Serologic Responses to Epitopes of the Major Surface Glycoprotein of *Pneumocystis jiroveci* Differ in Human Immunodeficiency Virus–Infected and Uninfected Persons

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The major surface glycoprotein (Msg) of *Pneumocystis jiroveci* (P. jiroveci) is important in the immunopathogenesis of *Pneumocystis* pneumonia (PcP), but is difficult to study in humans. We generated 3 overlapping recombinant Msg fragments (MsgA, MsgB and MsgC), and analyzed their reactivity with serum samples from 95 healthy blood donors and 94 human immunodeficiency virus (HIV)–infected persons. Reactivity to the Msg fragments varied with HIV infection and prior episodes of PcP but not with geographic origin. Recognition of MsgA was lower—and recognition of MsgB was significantly lower—in HIV+ serum compared with donor serum. Serum samples from HIV-positive patients with prior PcP recognized MsgC more frequently than did serum samples from those without PcP. None of the serum samples drawn from 9 patients before they had developed PcP recognized MsgC. These data suggest that these novel recombinant proteins are useful for the analysis of antibody responses to Msg.

*Pneumocystis jiroveci*, a fungal opportunistic pathogen in humans that previously had been known as “*Pneumocystis carinii* f. sp. *hominis*” [1], is the causative agent of *Pneumocystis* pneumonia (PcP), a leading cause of serious illness in immunocompromised patients [2, 3]. The use of potent anti–human immunodeficiency virus (HIV) drugs has dramatically reduced the frequency of opportunistic infections, including *P. jiroveci*, in HIV-positive patients [4]. Furthermore, the use of highly active anti-retroviral therapy (HAART) is associated with reconstitution of the immune system. Although the effects of HAART are generally measured by the increase in T cell numbers, data on reconstitution of organism-specific immune responses have been limited [5].

There is a high prevalence of anti–*P. jiroveci* antibodies in healthy adults, with >70% of serum samples being positive for reactivity to *P. jiroveci* antigens [6–11]. This level of reactivity is consistent with widespread exposure to *P. jiroveci*, which occurs during childhood. Serum antibodies reactive to *P. jiroveci* antigens are commonly found in children <4 years of age [8, 12]. It is known that many proteins of *P. jiroveci* can be recognized by serum antibodies [6, 13, 14], but little information exists on (a) the responses to specific *P. jiroveci* antigens, (b) how those responses change over time, and (c) whether these responses are protective. This is, in part, due to the fact that the antigen preparations used in previous studies were crude homogenates from human or rodent lungs and did not allow the identification of specific antigenic proteins [15]. Furthermore, serum samples isolated in different parts of the world exhibited, in Western blot analysis, different patterns of reactivity to *P. jiroveci* antigens, suggesting either that there may be variability between different *P. jiroveci* preparations or that there are geographic differences in reactivity to *P. jiroveci* antigens [6].

One of the *P. jiroveci* proteins that is consistently recognized by serum antibodies and helper T cells is a 95-kDa protein called the “major surface glycoprotein” (Msg), or “glycoprotein A (gpA).” This antigenic protein shares epitopes with the 120-kDa antigen of rodent *P. carinii* [14–18], elicits protective B and T cell responses [19–21], and plays an important role in the interaction between *Pneumocystis* organisms and the host lung [2, 15]. Msg is a family of related proteins that are encoded by multiple genes in the *Pneumocystis* genome [22, 23]. It appears that only 1 Msg is expressed at a given time, suggesting that *Pneumocystis* species may evade the host immune system by switching the isoform of the protein expressed [23–27]. Such antigenic variation is commonly used by microorganisms such as trypanosomes and the spirochete borrelia [28–30]. Analysis of antibody reactivity to Msg is hampered both by the crude antigen preparations described above and by the inherently diverse nature of Msg molecules. For example, native Msg is...
recognized in only 30%–40% of serum samples from healthy individuals [6, 8, 31]. There is a need to simplify antigen preparations to analyze the immune response to a single Msg molecule. The identification of antigenic epitopes on a single isof orm of Msg would allow tracking of the response to that specific epitope during an infection with Pneumocystis species, in convalescence following an episode of PCP, and in evaluation of the antigen-specific immune reconstitution of HIV-positive patients after HAART. In the present study, we have taken the first steps toward this goal, by generating a panel of recombinant Msg fragments that correspond to a single Msg molecule, for use as a standardized set of reagents in immunological assays. We have characterized the specificity of the fragments and have analyzed the patterns of reactivity of healthy and HIV− serum samples to the recombinants.

