INFECTION AND IMMUNITY, Jan. 1996, p. 290–297 Vol. 64, No. 1
0019-9567/96/$04.00+0

Antigenic Differences Associated with Genetically Distinct *Pneumocystis carinii* from Rats

JOSE VASQUEZ,[1] A. GEORGE SMULIAN,[2] MICHAEL J. LINKE,[2] AND MELANIE T. CUSHION[2]*

Department of Internal Medicine, James Quillen College of Medicine, Johnson City, Tennessee 37614-0622,[1] and Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0560, and the Veterans Affairs Medical Center, Cincinnati, Ohio 45220[2]

Received 28 June 1995/Returned for modification 18 August 1995/Accepted 19 October 1995

***Pneumocystis carinii* is a family of organisms found in a wide variety of mammalian lungs. In immunocompromised hosts, the organisms are able to produce an oftentimes fatal pneumonia. The existence of distinct types of *Pneumocystis* populations is strongly supported by antigenic and genetic evidence. In the present study, we assessed the antigenic profiles of two genetically distinct *Pneumocystis carinii* populations, *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti*, as well as two types of *P. carinii* f. sp. *carinii* defined by electrophoretic karyotyping (forms 1 and 2). The separated and blotted proteins of the organism preparations were probed with four monoclonal antibodies (MAbs) generated to the major surface glycoproteins of rat-derived *P. carinii*, one anti-human *P. carinii* MAb, and two polyclonal antisera made with rat-derived *P. carinii* as the immunogen. Differences in reactivities between the *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations were detected with two of the MAbs, and both of the rat *P. carinii* polyclonal antisera in the 45- to 55-kDa molecular mass range, but not with the human *P. carinii* MAb. The reactivities of the 16 *P. carinii* f. sp. *carinii* preparations were the same with two exceptions. Two preparations of form 1 showed strong reactivity with the anti-MSG MAb RA-C11. The ratios of cyst forms to trophic forms evaluated by microscopy were not associated with any of the differences observed in the antigenic profiles. The antigenic differences between *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* are consistent with the distinction of these two populations made by molecular genetic techniques, while the two differences detected among the *P. carinii* f. sp. *carinii* preparations suggest the organism may be able to modulate antigenic epitopes. The use of immunoblotting to differentiate infecting organism populations and assess antigenic modulation holds promise for future epidemiologic studies.**

Pneumocystis carinii strains are opportunistic eukaryotic organisms found in the lungs of a wide variety of mammalian hosts. These microbes are able to cause a potentially fatal pneumonia in immunocompromised hosts. In human beings, *P. carinii* pneumonia is of particular concern in individuals with AIDS. Although originally considered a protozoan parasite, studies comparing rRNA and DNA sequences from several genes demonstrate that *P. carinii* is more closely related to fungi (7, 8, 24, 34, 40, 44).

P. carinii isolated from humans, rats, ferrets and other mammals can be differentiated by genetic and antigenic techniques (1, 11–13, 17–20, 32). Moreover, two genetically distinct types of *P. carinii* have been identified in a single mammalian host, the rat (3, 5, 22), and were designated prototype and variant (3, 5). Prototype and variant *P. carinii* organisms differed in electrophoretic karyotype, the presence or absence of an intron in the 18S rRNA gene, the sequence of the 18S rRNA gene, homology with a probe containing genes encoding the repeated major surface glycoprotein (MSG) (5), and the sequence of the large subunit of the mitochondrial rRNA genes (27). Prototype organisms were found more frequently than variant organisms as the source of infection in the rat colonies surveyed, but the two types were able to coinfect the same animal (3, 5, 22). Initially, four different karyotype patterns were identified among prototype *P. carinii* in a survey of commercial rat vendors in the United States and were designated forms 1 to 4 (3). Four additional prototype forms (forms 5 to 8) have been reported recently from additional rat colony surveys (28, 43). Most prototype organisms from these colonies produced from 1 or form 2 karyotypes.

In compliance with the nomenclature changes suggested by the participants at the *Pneumocystis* portion of the International Workshop on *Pneumocystis*, *Cryptosporidium*, and Microsporidia, Cleveland, Ohio, June 1994, we have adopted the trinomial names *Pneumocystis carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* (corrected from the nongenitive *rattus*) to differentiate prototype from variant *P. carinii* (25). *P. carinii* f. sp. *hominis* is used to designate human-derived organisms. The binomial, *P. carinii*, is a more global term referring to organisms isolated from any species of mammalian host.

Variation in antigenic reactivities of *P. carinii* organisms isolated from a single host, the human being, has been previously reported by Kovacs et al. (17), raising the question of antigenic modulation or infection with more than one type of organism within a single host. However, the lack of available genetic typing techniques for *P. carinii* at the time of this study prevented any association of phenotype with genotype. More recently, we and others were able to show the presence of two genetically defined *P. carinii* populations within a single immunosuppressed rat (3, 5, 22). In the present study, our goal was to assess the antigenic profiles of these genetically defined *P. carinii* populations. Because *P. carinii* cannot be propagated in vitro, different genetic typing techniques (e.g., sequence polymorphisms, electrophoretic karyotyping) have been employed to study *P. carinii* populations from an epidemiologic perspective. Nonetheless, they are time-consuming and require specialized instrumentation and techniques not always found in the clinical laboratory setting. Because neither morphology nor metabolism appears to be helpful in discriminating different *P.*

* Corresponding author. Mailing address: University of Cincinnati College of Medicine, 231 Bethesda Ave., Cincinnati, OH 45267-0560. Phone: (513) 861-3100, ext. 4843. Fax: (513) 559-5646. Electronic mail address: cushiomt@ucbeh.san.uc.edu.

carinii populations, we assessed the ability of antigenic markers to differentiate between *P. carinii* organisms isolated from rats. Such markers could be used as epidemiologic typing tools to study the disease in other mammals, particularly humans. In addition, identification of the antigenic composition of the defined *P. carinii* population would permit evaluation of the role of antigenic modulation during the progression of infection.

At least two antigenically important molecules have been described from *P. carinii* prepared from different sources. These were chosen for evaluation in the present study. The first one is the MSG (18), also called glycoprotein A (10, 12), a large glycoprotein complex located on the surface of organisms isolated from rats, humans, and other animals (13, 17, 19, 37, 41, 42). Under reducing conditions, it migrates as a prominent band between 95 and 140 kDa. The functions of MSG are poorly understood, but these glycoproteins seem to mediate the attachment of the trophic form to the host type I pneumocyte (26) and alveolar macrophages (9). The MSGs are a family of closely related proteins encoded by multiple genes distributed throughout the organism's genome (18, 35, 36) that are found on every chromosome of *P. carinii* organisms separated by pulsed-field gel electrophoresis (3, 5, 18). These proteins are immunogenic and are able to elicit humoral and cellular immune responses (10, 11, 14).

