XX. Animal models of pneumocystosis

Eduardo Dei-Cas a,b,*, Monique Brun-Pascaud c, Vivi Bille-Hansen d, Aurelien Allaert b, El Moukhtar Aliouat e

a Faculty of Medicine and Regional University Hospital Centre, 1 Place Verdun, 59045 Lille, France
b Dept. of Microbiology of Ecosystems, Pasteur Institute of Lille, 1 rue du Prof-Calmette BP245, 59019 Lille, France
c INSERM U13, Hopital Bichat, 170 Boulevard Ney, 75877 Paris Cedex 18, France
d Dept. of Pathology and Epidemiology, Danish Veterinary Laboratory, Bilsowsej 27, 1790 Copenhagen V, Denmark
e Glaxo-Wellcome SA, PTM, 2 Severo Ochoa, 28760 Tres Cantos (Madrid), Spain

Abstract

As in vitro culture systems allowing to isolate Pneumocystis samples from patients or other mammal hosts are still not available, animal models have critical importance in Pneumocystis research. The parasite was reported in numerous mammals but P. carinii pneumonia (PCP) experimental models were essentially developed by using rats, mice, rabbits and ferrets. The rat treated with corticosteroids for 9–12 weeks is a useful PCP model. Like laboratory rats, conventional mice develop PCP after prolonged corticosteroid administration. The ferret (Mustela putorius furo) also develop PCP under corticosteroid regime. Whilst bronchoalveolar lavage (BAL) is really difficult to perform on live laboratory rodents, serial BAL sampling can be performed on live ferrets. Rabbits currently develop spontaneous PCP at weaning without corticosteroid administration. For this reason this model has been used for studying the host immune response as well as Pneumocystis-surfactant interactions. Pigs and horses also develop spontaneous PCP. Treated with corticosteroids, piglets develop extensive PCP and could be used as a non-rodent model. Pneumocystis was detected in many non-human primates. Primates could represent a source of parasites taxonomically related to P. carinii sp. f. hominis. Moreover, primates might be used as experimental hosts to human Pneumocystis. A marked variability of parasite levels among corticosteroid-treated animals and the fact that the origin of the parasite strain remains unknown, are important drawbacks of the corticosteroid-treated models. For these reasons, inoculated animal models of PCP were developed. The intratracheal inoculation of lung homogenates containing viable parasites in corticosteroid-treated non-latently infected rats resulted in extensive, reproducible Pneumocystis infections. Extensive PCP can be obtained within 5–7 weeks, whilst 9–12 weeks are needed in the classical model. The severe combined immunodeficiency (SCID) mouse inoculated by nasal route and the athymic nude rats intratracheally inoculated were used to test the infectivity of Pneumocystis samples coming from cultures or from different hosts. They were also used to test the anti-Pneumocystis activity of antimicrobial molecules. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Pneumocystis; In vivo model; Nasal inoculation; Intratracheal inoculation; Nude rat; Severe combined immunodeficiency mouse

* Corresponding author. Tel: +33 (320) 87 71 55; Fax: +33 (320) 87 79 08; E-mail: eduardo.dei-cas@pasteur-lille.fr

0928-8244/98/$19.00 © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.
PII: S0928-8244(98)00074-1
1. Introduction

As continuous cultures or in vitro systems allowing the isolation and multiplication of Pneumocystis samples from patients or other mammal hosts are still not available, animal models have critical importance in Pneumocystis research. Moreover, human parasites are difficult to obtain. For these reasons, Pneumocystis research is largely depending on Pneumocystis experimental hosts. Also, the lack of efficient culture systems has led many groups to develop mainly molecular biology research strategies, especially for working on human Pneumocystis isolates. However, even for displaying molecular approaches, reliable parasite animal sources are often necessary; for instance, great quantities of pure parasites are needed to develop pulsed field gel electrophoresis (PFGE) studies. Pneumocystis was reported in numerous domestic or wild mammals [1,2] but P. carinii pneumonia (PCP) experimental models were essentially developed by using rats, mice, rabbits and ferrets. A marked host species-related genetic diversity was usually found among Pneumocystis isolates derived from both natural and experimental hosts [3].

