Serological study of *Pneumocystis carinii* infection in the absence of immunosuppression

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SUMMARY Serum samples from 145 children with no known immunosuppressive illness were examined by indirect immunofluorescence for antibody to *Pneumocystis carinii*. Positive antibody titres ($\geq 1:8$ dilution) were found in 69 children (48%). Antibody could not be detected in the remaining children. Previous studies have shown that at least 75% of children have antibodies to *P. carinii* by the age of 4 years. This study shows a lower percentage of children with detectable antibody. This may be related to geographical variation of antigen or possibly to the widespread use of co-trimoxazole.

Pneumocystis carinii pneumonia occurs almost exclusively in the immunocompromised host. Isolated *P. carinii* organisms have been identified, however, in the lungs of individuals with no evidence of disease.¹ It is thought that clinical infection occurs as a result of reactivation of latent organisms producing replication and subsequent disease. There have been problems encountered in the development of specific methods for detecting infection histologically and serologically and these have caused the reliability of serological diagnosis in particular by either complement fixation or indirect immunofluorescence to be questioned.

Previous studies have suggested that there is a high prevalence of latent infection, with almost 100% of normal children tested in a Dutch study having positive antibody by the age of 2.² A similar study carried out by Pifer *et al* found that by the age of 4 years two thirds of normal children had antibody to *P. carinii* in titres of 1:16 or greater.³ Both of these authors concluded that their results indicated subclinical infection was highly prevalent in normal children but that disease was manifested in the compromised host only.

As part of a prospective study of pneumocystis antibody titres in children with acute lymphoblastic leukaemia we wished to find whether these observations applied to non-immunosuppressed children in the United Kingdom.

Patients and methods

Serum samples from 145 children were studied. Specimens were obtained at the same time as other

routine blood tests from children attending the diabetic clinic at the Royal Victoria Infirmary, Newcastle (50 children), and from children attending the paediatric outpatient clinics at the Royal Hospital for Sick Children, Edinburgh (95 children). The latter group comprised children attending the cardiology and haematology departments. No child was known to be suffering from any immunosuppressive illness and none was on steroid medication. The age range was 0.45 to 13.9 years (mean 7.4 years and median 7.35 years). There were 82 boys and 63 girls in the study.

Serum samples were tested at the microbiology department of the Royal Marsden Hospital, London, using an indirect immunofluorescence test, full details of which have been reported previously.⁴ Briefly, the substrate used was a paraffin section of human lung heavily infested with pneumocysts. The tissue was fixed in Bouin's fluid to destroy existing immunoglobulin in the alveolar exudate and to preserve polysaccharide antigen. The serum samples were initially screened at a 1:8 dilution with fluorescein labelled antihuman immunoglobulin (Wellcome reagents). Positive samples were subsequently titrated for IgG antibodies in doubling dilutions from 1:8 upwards. A positive reading was recorded when the entire content of the alveolar spaces (which include all stages of the parasites' development) was brightly stained.

Results

Seventy six children (52%) had no detectable antibody to *P. carinii*. Thirty six, 19, and 14 (25%,

Figure *Pneumocystis* antibody titres at different ages.

13%, and 10%) had antibody present at dilutions of 1:8, 1:16, and 1:32, respectively. The results were analysed to observe any variation in antibody production with age, and these calculations are shown in the Figure. There was a trend to more of the older children having higher levels of antibody but this did not achieve significance.

Discussion

These findings conflict with the earlier studies by Meuwissen² and Pifer.³ Studies using axenic rats have shown that *P. carinii* is naturally acquired as an airborne organism in a de novo infection.⁵ *P. carinii* seems to be a widely prevalent organism, but geographic variations in its distribution may occur and this could explain the different results obtained in this study, which was carried out in children from the north of England and Scotland in the autumn of 1984, and the two previous studies, which were carried out in Dutch children in 1977 and in children from Memphis, Tennessee, in 1978.

Co-trimoxazole is a commonly used antibiotic in the UK and is often used in the treatment of upper and lower respiratory tract infections. The use of prophylactic co-trimoxazole is effective in preventing pneumocystis pneumonia in children immunosuppressed as a result of acute lymphoblastic leukaemia.⁶ The widespread use of this antibiotic within the community could possibly reduce the number of primary infections that occur, and this could explain the different results obtained. In

contrast co-trimoxazole has not been widely used in general practice in the United States until fairly recently.

The incidence of *P. carinii* pneumonia is difficult to ascertain. A study from the USA by Siegel *et al* found an incidence of *P. carinii* pneumonia of 6.8% in a group of 844 children undergoing treatment for acute lymphoblastic leukaemia.⁷ Darbyshire *et al* in a report from the UK documented an incidence of proven *P. carinii* pneumonia of 9% in a group of 199 children with acute lymphoblastic leukaemia.⁸ Thus it would seem that there is no great difference in the incidence of pneumonia due to pneumocystis in the immunocompromised children from the USA and UK despite the different distribution of antibody titres in the population.

All three studies used an indirect immunofluorescence method for the detection of antibodies. This study used human lung heavily infested with pneumocysts. The method of extraction was designed to destroy existing immunoglobulin in the alveolar exudate and preserved polysaccharide antigen. In contrast Pifer *et al* used cysts grown in embryonic chick epithelial lung cell culture.³ The cysts were harvested and purified on a sucrose density gradient. Meuwissen *et al* also used human lung tissue as the slide antigen,² but there were differences in the purification and extraction of the material. These differences in methodology may have affected the sensitivity and specificity of the detection of *P. carinii* antibody and could account for the different results obtained.

This study was performed to define the distribution of *P. carinii* antibody in the UK child population as a prelude to a study in children with leukaemia. Initial results from the study on leukaemia show a very similar distribution of *P. carinii* antibody titres at the time of diagnosis as occurs in this 'normal' group. Thirty nine out of 85 (47%) children with leukaemia below the age of 4 years had no detectable antibody. Over the age of 4 years 41 out of 78 children (47%) were also antibody negative. Further studies on this group of children with acute lymphoblastic leukaemia are in progress and will be reported later.

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