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Review article

Animal pneumocystosis: a model for man

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Abstract – *Pneumocystis carinii* is an important pulmonary pathogen responsible for morbidity and mortality in patients with AIDS. Apart from AIDS, cases of pneumocystosis have been reported in patients receiving immunosuppressive therapy associated with organ transplantation without chemoprophylaxis and in malignant blood diseases. In vitro models are only of limited interest because there is no continuous in vitro culture. The in vivo models have contributed a great deal to the understanding of human *Pneumocystis carinii pneumonia*. Indeed, animal models remain of prime interest for many purposes, principally comparative medicine, pathogenesis, epidemiology and immunology. Among animal models, the rabbit is a very susceptible host to *P. carinii* infection, and does not need glucocorticoid treatment. Moreover, antigenic and genomic data suggest that rabbit-derived *Pneumocystis* strains are more closely related to human *Pneumocystis* than those of mice or rats. We have therefore shown that the rabbit model permits the study of the pulmonary surfactant modification due to *P. carinii* infection. This model should be a very interesting model for pathogenesis or immune response studies in immunocompetent animals. The rabbit model could also be used for epidemiological studies. *P. carinii* transmission appears to be very rapid via contact of *Pneumocystis*-free rabbits with infected rabbits. These *Pneumocystis*-free animals could be helpful for characterizing the source and the reservoir and studying parasite transmission. © Inra/Elsevier, Paris.

in vivo model / transmission / rabbit / *Pneumocystis carinii* pneumonia / AIDS

Résumé – La pneumocystose animale : un modèle pour l'homme. *Pneumocystis carinii* demeure l'un des agents pathogènes pulmonaires le plus important chez les patients atteints du SIDA. D'autres conditions telles la malnutrition, la prématurité, les traitements immunomodulateurs des cancers sont associés au développement d'une pneumonie à *P. carinii*. Ce pathogène est donc un important agent de mortalité et de morbidité chez la plupart des sujets immunodéprimés. En raison de l'absence de modèles in vitro, les avancées de la recherche sur *P. carinii* se réalisent par l'expérimentation animale. Les modèles animaux sont très intéressants pour l'étude de la pneumocystose tant sur le plan épidémiologique qu'en pathologie comparée. Parmi les modèles in vivo disponibles, le lapin est

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l'un des hôtes le plus réceptif à *P. carinii*. Parasités par ce microorganisme, les lapereaux développent vers 28 jours une pneumocystose sans traitement immunodépresseur. De plus, les données antigéniques et génétiques suggèrent que les souches de *P. carinii* du lapin sont plus proches de celles de l'homme que ne le sont les souches provenant des rongeurs. Nous avons montré que chez le lapin, des modifications du surfactant alvéolaire suivent le développement parasitaire. Le lapin est un modèle intéressant pour les études de pathogénicité, de la réponse immunitaire et d'épidémiologie. En effet, la transmission de *P. carinii* apparaît être très rapide via le contact d'animaux infectés avec des animaux indemnes de *P. carinii*. Ce modèle expérimental permettra d'identifier la forme infectante du parasite.

pneumonie à *Pneumocystis carinii* / transmission / lapin / modèle in vivo / sida

1. INTRODUCTION

Pneumocystis carinii was first described in 1909 by Carlos Chagas in the lungs of guinea pigs infected with *Trypanosoma cruzi* [34]. In the following year Carini observed the same microorganism in the lungs of rats infected with *Trypanosoma lewisi* [27] and in 1912 Delanoe and Delanoe [52] reported the presence of the same microorganism in the lungs of rats not infected with *Trypanosoma* spp. Then in 1914 the same authors [53] reported their findings in the lungs of guinea pigs inoculated with different species of *Trypanosoma*. They observed pulmonary cysts comprising eight developing intracystic bodies which were in all ways identical to those reported by Carini in rats infected with *T. lewisi*.

P. carinii was subsequently studied by few authors. The first clinical descriptions of interstitial pneumonia were reported in the 1920s in humans. They originated from German hospitals where premature and malnourished infants were hospitalized in the first few months of life [60]. However, it was not until the 1950s that Vanek et al. considered the parasite as the etiological agent of pneumonia by its presence in the alveolar exudate in premature and malnourished infants [188, 189]. The effectiveness of pentamidine isothionate in the treatment of this type of pneumonia was demonstrated in 1958 [90]. During the 1970s, *P. carinii* was reported to be responsible for lung disease in immunodepressed patients following chemotherapy for the treatment of cancer or after organ transplantation [19, 199]. With the acquired immune deficiency syndrome (AIDS) pandemic in the 1980s, *P. carinii* emerged as one of the main agents among the most common AIDS-defining opportunistic infections: 60–80 % of AIDS patients develop *P. carinii* pneumonia (PCP) [47, 83, 134]. *P. carinii* and *Toxoplasma gondii* are the main pathogens causing hospital admission of AIDS patients in Europe and North America, although in sub-Saharan Africa the incidence of PCP is not as high [9, 28, 55, 131]. However, an increasing number of patients with this disease has recently been reported in Africa [70, 92]. Standardization of prophylactic protocols in developed countries has resulted in a 20–30 % decrease in PCP in HIV-positive patients [94]. Before 1991 patients were also detected at a very late stage of the disease, and most of them presented with pneumocystosis revealing HIV infection. Nevertheless, since 1991 PCP appears to be more serious because it occurs at the period of most severe immunodepression. The mean survival of patients with pneumocystosis thus appears to be lower than in the past. Half the patients die in the 20 months following onset of the disease (1993 data [162]). Thus, despite very effective prophylaxis, PCP remains the opportunist infection which triggers the onset of AIDS (*table I*).

Cases of extrapulmonary pneumocystosis were occasionally reported between 1960 and 1990 [137]. Extrapulmonary infections occur mainly in the liver, lymph nodes, muscles and, more rarely, in the kidneys, thymus, pancreas, eyes and middle ear. They usually occur in terminal stage AIDS patients [26, 135, 145]. The incidence of extrapulmonary pneumocystosis might increase with continued use of aerosolized pentamidine [135]. The use of aerosolized pentamidine for prophylaxis has largely decreased since it has been proved less effective against pneumocystosis and cannot prevent toxoplasmosis.

Apart from AIDS, cases of pneumocystosis have been identified in patients receiving immunosuppressive therapy associated with organ transplantation without chemoprophylaxis and in malignant blood diseases. Two studies observed that, in the absence of primary prophylaxis, 43 % of children with acute lymphoblastic leukaemia, 26 % of patients with Hodgkin's disease and 9–16 % of grafted patients developed PCP [163, 205]. The mortality in such patients was approximately 50 %.

Table I. Evolution of the incidence (%) of the most frequently occurring opportunistic infections in AIDS patients in France established from Lot et al. data [112].

