GV-SOLAS
Gesellschaft für Versuchstierkunde
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Expert Information

From the Working Group on Hygiene

Implication of infectious agents on results of animal experiments

Pneumocystis spp.

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Contents

Pneumocystis spp.

Background

- First discovered by Carlos Chagas in 1909 and misidentified as a new schizogonic state of *Trypanosma cruzi* in the lungs of guinea pig.[1]
- In 1910, Antonio Carini identified similar cysts in the lungs of *Rattus norvegicus* infected by *Trypanosoma lewisi*.[2]
- In 1912, pulmonary cysts were named *Pneumocystis* (*P.*) *carinii* and described as a new biological entity by Delanoë.[3]
- *P. carinii* is the type species of the genus.[4]
- Since 1988, phylogenetic analyses have indicated that *Pneumocystis* belongs in the group of Fungi; the *Pneumocystis* genus was placed in the fungal phylum *Ascomyceta*.[5,6]
- Today, there are 12 *Pneumocystis* taxa, including 5 accepted *Pneumocystis* species: *P. carinii* (*Rattus norvegicus*), *P. jirovecii* (humans), *P. murina* (*Mus musculus*), *P. oryctolagi* (*Oryctolagus cuniculus*), *P. wakefieldiae* (*Rattus norvegicus*).[5,6]

Prevalence

- A high prevalence of *Pneumocystis* infection in a wide range of mammals can be assumed, but in natural ecosystems *Pneumocystis* organisms rather parasitize in the lung than causing interstitial pneumonia.[6]
- In prevalence studies, *Pneumocystis* spp. was detected by PCR in immunodeficient rats and mice in about 1% of the samples.[7]
- *P. oryctolagi* is highly prevalent in rabbit colonies.[8-10]
- Prevalence of *Pneumocystis* organisms of 36,9% in 564 slaughtered pigs in Brazil.[11]

Host species

- *Pneumocystis* spp. could be identified from a wide range of domestic, synanthropic and wild animals including laboratory animals and primates based on DNA sequences.[6,12]

Properties

- *Pneumocystis* spp. are ascomycetous opportunistic pathogens with the ability to sexually reproduce. They show life-cycle stages in the lungs of infected hosts that involves an ameboid, mononuclear trophic (vegetative) form, three consecutive sporocytic stages and a cystic stage (ascus).[5,6]
- *Pneumocystis* spp. show morphological, phenotypic, and immunological differences.[5,6,13]
- *Pneumocystis* spp. show a strong host specificity that precludes *Pneumocystis* inter-species transmission.[14]
- Detection of *Pneumocystis* DNA from the air at conventional animal facilities, where *Pneumocystis*-infected laboratory animals are housed, and detection of mature cysts in the bronchial lumen[15] indicates that *Pneumocystis* could be transmitted by the airborne route.[16-19]

- Cystic forms, but not the trophic forms can be transmitted by aerial route.[20,21]
- Respiratory or airborne transmission of *Pneumocystis* organisms between mammals of the same species, even between immunocompetent hosts and from mother to offsprings.[5,6,14,22,23]
- Transmission of *Pneumocystis* organisms from animals with *Pneumocystis* pneumonia to immunocompromised and immunocompetent recipients.[22,24-27]
- Transplacental route of transmission of *Pneumocystis* organisms in humans[28] and rabbits[29,30] described, but not in immunosuppressed mice and rats.[15]
- No evidence of *Pneumocystis* spp. environmental growth.[5]

Susceptibility

- High risk for all congenitally immunodeficient hosts and for experimental models of immunosuppression.
- Common in immunodeficient strains and orthologous genetic knockout mice as well as to lesser degree in aging immunocompetent mice.[31,32]
- Infections occur in the context of deficits in cellular immunity like decrease in the number of CD4+ T cells33-35, while mice depleted of CD8+ T cells are not susceptible to infection.[36]

Organotropism

- Co-phylogeny is the evolutionary pattern for *Pneumocystis* spp.; *Pneumocystis* exhibit an adaption to colonize the lungs of immunocompromised hosts.[6]
- Replication of *Pneumocystis* spp. also in the lungs of immunocompetent hosts.[5]

Clinical disease

- Subclinical Pneumocystis infection in immunocompetent hosts.[27]
- Induced immunosuppression of subclinically infected mice by the administration of cortisosteroids results in development of *Pneumocystis* pneumonia.[37]
- *Pneumocystis* pneumonia is common in a variety of immunodeficient strains of mice and rats and includes dyspnoea, cyanosis, wasting, weight loss, hunched posture, dry and scaly skin.[31,38]
- Significant clinical disease: pneumonia with obstruction of alveoli with organisms and subsequent respiratory failure in any species with acquired or congenital immune deficits.[39,40]
- Reduce the lifespan of immunodeficient mice.[41-44]
- Older immunodeficient mice are more severely affected than younger mice.[42]
- Spontaneous and usually benign pneumocystosis in rabbits at weaning[6,10] and in young piglets.[11]
- Dyspnoea and growth retardation in pigs.[45]
- *Pneumocystis* pneumonia is rarely reported in wild mammals.[6]

