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Cellular Responses to a 55-Kilodalton Recombinant *Pneumocystis carinii* Antigen

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The host-parasite interaction in *Pneumocystis carinii* pneumonia is poorly understood. In recent years, two major groups of *P. carinii* antigens have been identified. One class of antigens is characterized by a broad band of immunoreactivity between 45 and 55 kDa in *P. carinii* derived from rats. This antigen complex is the *P. carinii* antigen most commonly found in respiratory tract specimens and most frequently recognized by the host immune response. The availability of a recombinant antigen has permitted studies focusing on the cellular and humoral responses to a single antigen within this class, p55. In this study, we have demonstrated that the p55 antigen elicits a cell-mediated immune response in animals previously exposed to *P. carinii*. Under conditions of natural exposure, the 5′ portion of the molecule, p55(1-200), appears immunologically silent, failing to elicit lymphocyte proliferation or cytokine secretion. Following active immunization, the 5′ portion is capable of stimulating lymphocyte proliferation. The 3′ portion, p55(268-414), has at least one immunodominant region which contains a 7-amino-acid repeat motif. The cells responding to p55 include a CD4$^+$ T cell which secretes a Th1 cytokine pattern. A detailed understanding of the host-parasite interaction will facilitate the development of immunoprophylaxis and immunotherapy for *P. carinii* infection.

Pneumocystis carinii is an important opportunistic pulmonary pathogen, but the immune response and pathogenesis of infection caused by this organism are poorly understood. In recent years, two major groups of *P. carinii* antigens have been identified (11, 17, 18, 23, 43). One group consists of a major surface glycoprotein (MSG) complex with a molecular mass of 95 to 120 kDa, depending on the host species and method of purification (8, 9, 24, 25). MSG and other *P. carinii* antigens contain species-specific as well as cross-reactive antigens. Molecular analysis has revealed that MSG is actually a family of closely related proteins encoded by multiple genes (13, 19, 38, 42). MSG plays an important role in the interaction of *P. carinii* with host cells (22, 29, 30) and stimulates both humoral and cellular immune responses (6, 28, 36, 40).

The other antigen group is characterized by broad bands of 45 to 55 kDa and 35 to 45 kDa in *P. carinii* strains derived from rats and humans, respectively (11, 17, 18, 23, 43). These bands have shared as well as species-specific epitopes and appear to have similar biochemical properties. This antigen complex is the *P. carinii* antigen most commonly found in respiratory tract specimens and most frequently recognized by the host immune response (28, 36); thus, it serves as an important marker of infection.

The cellular localization on *P. carinii* and function of this antigen group are unknown, and attempts at purification have been unsuccessful. However, recent progress in cloning and sequencing the gene encoding a 55-kDa antigen (p55) of rat *P. carinii* offers insights into the possible function of this moiety (35, 37). The predicted amino acid sequence includes the presence of a secretory signal peptide at the amino terminus, an N-linked glycosylation site, and a potential phosphatidylinositol anchor at the carboxy terminus, suggesting that this antigen is membrane associated. The presence of an Arg-Gly-Asp (RGD) sequence at residues 80 to 82 is a potential site of attachment to host cells (33). There is also a repeated motif rich in glutamic acid residues; in other microbes (e.g., members of the genus *Plasmodium*), such repeated motifs are immunodominant and are thought to divert the host immune response from other hidden or cryptic epitopes which are more important in protective immunity ("smoking-gun effect") (1).

In previous reports, we have shown that rats which were naturally infected with *P. carinii* exhibited humoral and cellular immune responses to p55 (35, 37). We undertook the present study with the following aims: to compare the cellular immune responses to p55 following naturally acquired infection and active immunization, to analyze the cytokine production and phenotype of the responding cell populations, and to localize the major site(s) of reactivity on the p55 antigen.