	1990	1991	1992	1993	1994	1995	1996	Tendancy
Pneumocystosis	29.6	28.2	24.6	21.3	18.6	18.8	20	↘
Cerebral toxoplasmosis	15.8	15.9	15.9	12.8	11.3	10.4	10	↘
Esophageal candidosis	13.6	12.7	13.1	13.8	15.4	16.4	17.7	↗
Kaposi sarcoma	18.3	17.1	15.8	14.2	14	12.3	12.6	↘
Cytomegalovirus	5.1	5.3	6.2	6	7.6	8.9	8.4	↗
Cryptosporidiosis	3.4	3.5	3.6	4.5	4.2	3.5	3.7	↘
Cryptococcosis	2.1	2.5	2.4	1.7	2.3	2.9	3	↗
Mycobacteriosis	1.5	2.2	2.9	4.4	4.5	4.6	4	↗
Tuberculosis	/	/	/	6.2	6.7	5.8	6.6	→

In this review, we present the state of knowledge concerning mammal pneumocystosis, in in vitro and in vivo models, and the impact of animal models for a better understanding of human pneumocystosis.

2. *PNEUMOCYSTIS CARINII*

2.1. Taxonomy and diversity

The taxonomic position of *P. carinii* remains controversial. The first difficulty is to determine whether *P. carinii* should be grouped with the protozoa or fungi or whether it should be included in another group of eukaryotic organisms. The numerous mammal hosts of *P. carinii* suggest a long phylogenic coevolution of the parasite [26, 135]. The second difficulty is the definition of the genus *Pneumocystis*. It is particularly important to determine whether there is a single species or whether it is possible to distinguish several genetically isolated species.

2.1.1. Taxonomy

As the biological, epidemiological and therapeutic characteristics of protozoan and fungal diseases are different, separate axes of research could be undertaken according to the phylogenetic lineage of *P. carinii*.

The amoeboid appearance of the parasite stage, commonly called a 'trophozoite' and the presence of cytoplasm extension suggest that *P. carinii* is a protozoan. Moreover, *P. carinii* is sensitive to drugs that are effective against protozoan parasites, such as pentamidine and the association of trimethoprin-sulfamethoxazole (TMP/SMX). The parasite does not develop in vitro in culture medium for fungi [50] and, as for Apicomplexa, it is able to synthesize folates de novo from para-aminobenzoic acid [102]. Moreover, ergosterol, the sterol found in the membranes of most fungi, has not been detected in *P. carinii*. The lack of ergosterol may account for the clinical inefficacy of commonly used antifungal agents that act by binding to ergosterol (polyenes) or by blocking synthesis of ergosterol (azoles).

On the other hand, tinctorial affinity and the structure of the walls of the cysts suggest fungal affinity [123]. Similarly, the presence of lamellar mitochondrial cristae are suggestive of fungi [158]. However, many protozoans (Cryptomonadines, Euglinidae and zooflagella) also have lamellar mitochondrial cristae [47].

Moreover, although the enzymes thymidilate synthase and dihydrofolate

reductase exist as the same bifunctional protein in the protozoans studied to date, in *P. carinii* these enzymes consist of two separate monofunctional polypeptide chains, the synthesis of which is coded by genes situated on different chromosomes [114]. Finally, there are immunological affinities between *P. carinii*, *Saccharomyces cerevisiae*, *Aspergillus fumigatus* and other fungal species, but also with *Toxoplasma gondii* [78, 115].

The comparison of nucleic and protein sequences has been used to define the taxonomic position of *P. carinii*. The genes coding for small subunit ribosomal RNA (rRNA) (16S), and the large subunit of mitochondrial rRNA (mt LSU rRNA gene) of *P. carinii* have some similarities to the genes coding for rRNA of different fungal species (*Neurospora crassa*, *Saccharomyces cerevisiae* and *Candida albicans*) [151, 176]. Studies conducted on the 5S rRNA sequence suggest that *P. carinii* might be associated with the *Rhizopoda/Myxomycota/Zygomycota* group but not with common fungi such as *Ascomycota* or *Basidiomycota* nor with protozoa [200]. The ATPase peptide sequence of *P. carinii* is 66 % identical to that of fungi and only 34 % to that of a protozoan such as *Leishmania* [128]. In addition, the nucleic sequence of chaperonine, a cytoplasm protein of *P. carinii*, is 83 % identical to that of yeasts, and the degree of similarity reaches 90 % if the amino acid sequences are taken into account [173]. Finally, the construction of a phylogenic tree from the protein sequence of β-tubulin places *P. carinii* in the same group as *Candida*, *Saccharomyces* and *Schizosaccharomyces* spp. [10].

Most authors agree in attributing a fungal pattern to *P. carinii* but for some authors *P. carinii* is an *Ascomycota* [135, 176] or for others it is a *Basidiomycota* [76, 187]. In agreement with the fungal pattern, some authors recommend replacing the terminology of the different forms of the parasite (trophozoite, intracystic bodies) by a terminology suggesting that *P. carinii* is a fungus-like organism: the trophozoite should be referred to as the yeast cells, the cysts as sporangia, and intracystic bodies as spores [160].

2.1.2. Genetic diversity of P. carinii

The genetic diversity of *P. carinii* has been analysed at karyotype, nucleic, isoenzyme and antigen levels. Another approach has been the study of cross infections of *P. carinii* originating from different host species.

2.1.2.1. Karyotype polymorphism

Karyotype polymorphism has been distinguished by pulsed field electrophoresis of chromosomes of *P. carinii*, sometimes associated with hybridization (*table II*) [41, 81, 203]. A large amount of information has resulted from these karyotype studies: i) the number of chromosomes is different in *P. carinii* according to the host species [81, 201, 202]; ii) the number of chromosomes is not modified in vitro [81, 201, 202]; and iii) parasites with different karyotypes are found in the rat [41, 44, 81].

It should be emphasized that the number of chromosomes observed by pulsed field electrophoresis varies considerably in the same host species according to author. This can be explained by the technical conditions used for chromosome migration which have a direct influence on the karyotype observed [161, 169] and by the fact that laboratories use animals from different lines [41, 44].

2.1.2.2. Polymorphism of nucleic sequences and isoenzymes

The inter- and intra-specific diversity of *P. carinii* has been distinguished by sequencing numerous genes: genes coding for mitochondrial rRNA [87, 107, 113, 148, 196], genes encoding for cytoplasmic rRNA (rRNA 16S, 5S and 26S) [110, 111, 200], ITS sequences (internal transcribed spacers) [11, 91, 95, 107, 113, 142], tandem repeat sequence (5'-TTAGGG-3') from the

Table II. Comparison of pulsed field electrophoresis karyotypes of *Pneumocystis carinii* derived from rat cell cultures and rat, mouse, ferret and human lungs.

Authors	*P. carinii* source	Number of chromosomes	Size of chromosomes (kb)
Hong et al. [81]	rat	15–22	300–700
	human	10–12	300–700
Weinberg and Bartlett [201]	rat	8	350–700
	cell culture of rat	8	350–700
	ferret	5	580–900
Weinberg and Durant [202]	rat	14	315–680
	cell culture of rat	14	315–680
	mouse	15	315–610
	ferret	9	410–760

telomeric region of genes encoding the major surface glycoprotein (MSG) [103, 186, 193], genes encoding α- and β-tubulin [108, 202], the *arom* gene [11, 12] and the gene encoding thymidylate synthase [96, 107, 124, 126].