2. Corticosteroid-treated animal models

The conventional laboratory rat (Rattus norvegicus) submitted to subcutaneous or oral administration of corticosteroids for 9–12 weeks, was a very useful animal model of PCP [1,4,5]. Standard regimens consist of cortisone acetate (125 mg kg$^{-1}$) subcutaneously injected twice weekly, or dexamethasone in the drinking water (2 mg l$^{-1}$). Even today, the corticosteroid-treated rat remains the most used experimental host of Pneumocystis. It is usually considered both as the best source of parasites and as an interesting model to approach parasite transmission, PCP pathophysiology, host-parasite relationships, Pneumocystis genetic polymorphism [6] as well as to evaluate the effectiveness of therapeutic and prophylactic protocols. The use of the rat model was hampered by improvements of animal barrier facilities done in order to reduce bacterial, fungal or viral infections. The resulting ‘virus antibody-free’ rats were also found to be free of P. carinii infection [7]. However, the use of animal models protected from other infectious agents reduces the variability of P. carinii infection levels, sometimes observed in corticosteroid-treated rats. In fact, P. carinii infected rat colonies, but also virus free rats, remain of large interest in Pneumocystis research and their continuous production should be guaranteed.

As laboratory rats, conventional laboratory mice (Mus musculus) develop PCP after prolonged corticosteroid administration [8]. Subcutaneous injections of cortisone (50–100 mg kg$^{-1}$, twice weekly) can be used but prednisolone in drinking water (40 mg l$^{-1}$) revealed to be an efficient and practical solution [9]. Mice have been used both as parasite source and as experimental hosts for studies on Pneumocystis transmission, genetic polymorphism or host-parasite relationships. Interesting contributions to the knowledge of the host response have been made by using an elegant mouse model depleted of CD4+ T-cells by a monoclonal antibody and inoculated intratracheally with P. carinii [10]. Pneumocystis was detected in other small rodents [1] but interestingly, the hamster (Cricetus auratus) appears to be resistant to corticosteroid-induced PCP [11,12].

The ferret (Mustela putorius furo) also develops PCP under administration of corticosteroids [13]. This small carnivore is of interest for its resistance to body weight loss due to corticosteroids, for the large amount of lung (10 g vs. 1 g in rat) and for its immunological response relatively similar to that of man [13]. Moreover, whilst bronchoalveolar lavage (BAL) is really difficult to perform on live laboratory rodents, serial BAL sampling can be performed on live ferrets, allowing to follow the course of the infection. PCP is induced in ferrets by cortisone acetate (10–20 mg kg$^{-1}$ subcutaneously injected five days a week for 6–10 weeks) or by dexamethasone (2–5 mg l$^{-1}$ in the drinking water) [14]. Corticosteroid-treated ferrets were used as parasite source for studying Pneumocystis antigenic variation, especially of its major surface glycoprotein (MSG), genomic polymorphism and cross infection among different host species.

In the corticosteroid-treated models, chlorotetracycline or tetracycline (0.5–1 g l$^{-1}$) is often added in the drinking water in order to prevent bacterial superinfections which often occur. In these models, immunosuppression can be enhanced by addition of a low protein (8%) diet [1].
3. The rabbit at weaning: a corticosteroid-free model of pneumocystosis

Extensive corticosteroid-induced PCP was reported in rabbits (Oryctolagus cuniculi) as early as the 1950s by Sheldon [15]. However, interestingly, in 1989 it was reported that without corticosteroid administration, rabbits currently develop a spontaneous PCP at weaning (about 1 month after birth) [16]. This spontaneous, natural Pneumocystis infection which has been constantly observed in weanling rabbits of several strains [17], provokes lung histopathological changes typical of PCP, often associated with blood biochemical abnormalities [16]. The infection evolves during 7–10 days; afterward, parasite levels decrease gradually, becoming very low in 60-day old rabbits. Almost all animals recover within 3–4 weeks. The striking regularity of the pattern of this natural infection (abrupt onset at the weaning time, extensive diffuse pulmonary involvement evolving relatively shortly to complete, spontaneous healing) has allowed the development of kinetic studies of the host immune response against PCP [18,19]. This work is facilitated by the fact that PCP develops in this model without administration of corticosteroids, as these drugs affect the immunological mechanism. Corticosteroids also influence the production and composition of pulmonary surfactant. For this reason, the corticosteroid-free rabbit model was recently used to investigate Pneumocystis-surfactant interactions [20,21].