MATERIALS AND METHODS

Recombinant protein production. We have previously reported the isolation of a partial cDNA, pSK 10.2, encoding the 3′ portion of the 55-kDa antigen, and the generation of a cDNA, pSK 0-748, encoding the 5′ portion of the protein, by reverse transcription PCR (37). Two overlapping fragments, a *Pvu*I-*Kpn*I digestion fragment of pSK 10.2 and an *Eco*RV-*Stu*I fragment of pSK 0-748, were gel purified. A full-length cDNA was generated by denaturing these overlapping cDNA fragments, followed by annealing and extension in the presence of the Klenow fragment of DNA polymerase. The full-length cDNA was then amplified with primers (sense, GGAATTC CATATGAAGATATCTTTTCTTGCTATTTTTATA; antisense, TAGGGCGAATTGGGATCCGGGCCCCCCCTC) and *Taq* DNA polymerase. The primers contained unique *Nde*I and *Bam*HI restriction endonuclease recognition sequences 5′ to the region of homology to the p55 cDNA in order to permit directional cloning of the full-length cDNA into the expression vector pET 19b (Novagen, Madison, Wis.).

For expression of recombinant protein, the full-length cDNA p55(1-414) and the partial cDNA encoding the 5′ portion of the molecule, p55(1-200), were cloned into the

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plasmid pET 19b between the *Nde*I and *Bam*HI sites. The 3′ portion of the molecule, p55(268-414), was expressed in pUR as a β-galactosidase fusion protein and purified as previously described (35, 41). The authenticity of the constructs was verified by restriction mapping and sequencing across the junctions by dideoxy chain termination sequencing. pET fusion constructs were purified by metal chelation affinity chromatography over a Ni-nitrilotriacetic acid agarose column (Qiagen Inc., Chatsworth, Calif.). Recombinant fusion proteins were quantitated by the Coomassie blue protein assay (Pierce, Rockford, Ill.), and purity of the recombinant proteins was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Purified proteins were dialyzed and lyophilized for subsequent use in immunological assays. Following lyophilization, the purified proteins were reconstituted and tested for the presence of endotoxin by the *Limulus* amoebocyte assay (Whitaker Bioproducts, Walkersville, Md.). No detectable endotoxin (<0.125 U/ml) was found.

Animals. Adult Lewis rats, separated into two groups, were obtained from Charles River Laboratories (Wilmington, Mass.). One group consisted of 6-week-old rats raised in a protected environment free from exposure to common viruses and *P. carinii*. Upon arrival, this group of animals was placed in a Bioclean Porta-Room to prevent exposure to other sources of *P. carinii*. The second group of animals consisted of retired breeders which were 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 verified by Western blot (immunoblot) analysis.

Six-week-old virus-negative Lewis rats, housed under barrier conditions, were immunized subcutaneously with 50 μg of p55(1-200) protein in complete Freund's adjuvant. Control animals were immunized with complete Freund's adjuvant alone. Rats were sacrificed at 2 weeks, and serum was collected and stored at 4°C. Anti-*P. carinii* and anti-p55 antibodies were detected by Western blot as previously described (19).

Cell cultures. Single-cell suspensions of spleen cells were prepared from individual animals. The number of splenic mononuclear cells was quantitated with a hemacytometer, and cells were checked for viability by trypan blue exclusion. Viability was consistently 95% or better. For enriched T-cell populations, the spleen cell suspensions were separated over nylon wool columns. Following passage over a nylon wool column, the T-cell population was greater than 85% pure by fluorescence-activated cell sorter (FACS) analysis.

Lymphocyte proliferation assays. Spleen cell suspensions or enriched T-cell populations were prepared as previously described (37). Briefly, cells, plated at a concentration of 10^5/well, were cultured in 96-well, round-bottomed microtiter plates (Falcon, Lincoln Park, N.J.) in 0.1 ml of 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 bovine serum. Enriched T-cell populations were cocultured with (per well) 1.5×10^5 syngeneic spleen cells which had been treated with 25 μg of mitomycin (Sigma, St. Louis, Mo.) per ml for 30 min at 37°C, to act as antigen-presenting cells. All cultures were done in triplicate. Cells were cultured for 3 to 7 days in the presence of concanavalin A (1 μg/well), full-length recombinant p55 antigen (1 μg/well), the 5′ portion of p55, the 3′ portion of p55 antigen (1 μg/well), or a synthesized peptide (16-mer) of two repeat-motif units at 37°C with 5% CO_2. Following this incubation, cultures were pulsed with 1 μCi of [^{3}H]thymidine (2 Ci/mmol; New England Nuclear, Boston, Mass.) for 18 h and then cells were harvested 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 are expressed as mean counts per minute or as a stimulation index (mean counts per minute with antigen or mitogen/mean counts per minute in media).

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) 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 FACS (FACS III; Becton Dickinson, Mountain View, Calif.).