The sequence encoding mt LSU rRNA was compared in parasites isolated in nine host species (rat, mouse, shrew, rabbit, ferret, pig, horse, monkey and man). The different sequences varied from 4 to 27 %. These variations are greater when the host species are phylogenetically distant [96, 148].

The polymorphism of sequences is not limited to the mt LSU rRNA gene. Comparison of sequences has emphasized that the genetic divergence between *P. carinii* from different host species is greater than that observed between different species of other genera of fungi [96]. Genotype and phenotype findings suggest that the genus *Pneumocystis* might include several species. Nevertheless, it is not certain that different taxons of *P. carinii* can exchange genes and therefore, in order to avoid creating spurious species, *P. carinii* has not yet been divided into species but a trinomial nomenclature has been used (*table III*). Nevertheless, it is hardly credible that several taxa of *P. carinii* can cohabit in the same mammal. Indeed, it has been established that laboratory rats have at least two different taxa which are characterized genetically and antigenically [178]. The presence of these taxa of *P. carinii* in the same host thus presents difficulties in terms of taxonomy.

It was also possible to demonstrate the intra-species diversity by direct sequencing of PCR products [41, 91, 97, 106, 107, 142, 191] and by studying single strand polymorphism (SSCP) after PCR amplification [79, 121]. The same authors were able to show the existence of several strains of *P. carinii* in the same infected host (rat, ferret, shrew and man). Six to ten variants were identified in man from ITS sequences [91, 113] and four from mt LSU rRNA sequences [97, 107]. Other variants were characterized: 16S and 5.8S rRNA [96, 142], the arom gene [11], the genes of α- and β-tubulin [177, 202] and GMS genes [103, 181]. Moreover, our teams have observed two different types in rabbit *P. carinii* mt LSU rRNA sequences (unpublished data).

Differences have been detected in *P. carinii* using several enzyme systems (glucose 6-phosphate isomerase, malate dehydrogenase), not only according to the host species but also within parasite isolates of rats, mice and rabbits [124, 126].

Table III. Trinomial nomenclature of *Pneumocystis carinii* (from Stringer and Walzer [175])

Trinomial nomenclature*	Other nomenclature	Host species
Pneumocystis carinii f.sp. *carinii*	*P. c. carinii*	rat (prototype)
Pneumocystis carinii f.sp. *rattus*	*P. c. rattus*	rat (variant)
Pneumocystis carinii f.sp. *hominis*	*P. c. hominis*	human
Pneumocystis carinii f.sp. *mustelae*	*P. c. mustelae*	ferret
Pneumocystis carinii f.sp. *muris*	*P. c. muris*	mouse
Pneumocystis carinii f.sp. *equi*	*P. c. equi*	horse
Pneumocystis carinii f.sp. *suis*	*P. c. suis*	pig
Pneumocystis carinii f.sp. *oryctolagi*	*P. c. oryctolagi*	rabbit

* f.sp. designated as 'special forms' (*formae speciales*) of *P. carinii*, following the recommendation of the Botanical Code for physiological variants of parasitic fungal species characterized by their adaptation to different hosts.

2.1.2.3. Antigenic polymorphism

The initial studies showed that the anti-*Pneumocystis* antibodies produced in a given species principally recognized the antigen sites of *P. carinii* from the same species [198]. The antigen variability has mainly been studied by immunoblotting using polyclonal [17, 75, 101] or monoclonal antibodies [7, 64, 74, 191]. The action of monoclonal antibodies directed against the major surface glycoprotein (MSG) of *P. carinii* has been explored in the rat, mouse, ferret and man. These studies have demonstrated parasite antigen variation according to the host species [17, 61, 65, 67]. Moreover, using immunoblotting and lymphocyte proliferation tests some authors have observed that monoclonal antibodies directed against MSG present different reactions according to *P. carinii* strain [7, 184, 191]. These experiments have made it possible to understand better the interactions between T lymphocytes and the parasite and they suggest the existence of variable expression of the MSG in different isolates [7, 184]. The antigen variations in the MSG might constitute an escape mechanism from the host defences [69, 103, 194].

2.1.2.4. Cross infections

Host specificity has also been explored by cross inoculation of *P. carinii* from different host species. Sethi [164] proposed the severe combined immunodeficient (SCID) mouse as a means of isolating and maintaining *P. carinii* of human origin. Similarly PCR amplification and sequencing of ITS and mt LSU rRNA from a human isolate of *P. carinii* have revealed hybrid *P. carinii rattus–P. carinii hominis* sequences. These hybrid sequences suggest that *P. carinii rattus* is able to co-infect man and/or recombine with *P. carinii hominis* [113]. However, this research suggesting that cross infection is possible is actually controversial. Indeed, several recent studies have shown that parasites extracted from a given host species are only able to cause pneumocystosis in the same species [4, 68]. Moreover, using mouse or ferret *P. carinii*-specific monoclonal antibodies and PCR amplification of a gene fragment coding for the MSG of *P. carinii* from the ferret has shown that these co-infected hosts only harbour the parasite population specific to their species [68]. Identical results have been observed in mouse and rat models by Aliouat [2]. None of these experiments showed the existence of a species reservoir for the human pneumocystosis agent and therefore this disease should not be considered zoonotic.

2.2. Biological cycle

The ultra-structural data on human lungs or animals infected with *P. carinii* obtained

by electron microscopy have revealed two parasite forms (trophozoites and cystic forms). The trophozoite is an amoeboid mononucleate form of 2–5 µm in diameter *(figure 1)*. The presence of filopodia in amoeboids allows the trophozoite to attach itself to the surface of the lung epithelial cells, especially to type I pneumocytes. In the attachment zone the parasite surface is closely apposed to the epithelial cell. The

