Antibody Response to a Major Human *Pneumocystis carinii* Surface Antigen in Patients without Evidence of Immunosuppression and in Patients with Suspected Atypical Pneumonia

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IgG and IgM antibodies to a purified human *Pneumocystis carinii* surface antigen (gp95) were measured in 694 serum specimens from two different population groups using an EIA technique. In a population of 441 patients with no evidence of immunosuppression, the percentage of persons positive for IgG antibodies to gp95 was significantly lower in the age group 1 to 9 years (30 %, 23/77) compared to persons 10 to 19 years old (56 %, 49/88). In the age group 1 to 14 years there was a significant correlation between the percentage of persons with IgG antibodies to gp95 and age. In 106 consecutive patients under evaluation due to atypical pneumonia, 76 patients showed no change in the titre of antibodies to *Legionella* spp. or *Mycoplasma pneumoniae* in two consecutive serum samples. Three of these 76 patients (4 %) demonstrated an increase in the level of IgG antibodies to gp95 in the paired samples. One of these patients had a verified *Pneumocystis carinii* pneumonia, and the two others were elderly men in whom no microbiological diagnosis of the pneumonia was established.

Thus, it is concluded that IgG antibodies to gp95 develop in the majority of nonimmunosuppressed persons before the age of 13. Furthermore, *Pneumocystis carinii* pneumonia should be considered in the differential diagnosis in patients suspected of having atypical pneumonia.

*Pneumocystis carinii* is a major pulmonary pathogen in immunosuppressed patients, particularly those with AIDS (1). In immunocompetent patients with no underlying systemic disease, *Pneumocystis carinii* pneumonia (PCP) has only been described in a few sporadic cases in adults and neonates, and recently in a cluster of five elderly patients (1–3). Since it has not been possible to grow *Pneumocystis carinii* reliably in vitro, the natural history of infection with *Pneumocystis carinii* immunocompetent patients remains speculative. Serological techniques have been the only practical means of investigation in relation to the epidemiology and diagnosis of infection with *Pneumocystis carinii* (4–8). Findings in studies using immunofluorescence techniques with whole *Pneumocystis carinii* organisms as antigen suggest that the majority of the population acquires infection with *Pneumocystis carinii* after infancy (5, 6, 8). Western blot analysis has shown a more complex spectrum of changes in antibody responses against a variety of *Pneumocystis carinii* antigens, but these changes are difficult to quantitate (4, 9).

We have purified the major surface antigen of human *Pneumocystis carinii* (gp95), and developed an enzyme immunoassay (EIA) with which quantitation of serum antibodies to this antigen is possible (10). Applying this assay in a population of HIV infected patients with pneumonia, it was demonstrated that an increase in IgG antibodies to gp95 occurred almost exclusively in patients with PCP (11).

The aim of the present study was to further increase our understanding of the interaction between human *Pneumocystis carinii* and its host. The presence of antibodies to gp95 in patients...
with no evidence of immunosuppression divided into various age groups was investigated. Furthermore, serial serum samples from patients under evaluation due to atypical pneumonia were investigated to see if there was serological evidence of infection with Pneumocystis carinii in the patient population.

Materials and Methods

Pneumocystis carinii Antigen. Human Pneumocystis carinii was obtained from lung tissue from a patient with AIDS and PCP after autopsy (12). The ground Pneumocystis carinii was treated with Lyticease (Sigma, USA), and gp95 was purified in a two-step procedure using high-performance liquid chromatography (size dependent and charge dependent) as previously described (10).

Enzyme Immunoassay. A previously described EIA (11) was used in a modified form to detect both IgG and IgM antibodies. Purified human Pneumocystis carinii antigen (gp95, 1 µg/ml) was used as antigen. Human sera were diluted (1/100). As a second antibody we used peroxidase-conjugated goat anti-human IgG (heavy and light-chain specific) (Jackson Immuno Research Laboratories, USA) diluted 1:25,000 or IgM (µ-chain specific) (Tago, USA) diluted 1:40,000. All samples were tested in duplicate both with and without Pneumocystis carinii antigen. A positive and a negative sample were tested on each plate. Optical density (OD) values for each sample were determined by subtracting the mean of the duplicate wells containing no antigen from the mean of the duplicate wells containing antigen. Results were expressed as the OD ratio (OD sample/OD positive control). The OD range for the IgG positive control was 0.8-1.0, and for the IgM positive control 0.4-0.6. Each experiment also included an IgG negative control with an OD of less than 0.15. This negative control did not react with gp95 on Western blot. On the basis of preliminary immunoblot studies, an IgG positive sample was defined as having an OD value of >0.2 with an OD ratio of >0.25 (11), and an IgM positive sample was defined as having an OD value of >0.2 with an OD ratio of >0.4 (unpublished data). All samples found to be positive in the IgM EIA were tested for rheumatoid factors since the µ-chain specific antibody may also detect rheumatoid factors (13). Serial samples from the same patient were tested on the same plate. The investigator analyzing the results of tests for serum IgG antibodies to gp95 in 441 patients with no evidence of immunosuppression (group 1) divided into different age groups are shown in Figures 1 and 2. Infants younger than one year were excluded from the data in Figures 1 and 2 to avoid bias from maternal antibodies. However, only 8 of 24 (33 %) infants younger than one year had detectable IgG antibodies to gp95, and this frequency of positive subjects was not different from that in children 1 to 9 years old (30 %). The percentage of samples with IgG antibodies to gp95 varied between the different age groups (Figure 1). The greatest increase in the percentage of positive subjects was seen in children 1 to 9 years old (30 %, 23/77) compared to subjects 10 to 19 years old (56 %, 49/88) (p < 0.001, Fisher’s test). To further study at which age the increase in the percentage of subjects positive for gp95 occurred, subjects between 1 and 14 years were divided into two-year age groups (Figure 2). A steady increase in the percentage of positive subjects related to older age was found (r = 0.95, p < 0.05, Spearman test). There was a non-significant trend towards a decrease in the percentage of subjects positive for IgG antibodies to gp95 from age 10-19 years to age >70 years (r = -0.64, p = 0.12, Spearman test). IgM antibodies to gp95 were found in 6 of the 441 (1 %) patients. None of these patients had rheumatoid factors in their serum. Of the subjects positive in the IgM EIA one was situated in each of the age groups presented in Figure 1 up to 60 years of age. None of the 24 children less than one year of age had IgM antibodies to gp95.

The 106 patients suspected of having atypical pneumonia (group 2) were also tested for antibodies to gp95. Nine (30 %) of the 30 patients with serological evidence of Legionella spp. and Mycoplasma pneumoniae infection had IgG antibodies to gp95 in both the first and second samples. No change in the OD ratio between the first and second serum samples was observed in any of the patients. Of the remaining 76 patients, who had no serological evidence of infection with Legionella spp. or Mycoplasma pneumoniae and no detectable cold agglutinins, 37 (49 %) were found to have IgG antibodies to gp95. In 3 (4 %)