Bioassay of cytokines. For determining cytokine levels, spleen cell cultures were incubated with the three p55 constructs essentially as described elsewhere (40). Briefly, 10^6 cells per ml were plated with equimolar concentrations of the three constructs for various times. Supernatants from the cultures were collected, filtered through a 0.45-μm-pore-size filter, and assayed for cytokine levels. The presence of interleukin 2 (IL-2) was detected by using the murine IL-2-sensitive cell line CTLL-2 (American Type Culture Collection, Rockville, Md.). 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 with 5% CO_2 for 18 h and pulsed with 1 μCi of [^{3}H]thymidine (2 Ci/mmol; New England Nuclear) per well, and cells were harvested 6 h later. Units of IL-2 per ml 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 gamma interferon (IFN-γ) was determined by a rat IFN-γ enzyme-linked immunosorbent assay (ELISA) kit (Gibco, Gaithersburg, Md.) according to the manufacturer's instructions. Briefly, supernatants from the spleen cell suspensions incubated for 24 to 72 h with p55(1-414) or p55(1-200) were transferred in duplicate to microtiter wells precoated with a monoclonal antibody recognizing rat IFN-γ. Samples were incubated for 1 h at 37°C and then washed, after which a preformed detector complex, consisting of a biotinylated second mouse monoclonal antibody to rat IFN-γ and a streptavidin-alkaline phosphatase conjugate, was added to the samples. Incubation continued for 1 h at 37°C prior to the addition of the *p*-nitrophenyl phosphate substrate. Sample incubation continued until color development was complete, and the A_{405} was measured. A standard curve of concentrations was obtained by plotting absorbance versus the corresponding concentrations of the known standards.

The presence of IL-1 was detected by an IL-1-dependent cell line (40). Briefly, D10.G4.1 cells (American Type Culture Collection) were cultured at 10^5 cells per ml in Clicks media (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 concanavalin A per ml. Serial twofold dilutions of the supernatants from spleen cell suspensions incubated for 0.5 to 5 days with the three p55 constructs were prepared in triplicate and added to the cells. Cultures were incubated for 3 days at 37°C with 5% CO_2. In the last 6 h of incubation, the cells were pulsed with 1 μCi of [^{3}H]thymidine (2 Ci/mmol; New England Nuclear) per well, harvested onto glass fiber filters, and counted with a liquid scintillation counter.

The presence of tumor necrosis factor alpha (TNF-α) was detected by the conventional L929 cell cytotoxicity photometric assay in the presence of 1 μg of actinomycin D per ml (40). Twofold serial dilutions of the supernatants were incubated 16 to 18 h with L929 cells in 96-well microtiter plates in Dulbec-

TABLE 1. Proliferative response to p55 by Lewis rat T cells

Day[a]	[^{3}H]thymidine incorporation, cpm (mean ± SD[b]), on incubation with:		
	Media	Concanavalin A	p55(1-414)
3 (+)	1,628 ± 380	12,144 ± 3,063	4,233 ± 2,105
3 (−)	1,022 ± 275	12,357 ± 2,814	3,227 ± 1,559
4 (+)	1,330 ± 314	29,265 ± 2,891	15,380 ± 5,405[c]
4 (−)	1,438 ± 516	25,860 ± 3,880	3,650 ± 1,350
5 (+)	1,341 ± 421	32,231 ± 9,570	29,636 ± 3,811[c]
5 (−)	1,014 ± 288	29,664 ± 4,249	3,874 ± 516
6 (+)	879 ± 255	8,890 ± 2,044	24,524 ± 1,914[c]
6 (−)	1,203 ± 185	8,431 ± 1,378	2,444 ± 1,464
7 (+)	1,663 ± 551	8,966 ± 3,849	14,135 ± 6,442[c]
7 (−)	1,373 ± 661	6,486 ± 556	3,550 ± 600

[a] +, T cells from *P. carinii*-exposed rats; −, T cells from unexposed rats.
[b] Mean for three independent experiments; $n = 18$.
[c] $P \leq 0.05$ in comparison with the value for *P. carinii*-negative animals.

co's modified Eagle medium (Gibco) containing 5% fetal calf serum. After the plates had been stained with crystal violet (0.05% in 20% ethanol), washed, and dried, 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.

