Thorax 1982;**37**:481–485

Editorial

Pneumocystis carinii: the continuing enigma

Pneumocystis carinii occurs in patients who are marasmic, congenitally immunodeficient, or immunosuppressed, usually because of chemotherapy. Clinically the disease is most common in patients with acute lymphocytic leukaemia.[1] The organism attracted relatively little interest until the end of 1981, when a new syndrome was described.[2–9] This was a combination of *Pneumocystis carinii* pneumonia and Kaposi's sarcoma, occurring mainly in homosexual men. Kaposi's sarcoma is primarily a disease of the skin and occurs most commonly in Africa. In the West it usually occurs in elderly men and is seldom lethal. The disease has been shown to have an increased incidence in patients having immunosuppressive treatment.[10] Clinically there are multicentric red-blue tumours in the skin, histologically resembling benign capillary haemangiomas. With time, usually after many years, the disease may progress so that the cellularity increases and the tumour resembles a fibrosarcoma or a haemangioendothelioma. In the homosexual patients with the new syndrome the disease is rapidly progressive. Interestingly, the association between Kaposi's sarcoma and *Pneumocystis carinii* infection has occurred almost exclusively in New York State and California, where there have now been 89 cases of Kaposi's sarcoma, 91 of pneumocystis pneumonia, and 18 of the combined condition.[9] Forty per cent of these patients have died. Most of the patients apparently die from their pneumonia, though a smaller percentage have succumbed to the sarcoma.

The homosexual group is unusual in that the Kaposi's sarcoma and pneumocystis pneumonia are occurring in a population of previously healthy young men. In these patients there is a fairly consistent clinical pattern, skin or mucosal lesions occuring in the patients with Kaposi's sarcoma and in the others lymphadenopathy, fever, weight loss, diarrhoea, dyspnoea, and non-productive cough.[9] Considerable delay (three to six months) often occurred between the initial onset of symptoms and diagnosis in both groups. In addition to *Pneumocystis carinii* there were other opportunistic organisms—particularly cytomegalovirus, which was present in nearly every case.[8] These organisms included *Candida albicans* in the mouth or oesophagus, *Herpes zoster* or *simplex*, *Aspergillus* sp, *Cryptococcus* and Gram-negative bacteria. The homosexual patients were immunosuppressed with no lymphocytic proliferative responses to soluble antigens, a reversal of the normal ratio of helper T cells to suppressor and cytotoxic T cells, and an absolute lymphopenia. Humoral response was normal and in fact many patients had a raised level of IgA and, in a few cases, of IgM and IgG. Circulating immune complexes were also increased and these were thought to be responsible for the cotton-wool retinal lesions, which may be microinfarctions. Although most of the patients were homosexual, some were heterosexual and these took drugs,[3] particularly alcohol, heroin, methadone, and cocaine. The reason for the immunosuppression in homosexuals is unknown but cytomegalovirus, which can cause immunosuppression, could be implicated. This is not the entire answer, however, since this infection is common among homosexuals[11] whereas immunosuppression is not. Drugs, especially the nitrites, used as sexual stimulants in homosexuals, have been blamed for the immunosuppression. Heroin abuse alone can also cause immune deficiencies.[12]

Against this background of a dramatic increase in pneumocystis pneumonia, we must take a closer look at the organism. Even though it has been recognised in Britain since 1955[13] relatively little is known about *Pneumocystis carinii.* It is an organism of uncertain affinities. The presence of membrane-bound cell organelles inside individual organisms means that it is a eukaryotic cell and therefore has no relationship with viruses or bacteria. Its taxonomic position is problematical but the organism has been assigned to either the fungi or the protozoa.[14 15] The fact that the organism does not easily fit into the present classification of fungi or protozoa suggests either that the classification is deficient or, less likely, that relevant taxonomic features have been missed by the many workers who have examined *Pneumocystis carinii.* We believe it to be a protozoan, probably a sporozoan. No viable intracellular stages have been identified and the conoid structure characteristic of most sporozoa appears to be absent. The organism alternates between trophic and cystic phases.[16] The trophozoite is small, ranging from 2 to 12 μm, and pleomorphic,