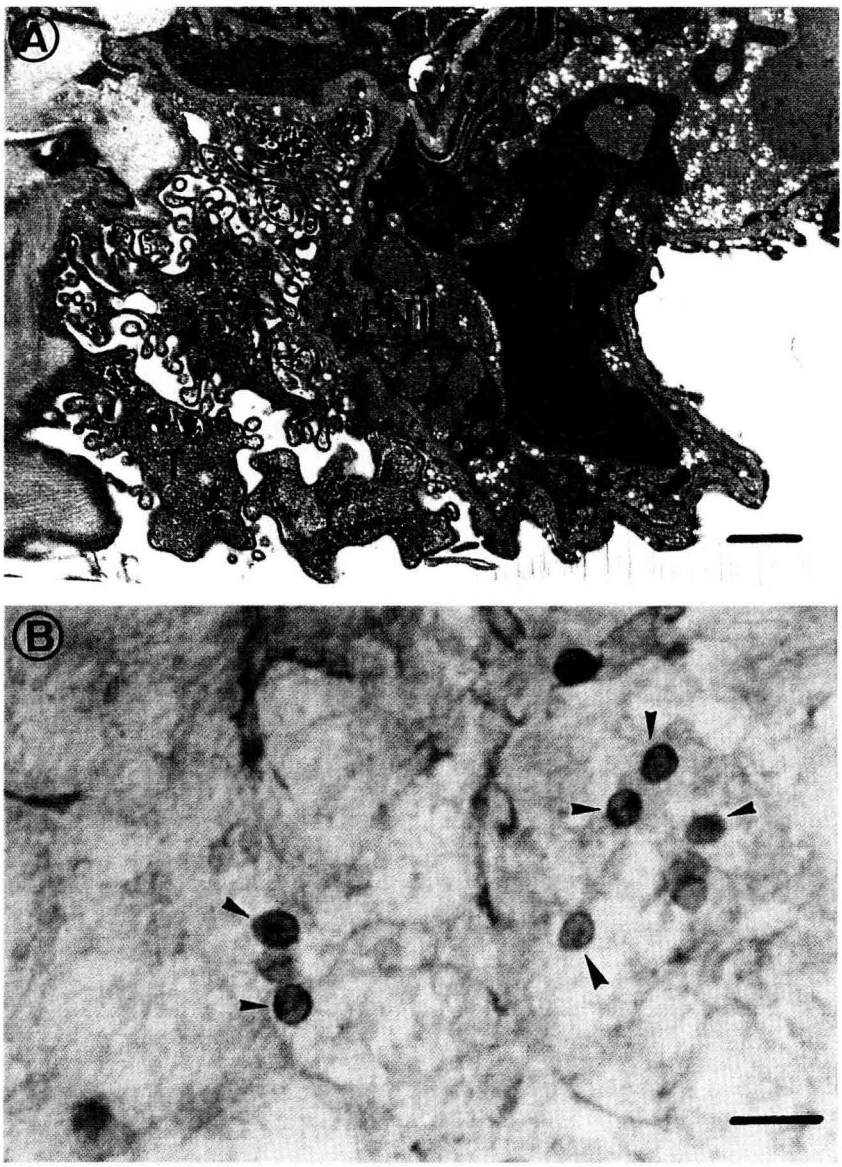

Figure 1. A. Ultrastructure of *Pneumocystis carinii* in the rabbit lung. PNII, lung epithelial cell of type II; T, trophozoite. Bar = 500 nm. B. Cystic forms of the parasite in the rabbit lung stained with toluidine blue O. Bar = 10 µm.

development of numerous vesicles in the cytoplasm of either the *P. carinii* trophozoite or the host cell suggests that this zone is metabolically very active [49, 136].

There are two cystic forms, precysts and mature cysts. The precyst is 3–5 μm in diameter and is characterized by a round outline and considerably reduced numbers of tubular expansions. Its structure and development define it as the intermediate form between the trophozoite and the mature cyst. It is possible to see early, intermediate and late precysts at this stage.

The early precyst is uninucleate and has a wall similar to that of trophozoite. The intermediate cyst has 2–8 nuclei. The cystic forms have a thick wall consisting of a plasma membrane, an electron-lucent middle layer and an electron-dense outer layer.

The late precyst has a thicker wall surrounding the cytoplasm in which the eight uninucleate intracystic bodies become individualized by invagination of the plasma membrane.

The mature cyst is 4–6 μm in diameter. It has a wall similar to that of intermediate and late precysts. It contains eight well-individualized uninucleate intracystic bodies.

Cushion et al. [43] and Yoshida [208] have proposed a hypothetical cycle based on this ultrastructure but the chronology and dynamics of the cycle remain largely unclarified [43, 208].

The eight intracystic bodies might be haploid and might give rise to trophic forms after excystation. The trophic forms become diploid by fusion. The nucleus of the precyst then undergoes meiotic followed by mitotic division, producing the eight haploid nuclei. The eight intracystic bodies might be formed by invagination of the precystic plasma membrane around each nucleus followed by an asexual cycle during which the trophozoites multiply by binary fission. Recent reports appear to confirm at least part of this cycle. Some studies suggest that trophozoites are haploid and can multiply by binary fission [157, 207]. A recent report on binucleate trophozoites supports the hypothesis of fusion or conjugation of two *P. carinii* cells to give rise to a zygote. The discovery of synaptonemal complexes at the early precyst stage is evidence of matching and alignment of homologous chromosomes, suggesting meiotic division [123].

The proposed cycle probably takes place entirely in the host lung. However, recent studies have reported the presence of DNA similar to that of *P. carinii rattus* and *hominis* in the environment and that *P. carinii* may be a component of the air spora [195]. The parasite cycle must therefore have a free phase, possibly involving distinct parasite forms which are smaller than the other forms described in *P. carinii*. These forms might be spores. During the free phase the parasite might thus undergo development which produces the infective forms which have not been yet identified. According to some authors, intracystic bodies, sometimes called sporozoites, are the most likely infective forms [47, 160].

2.3. Host–Parasite interactions

The alveolar epithelium comprises two types of cell. The majority of the alveolar surface is coated with large flattened cells called type I pneumocytes. The second cell type is the type II pneumocyte. These are rounded cells with vacuolized cytoplasm. The type I pneumocytes constitute the fine air–blood barrier while type II pneumocytes secrete and renew the surfactant.

The clearly identified habitat of *P. carinii* is the pulmonary aveoli. The trophozoites are specific to type I pneumocytes. Two interactions have thus been characterized, the interaction with the alveolar cells and the interaction with the alveolar surfactant.

2.3.1. Interaction of P. carinii *with alveolar cells*

The adhesion of pathogens (viruses, bacteria, fungi and protozoans) to host cells is

generally a critical phase in the onset of infection. The mechanisms of adhesion of microorganic pathogens to the plasma membrane of host cells occur either by direct recognition via receptor–ligand binding or by the intermediary of a molecule secreted and recognized both by the microorganism receptor and that of the host cell.

Integrins (particularly fibronectin and vitronectin) are secreted by the host cells and are found within the extracellular matrix. Fibronectin (Fn) has an important role in the adhesion of *P. carinii* to the surface of host cells [3, 109, 153]. The major surface glycoprotein (MSG) of *P. carinii* is probably the Fn receptor. The binding of *P. carinii* to type I pneumocytes would thus occur via vitronectin (Vn). The host–parasite interaction via Vn would therefore involve the intermediary of a receptor other than MSG, i.e. by means of a receptor specific to the heparin-binding region of Vn [109, 206].

Lectins of the cells and of the parasites probably have a role in the adhesion to type I pneumocytes and macrophages. The 'mannose' residues of MSG are recognized by the mannose receptors on the surface of macrophages [59, 139].