Materials and Methods

Cloning, expression, and purification of Msg fragments. Oligonucleotides were designed on the basis of the known sequence of the msg gene of P. jiroveci [32] and were used in polymerase chain reaction (PCR) to generate 3 overlapping fragments of the msg gene. The sequences of the oligonucleotides used were as follows: 5′ AACTACCTTAAAAACCTTCAA 3′ (forward) and 5′ TTAGGATCTGGATTCTGA 3′ (reverse), to generate msg15-1119; 5′ CGAAGCTTATGACTGCGAG 3′ (forward) and 5′ TCCTCGGAGCTCTACTTTTGAG 3′ (reverse), to generate msg729-2282; and 5′ TTCCAAAACGCTACGTGTAA 3′ (forward) and 5′ TCAATTGATGCTGAAGAGATG 3′ (reverse), to generate msg2015-3332. The template used to generate msg729-2282 and msg2015-3332 was an Agt11 clone of human-derived msg, whereas the template used to make msg15-1119 was genomic DNA from P. jiroveci–infected human lung. The sequence of the PCR products was confirmed, and they were cloned into the pET30 expression vector (Novagen) in the pET30 vector without insert was used as a control antigen. The recombinant proteins were expressed in inclusion bodies within E. coli. The cultures expressing recombinant Msg fragments were harvested by centrifugation, the cell pellet was sonicated and washed 3 times in binding buffer without urea (5 mM imidazole, 0.5 M NaCl, and 20 mM Tris-HCl [pH 7.9]), and the final pellet was dissolved in binding buffer with 6 M urea. The recombinant preparations were purified by affinity chromatography using HISBinding resin (Novagen), with the urea being removed during the wash stages. Eluted proteins were dialyzed overnight against PBS (pH 7.4), were filter sterilized, and were frozen at −70°C. Protein concentration was determined by A_{280} using a standard curve generated with bovine serum albumin.

Western blot analysis. Recombinant Msg fragments were run on SDS-PAGE gels (Invitrogen), were transferred to nitrocellulose, and were blocked in 1% nonfat milk in TTBS (20 mM Tris(hydroxymethyl)aminomethane (THAM), 0.5 M NaCl, 0.05% and Tween-20) for 1 h at room temperature. Blots were cut into strips, and individual strips were incubated, either overnight at 4°C or for 2 h at room temperature, either with serum from healthy donors or HIV−positive patients (1:50 dilution in TTBS) or with a rabbit polyclonal antisera (1:5000 dilution in TTBS) generated against a cell-wall preparation of P. jiroveci. The strips were washed 3 times with TTBS and were incubated either with horseradish peroxidase–labeled goat anti-human IgG or with horseradish peroxidase–labeled goat anti-rabbit IgG (Kirkegaard & Perry Laboratories) (1:5000 dilution in TTBS), for 1 h at room temperature. The strips were washed 3 times in TTBS, color was developed by use of TMB membrane substrate (BioFX), and the reactions were stopped by H2O. To identify the appropriate bands for analysis and to serve as a positive control in our assay, 1 strip from each gel was blocked in 1% nonfat milk, as described above, was probed with horseradish peroxidase–labeled S protein (Novagen) (1:5000 dilution in TTBS) for 1 h at room temperature, was washed 3 times, and was color developed as described above. S protein reacts with the S-tag encoded within the pET30 vector and is expressed as a fusion protein in our constructs, and therefore it serves as a positive control. It does not react with E. coli proteins. As a negative control, 1 strip from each gel was blocked in 1% nonfat milk, as described above, was incubated with horseradish peroxidase–labeled goat anti-human IgG, and was washed and color developed as described above. The strips probed with human serum were compared to the positive and negative control strips. Each assay was read by 2 independent readers. Results were usually unequivocal, but, in cases of ambiguous results, the assay was repeated at least once, and the predominant result from all readings of those samples was reported.