The other major antigenic complex has a molecular mass of 45 to 55 kDa in *P. carinii* f. sp. *carinii*. This moiety migrates as a broad-based band or can appear as two distinct bands on immunoblots (42). In contrast to MSG, this antigen complex stains poorly with Coomassie blue and other protein stains. A similar antigen complex has been identified in *P. carinii* f. sp. *hominis*, but it has a somewhat lower molecular mass (35 to 45 kDa) (19). The 45- to 55-kDa antigen complex elicits a vigorous humoral and moderate cellular immune response and is the *P. carinii* f. sp. *hominis* antigen complex most commonly recognized by serum antibodies of individuals exposed to the organism (30). The function and cellular location of this antigen complex are unknown. A gene encoding a protein in this complex has been cloned and sequenced from rat-derived *P. carinii* (29) and was mapped to a single band with a size of approximately 600 kb on rat *P. carinii* f. sp. *carinii* chromosomes separated by pulsed-field gel electrophoresis.

(This work was presented in part at the International Workshop on *Pneumocystis*, *Cryptosporidium*, and Microsporidia, Cleveland, Ohio, June 1994 [38a].)

MATERIALS AND METHODS

***P. carinii* organisms.** Inducement of *P. carinii* pneumonia and preparation of organisms from rats have previously been described (3, 5). Frozen lung homogenates from infected immunosuppressed rats that had been electrophoretically karyotyped previously were used in this study (Table 1). We chose two representative forms of the eight *P. carinii* f. sp. *carinii* forms defined by pulsed-field gel electrophoresis, forms 1 and 2. Form 1 was found as the infecting organism in most of the rat colonies surveyed, while form 2 was identified in the facilities of a single vendor (3). Ten samples of form 1 and 9 samples of form 2 were evaluated. *P. carinii* f. sp. *ratti* was represented by three separate preparations in which it was the predominant population. To date, only one genetically defined form of *P. carinii* f. sp. *ratti* has been identified, and this organism has almost always been found in the context of a mixed infection with *P. carinii* f. sp. *carinii* (5, 28). Most preparations were obtained from rats with naturally acquired infections, but in some cases, organisms were obtained from viral antibody-negative, *P. carinii*-naive immunosuppressed rats that had been inoculated with either form 1 or form 2 *P. carinii* f. sp. *carinii*. Inoculations were performed as previously described (2), and organisms were obtained in the same manner as with the other preparations.

Protein electrophoresis and immunoblot assays. *P. carinii* homogenates were solubilized with treatment buffer (0.125 M Tris-HCl [pH 6.8], 4% sodium dodecyl sulfate [SDS], 20% glycerol, 10% 2-mercaptoethanol) and electrophoresed through SDS-polyacrylamide gel electrophoresis (PAGE) (10% polyacrylamide) gels (19). Protein concentrations were standardized by densitometric analyses of Coomassie blue-stained gels, and equivalent amounts of protein of each sample were loaded for immunoblotting comparisons. The proteins were transferred electrophoretically from SDS-PAGE gels onto a nitrocellulose membrane, which was subsequently stained with Ponceau red to confirm the transfer and blocked in 1% skim milk at room temperature for 30 min (19, 38). The blot was then incubated overnight at 4°C with the antibody, diluted 1:1,000 or 1:500 for the monoclonal or polyclonal antisera, respectively, in 0.02 M Tris–0.5 M NaCl (pH 7.5) (TBS). The blot was then washed twice with TBS–0.05% Tween 20 (TTBS) for 10 min each time, incubated with the appropriate peroxidase-labeled conjugate at room temperature for 2 h (diluted 1:1,000 in TBS), and washed again twice with TTBS for 10 min each time. Reactivities with MSG antigens (~100 to 120 kDa) and p55 (~45 to 55 kDa) were evaluated by visual inspection and assigned scores of 0, 1+, or 2+, with 2+ representing the most intense reaction.

Antibodies. The availability of monoclonal antibodies (MAbs) directed against the MSG as well as anti-*P. carinii* polyclonal antibodies facilitated our studies designed to assess antigenic markers in *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations. Five MSG-reactive MAbs and two polyclonal antisera were selected as probes in these studies (21). The antibodies were a gift from Peter Walzer, Cincinnati, Ohio.

Production of MAbs. Four of the MAbs (RA-F1, RA-C6, RA-C7, and RA-C11) were generated against *P. carinii* f. sp. *carinii* MSG purified by SDS-PAGE and electroelution (21). The fifth MSG-reactive MAB (HB-G6) was generated against SDS-solubilized *P. carinii* f. sp. *hominis*. Two polyclonal antisera, 44866 and R-2, were generated in rabbits to *P. carinii* f. sp. *carinii*; the 44866 antiserum was produced against SDS-solubilized organisms (19), while a soluble cell wall fraction (21) was used to immunize rabbits for the production of antiserum R-2.

Sources of organisms. The organisms that provided the immunogens for RA-F1, RA-C6, RA-C7, and RA-C11 and polyclonal antiserum 44866 were isolated from rats obtained from Harlan-Sprague-Dawley, Madison, Wis. (room 205H). This colony is no longer in existence, but electrophoretic karyotyping of organisms prepared from this colony during the same period of the immunizations produced profiles similar to those of *P. carinii* f. sp. *carinii* form 2 identified in our later studies (3). Organisms used to prepare the immunogen for polyclonal antiserum R-2 were isolated from Lewis rats, which have historically displayed the *P. carinii* f. sp. *carinii* form 1 karyotype (3). *P. carinii* f. sp. *hominis* organisms

TABLE 1. *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* forms used in these studies

Form	Vendor	Rat strain	Sample no.
P. carinii f. sp. *carinii*			
1	Charles River, Wilmington, Mass.	Long Evans	1140
			1145
		Brown Norway	1151
			1153
			1159
			1160
	Charles River, Hollister, Calif.[a]	CD	89
			155
			169
			171
2	Hilltop Laboratories, Scottdale, Calif.	Fischer 344	1073
			1080
		Wistar	1115
			1117
			1120
	Charles River, Hollister, Calif.[b]	CD	177
			178
			179
			180
P. carinii f. sp. *ratti*	Hilltop Laboratories, Scottdale, Calif.	Fischer 344, Sprague-Dawley	1087
			959
	Charles River, Wilmington, Mass.	Lewis	1027

[a] Viral antibody-negative *Pneumocystis*-free CD rats were inoculated with form 1 *P. carinii* f. sp. *carinii* obtained from a Sasco Sprague-Dawley rat as described in Materials and Methods.

[b] Viral antibody-negative *Pneumocystis*-free CD rats were inoculated with form 2 *P. carinii* f. sp. *carinii* obtained from a Hilltop Sprague-Dawley rat as described in Materials and Methods.

