Review

Pneumocystis carinii: an atypical fungal micro-organism

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The purpose of this review is to assist mycologists in having a better understanding of Pneumocystis carinii and the disease that it causes. Now considered to be a fungus, P. carinii is unusual in its life cycle and relationship with the host. P. carinii pneumonia (PCP) pathogenesis, immunology and host defence mechanisms are examined, as well as epidemiological and control strategies. Most pneumocystosis pathophysiological changes result from the parasite’s attachment and proliferation in the lungs, resulting in a filling of the alveoli with masses of the micro-organism. Pathological changes include an increase in alveolar capillary membrane permeability and injury to the alveolar epithelium, which may be mediated by the release of degradative enzymes from the pathogen. A host response takes place by hypertrophy, and hyperplasia involving type II epithelial alveolar cells. P. carinii interacts with pulmonary surfactants by binding to the hydrophilic proteins A and D, and by modifying their phospholipid composition. Alveolar macrophages and CD4+ T cells play a key role in the host’s defence against Pneumocystis. The epidemiology of PCP remains poorly understood. Airborne transmission has been established, but the actual infective form and its source remains unknown. Studies concerning P. carinii genetic diversity have shown that the parasite polymorphism is related, at least partially, to the host species. A strong host-species specificity in P. carinii has been found. From an epidemiological perspective, there appears to be no animal reservoir for the agent of human PCP. Thus, this disease should not be considered to be zoonotic. Although a significant decrease in the incidence of pneumocystosis has been obtained when employing chemoprophylaxis, anti-P. carinii drugs are not completely successful, often inducing deleterious side-effects. For these reasons, new prophylactic and therapeutic strategies need to be developed. One approach could be based on the anti-P. carinii effect of yeast killer toxins and antibiotic anti-idiotypic antibodies.

Keywords control, Pneumocystis carinii, pneumocystosis, transmission

Fungal nature of Pneumocystis carinii

Pneumocystis carinii was discovered by Carlos Chagas in 1909. In Brazil, he discovered cystic forms in the lungs of guinea pigs and humans that were infected by Trypanosoma cruzi. Chagas believed that these forms were cystic stages of Trypanosoma [1]. In 1912, P. carinii was observed in the lungs of infected rats living in Paris, which were described as a new micro-organism by Delanoë and Delanoë [2]. They named the organism P. carinii in honour of Antonio Carini, an Italian biologist who described the parasite in the lungs of rats infected by Trypanosoma lewisi that he had studied in Brazil [3].
Subsequently, most authors placed *Pneumocystis* among the protozoa based on its morphology and response to antiprotozoal drugs [4].

All of the known growth stages of *P. carinii* are found in the lungs of the host. In Fig. 1, the characteristic stages of the parasite, an amoeboidal mononucleate trophozoite (Fig. 1a), a precyst (Fig. 1b and c) and a mature cyst containing intracystic bodies that will evolve into trophozoite forms (Fig. 1d) can be seen.

In the past 6 years, analysis of its nucleic acids has led to *P. carinii* being reclassified as a member of the Kingdom Fungi [5–8]. Sequence similarities between *P. carinii* 16S-like rRNA and yeast rRNA have been reported [9, 10]. Likewise, the study of several continuous mitochondrial genomic gene sequences have shown homologies with fungal gene sequences [11]. More recently, an ATPase gene of *P. carinii* has been cloned and sequenced [12]. The level of identity between the predicted *P. carinii* pump peptide and fungal proton pump peptide sequences exceeded 66%. The H⁺ pump from the protozoan *Leishmania* spp. is only 34% identical in amino acid sequence. According to studies of SS rRNA sequences, *P. carinii* has been designated as a primitive fungus belonging to the Phylum Chytridomycota (e.g. *Phlyctochytrium*).
irregulare) or Zygomycota (e.g. Basidiobolus magnus [13].
However, analysis of 18S rRNA gene sequences suggests that P. carinii has affinities with ascomycetous fungi such as Neurospora crassa, Saccharomyces cerevisiae and Candida albicans [9,10,14]. Sequence analysis of the conserved eukaryote-β tubulin gene also suggested that P. carinii is an ascomycetous fungus [15]. In contrast, using primers targeted to the mitochondrial rRNA gene, P. carinii was suggested to belong to the ustomycetous red yeasts (e.g. Rhodotorula glutinis or Sporidiobolus pararoseus) [7].

Moreover, the P. carinii genes of two important enzymes, thymidylate synthase (TS) and dihydrofolate reductase (DHFR), were found to be located on different chromosomes [16,17]. As in non-protozoan organisms, P. carinii TS and DHFR activities reside in two polypeptide chains, clearly distinct and monofunctional [16]. In all protozoans studied to date, these two enzymatic activities exist in one bifunctional protein, which is coded by the same gene [18].