Serum from volunteers. Samples were obtained from several different sources. Serum samples from 95 healthy adult blood donors were obtained from the Hoxworth Blood Bank in Cincinnati, Ohio; these samples were stored at −20°C. Four cohorts of serum samples, which had been obtained during 1990 from persons residing in different geographic regions, also were tested [6]. These serum samples were (1) from HIV-seronegative Haitian women enrolled in a study of transmission of HIV infection; (2) HIV-seronegative men from a rural area of South Africa; (3) HIV-seronegative patients attending an outpatient clinic at the University Hospital, College of Medicine, Seoul National University, in South Korea; and (4) blood donors from Hoxworth Blood Bank, Cincinnati, Ohio. All of these samples had previously been tested for reactivity to a crude preparation of P. jiroveci isolated from human lung [6]. Samples from 94 HIV-positive patients were obtained from serum banked at the University of Cincinnati Infectious Diseases Center. The medical records of these subjects were abstracted to obtain the CD4 lymphocyte counts. HIV RNA levels closest to the time when the samples had been collected (±3 months), and clinical history.

Antibody-elution assay. Recombinant MsgB was run on SDS-PAGE gels, was transferred to nitrocellulose, and was blocked in 1% nonfat milk in TTBS for 1 h at room temperature. The location of the recombinant protein was identified by reactivity to S protein, and strips corresponding to the molecular mass of the recombinant were cut out and were incubated either overnight at 4°C or for 2 h at room temperature, with a rabbit polyclonal antisera (diluted 1:50 with TTBS) generated against a cell-wall preparation of human P. jiroveci. The antisera was removed and stored, and the strips were washed 3 times with TTBS. After the final wash, the
Figure 1.  msg recombinants used in the present study, compared with the full-length msg gene. No. of nucleotide base pairs is shown.

strips were incubated with 0.1 M glycine (pH 2.5) for 5 min, to release the bound antibodies. The released antibodies were removed, and the pH was brought to neutrality by the addition of Tris-(hydroxymethyl)aminomethane hydrochloride (pH 8.5). The blots were rinsed once with TTBS, the antiserum was reapplied, and the bound antibodies were eluted as described above, 3 times. The eluted antibodies were tested for reactivity to a crude preparation of P. jiroveci isolated from infected human lung, by Western blot analysis.

Statistics. Statistical analysis was performed by Graphpad InStat (Graphpad Software). Serum samples from healthy blood donors were compared with those from subjects with HIV infection, to determine the rates of reactivity. Serum samples from HIV-seronegative subjects from Haiti, South Africa, Korea, and Cincinnati were compared, to determine the rates of reactivity. We calculated that a sample size of 22 subjects per group would be expected to have an 80% power to detect a 50% difference in the rate of reactivity ($P < .05$). The samples were correlated by a Kappa test. Statistical significance ($P < .05$) of categorical variables was determined either by $\chi^2$ test or, when appropriate, by Fisher's exact test.

Nomenclature. The nomenclature used in the present study conforms to the system proposed for Pneumocystis species [33].

