INFECTION AND IMMUNITY, Apr. 1993, p. 1586–1588
0019-9567/93/041586-03$02.00/0

Vol. 61, No. 4

Exacerbation of *Pneumocystis carinii* Pneumonia in Immunodeficient (*scid*) Mice by Concurrent Infection with a Pneumovirus

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Received 12 November 1992/Accepted 25 January 1993

***scid* mice naturally infected with *Pneumocystis carinii* and inoculated with a normally apathogenic pneumovirus had significantly higher *P. carinii* cyst counts and developed significantly more severe *P. carinii*-related disease than did sham-inoculated, *P. carinii*-infected *scid* mice. *P. carinii*-free, virus-infected *scid* mice survived for 2 months despite high pulmonary virus titers. These results show that a respiratory virus infection can exacerbate *P. carinii* disease in an immunocompromised-rodent model.**

Pneumocystis carinii is clinically important in immunosuppressed individuals and is the most frequent opportunist and leading cause of death among AIDS patients (9). *P. carinii* pneumonia also occurs in immunodeficient animals, including athymic rodents and rodents with severe combined immunodeficiency (5, 8, 10), causing high morbidity and eventual mortality. An exacerbating or contributing viral effect on the expression of *P. carinii* pneumonia is suspected in humans and animals but has not been extensively studied. Some evidence suggests that the corticosteroid-treated-rat model of *P. carinii* infection relies on concurrent or prior infection with naturally occurring rodent viruses (1). We have recently shown that pneumonia virus of mice (PVM), a normally apathogenic pneumovirus distantly related to respiratory syncytial virus (2), exacerbates pneumonia in *P. carinii*-infected C3H/HeSnJ *scid* mice (6). However, those studies did not define the relative contribution of each agent to the pathogenesis of the resulting pneumonia. The current studies were undertaken to confirm and extend the prior finding and to study the pathogenesis of the disease.

Seventy-two C3H/HeSnJ (C3H) *scid* mice, 26 C3H congenic +/+ mice, 42 BALB/cByJ (BALB) *scid* mice and 36 BALB congenic +/+ mice from The Jackson Laboratory (Bar Harbor, Maine), and 10 *P. carinii*-free C.B-17/IcrT acfDF (C.B-17) *scid* mice (histocompatible with BALB mice) and 4 *P. carinii*-free C.B-17 congenic +/+ mice from Taconic Laboratory Animals and Services (Germantown, N.Y.) were used at 2 to 3 months of age. They were housed in sterile Micro-Barrier (Allentown Caging, Allentown, N.J.) or Micro-Isolator (Lab Products, Maywood, N.J.) cages that were manipulated within a class II biological safety cabinet.

Mice were inoculated intranasally with 1,000 fluorescent-focus units (FFU) of PVM (12), kept alive for the intervals detailed below, killed with CO_2 gas, and exsanguinated. The accessory lung lobe was aseptically removed and frozen at −70°C. The remaining lobes were inflated in situ with 10% buffered formalin and immersed in formalin overnight. Three transverse 2- to 3-mm sections were taken from the center of the right anterior, middle, and posterior lung lobes and from corresponding areas of the left lung. Lungs were paraffin embedded, and 5-μm sections were mounted on 3-aminopropyltriethoxysilane-coated glass slides. Six sections per mouse were stained with each of the following: hematoxylin and eosin, avidin-biotin complex immunoperoxidase (12) to visualize PVM antigen, and Grocott's silver stain (7) to visualize *P. carinii* cysts. All slides were coded and evaluated blindly. Lesion severity scores were assigned by the method of Walzer et al. (11), viral antigen was quantified by the method of Reid (4), and *P. carinii* cyst counts were performed by the method of Roths et al. (5). Infectious PVM in accessory lung lobes was detected and quantified as previously described (12), and virus titers, expressed as FFU per gram, were calculated by the method of Lorenz and Bogel (3). Parametric data were analyzed by Student's two-tailed *t* test, and nonparametric data were analyzed by the Mann-Whitney U test.

Ruffled fur, dehydration, dyspnea, and death were observed only in PVM-inoculated (*P. carinii*-infected) C3H *scid* and BALB *scid* mice beginning on day 27. Lungs from C3H *scid* mice necropsied on days 10, 15, 20, 25, and 28 after virus inoculation and BALB *scid* mice necropsied on days 10, 20, and 28 showed similar trends, with progressively more severe lesions and more severe disease in virus-infected than in sham-inoculated *scid* mice (Fig. 1A and C). Microscopic evaluation of lungs from both sham- and virus-inoculated *scid* mice revealed alveolar septae that were thickened by infiltrating macrophages, alveoli and alveolar septae that contained sparse polymorphonuclear leukocytes, and moderate peribronchiolar accumulations of mononuclear leukocytes. Intra-alveolar macrophages with vesiculated or foamy cytoplasm and extracellular eosinophilic material, typical of *P. carinii* infection, were also seen. Virus-infected *scid* mice also had prominent perivascular and peribronchiolar infiltration of mononuclear leukocytes, and alveolar lumina contained exfoliated pneumocytes, macrophages, lymphoid cells, polymorphonuclear leukocytes, and occasionally erythrocytes. By day 28, some PVM-inoculated *scid* mice had consolidation that involved more than 50% of the lung mass.

