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Development and resolution of *Pneumocystis carinii* pneumonia in severe combined immunodeficient mice: a morphological study of host inflammatory responses

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Summary. The development and resolution of naturally-acquired *Pneumocystis carinii* pneumonia was studied in a severe combined immunodeficient (SCID) mouse model by light and electron microscopies. Initial infection was evident in 3-week-old SCID mice and started as focal alveolar colonization in the areas near terminal airways. Pronounced pulmonary inflammation occurred in animals of 10 weeks or older and the infection intensity reached a plateau in animals 12 weeks of age. At this stage of disease, the histopathological features of *P. carinii* infection in SCID mice were similar to those of immunodeficient man. Reconstitution of SCID mice with immunocompetent spleen cells at day 0 induced substantial pulmonary inflammation that was evident already by day 7 and most severe and extensive by day 12. The clearance of *P. carinii* did not begin until after day 12 and was almost completed by day 17. Alveolar macrophages in mice killed between days 12 and 15, at the time when *P. carinii* are being rapidly cleared, appeared active but phagocytosis of *P. carinii* was not commonly observed by either light or electron microscopy. These results suggest that (1) the presence of non-lymphoid inflammatory cells in SCID mice is not sufficient to control *P. carinii* infection; (2) the clearance of *P. carinii* from the lungs of reconstituted SCID mice requires local recruitment of large numbers of inflammatory cells with an active appearance; and (3) intracellular killing of *P. carinii* by phagocytosis does not appear to be a major mechanism in host defences against *P. carinii* infection in this model.

Keywords: pneumonia, *Pneumocystis carinii*, mice, morphology, immunodeficiency

Pneumocystis carinii (PC) pneumonia (PCP) is a major cause of morbidity and mortality in immunocompromised hosts. The mechanism of host defences against PC infection is not clear. Earlier studies have suggested that B lymphocytes and humoral immunity are important (Walzer *et al.* 1987; Gigliotti & Hughes 1988). More recent studies have shown that $CD4^+$ T lymphocytes are critical both in prevention of and recovery from PC infection (Harmsen & Stankiewicz 1990; Shellito *et al.* 1990). However, $CD4^+$ and B

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lymphocytes are unlikely to function directly as effector cells in PC killing. Studies of how these cells may contribute to host defences are, therefore, important for a better understanding of the immunopathogenesis of PC infection.

Several elegant pathological studies have been conducted in an attempt to clarify the immunopathogenesis of PCP. However, most of those studies either used animals that received long-term treatment with immunosuppressive agents (Lanken *et al.* 1980; Long *et al.* 1986; Millard *et al.* 1990; Stokes *et al.* 1987; Walzer *et al.* 1980; Yoneda & Walzer 1980, 1981), or were carried out in immunocompromised patients at a late stage of disease (Hasleton *et al.* 1981; Saldana *et al.* 1989; Weber *et al.* 1977). In addition, most studies have focused exclusively on the mechanism of PC-induced host tissue damage. There have been several recently published studies describing PCP in animals with genetic or acquired immunodeficiencies (Baskerville *et al.* 1991; Roths *et al.* 1990). However, these studies lack detailed descriptions of both the development of PC infection and the time course of resolution of naturally-acquired PCP in immunocompromised hosts. The findings that the C.B-17 *scid/scid* (severe combined immunodeficient, SCID) mouse develops detectable naturally acquired PC infection at about 4 weeks of age (Harmsen & Stankiewicz 1990; Roths *et al.* 1990) suggests that this strain of mice may be a model for studying the development of PCP. Reconstitution of these animals with spleen cells from immunocompetent mice can completely clear the infection (Harmsen & Stankiewicz 1990). Therefore, this model could also be used to study the resolution of PCP in resistant hosts. The objectives of the present study were to examine (1) the development of naturally acquired PC infection in SCID mice, (2) the host inflammatory responses to PC infection in SCID mice that had or had not been reconstituted with spleen cells, and (3) the importance of phagocytes (particularly macrophages) in the resolution of PCP in reconstituted SCID mice.

Materials and methods

Mice

C.B-17 +/+ and C.B-17 *scid/scid* mice were obtained from the Trudeau Animal Breeding Facility. Mice homozygous for the autosomal recessive mutation *scid* are deficient in both B- and T-lymphocyte functions (Bosma *et al.* 1983). A foundation stock of SCID mice was originally obtained from Dr Leonard Schultz of the Jackson Laboratory, Bar Harbor, Maine. Mice were bred and housed in microisolator cages containing sterilized food and water. Periodic screening for an extensive series of common mouse pathogens revealed no evidence of infection within this colony (Harmsen & Stankiewicz 1990).