4. Pneumocystis infection in pigs and horses

Pigs also develop spontaneous PCP. Only a few reports have been published and only in a few sporadic individuals [1]. However, recent studies from Denmark show Pneumocystis to be the causative agent of lung disease in piglets aged 4–12 weeks from several different pig herds [22]. The condition includes symptoms as growth retardation, dyspnea and dry cough. In some of the herds concurrent non-specific diarrhea is seen. Treatment with various antibiotics has no effect. Postmortem findings include enlarged lungs with focal/diffuse consolidations, characterized by an interstitial pneumonia with alveolar foamy acidophilic, honey comb mate-

rial. Cysts are seen by Grocott’s methenamine silver stain. The lung disease occurs in a period with many stress factors, such as early weaning, overcrowding and change of food. The nature is transitory; it comes and goes spontaneously, indicating basic management problems in the herd.

In the search for a non-rodent animal model of PCP, Nielsen et al. [23] have treated piglets with corticosteroids to reactivate a putative infection. Piglets aged 3.5 weeks were housed in the same pen and divided into two groups. During 6 weeks the animals were injected intramuscularly with high doses of methylprednisolone acetate once a week, the controls with the same volume of a saline solution. During the test period all piglets had a good appetite. Gradually the corticosteroid-treated pigs showed lower gain in body weight and dyspnea. Compared to the controls they had lymphopenia, neutrophilia and the anti-Pneumocystis antibody titers decreased or remained at zero. Postmortem examination revealed an extreme reduction of thymus and a mild pneumonitis dominated by intra-alveolar ‘honey comb’ material. Reduced body weight, thymus involution, lymphopenia, no titers of Pneumocystis specific antibodies and the histopathology of the PCP were consistent findings of the piglets under corticosteroid treatment, and comparable to the parameters of the classic rat model. Piglets could be reservoir and infection source in the individual pig herds. It is likely that stress, malnutrition or other diseases predispose to the development of PCP in these mammals. Experimentally the lung disease may be provoked by prolonged administration of corticosteroids.

Pneumocystis was reported in foals, where a horse-derived P. carinii specific DNA sequence was found different from that of rat-, rabbit-, ferret- and human-derived Pneumocystis [24].

5. Pneumocystis infection in non-human primates

Pneumocystis was detected in marmoset (Callithricidae), owl monkeys (Aotus trivirgatus), chimpanzees (Pan troglodytes) and macaques (Macaca fascicularis) (see [7] for review). More recently, two detailed reports of Pneumocystis infection in simian immunodeficiency virus (SIV)-infected rhesus mon-
keys (*M. mulatta*) were published. In the first report, histopathological and ultrastructural observations on the parasite in these hosts were shown [25]. In the second paper, the sequence of the fragment of the *Pneumocystis* lsumtRNAr gene, mostly used for detecting the parasite by PCR, is presented [26,27]. In this last report, the amplified sequence was found to be significantly different from the rat-derived *Pneumocystis* product. Moreover, the amplified fragment from simian-derived *Pneumocystis* DNA, was shorter (303 bp) than that from rat-derived *Pneumocystis* (346 bp). In contrast, the simian-derived *Pneumocystis* amplified sequence showed only two nucleotide substitutions when compared with the homologous sequence of human-derived *Pneumocystis* reported by Sinclair et al. [28].

Current drugs or definition of therapeutic and chemoprophylactic protocols used for treating PCP patients were mostly tested by using *Pneumocystis* strains of rodent origin. However, we know now that *Pneumocystis* strains parasitizing humans, i.e. *P. carinii* sp. f. *hominis*, are genotypically and probably phenotypically different from animal-derived parasite strains. As far as culture systems of human *Pneumocystis* isolates are unavailable, primates could represent a source of parasites taxonomically more related to *P. carinii* sp. f. *hominis* than rodent-derived *Pneumocystis* strains. Likewise, it should be investigated if human *Pneumocystis* strains develop or not in primates used as potential experimental hosts. Thus, the study of the *Pneumocystis* infection in non-human primates presents a growing interest.

