamide gels by the method of Laemmli (25). The gels were stained with Pro-Green (Integrated Separation Systems, Champaign, Ill.); the bands containing MSG were excised, destained, and allowed to equilibrate in Tris-glycine elution buffer (192 mM glycine, 25 mM Tris-HCl [pH 8.3]) for 15 min at room temperature. Gel strips were cut into small pieces and placed in an Elutrap apparatus (Schleicher & Schuell, Inc., Keene, N.H.). Electroelution was performed overnight at 100 V. The following day, the eluate was recovered, assayed for protein content by Coomassie protein assay, dialyzed overnight against 0.1 M ammonium bicarbonate, and lyophilized.

(iii) Size-exclusion HPLC. MSG contained in the 100,000 × *g* supernatant from Zymolyase digestions of rat-derived *P. carinii* was purified by high-performance liquid chromatography (HPLC) as described previously (28). MSG was separated on the basis of size by using a Macrosphere GPC 150 column (Alltech Associates, Deerfield, Ill.) under isocratic conditions with 0.1 M KH_2PO_4 (pH 7.0)–0.2 M NaCl as the mobile-phase buffer. The flow rate was kept constant at 1.5 ml/min, and fractions were collected every 30 s. Aliquots were removed, and the presence of MSG was confirmed by SDS-PAGE. Fractions containing isolated MSG were pooled, dialyzed overnight against 0.1 M ammonium bicarbonate, and lyophilized.

Purified MSG was analyzed by SDS-PAGE and Coomassie blue staining (Fig. 1). A 120,000-molecular-weight protein was detected in each preparation and identified as MSG by its reactivity with monoclonal antibodies (data not shown). Proteins in the range of 97 to 100 kDa in the affinity-purified and HPLC-purified preparations also reacted with MSG-specific monoclonal antibodies. The lower-molecular-weight bands are not detected when SDS-polyacrylamide gels are run under nonreducing conditions and may be due to partial degradation of the MSG which occurs during purification. Alternatively, they may be processing intermediates or different forms of MSG. Recently, it has been reported that the MSG of rat-derived *P. carinii* represents a family of related proteins encoded by unique genes (23). Although the origin of these additional bands remains unclear, no significant differences in cytokine secretion or proliferation response was observed between the different MSG preparations.

Preparation of lymphocytes. Single-cell suspensions were prepared essentially as described previously (38). Briefly,

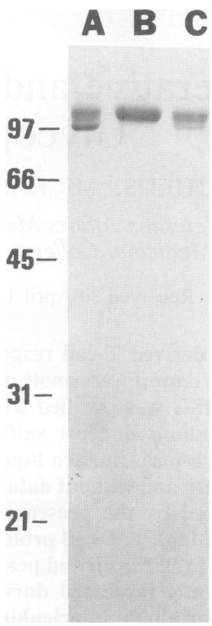

FIG. 1. Coomassie blue-stained SDS-PAGE analysis of MSG (2.5 μg per lane) purified by three different methods, namely, lectin affinity chromatography (lane A), electroelution from SDS-polyacrylamide gels (lane B), and size-exclusion HPLC (lane C).

spleens from six to eight rats per group per experiment were placed in PBS without divalent cations and then passaged through a stainless-steel screen to obtain a single-cell suspension. Erythrocytes in the cell preparation were lysed in 0.85% ammonium chloride. The cells were washed and resuspended in RPMI 1640 containing 2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 5 × 10^{-5} M β-mercaptoethanol, and 10% heat-inactivated fetal calf serum (RPMI complete). Cells were enumerated with a hemacytometer and checked for viability by trypan blue exclusion. Only cells with ≥90% viability were utilized. For enriched T-cell populations, the spleen cell suspensions were separated over nylon wool columns (8). All reagents, including purified *P. carinii* isolates and MSG preparation, had undetectable levels of endotoxin, as verified by the *Limulus* amebocyte assay (<0.125 U/ml; Whittaker M. A. Bioproducts, Walkersville, Md.).

