Pneumocystis carinii: molecular taxonomy and epidemiology

Pneumocystis carinii is an infection of the immunocompromised, including patients receiving immunosuppressive therapy for organ transplantation, patients with advanced malignancy and, in particular, patients with HIV infection. P. carinii causes a radiographically diffuse pneumonitis, progressive exertional dyspnoea, non-productive cough and fever; untreated it is fatal. Although inability to culture the organism effectively in vitro still remains a major obstacle, the application of molecular techniques to the study of P. carinii has advanced the understanding of the taxonomy and epidemiology of this micro-organism.

P. carinii is a fungus

Since its first description in the early 1900s, P. carinii has been regarded as a protozoan, based on its morphology, the inability to culture the organism in vitro and its response to anti/protozoal but not antifungal drugs. DNA sequence analysis of different regions of the genome of P. carinii has shown the organism to be a fungus [1, 2]. The first locus to be examined was that of the gene encoding the 18S ribosomal RNA from rat-derived P. carinii and this was found to show fungal homology. Subsequently, the cloning and sequencing of other P. carinii genes, including seven mitochondrial genes and genes encoding dihydrofolate reductase (DHFR), thymidylate synthase (TS), α- and β-tubulin, transcription factor IID, cation transporting ATPase, the AROM protein, actin and translation elongation factor EF3, have firmly established P. carinii as a member of the fungal kingdom [1, 2].

Host specificity of P. carinii infection

Antigenic differences between P. carinii isolates from different hosts have been demonstrated by electrophoretic characterisation of proteins and glycoproteins. Most antibodies elicited to P. carinii from one host species do not cross-react with antigen preparations of P. carinii from another host species. Although P. carinii is transmissible between animals of the same species by the airborne route, host specificity has been demonstrated by studies that have attempted, unsuc-cessfully, to transmit P. carinii from dogs to immunosuppressed guinea-pigs, from rats to immunosuppressed mice or hamsters, from mice to nude rats and from human patients to nude or immunosuppressed rats [1, 2].

Chromosome analysis, by pulsed-field gel electrophoresis has shown that rat-, mouse-, ferret- and human-derived P. carinii have karyotypes of a similar size range but different banding patterns. Sequence analysis of the gene encoding the mitochondrial large subunit rRNA (mt LSU rRNA) and the arom gene (encoding AROM, a pentafunctional protein that catalyses steps two to six of the prechorismate pathway of fungal aromatic amino-acid biosynthesis) has demonstrated that rat-, mouse-, rabbit-, ferret- and human-derived P. carinii are genetically distinct [3]. Analysis of other genes including β-tubulin, the nuclear rRNA operon and TS have also shown divergence among isolates of P. carinii from different host species. These cumulative data indicate that P. carinii infection is host specific and that human-derived P. carinii is genetically distinct from isolates from other mammalian host species. They also suggest that P. carinii infection in man is not a zoonosis acquired from a reservoir of parasites in the infected lungs of animals.

Genetic diversity among isolates of P. carinii from the same host species

Genetic diversity has been observed among isolates of P. carinii from the same host species. Two distinct types of P. carinii, named ‘prototype’ and ‘variant’, have been identified in the infected lungs of rats by differential migration of chromosomal bands on pulsed-field gel electrophoresis, differential hybridisation of chromosomal DNA to a P. carinii major surface glycoprotein gene probe, and the sequence (including the presence or absence of an intron) of the 18S rRNA gene [4]. Two genetically diverse types of P. carinii have been detected in the infected lungs of ferrets [3]. However, in most hosts the extent of genetic diversity amongst isolates of P. carinii from the same host species is lower than that between isolates from different host species.
Comparison of human-derived P. carinii isolates by sequence analysis of portions of the genes encoding the nuclear rRNA operon and mt LSU rRNA shows sequences that are identical, or that have between one and three single base polymorphisms [5]. Comparisons of human-derived P. carinii isolates, from the USA, Brazil, Zimbabwe and Britain, at the mt LSU rRNA locus, showed only a single base polymorphism. Furthermore, there was lack of sequence variation in the isolates from Britain over a 4.5-year period [6]. Sequence analysis of the arom gene of P. carinii from isolates from the same geographical locations also showed lack of genetic variation but indicated that infection in some individuals was not necessary clonal [3]. Sequence variation between human-derived P. carinii isolates at the internal transcribed spaced regions (ITS), which are located between the 18S, 5.8S and 26S rRNA gene sequences of the nuclear rRNA operon, demonstrated the existence of four different ITS sequence types and some samples appeared to be infected with two different types [7].