TABLE 2. Number of *P. carinii* preparations reacting with anti-*P. carinii* f. sp. *carinii* MSG MAbs and intensity of reactivity

Preparation	No. of preparations reacting with MAb at reactivity of[a]:															
	RA-F1				RA-C7				RA-C11				RA-C6			
	0	1+	2+	T	0	1+	2+	T	0	1+	2+	T	0	1+	2+	T
P. carinii f. sp. *carinii* Form 1			8	8			5	5	6		2	8	10			10
Form 2			6	6			4	4	8			8	9			9
P. carinii f. sp. *ratti*	1	2		3	1	2		3	3			3	3			3

[a] Intensity of reaction was graded on a visual scale of 0 (no visible reaction), 1+ (visible reactivity), and 2+ (intense reactivity). T, total number of preparations analyzed.

used in the production of HB-G6 were isolated from the lungs of an AIDS patient obtained at autopsy (15, 21).

Antibody reactivities. The MSG-reactive MAbs used in these studies were of the immunoglobulin G1 class. The reactivities of the MAbs with human- and rat-derived *P. carinii* were previously characterized by indirect immunofluorescence assaying (IFA) and immunoblotting (21). RA-F1 reacted by immunoblotting and IFA with both *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *hominis*; RA-C7 and RA-C6 reacted only with *P. carinii* f. sp. *carinii* by immunoblotting and IFA; RA-C11 reacted only with *P. carinii* f. sp. *carinii* organisms by immunoblotting. The reactivity of MAbs RA-F1, RA-C7, RA-C6, and RA-C11 with β-galactosidase–MSG fusion proteins produced from a λ gt11 *P. carinii* f. sp. *carinii* genomic expression library demonstrated their specificity for protein epitopes (31). HB-G6 reacted by immunoblotting with human- and rat-derived organisms and with human-derived organisms by IFA (21); IFA with rat-derived organisms was not done. The two polyclonal antisera reacted with the MSGs and other antigens from human- and rat-derived *P. carinii.*

C/T ratios. Association of antigenic expression with developmental form of *P. carinii* was evaluated by determining the cyst/trophic form (C/T) ratios in homogenates stained with Diff-Quik by bright-field microscopy. Trophic forms were defined morphologically by the presence of a single reddish-purple nucleus surrounded by blue cytoplasm. Cysts contained up to eight nuclei surrounded by blue cytoplasm and were circumscribed by a typical "exclusion halo" (4). At least 10 fields containing over 50 organisms per field were examined for each preparation.

Statistical analysis of immunoblot results and C/T ratios. The statistical package Epi Info (6) was used to calculate chi-square values and *P* values for comparison of MAb reactivities of *P. carinii* f. sp. *carinii* form 1 and form 2 versus those of *P. carinii* f. sp. *ratti*. When appropriate, data were combined to produce 2-by-2 contingency tables and Yates' correction for small samples was applied (e.g., form 1 versus form 2 comparisons). C/T ratio comparisons among the *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations were evaluated by analysis of variance.

Southern blot analysis and hybridization probes. Chromosomes of agarose-embedded *P. carinii* organisms were separated by the pulsed-field gel electrophoretic technique of a contour-clamped homogeneous electrical field, and the DNAs were transferred by capillary flow onto nylon membranes as previously described (3, 5). Two probes were used: Rp3-1, which contains at least two tandemly repeated copies of MSG genes from *P. carinii* f. sp. *carinii* (33, 35, 36), and 19c, a copy of the nuclear rRNA locus from *P. carinii* f. sp. *carinii* (5). Both probes were gifts from James R. Stringer, University of Cincinnati College of Medicine. Probes were labeled with ^{32}P according to previous protocols (3, 5). Hybridization conditions involved 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 0.015 M sodium citrate), 0.5% SDS, 100 μg of sheared herring sperm DNA per ml, and 5× Denhardt's solution for 16 to 18 h at 65°C. Washing was done in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 0.1% SDS at 65°C twice for 30 min each time and then in 0.2× SSC at 65°C twice for 15 min each time. The blots were exposed to Fuji RX film with intensifying screens, placed at −70°C for 18 to 24 h, and then developed.

RESULTS

Immunoblot reactivities of *P. carinii* preparations to anti-MSG antibodies. Reactivities with the four anti-MSG MAbs were the same for all *P. carinii* f. sp. *carinii* form 1 and 2 preparations, with one exception (Table 2). Of the eight form 1 preparations analyzed, two were found to react strongly with the MAb RA-C11 (Fig. 1), indicating that these two preparations contained organisms that expressed epitopes recognized by RA-C11 that were not expressed by organisms in the other form 1 preparations. The difference in reactivity was not due to variation in the amount of protein loaded, as shown in the photograph of the Coomassie-stained gel (Fig. 1A), nor were the reactivities due to contamination with other microbes or the presence of another *P. carinii* form or species. The MAbs have been shown previously to be nonreactive to a panel of common microbial contaminants (21), and microscopic or microbiologic detection methods did not identify any other contaminants. The two preparations that reacted with MAb RA-C11, preparations 1151 and 1160, originated from the same colony of Charles River Brown Norway rats that were sampled at the same time. Karyotypic and gene probe localization studies of this colony previously detected the presence of mixed infections of *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* in some rats (3, 5). However, no reactivity with RA-C11 was observed with any of the three preparations containing *P. carinii* f. sp. *ratti* or other electrophoretic karyotype forms evaluated (forms 1 to 4) (data not shown), implying that the reactivity was not due to a low number (undetectable by pulsed-field gel electrophoresis) of another type of *P. carinii* residing in the lung. Two other explanations for this phenotypic difference remained. Expression of the unique epitope of MSG was associated with a specific life cycle stage, or a subpopulation within the majority population of organisms could differentially express an MSG with an epitope at variance with the majority of organisms. The possibility of a life cycle stage-specific MSG was addressed by enumeration of the C/T ratio (described below). A genetic basis for antigenic modulation of

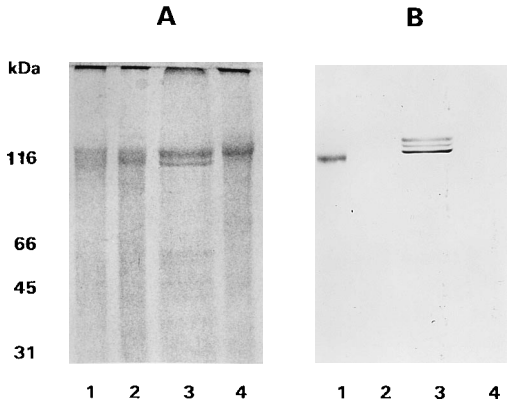

FIG. 1. Reactivity of two *P. carinii* f. sp. *carinii* form 1 preparations with MAB RA-C11. (A) Coomassie blue-stained gel. Lanes: 1, form 1 preparation 1160; 2, form 1 preparation 1140; 3, form 1 preparation 1151; 4, form 1 preparation 1153. (B) Immunoblot. Lanes correspond to those of panel A. Size estimations of the protein complexes were based on migration of known standards stained with Coomassie blue.