Lymphocyte proliferation assays. Proliferation assays were done as described previously (38). Enriched T-cell populations, at a concentration of 10^5 per well in RPMI complete, were cultured with 1.5 × 10^5 syngeneic spleen cells per well which had been treated with 25 μg of mitomycin (Sigma) per ml. This group of cells acted as antigen presentation cells. Cells were cultured for 3 to 7 days in the presence of ConA (1 μg per well) or MSG (1 μg per well), isolated by three different methods, at 37°C in 5% CO_2. After this incubation, the cultures were pulsed with 1 μCi of [^{3}H]thymidine per well (2 Ci/mmol; New England Nuclear, Boston, Mass.) for 18 h prior to harvest onto glass fiber filter strips with an automatic multiple-sample harvester (Mini-Mash II; Whittaker). The samples were then counted in a liquid scintillation counter. The data were expressed as mean counts per minute ± standard deviations of triplicate cultures.

FACS analysis. For T-cell analysis, 10^6 cells in 50 μl were incubated with a fluorescein-conjugated mouse anti-rat CD8 monoclonal antibody (PharMingen, San Diego, Calif.) and/or

phycoerythrin-conjugated mouse anti-rat CD4 monoclonal antibody (PharMingen, San Diego, Calif.) at 4°C for 20 min, washed three times in cold Hanks' balanced salt solution (HBSS), and resuspended in HBSS prior to flow cytometry analysis. The cells were analyzed by flow microfluorometry in a fluorescence-activated cell sorter (FACS III; Becton Dickinson, Mountain View, Calif.) as described previously (41).

Bioassays of cytokines. For cytokine levels, spleen cell cultures (10^6/ml) were incubated with MSG (10 μg/ml) isolated by HPLC and ConA affinity chromatography for various time points. Supernatants from the MSG-stimulated cultures of spleen cells were collected, filtered through a 0.45-μm-pore-size filter, and assayed for cytokines. The presence of IL-2 was detected by using the murine IL-2-sensitive cell line CTLL-2 (American Type Culture Collection, Rockville, Md.) as described previously (30). Briefly, serial twofold dilutions of the samples were placed in triplicate wells of a 96-well microtiter plate along with 5×10^3 CTLL-2 cells. Cultures were incubated at 37°C in 5% CO_2 for 18 h, pulsed with 1 μCi of [^{3}H]thymidine per well (2 Ci/mmol; New England Nuclear), and harvested 6 h later. Units of IL-2 per milliliter were calculated from the 50% end point of a reference curve constructed from a commercial IL-2 preparation free of lectin (Genzyme, Cambridge, Mass.).

The presence of IL-1 was detected by using the murine cell line D10.G4.1 (American Type Culture Collection) as described previously (20). Briefly, D10.G4.1 cells were cultured at 10^5 cells per ml in Clicks medium (Irving Scientific, Santa Ana, Calif.) supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, 5×10^{-5} M β-mercaptoethanol, 5% fetal calf serum, and 2.5 μg of ConA per ml. Serial twofold dilutions of the supernatants from spleen cell suspensions incubated for 0.5 to 5 days with MSG were prepared in triplicate and added to the cells. Cultures were incubated for 3 days at 37°C in 5% CO_2. In the last 6 h of incubation, the cells were pulsed with 1 μCi of [^{3}H]thymidine per well (2 Ci/mmol; New England Nuclear), harvested onto glass fiber filters, and counted with a liquid scintillation counter.