Pathology

- In mice and rats, lungs collapse poorly, and they have a rubbery consistency with pale, patchy areas of consolidation.[31,38]
- *P. carinii* infection causes interstitial pneumonia in young immunocompetent laboratory rats.[46,47]

- Slight infection: multifocal alveolar aggregates of cysts and interstitial/perivascular non-purulent infiltration.[51,52]
- Severe infection: consolidated lungs; extensive lung areas involved with alveolar aggregates of cysts (foamy eosinophilic, honeycombed material), proliferation of type II pneumocytes and severe interstitial fibrosis.[31,38]
- Severe *Pneumocystis* pneumonia is characterized by an intense neutrophilic inflammatory response resulting in gas exchange abnormalities, diffuse alveolar damage, and respiratory failure.[53]
- Interstitial pneumonia with proteinaceous exudation into the alveolar lumina, marked thickening of alveolar septa and infiltration with mononuclear leukocytes.[31,38]
- Vacuolated, eosinophilic material containing punctate cysts and alveolar macrophages are scattered in affected alveoli.[31,38]
- *Pneumocystis* pneumonia typical honeycomb intra-alveolar material is observed rarely in *P. oryctolagi* infected lungs.[10]
- Extrapulmonary infection of heart and spleen is reported in SCID mice.[54]
- Extrapulmonary detection of *Pneumocystis* spp. in pigs' bronchial lymph nodes, liver, spleen, kidney, and mesenteric lymph nodes.[45]

Morbidity and mortality

- Immunocompetent, healthy mammalian hosts act as *Pneumocystis* reservoir with low parasite burden.[5]
- Latent *Pneumocystis* parasitism seems to be less frequent in mice than in rats or rabbits.[6]
- Conventionally bred colonies may be persistently infected because of subclinical nature in immunocompetent hosts.[55]
- Once *Pneumocystis* infection is established in an immunodeficient mouse colony, the *Pneumocystis* organisms tend to persist for long periods of time.[42]
- Neonatal or young rodents, pigs and rabbits can harbour substantial numbers of *Pneumocystis* organisms.[29]
- High morbidity and mortality with chronic progressive pneumonia in immunosuppressed animals.

Zoonotic potential

- No zoonotic pattern of *Pneumocystis* spp. due to strong host specificity of *Pneumocystis* spp.[5] and revealed by cross-infection experiments.[56-58]

Interference with research

Oncology

- No data

Teratology

- No data

Infectiology

- Polymorphonuclear leukocytes in bacterial pneumonia may participate in host effector mechanisms against *P. carinii*.[59]

Immunology

- Control of lung infection and air shedding of *Pneumocystis* by immune response in immunocompetent mice and rats.[23,25]
- A mild infection resolves in 5 to 6 weeks in immunocompetent mice when an immune response is present. *Pneumocystis* organisms are rapidly eliminated without clinical signs of disease.[25]
- The adaptive host response to *Pneumocystis* infection involves humoral and cellular immune responses, alveolar macrophages, dendritic cells, neutrophils and cytokines to clear the infection, whereby CD4+ T lymphocytes tasks the central role in controlling the infection.[53,60,61]
- A deep inhibition of immune response mechanisms seems to be required for pneumocystosis in young, immune-naïve animals.[6]
- *P. carinii* induces activating and inhibitory innate cellular immune response mechanisms.[62]
- *Pneumocystis* spp. induces Th1, Th2, Th17 or T regulatory reactions.[32]
- Cellular immunity is important for protection of *P. carinii* infection in rat and mice.[49,63]
- CD4+ T lymphocytes are crucial to host defense against *P. carinii*[53] and protective immunity in the immunocompetent host.[64]
- Infected mice and rats are resistant to reinfection for 6 weeks and 3 months, respectively.*23,26*
- *P. carinii*-reactive CD4+ lymphocytes may contribute to the host's response in the mouse model by secretion of macrophage-activating cytokines (IFN-g and others) as well as by the production of signals that induce foster expansion of the antibody-forming pool of B cells and cytotoxic CD8+ lymphocytes.[65]
- Neutrophils, alveolar type II epithelial cells, B cells, CD8+ lymphocytes, antibodies and cytokines, such as IFN-g and TNF, participate in host effector mechanisms against *P. carinii*.[36,66-75]
- *P. carinii* induces TNF-a production from monocyte and macrophage cultures with a peak within 8 h of incubation.[76]
- *P. carinii* glycoprotein A stimulates IL-8 production and inflammatory cell activation in alveolar macrophages and cultured monocytes.[77]
- *P. carinii* induces expression of ICAM-1 and IL-6 in lung epithelial cells.[78,79]
- Formation of multinucleated giant cells in *P. carinii* pneumonia in Aß-/-, RAG1-/- and TCRß-/- mutant mice.[80]
- CD8+ T cells recruited to the lungs in response of infection have been associated with lung injury in murine models,[81,82] while other studies suggest a protective role of CD8+ T cells, particularly in the context of immunosuppression.[36,74]
- Both, CD4+ and CD8+ T cells accumulate in the lung and control the infection in immunocompetent mice.[83]
- CD8+ T cells in the lungs of immunodeficient mice fail to control the infection.[48,84]
- Passive transfer of a *Pneumocystis*-specific monoclonal antibody promotes clearance of *Pneumocystis* in corticosteroid-immunosuppressed rats and ferrets.[85]