Finally, P. carinii and some fungi share common epitopes for specific antibodies [19]. Currently, the general consensus is that P. carinii is a fungus related to ascomycetous yeasts such as S. cerevisiae. Regardless of its classification, P. carinii remains a unique parasitic micro-organism in that it possesses important features that distinguish it from other fungi.

**Pneumocystis carinii transmission by airborne route**

P. carinii is a pathogenic eukaryotic micro-organism that causes severe interstitial pneumonia in immunosuppressed mammals [3]. Patients treated with immunosuppressed drugs as well as immunocompromised patients infected by the human immunodeficiency virus (HIV), are particularly prone to developing P. carinii pneumonia (PCP). The nosocomial transmission of P. carinii to HIV-uninfected immunocompromised patients has been suggested [20–23] in the hospital setting.

The epidemiology of PCP remains poorly understood. Airborne transmission has been clearly established [3,24], but the actual infective form and its source causing human infections remain unknown. From animal experiments, particularly using murine hosts, it has been demonstrated that the airborne route is the mode of P. carinii transmission. Corticosteroid-treated germ-free rats developed PCP when housed with rats infected with P. carinii [3]. In these experiments, it was shown that germ-free rats did not become infected from unsterilized drinking water or feed. Non-infected SCID mice developed PCP following exposure to outbred mice having corticosteroid-induced PCP [25]. A single infected mouse was sufficient to infect at least 20 SCID mice during a single contact day. Recently, the airborne transmission of simian-derived P. carinii to simian immunodeficiency virus (SIV)-infected Rhesus macaques has been reported. Recipient and donor monkeys were maintained in separate rooms which shared a common ventilation system [26]. It is believed that P. carinii can be transmitted among humans in a similar manner.

P. carinii-free SCID mice can be infected by intranasal inoculation of P. carinii that have been harvested from parasitized mouse lungs [27]. In a similar manner, non-infected nude rats will develop PCP after intratracheal inoculation of the parasites originating from the lungs of infected rats [28,29].

Most investigators induce Pneumocystis infections in animals by administering corticosteroids to laboratory rodents. Exposure to infected animals is usually not needed. This suggests that highly infective forms of Pneumocystis are present in the environment. It has not been established where the fungus normally occurs in the environment and how the host acquires it.

**Putative airborne reservoir for pneumocystosis**

The presence of P. carinii trophozoite or cyst forms in the air has not been demonstrated. However, the detection in air samples of DNA gene sequences that are identical to those of P. carinii suggests that the parasite is probably located in the immediate environment. P. carinii DNA has been detected in room air samples where infected rats, or human patients, with PCP were present [30]. DNA sequences identical to both rat- and human-derived P. carinii have been detected in air samples collected in rural Oxfordshire, UK [31].

Only a limited amount of information is known about the influence of environmental factors on the viability and infectivity of P. carinii. Infective stages derived from the lungs of infected hosts could be resistant to destruction or inactivation by environmental factors, remaining potentially infectious for long periods. However, some evidence suggests that P. carinii rapidly loses its infectious ability outside of the host [3,32]. This is contrary to the belief that Pneumocystis can be acquired from environmental sources. P. carinii transmission probably involves a relatively close contact with a P. carinii infected host.

**Pneumocystis carinii attachment to host cell**

Adherence of P. carinii to type I pneumocytes appears to be the first step in the initiation of infection. Ultrastructural studies have shown a close in vitro attachment of trophozoites and cystic forms to cells growing in monolayer cultures [29,33–36]. These observations suggested that P. carinii-host cell interactions are required.
for parasite proliferation. The host cell may provide nutrients and stimulate the synthesis of parasite molecules involved with P. carinii growth and proliferation [33]. Interdigitation and membrane blurring between the parasite and the host cell have been described. Filopodia appear at the surface of trophozoites, allowing the parasite to anchor itself to the host cell. In vivo, close contact between the surface of filopodia and type I pneumocytes are routinely observed. Similar in vitro interactions have been demonstrated when P. carinii attaches itself to a variety of culture cells [33,34,37]. Filopodia have been shown to penetrate the host cell cytoplasm without disruption of the plasmalemma [29,34,37,38]. In vitro, rat-derived P. carinii cells were not able to proliferate in some cell lines [33]. The proliferation of P. carinii seems to occur when adhesion to host cells occurred, apparently following surface molecular interactions. However, P. carinii is able to grow in vitro without feeder cells [39,40].

P. carinii surface molecules have been demonstrated to be involved in the attachment process of the parasite to the host cell. As in other fungi where cell wall receptors interact with host proteins such as fibronectin, laminin or fibrinogen [41], cell surface molecules of P. carinii may play the same role in parasite binding to the host cell. Previous data have demonstrated that fibronectin is a mediator of P. carinii attachment to host cells [37,42]. Specific fibronectin-binding receptors in a major surface glycoprotein (MSG) were detected at the surface of the parasite [42]. Attachment to lung epithelial cells appears to be mediated by fibronectin-binding integrins [43]. Variation in the glycosylation process of P. carinii surface antigens (like MSG from human P. carinii) may influence the host–parasite relationships. A recent work has demonstrated that MSG consists of more than one form of glycoprotein, each having different binding affinities to the lectin concanavalin A [44]. The identification of parasite surface molecules which mediate P. carinii attachment to lung cells should provide insight into the pathogenesis of PCP. The genes coding for the family of MSG glycoproteins, which play a major role in parasite adhesion, have been cloned and sequenced from several P. carinii strains [45,46].