2.3.2. Interaction of P. carinii *with the alveolar surfactant*

The alveolar surfactant consists of 90 % phospholipids and 10 % proteins. It reduces the superficial water–air tension of the pulmonary alveoli. Its main functions are to reduce the elastic retraction force of the air–water interface, to stabilize alveolar units of unequal size and to contribute to the equilibrium of intrapulmonary fluids [104]. The components of the surfactant are synthesized by type II pneumocytes. Alveolar macrophages also participate in the elimination of excess surfactant.

Pneumocystosis is generally accompanied by acute respiratory insufficiency. The diagnosis may be established on the basis of the presence of *P. carinii* in different pulmonary samples, mainly the broncho-alveolar lavage fluid (BAL) or sputum. Pneumocystosis is characterized in the BAL by an increase in the levels of protein in the surfactant and changes in the composition of phospholipids [58, 149]. The former might result from the passage of serum proteins into the alveolar surfactant due to increased vascular permeability, and the latter might result directly from parasite activity. Indeed, *P. carinii* inhibits the secretion of phosphatidylcholine by type II pneumocytes [156]. During pneumocytosis the percentages of phosphatidylcholine and phosphatidylglycerol decrease and those of sphingomyelin and lysophosphatidylcholine increase [57]. The decrease in these phospholipids during pneumocystosis, and particularly in phosphatidylcholines, leads to disturbance within the alveolar units. Dipalmitoylphosphatidylcholine is the major phospholipid component and the one largely responsible for the surface activity [104].

In addition, *P. carinii* has several apoprotein A and D receptors for the alveolar surfactant and these interactions occur with high affinity [209]. These proteins might have a role either in the elimination of *P. carinii* by the macrophages via an opsonization mechanism in the parasite [138] or by an escape mechanism of the parasite from the host defense system [179].

3. PNEUMOCYSTOSIS

The main studies on pneumocystosis have been taking place since the beginning of the AIDS pandemic.

3.1. Clinical pattern

3.1.1. In man

Pneumocystosis is an opportunist infection which only develops in immunodepressed subjects. There can be two clinical patterns: slow evolution with radiological signs which are generally slight, and rapid

evolution, characterized by a dry cough, slight fever, clear radiological signs of diffuse interstitial pneumonia-type lesions and rapid evolution to acute respiratory insufficiency.

Disease is mainly limited to the lungs although rare extrapulmonary pneumocystosis occurs. According to the immune state of the patient, pneumocystosis is characterized histologically by numerous trophozoites adhering to type I epithelial cells and covering the alveolar walls, invasion of the alveolar lumen by mainly *P. carinii* and bacteria debris, and mononuclear infiltrate in severe disease.

Cases of extrapulmonary pneumocystosis are rare and occur after damage to the endothelium of pulmonary vessels. Parasite infiltration of other organs such as the liver, spleen, kidneys, bone marrow or knees, etc., has been described. Transport can take place by the blood or lymphatic system [35, 37, 135].

3.1.2. Other mammals

P. carinii infection has been described in several animal species but spontaneous pneumocystosis is rare.

Pneumocystosis has been described in nude rats and in SCID and nude mice. It results in a fatal wasting disease which sometimes decimates the affected colony [63, 204]. It has also been observed in young dogs (8 months–2 years) and the disease is characterized clinically by insidious evolution with dyspnea and cyanosis, finally resulting in death [180]. Sporadic cases of fatal interstitial broncho pneumonia have been observed in young horses (2–4 months), mainly the SCID horse [155]. Finally, outbreaks of pneumocystosis have been observed in some pig breeding colonies. It affects piglets aged 4–11 weeks and is characterized by dyspnea and weight loss [20, 100].

Histology examination of affected lungs shows diffuse interstitial pneumonia [20, 180] and foamy acidophilic material with desquamated epithelial cells and macrophages [20, 155].

3.2. Epidemiology

3.2.1. Factors influencing P. carinii *development*

Several factors have been found to be responsible for the development of *P. carinii* in man: absence of prophylaxis and number of CD4 lymphocytes lower than 200 cells/mm^3 blood (60–80 % of these patients have pneumocystosis) [82, 116, 163]. Moreover, some viral infections such as cytomegalovirus and Herpesvirus 6 appear to be additional risk factors for *P. carinii* infection for HIV+ patients [8, 23, 99, 117]. This result was described in a rat model infected with *P. carinii* and co-infected with Senda and RCV (rat coronavirus) by Cushion and Linke [40]. On the other hand, a seasonal and climatic influence (winter, continental climate) has been reported in the development of *P. carinii* pneumocystosis. The resurgence of these diseases may be due to *P. carinii* infections during the coldest months or may follow other upper respiratory tract infections. The weakened body offers an ideal milieu for the development of the parasite [82, 116].

3.2.2. Horizontal transmission of P. carinii

Nosocomial transmission of *P. carinii* to human immunodeficiency virus-uninfected patients has been suggested but the horizontal transmission of *P. carinii* has only been shown in animal models.

Airborne transmission (from an infected host to a potential host) has been proved for animals and suggested for humans [26, 31, 83, 154]. We have shown that the period of highest contagion following primary infection was between 8 and 18 days after experimental infection in naive rabbits [31]. This first description of the production of infective forms of *P. carinii*, 8 days after inocu-

lation, constitutes new information for epidemiological studies regarding the development of *P. carinii*.

Airborne interhuman nosocomial transmission is strongly suspected [14, 140]. The infective form of *P. carinii* in the environment and the sources of infection are still not proved [26, 50, 83]. *P. carinii* has been detected in the air near patients with pneumocystosis after aspiration/filtration on membranes and PCR amplification of DNA, and the DNA of *P. carinii rattus* near infected rats [14, 140]. This technique has also revealed airborne DNA of human, rat and ferret strains in rural areas. The possible presence of *P. carinii hominis* in areas where population density is low suggests the existence of an environmental source [195]. However, the filtration techniques cannot reveal whether detected forms are infective or not.

Contact of a debilitated subject with such reservoirs might generate a de novo infection. Indeed, several studies on the biodiversity of human strains indicate that recurrence of pneumocystosis may be due to a human strain different from that of the initial infection [106, 185]. The hypothesis of de novo infection is reinforced by various studies showing the absence of parasite in non-immune-depressed humans [147] and elimination of *P. carinii* in 75 % of rats in less than a year after the first pneumocystosis infection [190]. Pneumocystosis may also result from reactivation of *P. carinii* which is naturally present in a latent state in the lungs when immune depression occurs [83]. After the first infection, the parasite might remain in the lungs in a latent form. Several studies confirm this hypothesis: latent infection is detected by PCR [35, 98] and laboratory animals only develop pneumocystosis when an immune-depressed state is prolonged [83, 143].