- An increase in anti-*P. carinii* IgM and IgG antibody titers precedes clearance of *P. carinii* from the lungs of immunocompetent rats.[23]
- *P. carinii* f. sp. *muris*-specific immunoglobulin G as well as CD62low CD4- and CD8-positive T-cells cause the clearance of *P. carinii* f. sp. *muris* organisms from the lung in immunocompetent mice.[25]

Interactions with other infectious agents

- Superimposed viral infection can exacerbate *Pneumocystis* pneumonia in mice.[31]
- Dual infection with *Pasteurella (P.) pneumotropica* may result in suppurative bronchopneumonia and abscesses in immunodeficient mice.[75,86]
- SCID mice subclinically infected with *P. murina* develop severe respiratory tract lesions after inoculation with PVM and vice versa.[87]

Toxicology

- No data

Physiology

- *P. carinii* pneumonia leads to alterations in compliance and lung mechanisms.[88,89]
- During *Pneumocystis* pneumonia, alterations of quantity and quality of pulmonary surfactant have been reported.[90]
- *P. carinii* may alter the amount and type of surfactant produced: *P. carinii* pneumonia in rats leads to a decrease in surfactant phospholipids in bronchoalveolar lavage and to a lowering of the phospholipid/protein ratio.[91,92]
- Surfactant protein-A (SP-A) and SP-D levels increase during *P. carinii* pneumonia in the rat.[93,94]
- SP-A and SP-D can function as ligands between *P. carinii* and alveolar macrophages.[94,95]
- *P. carinii* organisms can directly inhibit secretion of surfactant phosphatidylcholine from alveolar type II cells.[96]
- Phosphatidylglycerol from the bronchoalveolar lavage of *P. carinii* pneumonia in rats is reduced and may account for the impairment of gas exchange observed in *P. carinii* pneumonia.[97]
- Attachment of *P. carinii* to alveolar macrophages occurs by a fibronectin- and calcium-dependent mechanism but does not trigger a phagocytic response.[98]
- *P. carinii* attachment increases expression of fibronectin-binding integrins on cultured lung cells.[99]
- *P. carinii* glycoprotein A (gpA) binds to macrophage mannose receptors, thereby mediating binding and uptake of *P. carinii* by alveolar macrophages.[100,101]
- Attachment of *P. carinii* to type I pneumocytes leads to their degeneration and to proliferation of type II pneumocytes. Following attachment of *P. carinii* to type I cells, surface glycocalyx is decreased and alveolar-capillary permeability is increased. As a consequence of dysplasia and disruption of the epithelium, underlying material gains access to the alveolar space and impairs normal lung function.[90,102-105]
- Trophic forms of *Pneumocystis* spp. attach to type-I epithelial alveolar cells, emit filopodia, develop progressively and may fill pulmonary alveolar cavities that leads to respiratory failure.[6,15]

- Rat-derived *Pneumocystis* seems to have a higher capacity for attaching in vitro to target cells than mouse-derived *Pneumocystis*.[106]
- *P. carinii* and IFN-gamma induce rat alveolar macrophages to produce nitric oxide.[107]
- Fibrinogen expression is induced in the lung epithelium during *P. carinii* pneumonia.[108]

Cell biology

- The host mitochondrial ATPase 6 gene is upregulated in *P. carinii*-infected rat lungs.[109]
- *P. carinii* infection alters GTP-binding regulatory proteins in the membranes of the lung parenchyma.[110]
- *P. carinii* inhibits the host epithelial cyclin-dependent kinase activity in lung epithelial cells.[111]

Assisted reproductive technology

- No data

Special considerations

- *Pneumocystis* spp. are unable to grow in vitro in fungal culture media and are not susceptible to most antifungal drugs.[6]
- *Pneumocystis* organisms possess chitin and beta-glucans in their cell wall and only one rRNA gene.[6]
- Stereoid-treated rats are the most frequently used *P. carinii* pneumonia models and most available data on *Pneumocystis* spp. are obtained from *P. carinii*.[6]
- *Pneumocystis* rabbit model has been used for studying *Pneumocystis*-surfactant interactions.[6]

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