**Pneumocystis carinii genetic diversity**

P. carinii MSG gene sequences differ in different strains studied from the same host species, and even within the same host. Other gene sequences were found to be different in human and animal isolates of P. carinii [47–49]. Some variable gene sequences, molecular karyotypes obtained by using Pulsed Field Gel Electrophoresis (PFGE) [50,51] or isoenzyme profiles obtained by Multi-locus Enzyme Electrophoresis (MEE) have been used as strain markers [48,52]. Genotypic or phenotypic markers have revealed a significant genetic diversity in the natural populations of *Pneumocystis*. Such markers are being used in research programmes dealing with PCP epidemiology.

**Coinfection with distinct Pneumocystis carinii strains**

Pulsed field gel electrophoresis studies have shown that coinfection with many *P. carinii* strains may occur [50,51,53]. Two distinct types of rat-derived *P. carinii* (named prototype and variant) were detected in the infected lungs of the same animal [54]. Four distinct karyotype patterns were identified among the prototype forms indicating that there may be even a greater genetic diversity among these forms than had been previously considered [55]. It has been shown that prototype and variant forms of rat-derived *P. carinii* can be phenotypically distinguished by their immunoreactivities to specific polyclonal and monoclonal antibodies [56]. Moreover, immunoblotting analyses and T cell proliferation assays have revealed differences in *P. carinii* MSGs of isolates obtained from different rat strains [57]. Two genetically distinct strains of *P. carinii* were identified in infected ferret lungs [58]. Point mutations or deletions in *P. carinii* conserved DNA regions were found among *P. carinii* isolates from the same host species based on experiments performed with both rat- and human-derived parasites [59].

**Host species specificity of Pneumocystis carinii**

Studies regarding *P. carinii* diversity using TS gene or isoenzyme markers have shown that genetic diversity of parasite isolates is related, at least partially, to the host species [48,52]. Prior to 1993, it had not been established whether *P. carinii* from a given host species was able to infect other host species. Parasites harvested from different host species (rat, mouse or rabbit) were inoculated into SCID mice or nude rats as a means to compare their relative infectivity [28]. Both SCID mice inoculated with mouse-derived *P. carinii* and nude rats inoculated with rat-derived *P. carinii* developed PCP. No parasites were found in the lungs of SCID mice inoculated with rat- or rabbit-derived *P. carinii* or in lungs of nude rats infected with mouse- or rabbit-derived *P. carinii*. Likewise, other investigators have shown that SCID mice were unable to support the growth and replication of ferret-derived *P. carinii* [60]. PCP in SCID mice or nude rats inoculated with simian-derived *P. carinii* has not been observed [61]. These findings demonstrate a strong host species specificity in rat-, mouse-, rabbit-, ferret- and simian-derived
P. carinii. From an epidemiological point of view, they suggest that there is no animal reservoir for the agent of human PCP, and that this disease should not be considered a zoonosis.

Different ‘species’ of Pneumocystis

P. carinii has been isolated from a wide variety of unrelated mammalian hosts to include human, monkey, rat, mouse, ferret, rabbit, swine, horse, guinea pig, goat, shrew, sloth, dog, cat, sheep, marmoset and vole [4]. Morphologic, antigenic and genomic differences have been reported among P. carinii strains from some of these hosts. Ultrastructurally, rabbit-derived P. carinii organisms could be distinguished from mouse-derived ones: their filopodia were clearly thicker and less numerous than those of mouse parasites [62]. However, morphological features are usually insufficient to distinguish P. carinii organisms from rats, mice or humans. The principal reports of specific differences between these Pneumocystis strains were made by immunological or molecular methods [7,15,48,52,63-68]. Data obtained by using these methods have shown that different host species are infected with genetically different Pneumocystis populations, imply that multiple Pneumocystis species and/or strains may exist. The Pneumocystis host species specificity, which has been found through cross infection experiments [27,28,60,69], is consistent with genetic data and supports the existence of different Pneumocystis species or varieties, at least in terms of host species.