The presence of TNF-α was detected by the conventional L929 cell cytotoxicity photometric assay in the presence of 1 μg of actinomycin D per ml (19). Twofold serial dilutions of the supernatants were incubated for 16 to 18 h with L929 in 96-well microtiter plates in Eagle minimal essential medium (GIBCO, Gaithersburg, Md.) containing 5% fetal calf serum. After staining the microtiter plates with crystal violet (0.05% in 20% ethanol) and washing and drying them, cell survival was quantitated by using a microtiter plate reader (Bio-Tek, Winooski, Vt.) at 595 nm. A unit of TNF-α was defined as the reciprocal of the dilution of commercially available recombinant TNF-α (Genzyme) producing 50% lysis.

mRNA isolation and analysis. Total RNA was isolated from spleen cell suspensions incubated in the presence of medium or medium supplemented with MSG (100 μg/ml) for various time points by using RNAzol (Tel-Test, Inc., Friendswood, Tex.). Briefly, cells were lysed in 2 ml of RNAzol per 10^7 cells by several passages of the lysate through a pipette. Two hundred microliters of chloroform was added to each 2 ml of homogenate. Samples were placed on ice for 15 min prior to centrifugation at 12,000 × *g* at 4°C for 15 min. The aqueous phase was collected and precipitated. The quantity and integrity of the RNA were routinely tested by A_{260} and ethidium bromide fluorescence of RNA electrophoresed through 1.2% nondenaturing agarose gels.

TABLE 1. Response to lectin affinity-purified MSG by Lewis rat T cells

Day	Rat group[a]	[^{3}H]thymidine incorporation (cpm [mean ± SD])[b]		
		Medium	ConA	MSG
3	+	1,636 ± 312	57,878 ± 9,179	32,300 ± 5,012[c]
3	−	1,063 ± 193	67,853 ± 10,495	5,126 ± 1,392
4	+	1,214 ± 300	55,792 ± 11,188	41,059 ± 9,427[c]
4	−	1,044 ± 377	62,240 ± 9,063	5,559 ± 2,787
5	+	976 ± 472	28,020 ± 8,589	28,659 ± 6,855[c]
5	−	806 ± 396	20,076 ± 7,899	4,055 ± 2,212

[a] +, rats with natural exposure to *P. carinii*; −, unexposed rats.
[b] Mean of three independent experiments (n = 18).
[c] $P \leq 0.05$ compared with the value of *P. carinii*-negative animals.

Northern blot (RNA) and slot blot analyses were done essentially as described previously (40). For Northern analysis, RNA (2 to 5 μg), denatured in formamide-formaldehyde, was fractionated through 1.2% formaldehyde-agarose gels. For slot blot analysis, the RNA (2 to 5 μg) was denatured with glyoxal-dimethyl sulfoxide at 50°C for 1 h. Both were transferred onto nylon membranes (0.45-μm pore size; Boehringer-Mannheim, Indianapolis, Ind.). Membranes were baked at 80°C for 1 h and hybridized to a γ-^{32}P-5′-end-labeled oligonucleotide (IL-1, IL-2, β-actin). Membranes were hybridized for 18 h; several stringency washes and autoradiography (Fuji) at −70°C with intensifying screens followed. Total RNA levels were assessed by the expression of β-actin mRNA determined by probing the same membranes with radiolabeled β-actin mRNA.

Statistics. The two-tailed Student's t test or analysis of variance, followed by a multicomparisons test, was used to determine if differences existed between data sets as appropriate. The alpha value for all statistical analysis was less than 0.05.

RESULTS

T-cell responses to MSG. Rats with natural environmental exposure to *P. carinii* as well as naive animals showed high proliferative responses to the mitogen ConA within 3 days of incubation (Table 1). However, the exposed rats displayed significantly greater responses to MSG than the unexposed rats throughout most of the experiment. The peak response to MSG occurred on day 4.