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

RESULTS

Humoral responses to p55. Antibody responses to p55 were analyzed under conditions of natural exposure and following immunization. Serum specimens from 10 unexposed and 10 *P. carinii*-exposed Lewis rats were assayed for the presence of antibodies to rat-derived whole *P. carinii*, p55(1-414), p55(1-200), and p55(268-414) by Western blot. No antibodies to whole *P. carinii*, p55(1-414), or any p55 fragment were detected in the naive unexposed rats. Anti-*P. carinii* antibodies were detected in 10 of 10 *P. carinii*-exposed animals. Two weeks following active immunization with p55(1-200), antibodies to *P. carinii*, the full-length p55(1-414), and the 5′ fragment, p55(1-200), were detected in the naive unexposed animals (data not shown).

Specific T-cell responses. The cell-mediated immune response to p55 was measured by the ability of splenocytes from Lewis rats to undergo blastogenesis. As shown in Table 1, splenocytes from both retired breeder rats which have been environmentally exposed to *P. carinii* and unexposed rats displayed good proliferative responses to the mitogen concanavalin A. Proliferative responses to p55(1-414) were significantly greater in splenocytes from exposed rats than in those from naive rats within 4 days of incubation, with peak responses occurring between day 5 and day 6. Proliferation was dependent on the presence of antigen and antigen-presenting cells. These data indicate that p55(1-414) is a specific T-cell antigen.

The proliferative responses obtained with p55(1-414) were then compared with those obtained with the different fragments of the protein at equimolar concentrations (Table 2). The 3′ portion, p55(268-414), elicited a pattern of proliferation similar to that of p55(1-414), which peaked at day 6. However, incubation with p55(1-200) did not invoke any proliferative response in purified T cells from the *P. carinii*-exposed Lewis rats. The most striking feature of the 3′ portion of the molecule is its 10 tandem repeats of a 7-amino-acid motif. To determine if the repeated domain induced part of the noted proliferative response to the 3′ portion, a 16-mer synthetic peptide consisting of two copies of the repeat motif (repeat peptide CPTEEEEKPTEEEEEY) was made. When the purified T cells from naturally exposed Lewis rats were incubated with the peptide, there was measurable proliferation which was not present in T cells from animals that had not been previously exposed to *P. carinii*. The stimulation index indicates that this portion is specifically recognized and may represent a T-cell epitope.

TABLE 2. Proliferative responses of T cells from Lewis rats to p55 as determined by stimulation index

Day	Response[a] to:			
	p55(1-200)	p55(268-414)	p55(1-414)	Repeat peptide[b]
5	2.7 ± 0.4	35.4 ± 6.6	23.1 ± 5.6	11.9 ± 3.1
6	2.8 ± 0.5	43.9 ± 7.5	33.1 ± 6.9	12.1 ± 2.6
7	3.8 ± 0.5	6.3 ± 2.1	6.2 ± 2.3	4.2 ± 2.2

[a] Values are presented as means ± standard deviations.
[b] The repeat peptide is a synthetic 16-mer consisting of two copies of CPTEEEEKPTEEEEEY.

Following immunization of naive unexposed animals with the 5′ fragment, p55(1-200), the proliferative responses of the purified T cells were altered. Table 3 demonstrates these results. In contrast to the results obtained with cells from animals under conditions of natural exposure, the 5′ portion, p55(1-200), elicited a significant proliferative response by day 4 in splenic T cells from immunized animals. In addition, the full-length p55(1-414) stimulated proliferation in the splenic T cells from immunized naive animals with a time course similar to that observed for the splenocytes from naturally exposed animals. Peak proliferative response of splenocytes from Freund's adjuvant-immunized control animals following incubation with p55(1-200) and p55(1-414) occurred between day 4 and day 5. Peak stimulation indices following incubation with p55(1-200) and p55(1-414) were 3.5 and 4.4, respectively. Proliferative responses seen following immunization with p55(1-200) were thus not due to nonspecific activation caused by the adjuvant.