Revised nomenclature for Pneumocystis carinii

At the Third Workshop on Opportunistic Protists in Cleveland, Ohio, USA, 24–29 June 1994, a consensus nomenclature for Pneumocystis was proposed [70]. Current data regarding genetic diversity in Pneumocystis were considered to be insufficient to distinguish speciation from clonal variation. Therefore, the following names were proposed to standardize the designation of the different types of Pneumocystis organisms found in the most commonly infected mammalian hosts (sp. f. refers to special form, after D. L. Hawksworth et al., 1983 Dictionary of the Fungi, 7th edn, CAB International, Kew):

Pneumocystis carinii sp. f. carinii Rat (prototype)
Pneumocystis carinii sp. f. rattus Rat (variant)
Pneumocystis carinii sp. f. hominis Human
Pneumocystis carinii sp. f. mustelae Ferret
Pneumocystis carinii sp. f. musiris Mouse
Pneumocystis carinii sp. f. equi Horse
Pneumocystis carinii sp. f. suis Pig
Pneumocystis carinii sp. f. oryctolagi Rabbit

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Pneumocystis carinii pneumonia pathogenesis

Studying the pathogenesis of P. carinii infections remains important for the development of new therapies and management approaches, even though the interplay of the parasite and host is complex. Unfortunately, data regarding the early phases of P. carinii infection are rare. The attachment of P. carinii to epithelial alveolar cells is probably a critical step in PCP pathogenesis. After making contact with the type I alveolar epithelial cell, the trophozoite extends filopodia into the host cell cytoplasm. Exchanges between P. carinii and the host cells could be facilitated by an activation of the plasmalemmal vesicular system of the host cell; actually, numerous vesicles have been observed in the host cell cytoplasm next to the attachment area [34,71]. Most PCP pathophysiological changes result from parasite attachment and proliferation with filling of the alveoli with masses of cells of the pathogen. The first impairment would be an increase of the alveolar-capillary membrane permeability related to the parasite attachment [4]. Subsequently, an injury to the alveolar epithelium occurs, ultimately leading to denudation of the basement membrane. Host cell injury could be mediated by the release of degradative enzymes from the parasite [72,73]. Then, a host reparative response takes place by hypertrophy and hyperplasia of type II epithelial alveolar cells [4], which will differentiate into type I cells. Moreover, P. carinii interacts with pulmonary surfactants by binding to hydrophilic surfactant proteins A (SP-A) and D (SP-D), and by modifying the surfactant composition. The mannose moieties of P. carinii MSG bind SP-A [158] or SP-D [159]. The consequences of these interactions are not known. The surfactant phospholipid rate decreases during PCP [74-77]. P. carinii could directly inhibit the phosphatidylcholine secretion by type II epithelial cells [78].

Immunology and host defence

The host immune response to P. carinii has been recently reviewed [68,73]. Alveolar macrophages and CD4+ T cells play a key role in the defence of the host against Pneumocystis. Pneumocystis MSG is a chemotactic factor for normal human monocytes. Macrophagic mannose receptor [79], fibronectin [80] and probably the complement receptor of the macrophage are involved in the adherence of P. carinii to this cell. However, P. carinii binding to the macrophage does not result always in either activation of this cell or parasite phagocytosis. In some experimental systems, the internalization and degradation of the parasite does not take place without the presence of anti-P. carinii serum [81]. Complement is not essential but could enhance antibody-mediated phagocytosis and free-oxygen radical production. Rabbits develop
spontaneous PCP at weaning [82,83] and rabbit alveolar macrophages are able to produce free-oxygen radicals following stimulation with rabbit P. carinii (Dridba et al., 1995, 35th ICAAC Congress, San Francisco, USA). P. carinii is also able to elicit an oxidative burst in normal rat [84]. The parasite appears to possess superoxide dismutase but lacks catalase activity [85]. Therefore, it would be susceptible to hydrogen peroxide-dependent killing mechanisms. P. carinii induces nitric oxide secretion by alveolar macrophages by stimulating the L-arginine-dependent pathway [86].

T cells play an important role in the host cellular immune response to Pneumocystis. AIDS patients often develop PCP when their CD4⁺ T cell counts fall to 200/\text{mm}^3 or lesser. A specific T cell proliferation response has been demonstrated in animals [87,88] and healthy humans previously exposed to P. carinii [89]. In contrast, the proliferative response is less marked in HIV-infected individuals, and it was not detected in patients with advanced AIDS. Adoptive transfer of splenic T cells has decreased delayed type hypersensitivity (DTH) reaction in normal recipients, and reduced the number of P. carinii parasites in infected tissues [90,91]. IFN-γ, produced primarily by activated CD4⁺ T cells, activates macrophages. Aerosolized IFN-γ induced activation of mononuclear cells and alveolar macrophages, resulted in marked reduction of PCP extension in CD4-depleted mice [92]. Systemic administration of IFN-γ was also effective against PCP in rats [68]. In PCP, IFN-γ could increase superoxide or NO production and activate macrophages for TNF-α release [68,88]. Alveolar macrophages from immunocompetent humans, or from HIV-infected patients with PCP, exhibited increased spontaneous TNF-α release when exposed to Pneumocystis [68,93,94]. The role of TNF-α was not clearly established [95], and no specific TNF-α receptor has been identified on P. carinii [73]. However, anti-TNF-α antibodies inhibited the clearance of P. carinii [73,88,96]. IL-1 and IL-6 could play a mediator role in the host defence against P. carinii [97–99]. The secretion of IL-1 is reduced in HIV-1 infected macrophages, possibly favouring the PCP development [68]. CD4⁺ T cells, but not CD8⁺ T cells, have been found to be essential for resistance to P. carinii pneumonitis in Balb/c mice depleted of CD4⁺ T cells, or in SCID mice reconstituted with splenic cells from immunocompetent mice [100–102]. However, infusions of thymus or spleen cells depleted of B cells did not resolve PCP in SCID mice [100]. Therefore, T cells do not seem to function alone in the host defence against P. carinii [68,88].