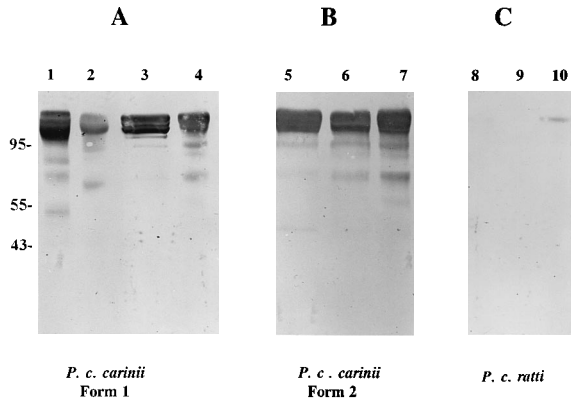

FIG. 2. Immunoblot of *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations probed with MAb RA-C7. (A) *P. carinii* f. sp. *carinii* form 1. Lanes: 1, preparation 1160; 2, preparation 1140; 3, preparation 1151; 4, preparation 1153. (B) *P. carinii* f. sp. *carinii* form 2. Lanes: 5, preparation 1073; 6, preparation 1117; 7, preparation 1080. (C) *P. carinii* f. sp. *ratti*. Lanes: 8, preparation 1027; 9, preparation 959; 10, preparation 1087. Size estimations of the protein complexes were based on blotted, prestained standards.

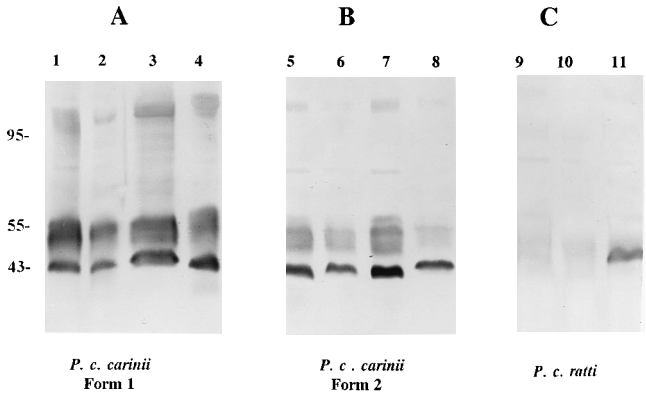

FIG. 3. Polyclonal antiserum 44866 reactivity with *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations. (A) *P. carinii* f. sp. *carinii* form 1. Lanes: 1, preparation 1160; 2, preparation 1140; 3, preparation 1151; 4, preparation 1153. (B) *P. carinii* f. sp. *carinii* form 2. Lanes: 5, preparation 179; 6, preparation 180; 7, preparation 177; 8, preparation 178. (C) *P. carinii* f. sp. *ratti*. Lanes: 9, preparation 1087; 10, preparation 959; 11, preparation 1027. Size estimations of the protein complexes were based on migration of blotted, prestained standards.

MSGs in *P. carinii* has been recently reported (35, 39) and was considered a possible explanation.

A difference in reactivity patterns of the two preparations of form 1 with MAb RA-C11 was apparent in Fig. 1. A single band of reactivity at approximately 116 kDa was observed with preparation 1160 (lane 1), while preparation 1151 (lane 3) produced three bands, all of which were larger than 120 kDa. Variations in molecular mass and band number of the MSG molecules have been observed by several laboratories and are likely due to the extant expression of numerous species of MSG encoded by the multigene family or in some cases may be due to processing intermediates of MSG molecules.

Differences in reactivities between the two genetically distinct rat-derived organisms, *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti*, were detected by two anti-rat-derived *P. carinii* MSG MAbs, RA-F1 and RA-C7 (Table 2). As shown in Fig. 2, reactivity of MAb RA-C7 with all preparations of *P. carinii* f. sp. *carinii* forms 1 and 2 (lanes 1 to 7) was strong (an example of the 2+ grade), while two of the three *P. carinii* f. sp. *ratti* preparations had weak reactivity (1+) with MAb RA-C7 (lanes 8 and 10), and one sample showed no reactivity (lane 9). We suspected this low level of reactivity in the *P. carinii* f. sp. *ratti* preparations was due to the presence of *P. carinii* f. sp. *carinii* in preparations 1027 (lane 8) and 1087 (lane 10). Further experiments were conducted to address this possibility (described below). The single MAb generated to human-derived *P. carinii*, HB-G6, did not show any significant differences between the *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* populations or between the form 1 and form 2 *P. carinii* f. sp. *carinii* preparations (data not shown). Negative controls with lung homogenate from noninfected immunosuppressed rats did not show any reactivity with these antibodies (data not shown).

Immunoblot reactivities of *P. carinii* to polyclonal antisera. The reactivities of the two polyclonal antisera used in these studies were evaluated in the regions of MSG and p55 migration, 100 to 120 kDa and 45 to 55 kDa. In the region of the MSG migration, all 9 of the form 2 preparations and all but 1 of a total of 10 form 1 preparations reacted with polyclonal antiserum 44866 (Table 3). Slightly less reactivity was observed with the other antiserum, R-2, when form 2 preparations were probed; three of seven preparations did not react. No difference in reactivity at the 45- to 55-kDa region was observed between form 1 and 2 populations with either antiserum.

Conversely, the two anti-rat *P. carinii*-derived polyclonal antisera exhibited differences in reactivity between the *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations in the 45- to 55-kDa region (Table 3), with all of the *P. carinii* f. sp. *carinii* preparations reacting with antiserum 44866 and only one of the *P. carinii* f. sp. *ratti* homogenates showing slight reactivity. R-2 antisera reacted with all 15 of the *P. carinii* f. sp. *carinii* preparations while reacting slightly or not at all with the *P. carinii* f. sp. *ratti* preparations. Reactions of representative preparations of the three types of *P. carinii* with polyclonal antiserum

TABLE 3. Anti-*P. carinii* f. sp. *carinii* polyclonal antiserum reactivities

Preparation	No. of preparations reacting with polyclonal antiserum at reactivity of[a]:															
	44866-MSG				44866-p55				R-2-MSG				R-2-p55			
	0	1+	2+	T	0	1+	2+	T	0	1+	2+	T	0	1+	2+	T
P. carinii f. sp. *carinii*																
Form 1	1	3	6	10			10	10		5	3	8		5	3	8
Form 2		2	7	9		1	8	9	3	2	2	7		1	6	7
P. carinii f. sp. *ratti*	1	2		3	2	1		3	1	2		3	1	2		3

[a] Intensity of reaction was graded on a visual scale of 0 (no visible reaction), 1+ (visible reactivity), and 2+ (intense reactivity). T, total number of preparations analyzed.