6. Inoculated animal models of pneumocystosis

Conventional laboratory rodents submitted to corticosteroid administration are the PCP animal models used most. However, they have important drawbacks. Thus, a marked variability of parasite levels among animals submitted to the same immunosuppression regime is often found. Moreover, as animals are not inoculated by the investigator, the origin or the identification of the parasite strain responsible for the resulting corticosteroid-induced PCP remains unknown. Actually, in classic corticosteroid-treated non-inoculated rodent models, PCP could develop
from either latent infection or unknown exogenous sources [29]. For these reasons, but also to avoid problems derived from the improvements of animal barrier facilities, inoculated animal models of pneumocystosis were developed over the last years.

The intratracheal inoculation of lung homogenates containing viable parasites in corticosteroid-treated non-latently infected Sprague-Dawley or Lewis rats resulted in extensive, more reproducible Pneumocystis infections than in the classical non-inoculated rat model [30]. Extensive PCP can be obtained within 5–7 weeks, whilst 9–12 weeks are needed in the classical model. Moreover, the inoculated rat model allows use of virus-free rats as required by many animal facilities [30].

Two other inoculated models of PCP were developed: the severe combined immunodeficiency (SCID) mouse inoculated by nasal route [31] and the athymic nude rats inoculated by intratracheal route [32]. These models were used to test the infectivity of Pneumocystis samples coming from cultures or from different hosts (cross infection experiments). Also, they were used to test the anti-Pneumocystis activity of antimicrobial molecules [33,34]. These interesting, highly reproducible inoculation models will be described briefly.

Pneumocystis-free 8-week old female SCID mice are anesthetized with a drug cocktail (ketamine hydrochloride 2 mg + diazepam 0.03 mg + atropine 0.01 mg for each mouse) given intraperitoneally and then inoculated intranasally with the parasite inoculum (about 2–3×10⁶ mouse Pneumocystis organisms per animal in 0.03 ml culture medium). To establish if nasally inoculated parasites reached the lungs, experiments were carried out where mice were nasally instilled with trypan blue-stained inocula and sacrificed 5–10 min post-inoculation. In these experiments, it has been established that the inoculum reaches the host lung effectively, only when recipient mice are anesthetized (Fig. 1). Extensive PCP is obtained 5 weeks post-inoculation. Higher parasite rates may be obtained by adding dexamethasone (4 mg l⁻¹) in the drinking water 1 week prior to parasite inoculation and throughout the experiment [35].

Non-latently Pneumocystis infected 5-week old corticosteroid-treated female nu/nu rats were used to set the nude rat model of PCP. The animals are given dexamethasone in their drinking water (1 mg l⁻¹) 2 weeks prior to parasite inoculation and throughout the experiment [35]. They are anesthetized with a drug cocktail (ketamine hydrochloride 8 mg + diazepam 0.12 mg + atropine 0.04 mg for each rat) given intraperitoneally and inoculated by the intratracheal route with the parasites. The trachea is exposed surgically and a 0.1-ml inoculum containing 5–10×10⁵ rat Pneumocystis organisms is injected into the trachea with 0.3 ml of air behind it. The incision is closed with non-resorbable sutures.

In dexamethasone-treated nude rats, a rapid increase of the parasite rate is observed between 1 and 3 or 4 weeks post-inoculation. Kinetic curves of the Pneumocystis infection developed by inoculated SCID mice or nude rats were given elsewhere [35]. All operations with these congenitally immunosuppressed experimental hosts must be done under sterile conditions. Currently, SCID mice or nude rats are sacrificed from day 40 post-inoculation in order to obtain uniform, extensive PCP. Their lungs are removed and parasites are counted on lung homogenate smears. They may be directly used for specific experiments, cryopreserved or inoculated to other susceptible hosts or to cultures [36].

References