Major surface glycoprotein of P. carinii

The major glycoprotein (MSG) of P. carinii, a mannose-rich glycoprotein, is abundant on the outer cell wall of the organism and is thought to play an important role in the interaction of the parasite with its host. High levels of divergence have been observed among the MSG gene sequences from P. carinii isolates from different host species. Rat-derived P. carinii MSG is encoded by a large multi-gene family of homologous but not identical genes, which are tandemly arranged on most of the P. carinii chromosomes, primarily in the subtelomeric regions [8]. Regulation of the expression of different isoforms of MSG may provide a mechanism for antigenic switching in P. carinii, in a strategy to evade the host immune response [9].

Acquisition and persistence of P. carinii infection

Reports of nosocomial clusters of P. carinii pneumonia among immunocompromised patients have challenged the concept of re-activation of latent infection, and studies with monoclonal antibodies and DNA amplification have failed to demonstrate P. carinii colonisation of bronchoalveolar lavage fluid or autopsy lung tissue from immunocompetent human subjects [2]. Experiments with SCID mice failed to reveal latent infection at extrapulmonary sites [10]. The dexamethasone immunosuppressed rat model of P. carinii pneumonia has been used to determine the period of persistence of P. carinii in the lungs after a primary episode of P. carinii pneumonia. At least 75% of animals had cleared the organism from their lungs within 1 year, indicating that persistence is limited [11].

Rats acquire P. carinii infection by an airborne route [12]; it is assumed that infection in man is similarly acquired. DNA sequences identical to those of P. carinii have been detected in samples of the air spora of rural Oxfordshire [13], animal facilities housing rats with P. carinii pneumonia [13, 14] and the rooms of patients with P. carinii pneumonia [14], suggesting that P. carinii may have an as yet morphologically unidentified environmental phase of its life cycle.

Seasonal variation in incidence of P. carinii pneumonia in HIV-infected patients has been reported, with late spring and late summer/early autumn peaks that may be related to variations in environmental humidity and temperature, which are important for fungal sporulation. Transient carriage of P. carinii may occur in the lungs of immunocompetent hosts. P. carinii DNA has been detected in the lungs of immunocompetent ‘sentinel’ rats housed near immunosuppressed P. carinii-infected rats and disappeared rapidly from the lungs of the ‘sentinel’ rats when they were isolated from the immunosuppressed infected animals. Immunocompetent health care workers in an AIDS unit exposed to patients with P. carinii pneumonia have been shown to have higher serum antibody levels to P. carinii than health care workers in a care of the elderly unit, suggesting that asymptomatic carriage results from environmental exposure.

Despite effective prophylaxis, recurrent episodes of P. carinii pneumonia are relatively common in HIV-infected patients. Sequence analysis of the mt LSU rRNA gene of P. carinii isolates from 10 patients with two episodes of P. carinii pneumonia showed that in five patients, genetically distinct isolates were associated with each episode [15]. In another study, repeat episodes of P. carinii pneumonia were examined by sequence analysis of the ITS regions: 10 different types of P. carinii were identified and in four of seven patients with recurrent pneumonia the sequence types found in the second episode were different from those of the first [16]. These cumulative data support the hypothesis that P. carinii infection in immunocompromised hosts may result from de novo acquisition of the organism from an exogenous source as well as re-activation of an endogenous infection.

In summary, molecular biological techniques have been used to show that P. carinii is a fungus and that different strains of the organism infect different mammalian species. Co-infection with genetically distinct strains of P. carinii occurs in some host species; in general, however, genetic diversity among isolates of P. carinii from a single host species has been shown to be lower than that between isolates of different host species. Clinical disease
in rats and man appears to arise by de novo acquisition of the organism by the airborne route, in the majority of cases, and not by re-activation of latent infection.

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References


Further reading