The proliferative responses obtained with MSG purified by lectin affinity chromatography were then compared with those achieved with MSG isolated either by SDS-PAGE with electroelution or size-exclusion HPLC (Table 2). Similar blastogenic responses were observed in all of the MSG preparations. The animals that were unexposed to *P. carinii*

TABLE 2. Response by Lewis rat T cells to different preparations of MSG

Day	[^{3}H]thymidine incorporation (cpm [mean ± SD]) prepared by[a]:		
	Elutrap[b]	HPLC[b]	Lectin affinity[b]
3	31,240 ± 3,090	26,040 ± 6,317	29,658 ± 8,932
4	39,033 ± 8,659	37,133 ± 7,498	37,539 ± 9,842
5	29,781 ± 5,378	26,720 ± 4,421	31,294 ± 5,319

[a] Mean of one experiment (n = 6).
[b] MSG purification technique.

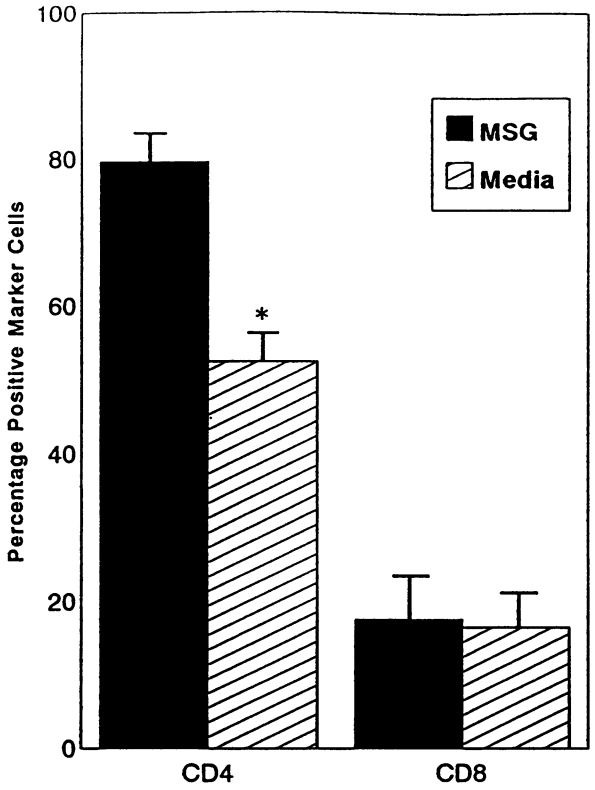

FIG. 2. Cellular composition of spleen cell suspensions from Lewis rats with environmental exposure to *P. carinii* incubated for 3 days with lectin-purified MSG or medium alone. Surviving cells were isolated on Ficoll-Hypaque gradients, and the percentage of marker-positive cells was determined by FACS analysis. The data represent the means of three separate experiments (n = 12). *, $P \leq 0.05$ compared with the value of cells incubated in medium alone.