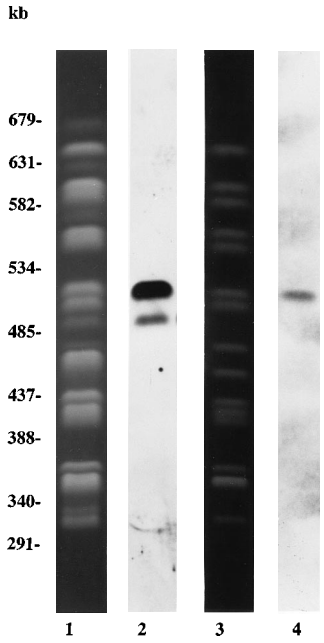

FIG. 4. Electrophoretic karyotypes of two preparations composed primarily of *P. carinii* f. sp. *ratti* hybridized with a probe to the nuclear ribosomal locus (19c). Lanes: 1, ethidium bromide-stained chromosome-size DNAs of *P. carinii* f. sp. *ratti* preparation 1027; 2, Southern-blotted DNAs corresponding to lane 1 probed with 19c; 3, ethidium bromide-stained chromosomes of *P. carinii* f. sp. *ratti* preparation 959; 4, Southern-blotted preparation 959 probed with 19c. Size estimations of bands were based on the migration of a lambda ladder (48.5-kb increments).

44866 are shown in Fig. 3. Of note is the intense staining in the 55-kDa region demonstrated by all of the form 1 and 2 preparations (lanes 1 to 8) and the dramatically less intense reaction of *P. carinii* f. sp. *ratti*-containing preparations. It was our hypothesis that the reactivity was due to minor populations of *P. carinii* f. sp. *carinii* in these samples. We explored the presence of *P. carinii* f. sp. *carinii* as a potential source of the antigens in the *P. carinii* f. sp. *ratti* preparations by pulsed-field gel electrophoresis and hybridization with single and repetitive probes.

Analysis of *P. carinii* f. sp. *ratti* preparations for the presence of *P. carinii* f. sp. *carinii*. We suspected that the presence of *P. carinii* f. sp. *carinii* organisms in preparations 1087 and 1027 was responsible for the slight reactivities observed in the immunoblotting studies, while the lack of reactivity of preparation 959 indicated that there was very little or no *P. carinii* f. sp. *carinii* in this sample. Since we had previously detected a mixed population in preparation 1087 (5), we analyzed preparations 1027 and 959 by electrophoretic karyotyping and hybridization with cloned probes to the nuclear rRNA locus and MSG genes of *P. carinii* f. sp. *carinii* (3, 5). The ribosomal locus was chosen because it could detect mixed infections due to hybridization to bands of different sizes in electrophoretic karyotypes of *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti*, while the other probe, Rp3-1, has been shown to hybridize to all chromosome-size bands of *P. carinii* f. sp. *carinii* under high-stringency conditions but not to bands of *P. carinii* f. sp. *ratti* (5).

The ribosomal probe hybridized to two bands in preparation 1027 but only one band in preparation 959 (Fig. 4), suggesting the presence of two types of *P. carinii* in 1027 (lane 2) but a single type in 959 (lane 4). Hybridization of the probe to a band approximately 530 kb in size in both of the preparations is indicative of the *P. carinii* f. sp. *ratti* organisms, while the second band about 500 kb in size found only in preparation 1027 corresponds to *P. carinii* f. sp. *carinii* (5). Lane 1 was purposefully overloaded to produce DNA quantities of the minor population of *P. carinii* f. sp. *carinii* present in preparation 1027 (note lighter-intensity bands at 679, 631, 570, 500, 480, and 330 kb) equivalent to those of the *P. carinii* f. sp. *ratti* population in lane 3. The similar intensities of the bands of hybridization at 500 kb in lane 2 (corresponding to *P. carinii* f. sp. *carinii*) and at 530 kb in lane 4 (*P. carinii* f. sp. *ratti*) attest to the equivalent organism concentrations.

The other probe, Rp3-1, did not hybridize at all with preparation 959 (Fig. 5, lanes 1 and 4) but did hybridize to the bands of *P. carinii* f. sp. *carinii* corresponding to the fainter ethidium bromide-stained bands in lanes 2 and 5, providing evidence of a population of *P. carinii* f. sp. *carinii* in this sample. The lack of hybridization with the *P. carinii* f. sp. *ratti* populations in preparations 959 and 1027 was not due to insufficient DNA loading, since both of the DNAs of the *P. carinii* f. sp. *ratti* preparations were equivalent, if not more abundant (Fig. 5, lanes 1 and 2) than those of the authentic population of *P. carinii* f. sp. *carinii* in Fig. 5, lane 3. In fact, the minor population of *P. carinii* f. sp. *carinii* was present at about a 10-fold lower concentration than the *P. carinii* f. sp. *ratti* population in preparation 1027, yet the Rp3-1 probe only hybridized with bands corresponding to *P. carinii* f. sp. *carinii*. Note the comigration of the faint ethidium bromide-stained bands present in lane 2 with those of the authentic *P. carinii* f. sp. *carinii* form 1 in lane 3 and the corresponding bands of hybridization in lanes 5 and 6. From these data, it appeared that at least two of the three *P. carinii* f. sp. *ratti* preparations contained detectable amounts of *P. carinii* f. sp. *carinii* organisms, while in preparation 959, a minor population was not detected by these methods. It is also apparent that the MSG genes of *P. carinii* f. sp. *ratti* have little homology with those of *P. carinii* f. sp. *carinii*. Thus, the minor populations of *P. carinii* f. sp. *carinii* in the *P. carinii* f. sp. *ratti* preparations 1027 and 1087 can account for the slight reactivities with MAbs RA-F1 and RA-C7 and antisera 44866 and R-2 in the 55-kDa region.

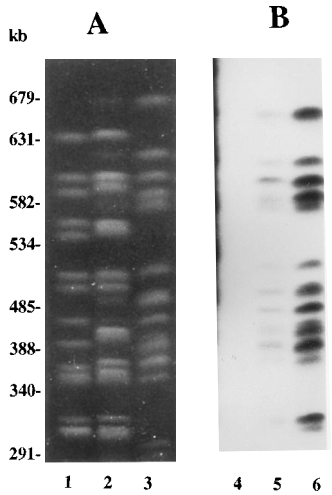

FIG. 5. Electrophoretic karyotypes of two preparations composed primarily of *P. carinii* f. sp. *ratti* hybridized with a probe to MSG. (A) Ethidium bromide-stained chromosome-size DNAs. Lanes: 1, *P. carinii* f. sp. *ratti* preparation 959; 2, *P. carinii* f. sp. *ratti* preparation 1027; 3, form 1 preparation 1029 (*P. carinii* f. sp. *carinii* from the same colony as no. 1027). (B) Lanes 4 to 6, Southern-blotted DNAs corresponding to lanes 1 to 3 probed with Rp3-1, a clone encoding at least two MSG genes. Size estimations of bands were based on migration of a lambda ladder (48.5-kb increments).