Cytokine secretion patterns. To investigate the production of IL-2 from Lewis rats naturally exposed to *P. carinii*, spleen cells were incubated with 25 μg of p55(1-414), p55(1-200), or p55(268-414) per ml and at 1 to 6 days of culture, supernatants were collected and assayed for IL-2 activity. As shown in Table 4, incubation with both p55(1-414) and p55(268-414) resulted in the production of high levels of IL-2 within 2 days of culture, with IL-2 levels peaking at day 4 and dropping after 5 days. The 5′ portion, p55(1-200), did not stimulate any significant

TABLE 3. Proliferative responses of T cells from Lewis rats following immunization with p55(1-200)

Day	[^{3}H]thymidine incorporation, cpm (mean ± SD[a]), on incubation with:		
	Media	p55(1-200)	p55(1-414)
3	923 ± 425	10,224 ± 2,478	4,936 ± 1,284
4	1,228 ± 504	24,406 ± 1,686	15,504 ± 3,858
5	1,159 ± 316	27,734 ± 2,301	28,399 ± 2,996

[a] $n = 8$.

TABLE 4. Cytokine production by spleen cells from Lewis rats in response to p55

Addition to incubation medium	Amt of indicated cytokine produced			
	IL-2[a]	IFN-γ[b]	TNF-α[c]	IL-1[d]
None	0.1 ± 0.2	179 ± 56	23 ± 5.0	1,246 ± 383
p55(1-200)	2.3 ± 0.3	563 ± 112	115 ± 12.6	6,132 ± 764
p55(268-414)	9.1 ± 1.1	ND[e]	278 ± 22.4	22,357 ± 1,250
p55(1-414)	8.3 ± 0.7	6,257 ± 299	316 ± 28.5	24,801 ± 1,379

[a] Peak IL-2 levels were detected at day 4 after stimulation by the CTLL-2 assay. Values are expressed as IL-2 units ± SEM on the basis of values obtained for control wells with known concentrations of IL-2.

[b] Peak IFN-γ levels were detected at day 2 after stimulation by rat IFN-γ ELISA (Gibco). Values are expressed as picograms per milliliter on the basis of absorbances of known standards.

[c] Peak TNF-α levels, expressed as picograms per milliliter ± SEM, were detected at 12 h after stimulation by the L929 assay.

[d] Peak IL-1 levels were detected at day 1 after stimulation by using the D10.G4.1 cell line. Values are expressed as counts per minute ± SEM of incorporated [^{3}H]thymidine.

[e] ND, Not determined.

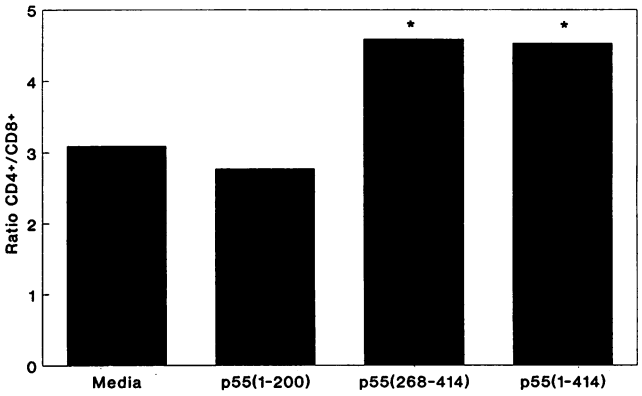

FIG. 1. Cellular composition of p55-stimulated cultures of spleen cells from Lewis rats environmentally exposed to *P. carinii*. Spleen cells were cultured for 4 days in media alone or with 25 μg of p55(1-200), p55(268-414), or p55(1-414) per ml, and the surviving cells were isolated on Ficoll-Hypaque gradients. The cell populations were then assessed by FACS analysis. The data are expressed as ratios of $CD4^+$ T cells/$CD8^+$ T cells. *, $P \leq 0.001$.

amount of IL-2 activity from the spleen cell suspensions of environmentally exposed rats. Splenocytes from *P. carinii* naive unexposed rats incubated with p55(1-414), p55(1-200), or p55(268-414) resulted in peak productions of 1.1 ± 1.3, 1.7 ± 0.1, and 2.5 ± 0.5 U, respectively.

IFN-γ is another cytokine produced primarily by activated T cells. It is an important mediator in the activation of macrophages for cytotoxicity and the destruction of intracellular parasites (e.g., *Toxoplasma gondii* and *Leishmania donovani*) (27). To examine whether p55(1-414) directs the secretion of IFN-γ, spleens were isolated from animals with previous environmental exposure to *P. carinii*. Cells were incubated with 25 μg of p55(1-414) or p55(1-200) per ml for 1 to 3 days. Supernatants were collected and assayed for IFN-γ by using a rat IFN-γ ELISA. Within 48 h of incubation, p55(1-414) was able to stimulate notable levels of secreted IFN-γ, which were significantly greater than those observed following incubation with the 5′ portion (Table 4). Incubation of spleen cells from rats without prior exposure to *P. carinii* with p55(1-414) resulted in the production of 1,429 ± 116 pg of IFN-γ per ml.