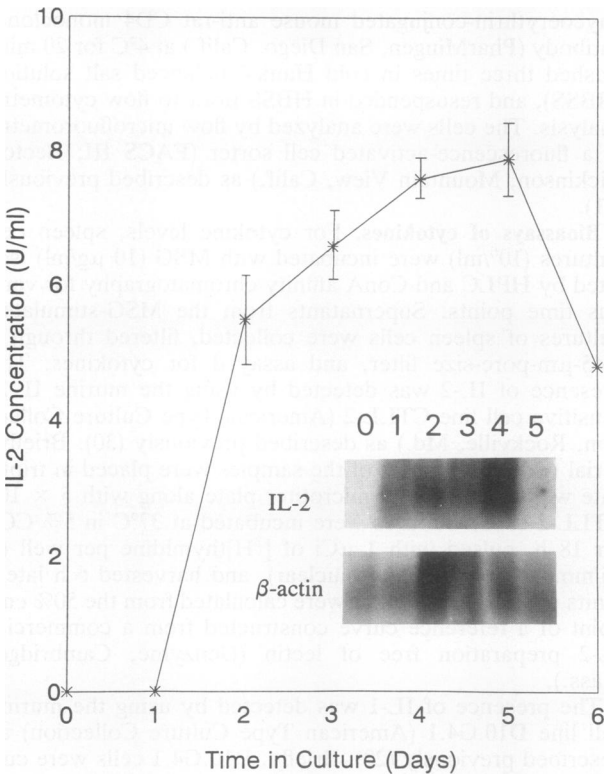

FIG. 3. MSG stimulates the release of IL-2 from spleen cell cultures of Lewis rats previously exposed to *P. carinii*. Cells were incubated for 0 to 6 days with MSG, and the culture supernatant was assessed for IL-2 activity with CTLL-2 cells. RNA was isolated from the same spleen cells and then subjected to Northern blot analysis. The blot was probed with γ-^{32}P-labeled oligomer specific for rat IL-2 and then with a γ-^{32}P-labeled oligomer specific for β-actin. IL-2 activity presented is the result of three separate experiments (n = 18), while the Northern blot is data from a single experiment.

did not demonstrate any significant proliferative response to any preparation of MSG (data not shown).

Cellular composition of MSG-stimulated cultures of spleen cells of Lewis rats. Spleen cells isolated from Lewis rats naturally exposed to *P. carinii* were cultured with 25 μg of MSG per ml, and after 3 days of incubation, the cells were centrifuged over Ficoll-Hypaque gradients. The percentages of recovered cells that expressed CD4 or CD8 were determined by FACS analysis. As shown in Fig. 2, spleen cells from retired breeder Lewis rats that had been incubated in medium alone displayed normal frequencies of CD4 and CD8 populations. There were also no significant differences in the frequency of CD8 cells between the groups. By contrast, there was a significant rise in the frequency of CD4 cells incubated with MSG, suggesting that these cells were active in the host response.

Production of IL-2 by spleen cells in response to MSG. Three separate experiments were completed for determining IL-2 levels by using MSG prepared by lectin affinity chromatography (two experiments) and HPLC (one experiment). Spleen cells were cultured with 25 μg of MSG per ml, and supernatants were collected daily for 6 days and assayed for IL-2 activity by using the IL-2-dependent cell line CTLL-2. As shown in Fig. 3, the supernatants from the spleen cell cultures of animals previously exposed to *P. carinii* produced high levels of IL-2 within 2 days of incubation with MSG. The IL-2 level remained high until 6 days of incubation, when the levels then began to drop. IL-2 mRNA was detected by Northern blot analysis after 1 day of incubation with MSG and displayed a strong signal for 4 days. By contrast, spleen cells of unexposed rats incubated with MSG exhibited no detectable IL-2 or expression of mRNA (data not shown).

Production of IL-1 in response to MSG. Figure 4 demonstrates the results of three separate experiments investigating IL-1 levels in the supernatants from spleen cell cultures that had been incubated with MSG (25 μg/ml) isolated by lectin affinity chromatography (two experiments) and HPLC (one experiment) for 6 days from animals previously exposed to *P. carinii*. The IL-1-dependent cell line D10.G4.1 was used to investigate the IL-1 levels. Serial dilutions of the supernatants were utilized for the cytokine levels. As can be seen, there were significant levels of IL-1 in the supernatants from these cultures after 24 h of incubation with MSG, and these levels decreased as incubation continued. When examining the mRNA levels for IL-1 by slot blot analysis, the spleen cell cultures expressed IL-1 mRNA after stimulation with MSG within 24 h. Supernatants of spleen cells from unexposed rats showed no detectable IL-1 or expression of mRNA (data not shown).