3.2.3. Vertical transmission of P. carinii

Vertical transmission of *P. carinii* has long been suspected in the rat [150] and in man [83, 135]. The occurrence of pneumocystosis in premature infants and the existence of cystic forms in the lungs of human foetuses [132] suggest the possibility of such transmission. Nevertheless the most recent results are controversial [85]. Identification of *P. carinii* infection in 7-day-old rabbits [48] and at birth [30] with mothers who had hardly any parasites suggests the placental route as another route of infection for this species. We have demonstrated the transmission in utero in the rabbit by PCR, classical histology methods and by immunofluorescence [30]. Moreover, these studies suggest that foetal infection with *P. carinii* occurs via the placenta. Is transplacental transmission of *P. carinii* particular to the rabbit? Several replies could be put forward to answer this question. Ito et al. [89] showed that the parasite is not transmitted by the transplacental route in the mouse. Thus vertical transmission may vary in frequency according to the animal species.

The question that arises is why vertical transmission is so common in the rabbit? Indeed, it is interesting to consider the doe placenta and to compare it to the human placenta. At 15 days' gestation the doe placenta is hemo-chorial, and thus comparable with the human placenta. After the 17th day it becomes hemo-endothelial [30]. The permeability of the placenta and the maternal/foetal blood exchange are greater than in the hemo-chorial type of foetus, which could be a permissive factor in foetal contamination. As the human placenta is hemo-chorial, the frequency of vertical transmission might therefore be lower. The exact moment of passage of parasites to the foetus has yet to be determined. In conclusion, recent studies have established for the first time that transplacental transmission is an additional route of infection for *P. carinii* (at least in the rabbit). The parasite is thus transmitted naturally from the does to the foetus during at least the last 6 days of gestation [30].

3.3. Immune response

Reports of subjects developing pneumocystosis have demonstrated the essential role

of the immune response to cellular mediation. The humoral response seems to be less determinant in the host defense against *P. carinii* [62, 135, 167]. It is involved in the maintenance of the microorganism during the latent stage of the infection or during parasite clearance in immune competent hosts [135].

3.3.1. Specific response to cellular mediation

Several reports indicate that CD4+ lymphocytes are essential to host defense against the parastite invasion. First, transfer of CD4+ lymphocytes from a *P. carinii*-infected mouse to another infected but athymic mouse (nude mouse) substantially reduced the parasite burden of the latter [143]. Second, cyclosporine, which specifically inhibits T lymphocytes, enhances the development of pneumocystosis in grafted patients [135]. Third, pulmonary proliferation of *P. carinii* in AIDS patients is considered to be the consequence of a decrease in the number of CD4+ lymphocytes [120]. The risk of occurrence of pneumocystosis is correlated with the number of circulating CD4 lymphocytes [26, 163]. However, CD8+ lymphocytes appear to have a less important role because depletion of CD8+ lymphocytes (injection of anti-CD8 antibodies) does not alter the ability of mice to eliminate *P. carinii* infection [77].

3.3.2. Specific response to humoral mediation

Anti-*P. carinii* antibodies (IgG, IgM) are frequently found in healthy individuals, demonstrating contact with *P. carinii* [75, 130]. In infected ferrets and rats, the transfer of monoclonal antibodies directed against the MSG of *P. carinii* slows the evolution of the infection [66]. Athymic mice infected with *P. carinii* do not synthesize anti-*P. carinii* antibodies. These animals are partially protected by the transfer of T helper lymphocytes (Th) of mice infected with the parasite. When the Th cells are introduced into athymic mice the B cells can then produce IgG [62]. The humoral response appears therefore to be involved in the protection of the body against *P. carinii* but in a less determinant way than the cell-mediated response.

3.3.3. Non-specific response

The alveolar surfactant interacts with the immune system. In fact the surfactant phospholipids have immune suppressant properties in relation to alveolar macrophages in order to avoid continuous pulmonary inflammation during inhalation of all airborne particles. The inflammatory response is the most important of the non-specific defense mechanisms against *P. carinii*. The inflammatory response against *P. carinii* is divided into two elements: the production of soluble compounds toxic for the parasite (cytokines, lysozymes, interferon, nitric oxide) and cellular elements (macrophages, polynuclear neutrophils). Several studies have illustrated the importance of non-specific immune mechanisms against *P. carinii*.

In vitro cultured rat alveolar macrophages are able to phagocytose the parasite, both in the presence and in the absence of opsonizing antibodies [119]. Granulocytes can have a substantial role in the defense against *P. carinii*. Neutrophils are in fact present in large numbers in the inflammatory infiltrates of AIDS patients. This high number of neutrophils is associated with fibrosis. The latter non-specific response appears to be more harmful than beneficial for the host. This is also the case for oxydative burst by the alveolar macrophages. The production of nitric oxide (NO) may amplify the inflammatory response [168]. The macrophages might have a preponderant role by acting either directly on *P. carinii* or by the intermediary of cytokine secretion. *P. carinii* can, in fact, induce the liberation of tumour necrosis factor alpha (TNF-α) by macrophages [182]. There might be a double mode of action of TNF-α against

P. carinii: i) a direct toxic effect of TNF-α on the parasite, but the presence of receptors for this cytokine has not yet been established; and ii) an indirect action by activation of the macrophages that liberate the toxic metabolites of oxygen [146]. The presence of infectious agents might enhance the virulence of *P. carinii*. The high frequency of concomitant infection by *P. carinii* and cytomegalovirus suggests possible interactions between these two organisms [8, 56]. Orenstein et al. [141] recently showed that *P. carinii* promotes HIV development. Indeed, these authors observed that the macrophages of people infected with HIV produce more virus when the person is co-infected with other pathogens such as *P. carinii*.

3.4. Control methods

Prophylaxis in patients with HIV has made it possible to reduce morbidity and thus to improve patient survival. Prophylaxis is envisaged over many months or many years, also the tolerance of the chemoprophylaxis is a major element in successful prevention.

3.4.1. Reference prophylaxis for pneumocystosis

The association of trimethoprim and sulfamethoxazole (TMP-SMX) is the most effective preventive and curative treatment and it is probably the most often prescribed against pneumocystosis [13, 36, 93]. This treatment is the association of a sulfamide, sulfamethoxazole, and a diaminopyrimidine, trimethoprim. Their mechanisms of action result in blocking the synthesis of nucleoproteins: sulfamides inhibit the synthesis of folic acid and trimethoprim inhibits the synthesis of dihydrofolate reductase. Their half-lives are approximately 12 h. The rapid diffusion of both drugs into various tissues and into the secretions provides good protection against PCP and extrapulmonary pneumocystosis. According to the 1996 report concerning prophylaxis for HIV patients, the dosages of TMP-SMX demonstrated to be effective are either 800 mg/day sulfamethoxazole associated with 160 mg/day trimethoprim, or 400 mg/day sulfamethoxazole associated with 80 mg/day trimethoprim. However, the lower dose is preferable because it induces less secondary effects [54].

Chemoprophylaxis against *P. carinii* pneumocystosis is undertaken under medical surveillance during the first trimester of pregnancy because the foetus cannot reduce the blood concentration of the drug and its derivatives during this period. The administration of TMP-SMX to infants from the age of 1 month is recommended for all children born to seropositive mothers, even before the diagnosis of HIV infection has been established. Prophylaxis is withdrawn if the child does not have HIV or maintained systematically until 1 year of age. After this age, the treatment continues if the number of circulating CD4 lymphocytes is lower than 500 per mm^3 (the normal number of CD4+ lymphocytes at birth is around 3 000 per mm^3).