Specific humoral immunity may play a role against P. carinii. Healthy individuals often develop antibodies against P. carinii by the age of two years [73,103]. IgG as well as IgA bind to the cyst wall and membranous structures of P. carinii [104]. A significant clearing of PCP was obtained in P. carinii infected SCID mice when administered hyperimmune serum [101]. Moreover, the administration of anti-MSG monoclonal antibodies reduced infection in corticosteroid-treated ferrets and rats [105]. Interestingly, an IgE-mediated allergy to P. carinii has been reported in AIDS patients [106].

**Pneumocystis carinii infections and deep mycoses**

Although the fungal nature of P. carinii is usually agreed on, PCP is rarely compared with deep fungal diseases. Nevertheless, it seems reasonable to compare P. carinii infections with deep seated mycoses. Some fungal diseases are caused by filamentous saprophytic species which become yeast-like when invading the host. That is the case of dimorphic fungi such as *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis* and *Histoplasma capsulatum* [107]. As in PCP, deep mycoses are primarily pulmonary processes because the pathogens enter through the respiratory tract. Unlike PCP, these mycoses are not transmitted from one infected host to another. In PCP, nasal or intratracheal inoculation of parasites leads to pulmonary lesions in non-latently infected animals (SCID mice or nude rats). In immunosuppressed hosts (patients or animal models), PCP seems to result from an exogenous source of P. carinii infection, but it might also develop from a latent asymptomatic P. carinii lung infection. In an experiment where P. carinii antibody-positive rats without PCP symptoms were placed in air-filtered containers prior to being immunosuppressed, they all developed PCP [6], thus, suggesting that reactivation of latent parasites can induce infection. On the contrary, other observations suggest that immunosuppressed susceptible humans develop PCP from widespread exogenous sources [108].

In deep mycoses, dissemination of the fungus commonly occurs from the lungs to other organs. Similarly, P. carinii is able to disseminate from infected lungs to induce secondary visceral lesions. Pulmonary P. carinii lesions may be unapparent when extrapulmonary lesions are detected as reported in deep mycoses having a respiratory origin.

From a pathologist’s perspective, PCP and deep mycoses are quite distinct. They induce different pathological changes in the infected host. Dimorphic fungi may elicit focal granulomatous reactions, fibrocaseous pulmonary nodules or abscess formation. In contrast, in PCP, the most typical histopathological changes consist of diffuse foamy, honeycombed eosinophilic exudate in the alveolar spaces, and infiltration with macrophages [109].

Finally, P. carinii shows strong host species specificity, whereas, other fungi do not, with the exception of dermatophytes [107].

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Extrapulmonary pneumocystosis

In the last ten years, an increased number of extrapulmonary pneumocystosis (EP) cases have been reported [110]. Most EP cases were found in AIDS patients having PCP treated by aerosolized pentamidine. Lymph nodes, spleen, kidneys, live, heart and bone marrow were the most commonly infected organs. However, the pathogenesis of EP is not yet understood [111]. We do not know whether EP results from an infection in which the virulence of P. carinii is higher than normal, or when there is an unusual evolution of the pulmonary infection. The pathogen can disseminate by haemogenous and/or lymphatic means.

In our laboratory, the frequency of EP in rodent models was investigated [112]. The most common site of EP was the heart in rats and SCID mice. The frequency of EP is probably higher than what we have found. The frequency of EP in humans is probably also underestimated, owing to the possible existence of subclinical EP and a low sensitivity of available diagnostic tools. A significant number of cases of EP without symptoms of PCP have been reported in the literature; no correlation between EP frequency and PCP extension seems to exist. The trend for P. carinii to disseminate from infected lungs to extrapulmonary sites should not be considered as a general rule. Highly infective strains were unable to induce infections when introduced into the bloodstream of animals. Parasites, which were able to infect SCID mouse or nude rat by the airborne route, lost their infectivity via the bloodstream [112]. Although hosts were severely immunosuppressed, blood or serum non-specific factors could inhibit the virulence of the parasite, as has been reported in other parasitic or fungal infections. For instance, Trypanosoma cruzi virulence is inhibited by both seric high-density and low-density lipoproteins [113]. In the same way, non-specific seric factors could be involved in the host response against Candida albicans infections [107].