Production of TNF-α in response to MSG. Figure 5 demonstrates the levels of TNF-α secreted in response to incu-

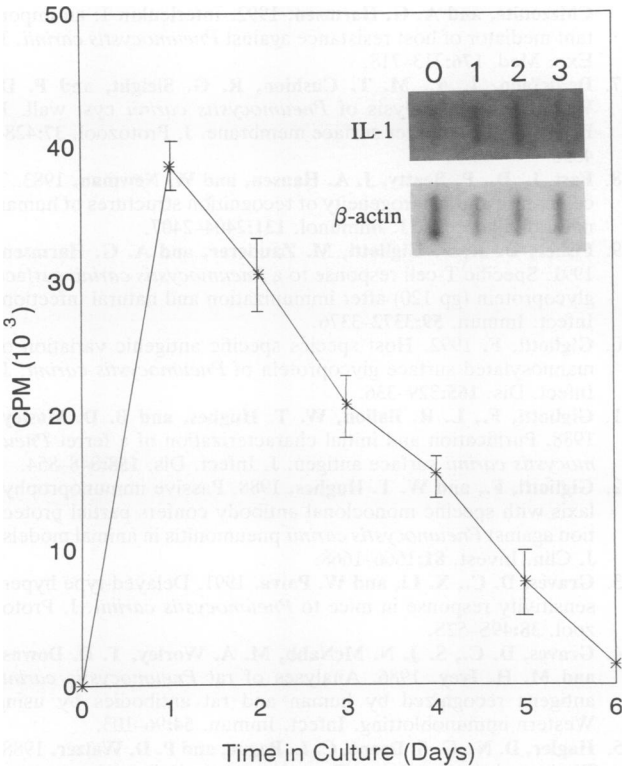

FIG. 4. Production of IL-1 from spleen cell cultures of Lewis rats naturally exposed to *P. carinii* after incubation with MSG. Spleen cells were incubated for 0 to 6 days with purified MSG, and the supernatants were assayed for IL-1 activity by using D10.G4.1 cells. RNA was isolated from the spleen cells and analyzed by slot blot analysis by using a γ-^{32}P-labeled oligonucleotide specific for rat IL-1 and then a γ-^{32}P-labeled oligomer specific for β-actin. IL-1 activity presented is the result of three separate experiments (n = 18), while the RNA slot blot is data from a single representative experiment.

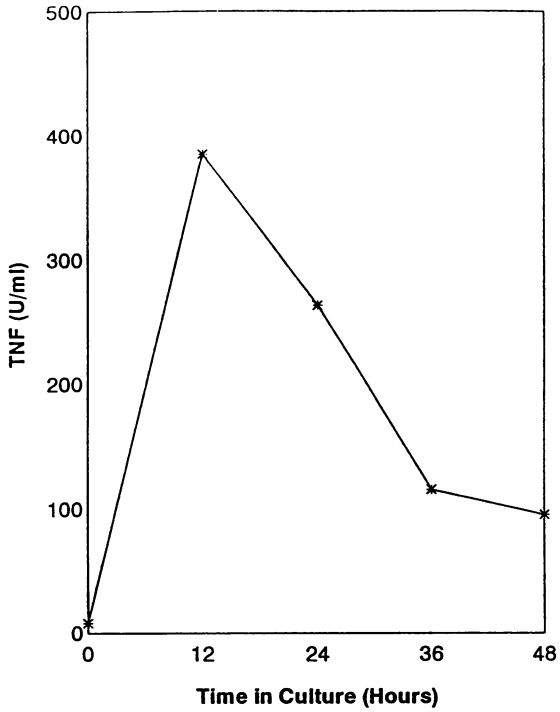

FIG. 5. Spleen cell cultures of *P. carinii*-exposed Lewis rats release TNF-α in response to incubation with MSG. Cell cultures were incubated for 0 to 48 h with MSG, and supernatants were assessed for TNF-α activity by using the cytolytic L929 cell assay. Data presented are the mean of three experiments (n = 18).

bation with MSG isolated by lectin affinity chromatography and HPLC. To begin investigating the cell population responsible for TNF-α secretion, spleen cell suspensions were either incubated with MSG or allowed to adhere, and nonadherent cells were removed and then incubated with MSG. Supernatants were then collected and assayed for cytokine levels. There was a peak of splenic macrophage-derived TNF-α production within 12 h of incubation which declined as incubation time increased. No difference was exhibited between the adherent cell population or the whole-spleen-cell population in the levels of TNF-α secreted in response to either lectin affinity- or HPLC-purified MSG. Spleen cell suspensions from animals that had no prior exposure to *P. carinii* did not exhibit significant levels of secreted cytokine when incubated with MSG (data not shown).