Long-term use of these drugs can lead to intolerance and side effects such as nausea, urticaria and stomach pain [24, 84, 93, 159] and occasionally toxicoderma in 16 % of patients receiving high doses of TMP-SMX [29]. Moreover, recent studies have suggested the use of other drugs for prophylaxis because resistant parasite strains might develop [15]. Indeed, Lane's work [105] has shown the development of strains resistant to sulfamides in mice. Such resistance occurs after alternation of four cycles comprising a treatment phase followed by a resting phase.

3.4.2. Other prophylaxes

The exclusive use of anti-folics has been recommended in the prophylaxis against PCP (sulfadimethoxine, sulfamethoxypyridazine). They block the formation of the

folic acid necessary for parasite metabolism. Their use must, nevertheless, be kept under high surveillance because they can cause mucocutaneous and hematologic events. These drugs are therefore contra-indicated in patients intolerant of sulfamides, and in the last weeks of pregnancy and in neonates (immature enzyme system) [86]. The other treatments and prophylactics are less effective and are only justified when there is intolerance of TMP-SMX. Pentamidine aerosols are frequently used in prophylaxis [38, 71]. They are, however, less effective when the CD4+ lymphocyte level is lower than 100 per mm^3. Other drugs used alone or in association have been shown to be effective against opportunist *P. carinii* and *Toxoplasma gondii* infections. However, the administration of dapsone or the association of clindamycin–primaquine does not prevent recurrence of *P. carinii* pneumocystosis (*table IV*) [13].

Other dihydrofolate reductase inhibitors such as PS-15 (a new biguanidine), epiroprim and pyrimethamine associated with dapsone have been shown to be effective [18, 24, 36, 129]. Similarly, albendazole (from the benzimidazole family) [14] and lasalocid (ionophore family) [144] can be active drugs against *P. carinii*. Oz and Hughes recently emphasized the advantages of a suppressive drug with antibacterial, antiviral and antiparasite properties, such as mycophenolate mofetil used in organ transplantation. The use of such drugs would make it possible to reduce considerably the side effects and toxicity due to the simultaneous administration of several active agents [143].

4. IN VITRO AND IN VIVO MODELS OF PNEUMOCYSTOSIS

4.1. In vitro culture of *P. carinii*

Several attempts to culture *P. carinii* have been performed on cell lines, on excised parasite-infected lungs and on axenic culture.

4.1.1. Co-culture of P. carinii *cultivated feeder cells*

Most cell lines are fibroblast or epithelial cell lines from mammals, such as line A 549 (human alveolar epithelial cells) [43], line L2 (rat alveolar epithelial cells) described by Burnstein [25] and Aliouat et al. [5], the Vero line (kidney fibroblasts from the African green monkey) [5] and epithelial rabbit lung cell [152]. It has been demonstrated that rat, mouse, human and rabbit-derived *P. carinii* are able to attach in vitro to several original cell lines: L2, Vero, A 549 cells and rabbit lung cell, respectively.

In vitro culture of *P. carinii* is achieved by inoculation of a freshly extracted parasite solution into a cell culture [5]. The results obtained in various cell systems are similar: the number of parasites increases about six to ten times within the first 3–4 days post-inoculation. Such an increase might essentially be due to the multiplication of the trophozoite form [47]. After 8 days of culture, the number of *P. carinii* decreases significantly [5, 25, 43]. The growth of the parasite is limited because their numbers gradually decrease after two or three passages [42]. However, the infective potency of the para-

Table IV. Relapse rate of pneumocystosis in patients receiving treatment.

Treatment	Frequency of pneumocystosis relapse (%)
Dapsone	11
Clindamycin–Primaquine	31
Trimethoprim–Sulfamethoxazole	3.4

sites obtained by this system is seven to ten times greater than that of the freshly extracted parasites [5]. These authors have shown that the extraction procedures can modify the infective potency of the parasite.

4.1.2. Lung explant culture

The development of *P. carinii* on excised parasite-infected lungs of rabbits has been presented by Dei-Cas et al. [46]. However, the multiplication rate of the parasite is low and slow (the number of parasites is increased six-fold after 41 days of culture). The extracted parasites are very contaminated with pulmonary debris.

4.1.3. Axenic culture

Significant *P. carinii* growth in cultures without feeder cells has also been reported. Two types of liquid medium have been used: a classical culture medium for cells enriched with cystein and supplemented with 2-mercaptoethanol and batocuprein [183] and a medium composed of neopeptone and N-acetylglucosamine (pH4) [39]. This type of culture permits parasite development as high as in the co-culture systems but the initial studies have not yet been confirmed.

In conclusion, to date there is no continuous culture system for *P. carinii*. Such a system would provide valuable data on the biology of the parasite and would aid the development of new therapeutic strategies and allow isolation of different parasite strains.

4.2. Animal models of pneumocystosis

Because there is an absence of in vitro continuous *P. carinii* culture models, many advances in research on *P. carinii* occur in animal experimentation. Animal models are very interesting for the study of pneumocystosis, both in terms of epidemiology and comparative disease. *P. carinii* has been observed in the lungs of several mammals [83].

4.2.1. Rodent models

The first models described in the study of pneumocystosis were rodents (rats and mice). However, these animals are not very susceptible to *P. carinii* and the infection only develops after prolonged administration of corticosteroids (6–14 weeks) in association with a hypoprotein diet [22, 39, 172]. These animal models are therefore not perfect because immunosuppressant treatment makes it difficult to study the biology of the parasite and the host–parasite interaction. New rodent models have appeared which have developed pneumocystosis induced by nasal or tracheal inoculation without association of corticosteroids. Murine lines with congenital immune deficit (Severe Combined Immunodeficiency SCID mice) which do not produce T or B lymphocytes [4, 154, 197], athymic mice and rats (nude) which do not produce T lymphocytes [4, 172] and T CD4+ lymphocyte-depleted mice [127, 167] have been used to provide an approach to the biology of *P. carinii* [86]. It is unquestionably with the mouse that the greatest progress has been made in the understanding of the relationship between *P. carinii* and the host immune system. The mouse is, to date, the best immunologically understood model.

4.2.2. Ferret and pig models

The ferret develops *P. carinii* pneumocystosis when it is treated with corticosteroids. This animal model tolerates immunosuppressant treatment better and over a longer period, with daily long-term administration of cortisone-acetate at 10–20 mg/kg, for 9–10 weeks. However, corticosteroid-treated rats or mice have been used in most experimental studies of *P. carinii* pneumonia, but there are several disadvantages in using rodents. Rats typically lose 20–30 % of their baseline weight during steroid treatment. Nevertheless, the ferret, like man, is 'resistant' to body weight loss by corticosteroids [174].