P. carinii DNA has been detected in the sera of immunosuppressed rats during active PCP [114] and in the sera of HIV-infected patients with PCP [115]. The detection of P. carinii DNA in these sera, however, was not correlated with the presence of viable parasites in the bloodstream. Only one study reported the recovery of P. carinii organisms by culture from blood samples [116].

Potential vertical transmission of pneumocystosis

Vertical transmission of P. carinii has been suspected for a long time in humans [117]. In contrast, P. carinii infections have not been documented to occur through the placenta in SCID mice [118]. Rabbits are an interesting model to investigate vertical transmission of Pneumocystis. We have reported that almost all untreated young rabbits are spontaneously and heavily infected by P. carinii at weaning (28-day-old rabbits) [83], but we do not know when nor how P. carinii infects them. We have reported P. carinii infections in 7-day-old rabbits [119], and we are attempting to detect parasites in rabbit placenta and fetuses by using PCR and in situ hybridization techniques.

Drugs against pneumocystosis

The drugs of choice for treating PCP remain trimethoprim-sulphamethoxazole (TMP-SMZ) or pentamidine which are also used in primary and secondary prevention of the disease in HIV-infected patients [120]. The TMP-SMZ combination often caused unwanted effects in these patients. Epiroprim, another folic acid inhibitor, has a significant effect against both P. carinii and Toxoplasma gondii in mice [121]. About 20% of HIV-infected patients receiving primary or secondary chemoprophylaxis will develop PCP [122]. Aerosolized pentamidine can promote atypical pulmonary and/or extrapulmonary P. carinii infections. Antifungal drugs have been demonstrated to be of little use. P. carinii is resistant to amphotericin B which may be explained by the absence of ergosterol in the pathogen’s cell membrane [123]. Other antifungal therapeutic agents, echinocandins and papulocandins, cyclic lipopeptide β-1,3 glucan synthesis inhibitors, are effective in vivo against P. carinii [124]. Recently, new semi-synthetic pneumocandins have been found to have a high anti-P. carinii activity in immunocompromised rats [125]. Less effective than TMP/SMZ and pentamidine, atovaquone, a hydroxynaphthoquinone that is apparently microbicidal to P. carinii, is useful in mild-to-moderate cases of PCP [126,127]. Other agents, such as clarithromycine-SMZ, have been found to be effective against PCP in rats [128]. Deferoxamine mesylate, an iron-chelating drug, is active in vitro and in vivo against P. carinii but very high doses are needed for rat or mouse PCP [129,130]. Comprehensive analyses of folate antagonists, primaquine, clindamycin and other agents used to control PCP were recently published in the second edition of the P. D. Walzer’s book: Pneumocystis carinii Pneumonia [131–133].

New strategies to control PCP

The development of new anti-P. carinii strategies remains a priority. Present research includes the identification of new metabolic targets and the study of the ‘yeast killer
The characterization of *P. carinii* specific metabolic targets has led to the study of key enzymes involved in the cell wall synthesis, and the investigation of carbohydrate, aromatic amino acids, sterol and antioxidant molecules.

The study of the phosphomannose isomerase of the pathogen, an enzyme involved in the synthesis of the cell wall compounds, is being developed [134]. The potential of *P. carinii* cell wall biosynthetic pathways as a chemotherapeutic target site is being explored using β-1,3 glucan inhibitors [124,125].

*P. carinii* dwells in the alveolar microenvironment, which is rich in phospholipids, where it could incorporate exogenous lipids [135,136]. Moreover, evidence for the de novo lipid synthesis of many lipid components by *P. carinii* has been discovered. For instance, *P. carinii*, like the rust fungi, can synthesize the unusual fatty acid cis-9,10-epoxy stearate methyl ester [137]. The absence of this molecule in the host makes it a potential chemotherapeutic target [138]. Isoprenoid metabolism has also been investigated. The cellular concentration of CoQ (ubiquinone) in the parasite was found at the lower end of the range of values typically reported for aerobic microorganisms, suggesting that *P. carinii* metabolism may not be strictly aerobic [135]. The main homologue of ubiquinone in normal rat lung is CoQ9, whereas *P. carinii* contains high CoQ10 concentrations with lower concentrations of CoQ9. In the parasite, the benzoquinone ring could be synthesized via the shikimic acid pathway. The AROM locus, which is involved in the aromatic amino acid synthesis in *P. carinii*, has been cloned and sequenced [58,139]. As the shikimic acid pathway is absent in mammals, it could be a potential chemotherapeutic target.

Cholesterol constitutes about 78% of the total sterols in *P. carinii* [135]. The parasite should be able to uptake directly cholesterol from the host lung. It is interesting that *P. carinii*, like other fungi, does not have ergosterol in the cell membranes. Moreover, *P. carinii* contains some C<sub>24</sub> and C<sub>29</sub> sterols (ergost-7-en-3-ol, ergostadien-3-ol, stigmaster-7-en-3-ol, sigmastadien-3-ol), undetected in the host lung and probably synthesized by the parasite. These minor sterols could have a metabolic role, and therefore, be used as a therapeutic target.