DISCUSSION

This study has shown that MSG purified from rat-derived *P. carinii* elicits a specific in vitro T-cell response in rats naturally exposed to *P. carinii* via the environment. The data reported here confirm and extend the findings of Fisher et al. (9) in two important ways. First, the *P. carinii* antigen preparation and responding splenocytes were obtained from the same animal host species. Such data are needed to accurately characterize the host immune response to this infection in light of emerging information showing differences in MSG from *P. carinii* infecting different animals (16, 23). Second, similar proliferative responses were obtained with MSG purified by three different techniques. This approach minimized the chance of contaminants or other factors related to the methods of preparation contributing to the proliferative response and thus enhanced the specificity of our findings.

There have been numerous studies, using both SCID mice and T-cell-depleted mice, which indicate that T cells, specifically CD4 cells, are critical in host defense against *P. carinii* infection (2, 17, 35, 36). In the present report, incubation of spleen cell suspensions from animals exposed to *P. carinii* with MSG resulted in an increase in the percentage of CD4 cells by FACS analysis. This phenomenon has also been documented in the case of *Mycobacterium tuberculosis*, in which an isolated 65-kDa mycobacterial antigen was shown to increase the percentage of CD4 cells while also affecting the proliferative response (3). This increase in CD4 cells after incubation with MSG indicates that these cells are involved in host T-cell recognition of *P. carinii*.

It is well known that T-helper cells produce IL-2 when stimulated with a specific antigen. In the present study, when the levels of secreted IL-2 were investigated, there was a significant increase in IL-2 present in the supernatants from the spleen cell suspensions of exposed rats in response to incubation with MSG. Although IL-2 has multiple effects on the immune system, one possible function for this cytokine would involve B-cell signaling and antibody production (1). *P. carinii* maintains an extracellular existence within

alveoli, and there is increasing evidence from cell transfer and passive immunotherapy studies for B cells in the host defense against the organism (12, 18, 35).

In response to activation by antigen, monocytes produce a number of biologically active cytokines, including IL-1 and TNF-α (20). Several reports have suggested that these cytokines are important in host defenses against *P. carinii* (2, 4–6, 17, 21, 32, 39). Chen et al. demonstrated that endogenous levels of IL-1 were significantly increased in SCID mice with pneumocystosis and that this cytokine exerted its greatest effects early in the disease (6). In the present study, MSG stimulated the production of IL-1 within 24 h of incubation in spleen cells of rats previously exposed to *P. carinii*; however, the spleen cell subpopulation responsible for this cytokine secretion is not known at this time. One role for IL-1 is to enhance T-cell activation and IL-2 production (29).

The present study has also shown that, after incubation with MSG, there is a significant release of TNF-α from splenic macrophages from animals exposed naturally to *P. carinii*. This TNF-α release occurred within 12 h of incubation with MSG. Recent studies investigating TNF-α levels in both CD4-depleted and normal mice demonstrated that this cytokine was released early in *P. carinii* infection and preceded the influx of inflammatory cells into the lungs (21). It has been suggested that *P. carinii* binds TNF-α and is directly lethal in vitro (32); however, other investigators showed that in immunocompromised hosts naturally exposed to *P. carinii*, TNF-α on its own does not directly kill *P. carinii* or activate effector cells to kill the organism (5). Thus, the precise role played by TNF-α in the host immune response to *P. carinii* requires further investigation.

In conclusion, the present report has shown that MSG elicits cellular immune responses and cytokine production in rats previously exposed to *P. carinii*. These data add to the growing body of information pointing to the importance of MSG in the host-parasite relationship in *P. carinii* infection.

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