Results

Specificity of the Msg constructs. Using oligonucleotides specific for the sequence of a cloned msg gene, we used PCR to generate 3 overlapping fragments spanning the entire length of the msg gene (figure 1). Whereas msg29-2282 and msg3015-3332 were generated by using the cloned msg gene as template, msg15-1119 was generated by using P. jiroveci DNA, isolated from infected human lung, as template. The nucletotide sequence of msg15-1119 (GenBank AY072779) is 86% identical to the corresponding sequence of the cloned msg gene used as a template for the remaining fragments, and the deduced amino acid sequence is 74% identical to that of the cloned gene. The sequence of msg15-1119 also exhibits 67%–70% identity, at the nucleotide level, and 57%–60% identity, at the deduced amino acid level, with the corresponding portion of other cloned msg genes (figure 2) [7, 32]. The 3 recombinant fragments of Msg—MsgA, MsgB, and MsgC—were expressed in E. coli and were purified away from contaminating bacterial proteins by affinity chromatography. A typical purification profile for MsgB is shown in figure 3; similar profiles were obtained for the other fragments and for the control protein (i.e., pET expression vector without insert) (data not shown). Whereas the purification of MsgA and MsgB consistently gave a single band in SDS-PAGE analysis, the profiles for purification of MsgC always had multiple bands in addition to the full-length fragment. Presumably, these were breakdown products that were copurified along with the full-length fragment because they express the S-tag used for purification. These extra bands were not taken into consideration when reactivity to the MsgC fragment was scored; only reactivity to the full-length band was recorded.

To investigate the utility that our recombinant fragments had for the analysis of human serum antibody reactivity, we first had to demonstrate the specificity of the fragments and that human serum could recognize all 3 fragments. We showed the specificity of the Msg fragments in 3 ways (figure 4). First, a polyclonal rabbit antiserum that was raised against a cell-wall preparation of Pneumocystis carinii was used to demonstrate the specificity of each fragment. The antiserum was reacted with each of the recombinant fragments, and the reactivity of the antiserum was determined by Western blot analysis. The results of these experiments are shown in figure 4, and the data are summarized in table 1.

Figure 2. Comparison of deduced amino acid sequences of major surface glycoprotein A (MsgA) and the corresponding sequences of the known Msg gene products [7, 32]. An asterisk (*) indicates identity; blank spaces have been introduced for optimal alignment.
None of the serum samples tested reacted with the control protein expressed by the empty vector (data not shown).

Comparison of serum samples from different geographic locations. To determine if recognition of the Msg fragments was a function of the geographic source of the serum samples, we tested 4 panels of serum samples that originated in different geographic locations, for the ability to recognize the recombinant fragments. Twenty-two serum samples each from the United States, Haiti, South Africa, and South Korea were tested, by Western blot analysis, for their ability to recognize the Msg fragments (table 1). Each of the 3 Msg fragments could be recognized by serum samples from each country, and the frequency of recognition did not vary significantly between the groups of serum samples; 77% (17/22) of US 1990 serum samples reacted with ≥1 of the fragments, whereas 59% (13/22) of Haitian serum samples, 68% (15/22) of South African serum samples, and 55% (12/22) of South Korean serum samples did so; furthermore, the recognition of any 1 of the 8 possible patterns was not statistically different between the panels of serum samples when compared by a Kappa test; for example, 36% (8/22) of 1990 US serum samples, 27% (6/22) of Haitian serum samples, 23% (5/22) of South African serum samples, and 18% (4/22) of South Korean serum samples recognized MsgA. To determine if the apparent variation in reactivity to the Msg fragments is due to the relatively small number of samples tested, we analyzed an additional 45 serum samples from Korea, for reactivity to MsgA (data not shown). When all 67 Korean serum samples were analyzed together, 39% (26/67) were positive for reactivity to MsgA, compared with 36.5% and 40% of US serum samples (in the 1990 and 2000 groups, respectively). The median CD4 count after ≤3 months of the collection of serum samples was 94 cells/mm³ (n = 86). The median HIV-1 RNA level was 87,000 copies/mL (n = 67). Few subjects—4.4% (3/67)—with HIV infection had an HIV-1 RNA level <400 copies/mL. Thirty-five subjects had experienced a prior episode of histologically proven P. jiroveci. The overall reactivity of the HIV+ serum samples was lower than that of the serum samples from healthy donors, in that 34% (32/94) of the HIV+ serum samples did not react with any of the fragments, whereas only 16% (15/95) of the HIV+ serum samples from healthy donors showed lack of reactivity (P = .003). The reactivity to individual fragments of Msg varied, depending on both the source of the serum and the identity of the fragment being tested. Both MsgA and MsgB were recognized more frequently by serum samples from healthy donors than by serum samples from HIV-positive donors. MsgA was recognized by 40% (38/95) of the serum samples from healthy donors.
Figure 4. Specificity of major surface glycoprotein (Msg) constructs, as shown by antibody reactivity with MsgA, MsgB, and MsgC but not with the control (pET). A, Reactivity of a polyclonal rabbit antiserum, raised against a cell-wall preparation of *Pneumocystis jiroveci*, to Msg constructs. Clear reactivity to MsgC was seen after increased development time (MsgC/*H11001*). B, Reactivity of MsgB-specific antibodies to native Msg. Rabbit antibodies eluted from MsgB (αMsgB), but not antibodies eluted from an irrelevant protein (neg.), reacted with a 95-kDa protein (*) in a crude homogenate of infected human lung. C, Reactivity of human serum to each Msg recombinant.