TABLE 4. Organism burden and C/T ratio for *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* preparations

Preparation	No. of *P. carinii* f. sp. *carinii* organisms (10^9)/ml	C/T ratio	No. of wks immunosuppressed
P. carinii f. sp. *carinii*			
Form 1			
1145	0.08	1:13	14.5
1151	1.53	1:15	12.0
1153	2.69	1:23	11.0
1160	5.09	1:11	12.0
1140	8.74	1:17	14.5
Form 2			
1120	0.59	1:8	14.5
1115	1.35	1:10	14.5
1117	2.30	1:20	14.5
1073	5.06	1:23	11.5
1080	6.60	1:20	11.5
P. carinii f. sp. *ratti*			
959	1.95	1:5	13.0
1087	3.35	1:18	11.5
1027	5.26	1:13	18.0

Comparison of C/T ratios. To determine if antigenic expression of a given *P. carinii* population was associated with the developmental forms present, with organism burden (severity of infection), or with length of immunosuppression, Diff-Quik-stained cyst and trophic forms in *P. carinii* preparations used in the immunoblotting analyses were enumerated, and total organism burden was estimated by counting the total number of *P. carinii* nuclei (Table 4). Comparison of the C/T ratios among the *P. carinii* f. sp. *carinii* preparations, ranging between 1:8 and 1:23, including those preparations that reacted with MAb RA-C11 (numbers 1151 and 1160), did not show any significant differences in C/T ratio or antibody reactivity. Two of the three *P. carinii* f. sp. *ratti* preparations (numbers 1087 and 1027) had ratios of 1:18 and 1:13, which were well within the range of all of the *P. carinii* f. sp. *carinii* preparations. The one *P. carinii* f. sp. *ratti* preparation which had no detectable *P. carinii* f. sp. *carinii* population had the lowest C/T ratio, 1:5. Since there was only a single preparation of this composition, no conclusions concerning the presence of more cyst forms in such preparations can be made, but further investigation into this finding is warranted. While most infections had heavy organism burdens, lower C/T ratios could be found in lighter infections (e.g., 1:8 in 1120) as well as in those with heavy infections (e.g., 1160). The highest C/T ratios (1:20 and 1:23) were found in preparations ranging from 2.3×10^9 to 6.6×10^9 organisms per ml. There was no statistical correlation between organism burden, C/T ratio, or length of immunosuppression.

DISCUSSION

Our appreciation of the complexity of infections caused by *P. carinii* in rats has grown towards acceptance of the causative agent as a heterogeneous population of organisms, sometimes including two distinct organism populations coexisting within the same host. Detection of infections caused by two types of *P. carinii* has been exclusively shown by genetic methods (5, 22). In the present study, we report phenotypic differences expressed by a single genetically defined *P. carinii* f. sp. *carinii* population and between two genetically distinct organism populations, *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti*. Two MAbs directed against purified MSG from rat-derived *P. carinii* (RA-C7 and RA-F1) showed significant differences in reactivity between *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti*. Likewise, two polyclonal antisera (44866 and R-2) detected differences between these two populations in antigen species migrating at 45 and 55 kDa. It is likely that there is a genetic basis for these phenotypes. In the present study, as well as in a previous study (5), hybridization of *P. carinii* f. sp. *ratti* chromosomes with a probe containing MSG genes isolated from *P. carinii* f. sp. *carinii* showed no homology with chromosomes of *P. carinii* f. sp. *ratti* under conditions of high stringency, supporting the notion that the genes encoding the MSG molecules from these two populations are quite different.

The expression of antigens specific to a genetically defined *P. carinii* population is also supported by previous studies of ferret-, rat-, human-, and mouse-derived *P. carinii*-specific antibody reactivities (11–14, 17, 19, 41). Thus, this study has shown that the antigen-specific expression of the two genetically distinct *P. carinii* populations found in rats is in keeping with the antigenic differences observed for *P. carinii* isolated from different species of mammals. Furthermore, we suggest that the antibodies that produced the dramatic differences in reactivity between *P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti* should be helpful in characterizing the organisms in infections caused by a single *P. carinii* population and in infections caused by two genetically distinct organisms. Historically, the rat model of *P. carinii* pneumonia has been a reliable predictor of the basic biology and drug response of organisms causing human infection. On the basis of this paradigm, it is likely that *P. carinii* pneumonia in human beings may be caused by mixed or single infections of genetically distinct organisms and that these organisms will express distinct MSG proteins that should be detectable by immunoblotting methods. Recent genotyping studies of bronchoalveolar lavage fluids from patients with *P. carinii* pneumonia have reported several different genotypes of infecting organisms on the basis of amplification of gene sequences by the PCR (16, 23), supporting the diversity of organisms infecting human beings.

Perhaps more intriguing was the finding of phenotypic variation within a single genetically defined population of *P. carinii* f. sp. *carinii*. Variation in antigenic reactivity was detected by a single MAb, RA-C11. This MAb reacted with 2 of 16 preparations of *P. carinii* f. sp. *carinii*, both of which were of the form 1 electrophoretic karyotype profile and both of which were found in the same colony of animals. There are several possible explanations for this observation. The first is that the reaction was due to another species or strain of *P. carinii* coexisting in the lung. This was ruled out by the nonreactivity of the *P. carinii* f. sp. *ratti* and other *P. carinii* f. sp. *carinii* preparations with MAb RA-C11. The second possibility is expression of the antigen by a specific life cycle stage (developmental form) of the organism. However, C/T ratios of the preparations used in the studies did not show any correlation with antibody reactivity. A third possibility would be genetic divergence between preparations 1060 and 1151 and the other *P. carinii* f. sp. *carinii* preparations. Both preparations 1060 and 11511 produced the same electrophoretic karyotype (form 1), and both samples were identical in sequences through portions of the 18S and mitochondrial large rRNA gene sequences (27). However, electrophoretic karyotyping is useful for detection of genetic differences such as gene duplications or other such events that would result in a change of size in chromosomes but it cannot detect subtle differences in gene sequences. Likewise, targeted amplification and sequencing of specific DNA sequences with the PCR could theoretically miss a minor population of organisms. Although the populations appeared identical at two ge-