and frequently bears tubular surface projections. These projections may assist in adhesion to alveolar cells[17] and may also increase the nutritional absorptive area. The pleomorphic nature of the organism suggests that it may be mobile but no evidence of ameoboid movement has been described. On exposure to a stimulus, as yet unknown, the trophozoite rounds up, the surrounding wall thickens, and a precyst is formed. The precyst undergoes some morphological changes, which produce several trophozoite-like intracystic bodies within a mature cyst and culminate in the release of these inclusions. The release of the intracystic bodies appears to be followed by binary fission. After release of the trophozoites the collapsed cyst has a characteristic crescent shape and is easily seen by both light and electron microscopy. This completely asexual process seems to allow *Pneumocystis* to multiply rapidly and also furnishes it with a resistant cystic stage. A summary of the proposed life cycle of the organism is shown in the figure.

The organism appears to cause interstitial oedema and fibrosis in the alveolar wall leading to hypoxaemia. The question of whether *Pneumocystis* causes pulmonary fibrosis, however, has not yet been completely resolved. This is because many patients have received radiotherapy or chemotherapy or have been given oxygen, all of which have been implicated in the causation of pulmonary fibrosis, though recently the theory that short-term oxygen treatment causes lung damage has been questioned.[18] Pulmonary fibrosis has been noted after pentamidine treatment used to treat the pneumocystis infection.[19] Despite the many factors that may be associated with pulmonary fibrosis, in cases of pneumocystis pneumonia the organism seems likely to be the important cause. A prospective study of children surviving pneumocystis pneumonia has been carried out at St Jude Children's Research Hospital, Memphis, Tennessee, a leading centre for research into the disease, where Sanyal *et al* followed 23 children who survived the acute stage of pneumocystis pneumonitis.[20] Seventeen patients had a decrease in pulmonary gas transfer factor but by six months there was complete resolution in all survivors. This finding is in keeping with recent evidence suggesting reversibility of pulmonary fibrosis. We know that there is increased collagenase and elastolytic enzyme activity and low levels of α_1-antiprotease[21] in the lungs of patients recovering from adult respiratory distress syndrome. In nine patients who died in the follow-up period there was no residual interstitial fibrosis or collagen deposition. Excluded from this study were a group of children who received ventilation and had high fractional inspired oxygen values (FIO_2) of 0·8–1 atm for more than one week. These patients had pulmonary fibrosis, which the authors attributed to oxygen toxicity.

The diagnosis of pneumocystis infection is estab-

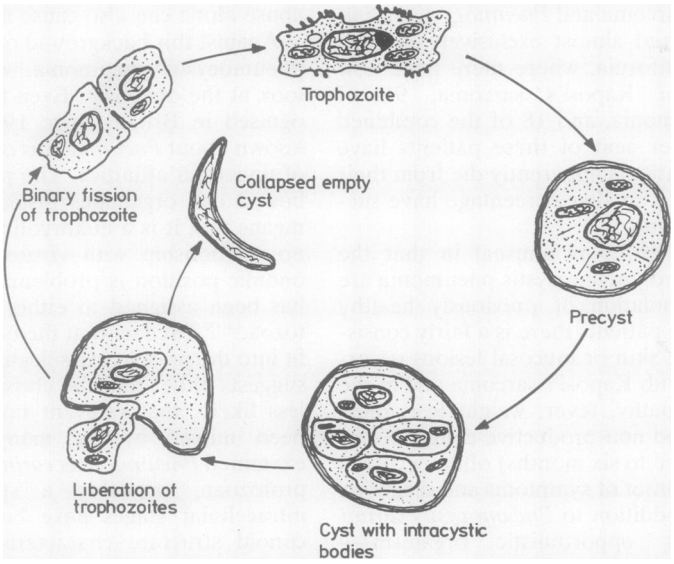

Diagram of life cycle of Pneumocystis carinii. *From Hasleton* et al.[16] *Reproduced by courtesy of the Journal of Clinical Pathology.*