Donors but by only 27% (25/94) of the HIV⁺ serum samples (P = .064). However, the difference in recognition of MsgB was significant in that 64% (61/95) of the serum samples from healthy donors and 45% (42/94) of the serum samples from HIV-positive donors recognized the fragment (P = .0086). There was no difference in frequency of recognition of MsgC—41% (39/95) of healthy and 39% (37/94) of HIV⁺ serum samples recognized the fragment.

The reactivity of HIV⁺ serum samples to individual fragments of Msg varied depending on whether the patient had experienced a previous bout of PcP (figure 5B). Of 34 PcP-positive patients, 20 (59%) recognized MsgC, compared with only 17 (28%) of 60 PcP-negative patients (P = .0046). Of particular interest is that none of the 9 HIV-positive patients who went on to develop PcP after their serum specimen was obtained had antibodies to MsgC. In the rates of reactivity to the other Msg fragments, there was no significant difference between the groups of serum samples (figure 5B). There was also no significant difference in frequency of reactivity to any of the fragments when the HIV⁺ serum samples were separated on the basis of either CD4⁺ T cell count (i.e., when those with <100 CD4⁺ T cells/mm³ were compared with those with >100 CD4⁺ T cells/mm³) or virus titer (i.e., when those with <100,000 viral copies/mL were compared with those with >100,000 viral copies/mL) (data not shown).

Discussion

In the present study, we have generated 3 overlapping recombinant fragments of human *P. jiroveci*-derived Msg, for use as standardized reagents in immunological assays. Each recombinant represents a single Msg fragment that can be analyzed independently. Given both the difficulty in obtaining sufficient quantities of antigens from *P. jiroveci* and the inherent variability in Msg isotypes, these fragments represent a unique opportunity to analyze immune responses to a single Msg fragment. The usefulness of these proteins for serological studies is underscored by the fact that each fragment can be recognized in a specific manner by both human and rabbit serum antibodies.

Western blot analysis of 95 randomly obtained serum samples from anonymous healthy donors demonstrates that each of the constructs can be recognized by human serum. Of these serum samples 84% (80/95) reacted with 1 of the Msg fragments; MsgB was recognized by 64% of the serum samples tested, whereas MsgA and MsgC were recognized by 40% (38/95) and 41% (39/95) of the serum samples, respectively. In general there was greater recognition of MsgB: 76% (29/38) of the serum samples that recognized MsgA and 69% (27/39) of the serum samples that recognized MsgC also recognized MsgB. This suggests that MsgB may contain epitopes that are more commonly recognized.

This high level of reactivity in a healthy population of adults is consistent with prior exposure to Msg as an antigen, probably as a consequence of early childhood exposure to *P. jiroveci*. It is interesting that such a large proportion (84%) of healthy samples reacted positively with the recombinant fragments, because it suggests either that the variability in Msg epitopes recognized by B cells is restricted and biased toward cross-reactive epitopes or that the responding subjects in the present study were exposed to the Msg that is represented in the recombinant fragments. The second scenario could be true if the subjects were exposed to a dominant, localized population of *P. jiroveci* expressing a single Msg. However, the fact that serum samples collected from different global locations, as well as serum samples collected locally but 10 years apart, exhibit es-
sentially the same patterns of reactivity to the Msg recombinants supports the former model. It has been suggested that *Pneumocystis* species has the potential to evade the immune system by switching the isoform of Msg that is expressed; however, in the absence of a continuous-culture system for the organism, this theory is difficult to test. The data presented herein suggest that there are a limited number of Msg epitopes that are recognized by serum antibodies and that exposure to Msg results in the germination of cross-reactive antibodies. One way of testing this hypothesis is to isolate a panel of recombinants that represent different isoforms of Msg and then to analyze the patterns of reactivity to those new fragments. Since reactivity to different isoforms of Msg could simply mean exposure to those different isoforms of Msg—not the presence of cross-reactive antibodies—antibody-elution experiments would help to elucidate the extent of cross-reactivity between different isoforms of Msg.