In both PVM-inoculated and sham-inoculated *scid* mice,

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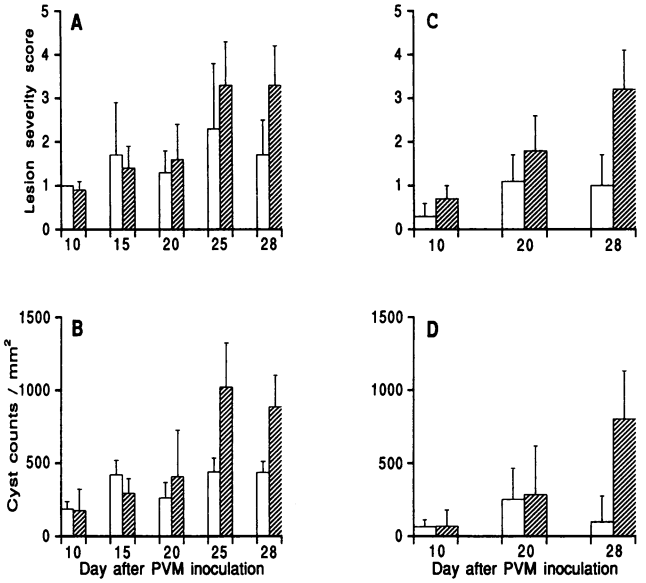

FIG. 1. Mean microscopic-lesion severity scores (A and C) and *P. carinii* cyst counts (B and D) for sham-inoculated (open bars) and PVM-inoculated (hatched bars) C3H/HeSnJ *scid* (A and B) and BALB/cByJ *scid* (C and D) mice. Each bar represents the mean for six sections from 3 to 11 mice per interval (± the standard deviation).

cyst densities increased with time (Fig. 1B and D), with significant differences on days 25 and 28 between virus- and sham-inoculated C3H *scid* mice and on day 28 between between virus- and sham-inoculated BALB *scid* mice ($P < 0.05$). Cyst counts for virus-infected and sham-inoculated C3H +/+ and BALB +/+ mice varied from 0.2 to 23.8 cysts per mm^2 over the 28-day study period with no trend over time.

Viral antigen was not seen in the lungs of any sham-inoculated *scid* mice (Table 1) or PVM-infected or sham-inoculated +/+ mice at any interval from 10 to 28 days. Among virus-infected *scid* mice, antigen was in the cytoplasm of type I and II alveolar epithelial cells, in macrophages, and in exfoliated cells in the alveolar spaces. The amount of viral antigen increased over time (Table 1) and was restricted primarily to macrophages late in the infection. Lungs of virus-inoculated C3H *scid* mice, double stained by PVM immunohistochemistry and by Grocott's silver stain, had no evidence of colocalization of viral antigen and *P. carinii* cysts (Fig. 2).

Virus titers in the lungs of PVM-inoculated C3H *scid* mice changed little over time, varying from 3.0 ± 0.4 $\log_{10}$ FFU/g on day 10 to 3.6 ± 0.3 $\log_{10}$ FFU/g on day 28. Virus concentrations in the lungs of PVM-inoculated BALB *scid* mice were 2.8 ± 0.0, 4.0 ± 0.9, and 4.3 ± 0.4 $\log_{10}$ FFU/g on days 10, 20, and 28, respectively.

In contrast to *scid* mice that were *P. carinii* infected at the time of virus inoculation, *P. carinii*-free C.B-17 *scid* mice developed only subtle clinical signs (slightly ruffled fur) at 58 days after virus infection. Microscopic lesions were widespread in lungs of virus-infected C.B-17 *scid* mice at this interval but were mild relative to those seen in lungs of *P. carinii*-infected C3H *scid* and BALB *scid* mice. The lesion severity scores were 0.0 ± 0.0 for sham-inoculated C.B-17 *scid* mice ($n = 5$) and 2.8 ± 0.5 for virus-infected C.B-17 *scid* mice ($n = 5$; $P < 0.01$). Lungs of PVM-inoculated, *P. carinii*-free C.B-17 *scid* mice contained twice as much viral antigen at day 58 as did lungs of virus-infected C3H *scid* or BALB *scid* mice at day 28 (Table 1), and the mean virus titer in lungs of PVM-inoculated C.B-17 *scid* mice ($n = 5$) was 5.2 ± 0.9 $\log_{10}$ FFU/g.