netic loci, the possibility of variation at other loci remains. Therefore, the population of organisms found in *P. carinii* f. sp. *carinii* form 1 preparations 1060 and 1151 could be composed of a heterogeneous population of organisms containing a major and minor population with indistinguishable electrophoretic karyotypes, the latter of which is genetically distinct and expresses the unique epitope reacting with RA-C11. The final possibility would be that the *P. carinii* f. sp. *carinii* population was genetically homogeneous but contained some organisms that expressed an MSG gene different from those expressed by the other organisms present in the lung. *P. carinii* f. sp. *carinii* form 1 preparations 1160 and 1151 reacted with MAbs in a manner identical to the other 14 *P. carinii* f. sp. *carinii* preparations, with the exception of RA-C11, suggesting the populations in these two preparations also express MSG epitopes common to the other *P. carinii* f. sp. *carinii* populations in addition to their unique epitopes. The latter hypothesis of differential expression of an MSG gene by a minor population was supported by recent studies that provide genetic evidence for the translocation and expression of distinct MSG genes at telomeric expression sites (35, 39). Likewise, characterization of mRNAs encoding several MSGs isolated from a single population of *P. carinii* (20) provides additional strong evidence for the ability of an organism population to express more than one MSG. Since the two populations of form 1 *P. carinii* f. sp. *carinii* reacting with MAb RA-C11 were identical in karyotype and at two genetic loci, it seems plausible that a population of organisms expressing an MSG gene encoding an epitope distinct from the majority population were detected by MAb RA-C11.

In summary, this study has shown phenotypic differences associated with genotypically distinct *P. carinii* populations with the only phenotypic marker available at this time, antigenic expression. We have shown that it is possible to differentiate infections caused by one or the other genetically defined populations of rat-derived *P. carinii* by immunoblotting techniques. The presence of a minor population of one species of organism, *P. carinii* f. sp. *carinii*, in an infection containing predominantly *P. carinii* f. sp. *ratti*, could be detected by MAbs RA-F1 and RA-C7 generated to the former organism, providing a rationale for the immunoblotting technique in epidemiologic situations. Additionally, reaction of 2 of 16 *P. carinii* f. sp. *carinii* preparations with one MAb, RA-C11, provides evidence for antigenic modulation or heterogeneity of infecting populations that will only be addressed by further analyses of the transcription, expression, and function of the MSG family of genes.

ACKNOWLEDGMENTS

We thank Marnie A. Ryan for technical expertise. We gratefully acknowledge Peter D. Walzer and James R. Stringer for the antibodies and gene probes used in this study.

This study was supported by a grant from the National Institutes of Health, RO1 AI 29839.

REFERENCES

1. **Armstrong, M. Y. K., and M. T. Cushion.** 1994. Pneumocystosis: animal models, p. 181–222. *In* P. Walzer (ed.), *Pneumocystis carinii* pneumonia, 2nd ed. Marcel Dekker, New York.
2. **Boylan, C. J., and W. L. Current.** 1992. Improved rat model of *Pneumocystis carinii* pneumonia: induced laboratory infections in *Pneumocystis*-free animals. Infect. Immun. **60:**1589–1597.
3. **Cushion, M. T., M. Kaselis, S. L. Stringer, and J. R. Stringer.** 1993. Genetic stability and diversity of *Pneumocystis carinii* infecting rat colonies. Infect. Immun. **61:**4801–4813.
4. **Cushion, M. T., J. J. Ruffolo, M. J. Linke, and P. D. Walzer.** 1985. *Pneumocystis carinii*: growth variables and estimates in A549 and WI38 VA 13 human cell lines. Exp. Parasitol. **60:**43–54.
5. **Cushion, M. T., J. Zhang, M. Kaselis, D. Giuntoli, S. L. Stringer, and J. R. Stringer.** 1993. Evidence for two genetic variants of *Pneumocystis carinii* coinfecting laboratory rats. J. Clin. Microbiol. **31:**1217–1223.
6. **Dean, A. G., J. A. Dean, A. H. Burton, and R. C. Dicker.** 1990. Epi Info, version 5: a word processing, database, and statistics program for epidemiology on microcomputers. USD, Inc., Stone Mountain, Ga.
7. **Edlind, T. D., M. S. Bartlett, G. A. Weinberg, G. N. Prah, and J. W. Smith.** 1992. The β-tubulin gene from rat and human preparations of *Pneumocystis carinii*. Mol. Microbiol. **6:**3365–3373.
8. **Edman, J. C., J. A. Kovacs, H. Masur, D. Santi, H. J. Elwood, and M. L. Sogin.** 1988. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature (London) **334:**519–522.
9. **Ezekowitz, R. A. B., D. J. Williams, J. Koziel, M. Y. K. Armstrong, A. Warner, F. F. Richards, and R. M. Rose.** 1991. Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor. Nature (London) **351:**155–158.
10. **Fisher, D. J., F. Gigliotti, M. Zauderer, and A. G. Harmsen.** 1991. Specific T-cell response to a *Pneumocystis carinii* surface glycoprotein (gp120) after immunization and natural infection. Infect. Immun. **59:**3372–3376.
11. **Gigliotti, F.** 1991. Host species-specific antigenic variation of a mannosylated surface glycoprotein of *Pneumocystis carinii*. J. Infect. Dis. **165:**329–336.
12. **Gigliotti, F., L. R. Ballou, W. T. Hughes, and B. D. Mosley.** 1988. Purification and initial characterization of a ferret *Pneumocystis carinii* surface antigen. J. Infect. Dis. **158:**848–854.
13. **Graves, D. C., S. J. N. McNabb, M. A. Worley, T. D. Downs, and M. H. Ivey.** 1986. Analysis of rat *Pneumocystis carinii* antigens recognized by human and rat antibodies by using Western immunoblotting. Infect. Immun. **54:**96–103.
14. **Graves, D. C., A. G. Smulian, and P. D. Walzer.** 1994. Humoral and cellular immunity, p. 267–288. *In* P. D. Walzer (ed.), *Pneumocystis carinii* pneumonia, 2nd ed. Marcel Dekker, Inc., New York.
15. **Hong, S.-T., P. E. Steele, M. T. Cushion, P. D. Walzer, S. L. Stringer, and J. R. Stringer.** 1990. *Pneumocystis carinii* karyotypes. J. Clin. Microbiol. **28:**1785–1795.
16. **Keely, S. P., J. R. Stringer, R. P. Baughman, M. J. Linke, P. D. Walzer, and A. G. Smulian.** 1995. Genetic variation among *Pneumocystis carinii hominis* isolates in recurrent pneumocystosis. J. Infect. Dis. **172:**595–598.
17. **Kovacs, J. A., J. L. Halpern, B. Lundgren, J. C. Swan, J. E. Parillo, and H. Masur.** 1989. Monoclonal antibodies to *Pneumocystis carinii*: identification of specific antigens and characterization of antigenic differences between rat and human isolates. J. Infect. Dis. **159:**60–70.
18. **Kovacs, J. A., F. Powell, J. C. Edman, B. Lundgren, A. Martinez, B. Drew, and C. W. Angus.** 1993. Multiple genes encode the major surface glycoprotein of *Pneumocystis carinii*. J. Biol. Chem. **268:**6034–6040.
19. **Linke, M. J., M. T. Cushion, and P. D. Walzer.** 1989. Properties of the major antigens of rat and human *Pneumocystis carinii*. Infect. Immun. **57:**1547–1555.
20. **Linke, M. J., A. G. Smulian, J. R. Stringer, and P. D. Walzer.** 1994. Characterization of multiple unique cDNAs encoding the major surface glycoprotein of rat-derived *Pneumocystis carinii*. Parasitol. Res. **80:**478–486.
21. **Linke, M. J., A. G. Smulian, P. Yoshihara, and P. D. Walzer.** 1994. Production and characterization of monoclonal antibodies specific for the major surface glycoprotein of *Pneumocystis carinii*. J. Eukaryot. Microbiol. **41:**99S–100S.
22. **Liu, Y., and M. J. Leibowitz.** 1993. Variation and in vitro splicing of group I introns in rRNA genes of *Pneumocystis carinii*. Nucleic Acids Res. **21:**2415–2421.
23. **Lu, J. J., M. S. Bartlett, M. M. Shaw, S. F. Queener, J. W. Smith, M. Ortiz-Rivera, M. J. Leibowitz, and C.-H. Lee.** 1994. Typing of *Pneumocystis carinii* strains that infect humans based on nucleotide sequence variations of internal transcribed spacers of rRNA genes. J. Clin. Microbiol. **32:**2904–2912.
24. **Pixley, F. J., A. E. Wakefield, S. Banerji, and J. M. Hopkin.** 1991. Mitochondrial gene sequences show fungal homology for *Pneumocystis carinii*. Mol. Microbiol. **5:**1347–1351.
25. **Pneumocystis Workshop.** 1994. Revised nomenclature for *Pneumocystis carinii*. J. Eukaryot. Microbiol. **41:**121S.
26. **Pottratz, S. J., J. Paulsrud, J. S. Smith, and W. J. Martin II.** 1991. *Pneumocystis carinii* attachment to cultured lung cells by pneumocystis gp120, a fibronectin binding protein. J. Clin. Invest. **88:**403–407.
27. **Ryan, M. A., and M. T. Cushion.** Conservation and variation among RNA gene sequences of candidate *Pneumocystis* strains and species. Submitted for publication.
28. **Ryan, M. A., and M. T. Cushion.** 1995. New forms of *Pneumocystis carinii carinii* and an increased presence of *Pneumocystis carinii rattus* detected in a survey of rat colonies in the United States, abstr. F-32, p. 92. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
29. **Smulian, A. G., J. R. Stringer, M. J. Linke, and P. D. Walzer.** 1992. Isolation and characterization of a recombinant antigen of *Pneumocystis carinii*. Infect. Immun. **60:**907–915.
30. **Smulian, A. G., D. W. Sullivan, M. J. Linke, N. A. Halsey, T. C. Quinn, A. P. MacPhail, M. A. Hernandez-Avila, S. T. Hong, and P. D. Walzer.** 1993.