lished by microscopy and consideration of the methods available is important. The laboratory must be given the largest possible sample of lung tissue. Walzer[1] showed that diagnosis was made most often from open lung biopsy. The advantage of this method is that the surgeon can inspect the lung and remove an abnormal area. The tissue should be divided into four pieces. One piece of tissue goes for bacteriological and virological examination, another for preparation of conventional paraffin sections, and the remaining two pieces for electron microscopy. One of the pieces for electron microscopy can be processed rapidly,[22] giving a diagnosis in three hours. This method does have a sampling error, however, and there may be difficulty in embedding the tissue. The other piece is processed for electron microscopy by a conventional method.[23] Open lung biopsy is fully justified in immunocompromised patients since the mortality from the disease is high.[8] The biopsy should not be left until the last moment, when the patient is in extremis.

Other methods used for obtaining material are examination of sputum, tracheal and pharyngeal aspirates, bronchopulmonary lavage, transbronchial lung biopsy, percutaneous needle biopsy, percutaneous needle aspiration, and endobronchial brush biopsy. Sputum and tracheal and pharyngeal aspirates are of little diagnostic use in the adult.[24] The remaining methods used will depend on the state of the patient, the preference of the physician, and the experience of the pathologist. The best stains for the organism are Gomori's methanamine silver or toluidine blue.[24] If open lung biopsy is not available or applicable, then brush biopsy or needle aspiration should be considered. Brush biopsy may give a false-negative result, as in case 2 described by Gottlieb.[2] Lung biopsy shows a characteristic picture with a honeycomb intra-alveolar exudate and a plasma cell infiltrate in the alveolar walls. Only one case with systemic spread has been documented.[25]

Serological tests are of little use in the diagnosis of pneumocystis infection in immunocompromised hosts as the disease progresses rapidly and there is poor seroconversion. As an epidemiological test in the normal population, however, complement-fixation or immunofluorescence tests are appropriate, provided that a suitable source of pneumocystis antigen is available. Some workers have used antigen derived purely from cortisone-treated animals whereas others have had access to antigen from frozen human lung.[27] Such antigens are of uncertain quality since the organism may be different in rats and in man and indeed may be antigenically different in immunosuppressed American patients and those found in European epidemics.[25] We will have to wait for serial propagation of human strains of *Pneumocystis* to obtain antigen of good quality and quantity. Partial success has been achieved in the culture of the organism[29] and a method of continuous serial propagation is likely to be developed soon.

The mode of transmission of this organism is not clearly understood. If pneumocystis infection is spread by oral droplets, then a cystic stage would be advantageous between hosts. The organism is of fairly widespread occurrence in mammals, especially rodents. This reservoir of infection has been implicated in some human outbreaks of pneumocystosis infection but, as stated above, recent serological evidence suggests that human *Pneumocystis carinii* is serologically distinct from the strains found in rats and mice.[30] Thus the human strain may be a distinctive organism having little infective capability in rodents. Some further evidence that animals cannot transmit the disease comes from a study of children with both pneumocystis and other pneumonias. Pet keeping was high in both groups.[31] Human *Pneumocystis* is difficult to propagate in rodents and requires the extreme procedure of administering immunosuppressive doses of corticosteroids.[26] Human infection by the organism is thought to occur almost naturally in infancy and this has led to the concept of dormancy and reactivation, much as in *Herpes* and tuberculous infections, in immunosuppressed individuals. No stages of *Pneumocystis* have been identified, however, in normal adult lungs and we must ask: if the organism is dormant, where is it residing? Several ultrastructural studies have shown ingestion of both trophozoites and cysts by macrophages.[14 16] If some organisms resisted lysis by the macrophage's lysosomes then a possible dormant stage, protected from most elements of the immune system, would be present before immunosuppression. An alternative would be that the trophozoite resides in the alveolar spaces and has a variable surface antigenicity, such as has been ascribed to trypanosomes, and thus evades immunological attack.[32 33] *Pneumocystis* has a coating external to its plasma membrane, which may perform this variable antigenic function.