P. carinii is also responsible for transient pneumocystosis in 40 % of piglets aged 4–10 weeks. Piglets are interesting for the study of pneumocystosis because they develop spontaneous pneumocystosis at this age without immunosuppressants and the pneumonia is comparable to the pneumonia in man [20, 165].

4.2.3. Rabbit model

The rabbit model for the study of pneumocystosis was first described by Sheldon in 1959 [166]. Although the data on the immune system are less advanced than in rodents (rats and mice), the rabbit appears to be an excellent model for the study of pneumocystosis.

The rabbit model presents at least two advantages. The first is that the rabbit is a very receptive host to *P. carinii*. When they are infected by the microorganism the weanlings develop pneumocystosis at around 28 days (on weaning) without immunosuppressants [1, 165, 171]. This more or less systematic pneumocystosis (80–100 % of the rabbits studied) results in a variable number of parasites, i.e. between 3 and 68 million cysts per animal on weaning. During the weeks following weaning, the rabbits recover spontaneously and in parallel produce specific antibodies [1, 47]. The second advantage is that the antigenic and genetic findings suggest that the rabbit *P. carinii* strains are closer to those of man than the strains of rodent origin [51, 72, 125, 170]. Primates might therefore be phylogenetically closer to lagomorphs than to rodents [73]. In fact, the comparison of sequences from 91 different proteins, each originating from four species (rabbits, rodents, primates and marsupials or birds) shows a greater proximity between rabbit and man than between man and rodents. Similarly, the work of Vuillaumier et al. [192] on the phylogenetic analysis of CFRT DNAc (cystic fibrosis transmembrane conductance regulator) confirms that this sequence in the rabbit is closer to that of man than to that of rodents. The unexpected divergence between rabbits and rodents is corroborated by analyses of the respiratory surface epithelium. These studies should, nevertheless, be complemented by the analysis of other nucleic and protein sequences. Ceré et al. [33] recently showed that it is possible to obtain *P. carinii*-free rabbits after treatment with the trimethoprim–sulfamethoxazole combination for about 6 months.

Using this *P. carinii*-free colony the same authors showed that primary experimental infection in non-immunosuppressed rabbits is expressed as pneumocystosis. A parasite peak appeared in these animals much earlier than in rodents (in 10–20 days), and they then observed a rapid decrease in the number of *P. carinii* [32]. This decrease in the level of infection corresponded to the appearance of anti-*P. carinii* antibodies [1]. Immunocompetent *P. carinii*-free rabbits placed in contact with rabbits inoculated with *P. carinii* were rapidly contaminated (in less than 4 days) and presented a parasite peak 15 days later, which was proportional to the number of parasites present in the inoculated source-rabbits [31]. In addition, preliminary trials have shown that immunocompetent *P. carinii*-free rabbits placed outdoors were rapidly contaminated, the parasites being isolated from the lungs 10–20 days after exposure (unpublished data).

5. CONCLUSION AND PERSPECTIVES

Nowadays in vitro models are only of limited interest because there is no continuous in vitro culture. However, limited parasite growth has been obtained within a few days in systems with or without feeder cells or parasitized lung. To assess the activity of anti-*Pneumocystis* molecules, different in vitro systems have been proposed to test the capacity of molecules specifically to inhibit growth or attachment of *P. carinii* [80]. Moreover, in vitro systems are used to provide great amounts of viable and puri-

fied parasites which may be conserved by freezing or used for biochemical, immunological, development and transmission studies [45]. Nevertheless, in vitro systems cannot be used for the isolation of *P. carinii* strains because they need a large number of parasites to permit the growth of the culture. The discovery of continuous in vitro systems should be of great interest for many reasons, especially for better knowledge of the *P. carinii* life cycle and the characteristics of the different forms, principally the infectious forms involved in the natural transmission of the parasite.

The in vivo models have contributed a great deal to the understanding of human PCP. Indeed, animal models remain of prime interest for many purposes, principally comparative medicine, pathogenesis, development of new therapies, epidemiology and immunology. Different species have been proposed as animal models. Although rodent models have been extensively studied, other models should be used more often.

In rodent models the infection develops only in immunosuppressed or in congenital immune-deficient animals. Rodent models have been used successfully to test drugs, and to evaluate immnune responses to infection [16]. HIV models have been proposed by implantation of human immune cells into SCID mice, these models should be interesting for PCP studies during HIV infection [21, 133]. Similarly, using knock-out mice will help us to study more precisely the immunological mechanisms against PCP. The principal advantage of the rat and mouse is that these two species are very cheap and easy to keep in an experimental unit. In addition, many cytokines or others biochemical products are available for the rat and mouse. The main problem in rodent models is that PCP is not observed in normal immunocompetent animals, so some experiments are perturbed (e.g. glucocorticoids modify pulmonary surfactant composition and it is difficult to know if surfactant modification is due to the drug or to *P. carinii* infection).

The rabbit model has been less extensively used. Antigenic and genomic data suggest that rabbit-derived *Pneumocystis* strains are more closely related to human *Pneumocystis* than those of mice or rats. In addition, the rabbit is also closer to the human species than rodents, and thus the genomic organization of rabbit MHC genes is more similar to that of humans than to that of rodents [88, 118]. Moreover, the rabbit is a very susceptible host to *P. carinii* infection, and does not need glucocorticoid treatment. We have therefore shown that the rabbit model permits study of the pulmonary surfactant modification due to *P. carinii* infection [6] and should be a very interesting model for pathogenesis or immune response studies in immunocompetent animals. Similarly the rabbit is the only non-immunocompromized animal that can be used for drug evaluation. Furthermore, drug testing on rabbits is quicker than on rodents because it can be achieved after an infection of less than 2 weeks. Rabbit could also be used for epidemiological studies. *P. carinii* transmission appears to be very rapid via contact of *Pneumocystis*-free rabbits with infected rabbits. Distant contamination is less efficient and does not account for the parasite burdens of the contact animals [31]. Non-immunosupressed *P. carinii*-free rabbits should be utilized as sentinels to recover *P. carinii* infective forms from the environment. These sentinels could help to characterize the source and the reservoir and to study parasite transmission. At the present time, transmission studies are realized by PCR to detect *P. carinii* DNA in air from the animal room housing infected rats or mice, and *P. carinii hominis* can be detected in samples of air collected in a rural location [195]. We showed that in utero transmission of *P. carinii oryctolagi* is a supplementary route of infection besides the airborne route [30]. As humans also have a hemochorial type of placenta, in utero transmission of *P. carinii hominis* might occur [132] even though there is no proof [85]. Rabbit should help to understand how

the in utero transmission occurs. However, the main limitation for rabbit models is that obtaining *P. carinii*-free rabbits is a long and expensive process which might limit the use of this attractive model as an alternative to rodent models.

Other animal species might be used as PCP models, e.g. pig, ferret or primates. Nevertheless, experiments with these species are difficult because there are very few experimental units for these species and the cost is very high in comparison with rodent and rabbit models.

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