TABLE 1. Immunohistochemistry scores for lungs of C3H, BALB, and C.B-17 *scid* mice inoculated intranasally with PVM

Day postinoculation	*scid* mouse strain	PVM infection	No. of mice	Mean IHC score[a] ± SEM
10	C3H	−	6	—[b]
		+	10	5.3 ± 6.7
	BALB	−	4	—
		+	6	0.5 ± 0.5
15	C3H	−	3	—
		+	7	9.7 ± 6.1
20	C3H	−	7	—
		+	11	24.9 ± 10.0
	BALB	−	4	—
		+	6	27.3 ± 11.0
25	C3H	−	4	—
		+	7	27.9 ± 23.4
28	C3H	−	6	—
		+	11	31.8 ± 22.3
	BALB	−	4	—
		+	11	28.3 ± 14.9
56	C.B-17	−	5	—
		+	5	64.6 ± 5.5

[a] IHC, immunohistochemistry. Viral antigen was quantified by the method of Reid (4).
[b] PVM antigen was not detected in any of the six sections per mouse tested.

Prior studies of PVM-associated exacerbation of *P. carinii* disease (6) had been done with C3H *scid* mice. The current study extends that finding to BALB *scid* mice. The development of significant differences in lesion severity and cyst density between sham- and PVM-inoculated *scid* mice of either strain occurred rapidly and quite late in the course of disease. Despite high pulmonary virus titers, PVM-infected C.B-17 *scid* mice that were *P. carinii* free were also clinically normal at 1 month and survived twice as long as virus-inoculated, *P. carinii*-infected C3H *scid* and BALB *scid* mice. Colocalization of *P. carinii* cysts and PVM antigen was not observed in lungs of C3H *scid* mice, suggesting that exacerbation was due not to direct interaction of the two agents but possibly to interaction via host cells and/or cytokines. However, the possibility of an unseen direct effect does exist because cysts, not trophozoites, were stained.

One goal of these studies was to determine the cause of disease and death in dually infected C3H *scid* and BALB *scid* mice. The relatively low virus titers and very high cyst densities in the lungs of mice of both strains at 28 days, combined with the results obtained with *P. carinii*-free C.B-17 *scid* mice, strongly suggest that disease and death were due to exacerbation of *P. carinii* pneumonia.

We thank Theodore Holford for help with the statistical analysis.
This work was supported by NIH grants RR07034, RR00393, and AI25765.

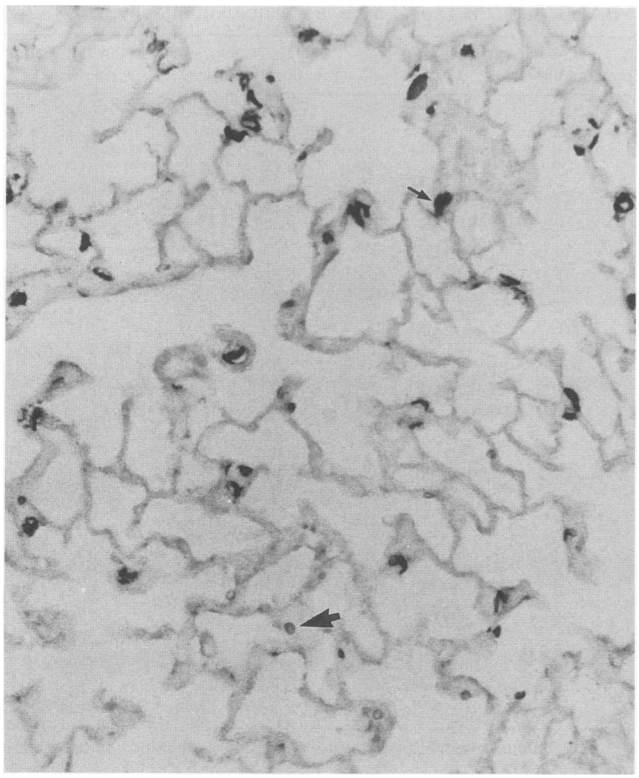

FIG. 2. Lung section from a *P. carinii*-infected, PVM-inoculated C3H/HeSnJ *scid* mouse at 1 month after virus infection. The section was double stained by avidin-biotin complex immunohistochemistry for PVM antigen and by Grocott's silver stain for cysts. PVM antigen-containing cells (small arrow) are abundant in this area, whereas only a few alveoli contain *Pneumocystis* cysts (large arrow). Magnification, ×100.

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