Some evidence of parasite superoxide dismutase exists [85]. The alveolar environment is rich in molecular oxygen as well as in reactive oxygen moieties originating from activated alveolar macrophages or polymorphonuclear cells. Therefore, the *P. carinii* enzymatic mechanisms to evade oxygen radical-generating systems can be considered as potential chemotherapeutic targets.

Finally, because the attachment of *P. carinii* to alveolar cells is an essential event in PCP development [140,141], a control strategy based on attachment inhibition could be designed. Unfortunately, no therapeutic drugs that can inhibit parasite attachment are known. For this reason, we are developing in our laboratory a new strategy to PCP control by using yeast killer toxins and antibiotic anti-idiotypic antibodies.

**Effect of a Pichia anomala killer toxin (PaKT) on Pneumocystis carinii**

PaKT is a yeast toxin secreted by a killer strain of *Pichia anomala*. PaKT was selected because of its wide antibiotic spectrum against unrelated pathogenic microorganisms including bacteria and pathogenic fungi [142]. Moreover, its activity can be neutralized by a monoclonal antibody (mAb KT4) and anti-idiotypic antibodies (antilids) produced against mAb KT4 are able to mimic the *in vitro* killer effect of PaKT. The PaKT secretion sites were localized in the cell wall of the killer yeast strain by using mAb KT4 in immunofluorescence assay and immunoelectron microscopy [143–146]. The susceptibility of *P. carinii* to PaKT has been investigated [147–149]. Rat-derived *P. carinii* cystic forms and trophozoites were preincubated either with PaKT or control buffers before being added to monolayer Vero cells or L2 lung cell cultures. Our results showed that PaKT induced a marked inhibitory effect on the *P. carinii in vitro* attachment. Similar data were obtained with mouse-derived parasites. As the inhibitory effect of PaKT was neutralized by mAb KT4 [150], the decrease in *P. carinii in vitro* attachment was due to the specific antimicrobial activity of the killer toxin.

In *in vivo* experiments were developed to establish if PaKT could inhibit the parasite’s infectivity in SCID mice. Parasites were preincubated in either PaKT or control buffers before being added to monolayer Vero cells or L2 lung cell cultures. Our results showed that PaKT induced a marked inhibitory effect on the *P. carinii in vitro* attachment. Although it is too early to affirm the existence of a real killer effect, *P. carinii* infectivity appeared to be clearly sensitive to PaKT activity. The decrease of *P. carinii in vitro* attachment and the inhibition of the parasite virulence in SCID mice was the result of either the death of parasite or an inhibitory effect of the PaKT on *P. carinii* adhesion mechanisms.

**PaKT-like anti-idiotypic antibodies and idiotypic immunization**

As PaKT could not be used as a systemic antibiotic, new molecules have been produced by applying the anti-idiotypic network theory [151]. It is possible to cause
rabbit antilids with PaKT antimicrobial properties. These antilids were demonstrated to be able to inhibit the in vitro growth of yeast, thus, mimicking the antimicrobial properties of the PaKT [152,153].

We are developing new preventive and therapeutic strategies directed against pathogenic micro-organisms susceptible to PaKT. The reliability of this approach is attested by two recent studies. The administration of mAb KT4 to syngeneic mice induced a significant protection against lethal inocula of C. albicans cells in relation to in vivo elicitation of antilids [154]. This was the first report of antimicrobial protection (idiotypic vaccination) using the properties of killer toxin-like antilids which presumably acted in vivo as antibiotics. A significant protection against candidal vaginitis was obtained in mAb KT4-vaccinated female rats [155].

Finally, a new molecular approach has been initiated. Nucleotide primers have been used to amplify DNA sequences corresponding to the variable domains of mouse immunoglobulin light and heavy chains [156], thus allowing the DNA encoding the antilids variable domains to be cloned and sequenced. The production of small synthetic peptides corresponding to artificial idotype and avoiding undesired side-effects to the host might constitute a new way for the treatment and prophylaxis of P. carinii infections.

**Conclusions**

In recent years, P. carinii has been classified as a member of the Kingdom Fungi, essentially on the basis of genomic studies. However, this fungal micro-organism remains atypical with respect to its phenotypic features, its biological life cycle, its relationships with the host and its susceptibility and resistance to most antifungal drugs.

Knowledge of the life cycle of P. carinii is limited by the lack of a continuous in vitro culture system. Although we are able to infect animals with P. carinii isolates, we do not know the infective stage involved in the natural transmission of the parasite. DNA sequences similar to those of P. carinii have been found in air samples. However, lung forms of the parasite seem to survive only for a short time in the environment.