Although the work presented herein represents an initial characterization of new recombinant reagents, the high level of reactivity to the Msg fragments in a healthy population presents a drawback to the use of the fragments in following active PcP infection, at least when Western blot analysis is used. It is very unlikely that the presence or absence of reactivity to the Msg recombinants will serve as a marker worth studying during infection; it is more probable that the titer of anti-Msg antibodies will be important as a measurable parameter of infection. To this end, the use of the Msg recombinants would be enhanced by the development of an ELISA system to measure quantitative antibody-titer differences between groups of subjects. Prospective studies are needed in which serum specimens are obtained over time from patients who do or do not develop episodes of PcP. Such studies will help to determine whether epitope recognition and the magnitude of the antibody response can be correlated with susceptibility to and recovery from PcP.

Analysis of serum samples from 4 different countries has shown that the recognition patterns of the Msg fragments do not significantly vary on the basis of the geographic origin of the serum samples. These serum samples had previously shown differential reactivity to various high-molecular-weight *P. jiroveci* antigens, including Msg [6]. However, the lack of variability in the response to the recombinant Msg fragments that has been seen in the present study suggests that there are a limited number of Msg epitopes that can be recognized by serum antibodies. It is possible that the results reported herein reflect the number of serum samples analyzed and that differences in reactivity patterns may emerge from analyses of larger samples. However, we have not been able to find any correlation between the reactivity of a given serum sample to any recombinant Msg fragment and its reactivity to the crude preparation of antigen analyzed in the previous study, suggesting that there are inherent differences in the preparations of antigen used in this and in previous studies. The difference in results may reside in the fact that, in the present study, we have directly analyzed the response to recombinant fragments of Msg, whereas the previous study had analyzed the response to a complex mixture of *P. jiroveci* antigens derived from infected human lungs. Definitive analysis of a single protein is very difficult when complex mixtures of proteins such as those in a lung extract is used. An alternative explanation may be that native Msg as expressed in *P. jiroveci* is glycosylated, and it is possible that the variability in reactivity to the native Msg is due to regional variations in glycosylation of the proteins. The recombinants that we have studied herein are expressed in *E. coli* and are not posttranslationally modified. Therefore, the responses that we have measured are directed at epitopes in the peptide backbone of Msg and are independent of glycosylation patterns. It would be interesting to engineer the Msg fragments for expression in *Pichia pastoris*, a yeast system that allows glycosylation of expressed proteins. Although the glycosylation pattern of *P. pastoris* may not be the same as that of *P. jiroveci*, expression of the Msg recombinants in this system would allow us to analyze the effect that glycosylation has on recognition by serum antibodies. It

**Table 1.** Recognition of major surface glycoprotein (Msg) fragments by human immunodeficiency virus (HIV)-negative serum from 4 distinct geographic regions