Recent studies suggest that another cytokine, TNF-α, is also an important component of host defense against *P. carinii* (4, 14, 16, 20, 39). To determine whether spleen cell suspensions isolated from Lewis rats with previous exposure to *P. carinii* exhibit increased TNF-α secretion on exposure to the antigen p55 or any portion thereof, these cells were incubated for 12 h with the antigen. Supernatants were collected, and the amount of TNF-α released was measured by the L929 cell cytotoxicity assay. As shown in Table 4, both p55(1-414) and p55(268-414) stimulated the release of high levels of TNF-α, 316 ± 28.5 and 278 ± 22.4 pg/ml (mean ± standard error of the mean [SEM]), respectively. Although the 5′ portion, p55(1-200), stimulated the release of 115 ± 12.6 pg/ml (mean ± SEM), this value was significantly less than the levels of TNF-α elicited by either p55(1-414) or p55(268-414). Spleen cells cultured in the absence of p55 did not release detectable TNF-α. Culture of splenocytes from rats without prior *P. carinii* exposure in the presence of p55(1-414), p55(1-200), and p55(268-414) stimulated the release of 68 ± 34, 19 ± 2, and 24 ± 46 pg of TNF-α per ml, respectively.

Endogenous IL-1 has been shown to be required for the clearance of *P. carinii* (5). By using the IL-1-dependent cell line D10.G4.1 for quantitation, IL-1 production elicited by the different portions of p55 was measured. The 5′ portion, p55(1-200), did cause the secretion of measurable amounts of active IL-1 within 24 h of incubation, although this level was still significantly less than those exhibited by either p55(1-414) or p55(268-414) (Table 4).

Cellular composition. Spleen cells from Lewis rats environmentally exposed to *P. carinii* were cultured in the presence of 25 μg of p55(1-414), p55(1-200), or p55(268-414) per ml and, after 4 days, surviving cells were isolated on Ficoll-Hypaque gradients. The population of T cells stimulated by each of the different portions of p55 was assessed by FACS analysis (Fig. 1). Over a 4-day period, the full-length protein, p55(1-414), resulted in the proliferation of predominantly $CD4^+$ cells. The same pattern was observed for the spleen cell suspensions incubated with p55(268-414). However, the percentage of $CD4^+$ cells in the suspension incubated with p55(1-200) did not increase significantly compared with that of those cells incubated in media alone. In all cases, the percentage of $CD8^+$ cells remained constant. $CD4^+/CD8^+$ ratios increased from day 3 through day 5, but by day 6, increased numbers of nonviable cells resulted in a high level of background staining.

DISCUSSION

Although previous studies have examined the host immune response to *P. carinii*, most have examined the response to whole organisms because of the difficulty in purifying specific *P. carinii* antigens. Several laboratories have shown that in both humans and rats, there is specific T-cell proliferation in vitro when the cells are challenged with whole *P. carinii* (7, 12). Work has been presented demonstrating that purified native antigen, MSG, also elicits a T-cell response in rats and SCID mice (6, 40); however, purification methods for antigen preparation differ. In addition, there is evidence that MSG belongs to a family of genes and that by purifying native antigen, one or numerous species of MSG may be represented (13, 19, 38, 42). The availability of a recombinant antigen has permitted studies focusing on the cellular and humoral responses to a single antigen of *P. carinii*, p55.

Under conditions of natural exposure, the 5′ portion of the antigen appears to be immunologically silent. Neither humoral nor cell-mediated responses are mounted to this portion of the molecule. In contrast, after active immunization, this portion

of the molecule is immunogenic and is able to stimulate a humoral and cell-mediated response. Similar cryptic or concealed epitopes in other microbes such as members of the genus *Plasmodium* and ectoparasites such as ticks, blowflies, and worms have been reported (26, 44, 45). Immunization with such a cryptic antigen has resulted in protective immunity in some instances (44). Epitopes might remain concealed because of the tertiary or quaternary structure of the protein (21), the physical location of the antigen (44), or diversion of the immune response by other immunodominant epitopes (1). The tertiary structure of insulin is important in the formation of the antigenic determinant recognized by insulin-specific T-cell hybridomas (10). Structural constraints associated with a given tertiary structure may be modified by association with other proteins or residues within the protein. This interaction can influence the quaternary structure, affecting its presentation to T cells by interfering with uptake of the protein by antigen-presenting cells or proteolytic degradation into peptides. Alternatively, the recombinant protein may be processed differently to the native antigen by antigen-presenting cells, resulting in antigenically different peptides.