P. carinii taxonomy and phylogeny remain important goals as a means to clarify environmental sources of the pathogen and generate high-performance methods to in vitro culture. The development of a continuous culture system might give a better understanding of the biology and life-cycle of P. carinii, thus improving methods for the evaluation of anti-P. carinii drugs, leading to new therapies and prophylaxis against PCP. In this area, the sensitivity of P. carinii to the antibiotic activity of the PaKT and the protective effect of mAb KT4 idiotypic vaccination suggests that the yeast killer phenomenon could be used as a method to control PCP [157]. PCP could be prevented in transplant recipient or leukaemia patients by idiotypic vaccination carried out before the iatrogenic induction of immunosuppressed states. With regard to HIV-infected patients, they could also be protected against PCP by carrying out idiotypic vaccination when their immunological response is still efficient. Above all, the yeast killer phenomenon which has led to the obtention of recombinant peptides with antifungal activity might be used in a new therapeutic way.

**Acknowledgements**

We thank Mrs Chantal Mullet, Miss Karine Guyot and Mr Philippe Delcourt, U42 INSERM, Villeneuve d’Ascq, France, for their valuable technical assistance. We thank Dr Derek Sullivan, School of Dental Science, University of Dublin, Ireland, for the critical reading of the manuscript. Our investigations were supported by the Agence Nationale de Recherche sur le SIDA (ANRS, reference no. 94635), the Centre Hospitalier Régional de l’Université de Lille (reference 93-11) and developed in the framework of the European Union BIOMED-1 Concerted Action entitled: ‘Pneumocystis and pneumocystosis. Impact of the biodiversity of Pneumocystis carinii on epidemiology, pathology, diagnosis, monitoring and prevention of pneumocystosis. New therapeutic approaches’ (reference no. PL941118).

**References**

10 Stringer SL, Stringer JR, Blase MA, Walzer PD, Cushion MT. 


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distinction of Pneumocystis carinii from rats and humans.

genetic variants of *Pneumocystis carinii* confounding laboratory

51 Cushion MT, Kaselis M, Stringer SL, Stringer JR. Genetic
stability and diversity of *Pneumocystis carinii* infecting rat

52 Mazars E, Ödberg-Ferragut C, Durand I, et al. Genomic and
isoenzymatic markers of *Pneumocystis* from different host

53 Weinberg GA, O’Gara MJ, Cushion MT. Coinfection of rats
with genetically diverse forms of *Pneumocystis carinii* demon-
strated by P. carinii inosine monophosphate dehydrogenase

54 Hong ST, Steele PE, Cushion MT, et al. *Pneumocystis carinii*

55 Weinberg GA, Dykstra CC, Durant PJ, Cushion MT. Chromo-
somal localization of 20 genes to five distinct pulsed field gel
karyotypic forms of rat *Pneumocystis carinii*. *J Eukaryot Micro-
bioI* 1994; 41: 117S.

56 Vasquez J, Smulian AG, Linke MJ, Cushion MT. Antigenic
differences among genetically distinct types of rat-derived

57 Andrews RP, Theus SA, Cushion MT, Walder PD. Comparison of
the antigenic recognition of the major surface glycoprotein of
*Pneumocystis carinii* isolated from different rat strains.
*J Eukaryot Microbiol* 1994; 41: 72S.

58 Banerji S, Lugli EB, Wakefield AE. Identification of two
generally distinct strains of *Pneumocystis carinii* in infected

efficiency and nucleotide sequence variation in various *Pneu-
omycystis* isolates from humans and rats. *J Eukaryot Microbiol*
1994; 41: 85S.

60 Gigliotti F, Harmsen AG, Haidaris CG, Haidaris PJ. *Pneumo-
cystis carinii* is not universally transmissible between mam-

61 Furuta T, Fujita M, Muki R, et al. Severe pulmonary pneu-
moctysis in simian acquired immunodeficiency syndrome
induced by simian immunodeficiency virus. Its characterization
by the polymerase chain reaction method and failure of experi-
mental transmission to immunodeficient animals. *Parasitol Res*

62 Dei-Cas E, Mazars E, Ödberg-Ferragut C, et al. Ultrastructural,
genomic, isoenzymatic and biological features make it possible
to distinguish rabbit *Pneumocystis* from other mammal

63 Baeru NL, Paulsrud JR, Bartlett MS, Smith JW, Wilde CE.
*Pneumocystis carinii* organisms obtained from rats, ferrets and

64 Edlund TD, Bartlett MS, Weinberg GA, Prah GN, Smith JW.
The β-tubulin gene from rat and human isolates of *Pneumo-

65 Fletcher LD, Berger LC, Peel SA, et al. Isolation and identifi-
cation of six *Pneumocystis carinii* genes utilizing codon bias.

66 Gigliotti F. Host species-specific antigenic variation of manno-
sylated surface glycoprotein of *Pneumocystis carinii*. *J Infect Dis*


101 Roths JB, Sidman CL. Both immunity and hyper responsiveness to Pneumocystis carinii result from transfer of CD4 + but not CD8 + T cells into severe combined immunodeficiency mice. J Clin Invest 1992; 90: 673-8.


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