Two main drugs have been used in the treatment of pneumocystis pneumonia. Probably the most commonly used treatment is a combination of sulphamethoxazole and trimethoprim. For the acute illness 20 mg trimethoprim and 100 mg sulphamethoxazole per kilogram body weight a day are given. For prophylaxis[34] 150 mg trimethoprim and 750 mg sulphamethoxazole per square metre are given. Prophylactic chemotherapy must be considered since the risk of developing pneumocystis pneumonia has been shown to increase with the intensity of chemotherapy. Thus in children with

acute lymphocytic leukaemia receiving one chemotherapeutic drug the incidence of pneumocystis infection was 5% whereas when four drugs were given the incidence was 22·4%.[35]

Not all patients, however, respond to the above regimen in the acute ilness and pentamidine has to be given, either on its own or in combination with sulphamethoxazole and trimethoprim.[2 3 9] Thus pentamidine is not the abandoned drug that some people may have felt it to be, especially as it has been shown to be directly lethal to the organisms,[36] which the combination of sulphamethoxazole and trimethoprim is not.[36] But pentamidine does have side effects, which include hypotension, hypoglycaemia, nephrotoxicity, folic acid deficiency, rashes, and infection site reactions.

We appear to have a new syndrome of pneumocystis pneumonia in immunosuppressed homosexuals. Clearly such patients do not announce themselves as homosexuals, just as patients having chemotherapy do not easily declare their infection. Physicians and pathologists must constantly keep the diagnosis of pneumocystis pneumonia in mind, and as more cases come to light some of the complexities of the organism may be unravelled.

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References

[1] Walzer PD, Perl DP, Krogstad DJ, Rawson PG, Schultz MG. *Pneumocystis carinii* pneumonia in the United States. Epidemiologic, diagnostic and clinical features. *Ann Int Med* 1974;**80**:83–93.

[2] Gottlieb MS, Schroff R, Schanker HM, *et al. Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. *N Engl J Med* 1981;**305**:1425–31.

[3] Masur H, Michelis MA, Greene JB, *et al.* An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* 1981;**305**:1431–8.

[4] Siegal FP, Lopez C, Hammer GS, *et al.* Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med* 1981;**305**:1439–44.

[5] Brennan RO, Durack DT. Gay compromise syndrome. *Lancet* 1981;ii:1338–9.

[6] Du Bois RM, Branthwaite MA, Mikhail JR, Batten JC. Primary *Pneumocystis carinii* and cytomegalovirus infections. *Lancet* 1981;ii:1339.

[7] Anonymous. Immunocompromised homosexuals. *Lancet* 1981;ii:1325–6.

[8] Durack DT. Opportunistic infections and Kaposi's sarcoma in homosexual men. *N Engl J Med* 1981;**305**:1465–7.

[9] Anonymous. Epidemiologic aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. *N Engl J Med* 1982;**306**:248–52.

[10] Klepp O, Dahl O, Stenwig JT. Association of Kaposi's sarcoma and prior immunosuppressive therapy: a 5 year material of Kaposi's sarcoma in Norway. *Cancer* 1978;**42**:2626–30.

[11] Drew WL, Mintz L, Miner RC, Sands M, Ketterer B. Prevalence of cytomegalovirus infection in homosexual men. *J Infect Dis* 1981;**143**:188–92.

[12] Brown SM, Stimmel B, Taub RN, Kochwa S, Rosenfeld RE. Immunologic dysfunction in heroin addicts. *Arch Int Med* 1974;**134**:1001–6.

[13] Baar HS. Interstitial plasmacellular pneumonia due to *Pneumocystis carinii*. *J Clin Pathol* 1955;**8**:19–24.

[14] Vavra J, Kucera K. *Pneumocystis carinii* Delanoë, its ultrastructure and ultrastructural affinities. *J Protozool* 1970;**17**:463–83.