An important aspect of the characterization of the T-cell epitopes of this *P. carinii* antigen is defining the nature of the T-cell responses induced or elicited. In the present study, we have demonstrated a specific in vitro T-cell proliferative response to the recombinant antigen p55(1-414) of rat-derived *P. carinii* in Lewis rats naturally exposed to the organism. We have further expanded these studies to include portions of p55 and the different reactions observed. The 5′ region of p55 did not induce a proliferative response, while the 3′ portion elicited a vigorous proliferative response in splenocytes from naturally exposed animals. A specific T-cell proliferative response was induced from the 5′ portion of p55 only after active immunization. The most prominent feature of the 3′ portion of p55 is its 10 tandem repeats of a glutamic acid-rich 7-amino-acid motif. A synthetic peptide containing two copies of this repeat did stimulate a specific T-cell response, indicating that this region is one T-cell epitope present in the protein. It will be of interest to know whether this 7-amino-acid motif is conserved in p55 homologs from *P. carinii* from different host species, thereby revealing an immunodominant, conserved T-cell motif.

Several laboratories have demonstrated the importance of $CD4^+$ lymphocytes in host defense against *P. carinii* (2, 14, 32). Other organisms, such as *Candida albicans* (31) and *Mycobacterium tuberculosis* (3), demonstrate a major role for $CD4^+$ T cells, which secrete a Th1 cytokine profile (IL-2 and IFN-γ), in immunity to infections. The predominant surface antigen of African trypanosomes, variant surface glycoprotein, is also an example of $CD4^+$, Th1-cytokine-secreting T-cell response (34). In this study, we demonstrate an increase in the proportion of $CD4^+$ T cells following incubation with p55(1-414) and p55(268-414). Additionally, incubation of the spleen cell suspensions with p55(1-414) induced IL-2 and IFN-γ secretion, suggesting possible activation and proliferation of Th1 cells in response to this *P. carinii* antigen. Th1 cells secrete the major cytokines involved in cell-mediated immunity. Interestingly, in AIDS patients, cell-mediated immunity is clearly defective. However, the delineation between a Th1 and a Th2 response in rats is not clear. At present, it is not known whether p55 elicits any Th2 cytokines such as IL-4, IL-5, or IL-6.

As important as $CD4^+$ cells are in resistance to and recovery from *P. carinii* infection, T cells are unlikely to function alone as effector cells. Through cytokine production, these T cells may regulate interactions with other effector cells and augment the humoral response. Two cytokines which are known to play an important role in resistance to *P. carinii* are IL-1 (5) and TNF-α (4, 14, 16, 20, 39). Both have been shown to be secreted in response to incubation with whole *P. carinii* in SCID mice (5) and rats (15) or following incubation with purified MSG in rats (40). The present study showed that both p55(1-414) and p55(268-414) cause the release of IL-1 and TNF-α in spleen cell suspensions from animals with environmental exposure to *P. carinii*. These cytokines are important in the early inflammatory response and may function to recruit cells into the area of infection.

A detailed understanding of the host-parasite interaction will facilitate the development of immunoprophylaxis and immunotherapy for *P. carinii* infection. In this study, we have demonstrated that the p55 antigen elicits a cell-mediated immune response in animals previously exposed to *P. carinii*. Under conditions of natural exposure, the 5′ portion of the molecule, p55(1-200), appears immunologically silent, failing to elicit lymphocyte proliferation or cytokine secretion. Following active immunization, epitopes within the 5′ portion are specifically recognized by T cells from these animals, resulting in a proliferative response. The 3′ portion, p55(268-414), has at least one immunodominant region, which contains a 7-amino-acid repeat motif. The cells responding to p55 include a $CD4^+$ T cell which secretes a Th1 cytokine pattern.

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