Geographic variation in the humoral response to *Pneumocystis carinii*. J. Infect. Dis. **167:**1243–1247.
31. **Stringer, J. R., M. L. Linke, and S. M. Sunkin.** Unpublished data.
32. **Stringer, J. R., S. L. Stringer, J. Zhang, R. Baughman, A. G. Smulian, and M. T. Cushion.** 1993. Molecular genetic distinction of *Pneumocystis carinii* from rats and humans. J. Eukaryot. Microbiol. **40:**733–741.
33. **Stringer, S. L., S.-T. Hong, D. Giuntoli, and J. R. Stringer.** 1993. Repeated DNA in *Pneumocystis carinii*. J. Clin. Microbiol. **29:**1194–1201.
34. **Stringer, S. L., J. R. Stringer, M. A. Blase, P. D. Walzer, and M. T. Cushion.** 1989. *Pneumocystis carinii*: sequence from ribosomal RNA implies a close relationship with fungi. Exp. Parasitol. **68:**450–461.
35. **Sunkin, S. M., and S. L. Stringer.** Translocation of surface antigen genes to a unique telomeric expression site in *Pneumocystis carinii*. Submitted for publication.
36. **Sunkin, S. M., S. L. Stringer, and J. R. Stringer.** 1994. A tandem repeat of rat-derived *Pneumocystis carinii* genes encoding the major surface glycoprotein. J. Eukaryot. Microbiol. **41:**292–300.
37. **Tanabe, K., S. Takasaki, J.-I. Watanabe, A. Kobata, K. Egawa, and Y. Nakamura.** 1989. Glycoproteins composed of major surface immunodeterminants of *Pneumocystis carinii*. Infect. Immun. **57:**1363–1366.
38. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
38a. **Vasquez, J., M. J. Linke, A. G. Smulian, and M. T. Cushion.** 1994. Antigenic differences among genetically distinct types of rat-derived *Pneumocystis carinii.* J. Eukaryop. Microbiol. **41:**114s.
39. **Wada, M., S. M. Sunkin, J. R. Stringer, and Y. Nakamura.** 1995. Antigenic variation by positional control of major surface glycoprotein gene expression in *Pneumocystis carinii*. J. Infect. Dis. **171:**1563–1568.
40. **Wakefield, A. E., S. E. Peters, S. Banerji, P. D. Bridge, G. S. Hall, D. L. Hawksworth, L. A. Guiver, A. G. Allen, and J. M. Hopkin.** 1992. *Pneumocystis carinii* shows DNA homology with the ustomycetous red yeast fungi. Mol. Microbiol. **6:**1903–1911.
41. **Walzer, P. D., C. K. Kim, M. J. Linke, C. L. Pogue, M. J. Huerkamp, C. E. Chrisp, A. V. Lerro, S. K. Wixson, E. Hall, and L. D. Schultz.** 1989. Outbreaks of *Pneumocystis carinii* pneumonia in colonies of immunodeficient mice. Infect. Immun. **57:**62–70.
42. **Walzer, P. D., and M. J. Linke.** 1987. A comparison of the antigenic characteristics of rat and human *Pneumocystis carinii* by immunoblotting. J. Immunol. **138:**2257–2265.
43. **Weinberg, G. A., C. C. Dykstra, P. J. Durant, and M. T. Cushion.** 1994. Chromosomal localization of 20 genes to 5 distinct pulsed field gel karyotypic forms of rat-*Pneumocystis carinii*. J. Eukaryot. Microbiol. **41:**117S.
44. **Ypma-Wong, M. F., W. A. Fonzi, and P. S. Sypherd.** 1992. Fungus-specific translation elongation factor 3 gene present in *Pneumocystis carinii*. Infect. Immun. **60:**4140–4145.

Editor: D. H. Howard