[15] Von Lichtenberg F. Enigmatic parasites of man and animal models: summation. *Ann NY Acad Sci* 1970;**174**:1052–6.

[16] Hasleton PS, Curry A, Rankin EM. *Pneumocystis carinii* pneumonia: a light microscopical and ultrastructural study. *J Clin Pathol* 1981;**34**:1138–46.

[17] Pifer LL, Hughes WT, Murphy MJ Jr. Propagation of *Pneumocystis carinii* in vitro. *Pediatr Res* 1977;**11**:305–16.

[18] Hasleton PS, Penna P, Torry J. Effect of oxygen on the lungs after blast injury and burns. *J Clin Pathol* 1981;**34**:1147–54.

[19] Burke BA, Good RA. *Pneumocystis carinii* infection. *Medicine (Baltimore)* 1973;**52**:23–51.

[20] Sanyal SK, Mariencheck WC, Hughes WT, Parvey LS, Tsiatis AA, Mackert PW. Course of pulmonary dysfunction in children surviving *Pneumocystis carinii* pneumonitis: a prospective study. *Am Rev Respir Dis* 1981;**124**:161–6.

[21] Bowden DH. Alveolar response to injury. *Thorax* 1981;**36**:801–4.

[22] Rowden G, Lewis MG. Experience with a three hour electron microscopy biopsy service. *J Clin Pathol* 1974;**27**:505–10.

[23] Harris M. Differential diagnosis of spindle cell tumours by electron microscopy: personal experience and a review. *Histopathology* 1981;**5**:81–105.

[24] Hughes WT. Current status of laboratory diagnosis of *Pneumocystis carinii* pneumonitis. *Crit Rev Clin Lab Sci* 1975;**6**:145–70.

[25] Rahimi SA. Disseminated *Pneumocystis carinii* in thymic alymphoplasia. *Arch Pathol* 1974;**97**:162–5.

[26] Walzer PD,, Powell RD jun, Yoneda K. Experimental *Pneumocystis carinii* pneumonia in different strains of cortisonized mice. *Infect Immun* 1979;**24**:939–47.

[27] Shepherd V, Jameson B, Knowles GK. *Pneumocystis carinii* pneumonitis: a serological study. *J Clin Pathol* 1979;**32**:773–7.

[28] Goetz O. Serologische Befundeinterstitieller Pneumonien aus den Vereinigten Staaten. *Archiv Kinderheilkunde* 1964;**170**:60–6.

[29] Pifer LL, Woods D, Hughes WT. Propagation of *Pneumocystis carinii* in Vero cell culture. *Infect Immun* 1978;**20**:66–8.

[30] Walzer PD, Rutledge ME. Comparison of rat, mouse and human *Pneumocystis carinii* by immunofluorescence. *J Infect Dis* 1980;**142**:449.

[31] Hughes WT, Price RA, Kim HK, Coburn TP, Grigsby D, Feldman S. *Pneumocystis carinii* pneumonitis in children with malignancies. *J Pediat* 1973;**82**:404–15.

[32] Vickerman K. On the surface coat and flagellar adhesion in trypanosomes. *J Cell Sci* 1969;**5**:163–93.

[33] Williams RO, Young JR, Majiwa PAO. Genomic rearrangements correlated with antigenic variation in *Trypanosoma brucei*. *Nature* 1979;**282**:847–9.

[34] Hughes WT, Kuhn S, Chaudhary S, *et al.* Successful chemoprophylaxis for *Pneumocystis carinii* pneumonitis. *N Engl J Med*

197;**297**:1419–26.

[35] Hughes WT, Feldman S, Aur JA, Verzosa MS, Huston HO, Simon JV. Intensity of immunosuppressive therapy and the incidence of *Pneumocystis carinii* pneumonitis. *Cancer* 1975;**36**:2004–9.

[36] Pesanti EL. In vitro effects of antiprotozoan drugs and immune serum on *Pneumocystis carinii*. *J Infect Dis* 1980;**141**:775–780.