<table>
<thead>
<tr>
<th>Fragment(s) recognized</th>
<th>United States</th>
<th>Haiti</th>
<th>South Africa</th>
<th>South Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000 (n = 95)</td>
<td>1990 (n = 22)</td>
<td>(n = 22)</td>
<td>(n = 22)</td>
</tr>
<tr>
<td>MsgA alone</td>
<td>7 (7)</td>
<td>4.5 (1)</td>
<td>9 (2)</td>
<td>0</td>
</tr>
<tr>
<td>MsgB alone</td>
<td>21 (20)</td>
<td>4.5 (1)</td>
<td>9 (2)</td>
<td>14 (3)</td>
</tr>
<tr>
<td>MsgC alone</td>
<td>10 (10)</td>
<td>9 (2)</td>
<td>9 (2)</td>
<td>18 (4)</td>
</tr>
<tr>
<td>MsgA + MsgB</td>
<td>15 (14)</td>
<td>14 (3)</td>
<td>4.5 (1)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>MsgA + MsgC</td>
<td>2 (2)</td>
<td>0</td>
<td>9 (2)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>MsgB + MsgC</td>
<td>13 (12)</td>
<td>27 (6)</td>
<td>14 (3)</td>
<td>14 (3)</td>
</tr>
<tr>
<td>MsgA + MsgB + MsgC</td>
<td>16 (15)</td>
<td>18 (4)</td>
<td>4.5 (1)</td>
<td>4.5 (1)</td>
</tr>
<tr>
<td>None</td>
<td>16 (15)</td>
<td>23 (5)</td>
<td>41 (9)</td>
<td>32 (7)</td>
</tr>
<tr>
<td>Total MsgA</td>
<td>40 (38)</td>
<td>36.5 (8)</td>
<td>26 (7)</td>
<td>23 (5)</td>
</tr>
<tr>
<td>Total MsgB</td>
<td>64 (61)</td>
<td>64 (14)</td>
<td>32 (7)</td>
<td>41 (9)</td>
</tr>
<tr>
<td>Total MsgC</td>
<td>41 (39)</td>
<td>55 (12)</td>
<td>36 (8)</td>
<td>45.5 (10)</td>
</tr>
</tbody>
</table>
| *a* Except for the United States “2000” samples, all serum samples had been drawn during 1990 and had been analyzed against crude preparations of *P. jiroveci* [6].
Figure 5. Frequency of reactivity to Msg fragments, in serum samples from (A) healthy donors and human immunodeficiency virus (HIV)-positive patients and (B) HIV-positive patients with or without prior exposure to PcP. The groups of serum samples were compared for reactivity to each fragment, by Fisher’s exact test. * and **P < .003 when HIV+/H11002 and HIV+/H11001 serum samples were compared (A), and *P = .0046 when PcP− and PcP+ serum samples were compared (B).

...would be interesting to compare the patterns of serum reactivity to the Msg fragments expressed in 2 different systems.

...Previous studies that used crude P. jiroveci antigens preparations to compare the frequency or level of antibodies in healthy subjects and in HIV-positive patients or other immunocompromised hosts have shown varying results [6, 8–15, 31, 34, 35]. These studies also revealed that HIV-positive patients who developed PcP could mount an antibody response; but the antigens were not strongly recognized. One report, which used a highly conserved portion of the carboxy terminus of human P. jiroveci Msg, found reactivity in all healthy control subjects and in HIV-positive patients with and without PcP [7].

The present study has shown that healthy adults have a significantly higher prevalence of serum antibodies to recombinant Msg than do HIV-positive patients, but this frequency of recognition mainly resides primarily in the MsgB fragment. Serum samples from HIV-positive patients who have recovered from PcP recognize MsgC significantly more frequently than do serum samples from HIV-positive patients who have not experienced PcP; none of the serum samples drawn from 9 patients before they had developed PcP recognized MsgC. These results are important because they demonstrate how the use of different segments of a single recombinant antigen can reveal previously unrecognized differences between population groups. It is possible that independent recognition of Msg fragments would be of value in the analysis of the diversity of epitopes either during various stages of HIV infection or after HAART.

...The results also suggest that antibodies play a role in host defenses against PcP in HIV-positive patients. Although there have been studies in humans [8, 15, 35–37], the most direct evidence for the importance of antibodies has come from animal models of PcP [19, 21, 38–46]. The results are also important because they raise the possibility that the B cell epitopes that elicit these antibody responses reside within MsgC. Mapping of the epitopes recognized by such antibodies will be facilitated by localization of these epitopes within a single Msg segment.

Acknowledgment

We gratefully acknowledge the excellent technical assistance of Judy Koch.

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