Experimental design

Thirty-four 3-week-old SCID mice (24 male and 10 female) were used to study the development of PCP. They were divided randomly into nine groups with three or four mice in each group. Groups of mice were killed at 3, 4, 5, 6, 7, 8, 10, 12 or 14 weeks of age.

For the study of resolution of PCP, SCID mice were reconstituted with spleen cells from immunocompetent mice. A total of 34 7-week-old SCID mice (17 male and 17 female) were used in two separate experiments. All mice were reconstituted with spleen cells from C.B-17 +/+ mice as described below. In each experiment, one group of two to four reconstituted mice was killed at 7, 12, 15, 17 or 21 days post-reconstitution (DPR). In addition, in one experiment, one group of two to four reconstituted mice was killed at the time of reconstitution (DPR 0) or at DPR 3 or 28. Additional groups of three reconstituted SCID mice were killed at DPR 10, 13, 15 or 17 for ultrastructural studies.

Reconstitution of SCID mice with immunocompetent spleen cells

Spleens were collected aseptically from 6–8-

week-old C.B.17+/+ mice, diced into small pieces, gently pushed through stainless steel screens into Hanks' balanced salt solution (GIBCO, Grand Island, NY), and triturated with a Pasteur pipette. After removal of debris, the cells were washed twice with phosphate buffered saline (pH 7.2, Sigma, St Louis, MO), counted, and resuspended in phosphate buffered saline at a concentration of 5×10^7 nucleated cells per millilitre. Recipient SCID mice were given 1 ml of the cell suspension by injection into a tail vein.

Pathology

Mice were anaesthetized deeply with halothane and exsanguinated at the predetermined times. For light microscopic studies, the thoracic cavity was opened to expose the lung and trachea. The trachea was cannulated with a polystyrene catheter (i.d. 0.51 mm; o.d. 1.56 mm), and the lungs were inflated with 0.5–0.8 ml of 10% neutral buffered formalin. The trachea was ligated and the lung was then removed *en bloc*, immersed immediately in the same fixative, and processed by standard paraffin embedding methods. Sections were cut 5 μm thick, and stained with haematoxylin-eosin (HE) or by Grocott's methenamine silver method (GMS). Selected formalin-fixed lung tissues were also processed and embedded in Immuno-Bed (Polysciences, Inc., Warrington, PA), and sections of 1–2 μm thickness were cut and stained with toluidine blue. The intensity of PC infection was graded in GMS-stained sections by examining the whole section at a final magnification of 100–400. An intensity score of 0–5 was assigned to each section based on the method of Lanken *et al.* (1980) with slight modification (0, no PC; 1, a few PC present in a few alveoli; 2, a few PC present in many alveoli; 3, moderate numbers of PC present in many alveoli; 4, one-third of alveoli filled with PC and 5, one-half or greater of alveoli filled with PC). The scores collected from mice killed at the same time point were compiled and their medians and ranges were calculated.

For ultrastructural studies, the thoracic cavity of mice was opened immediately after the death of animals, with care being taken not to damage the heart or major blood vessels. The pulmonary circulatory system was flushed with 50 ml of heparinized phosphate buffered saline (50 U heparin per ml) and the animal was then perfused steadily over about 5 min with 20 ml of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The lung was dissected out and fixed in the same fixative at 4°C overnight and then transferred into 1% glutaraldehyde in 0.1 M phosphate buffer. Tissue blocks of 1 mm^3 were selected from parts of the lungs showing gross lesions, if present. After washing in two changes of cold 0.1 M phosphate buffer, the tissues were processed by the procedure described elsewhere (Chen *et al.* 1989) and examined on a Jeol JEM-1200EX transmission electron microscope.

Results

Development of PCP in SCID mice

The intensity of PC infection in SCID mice of different ages is summarized in Fig. 1. The infection was evident but mild in 3-week-old mice, increased its intensity persistently with age and reached its peak by 12 weeks.

The lungs of 3–4-week-old SCID mice were

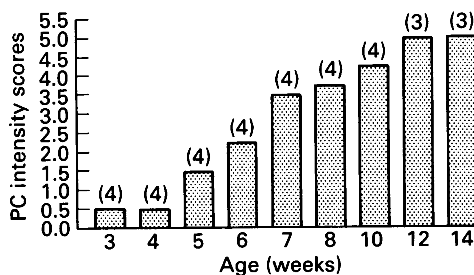

Fig. 1. Intensity scores of PC infection in the lungs of SCID mice at different ages. Data are presented as medians with numbers of mice in parentheses. Ranges are omitted for clarity and are within 0.5 score ± medians in all groups.

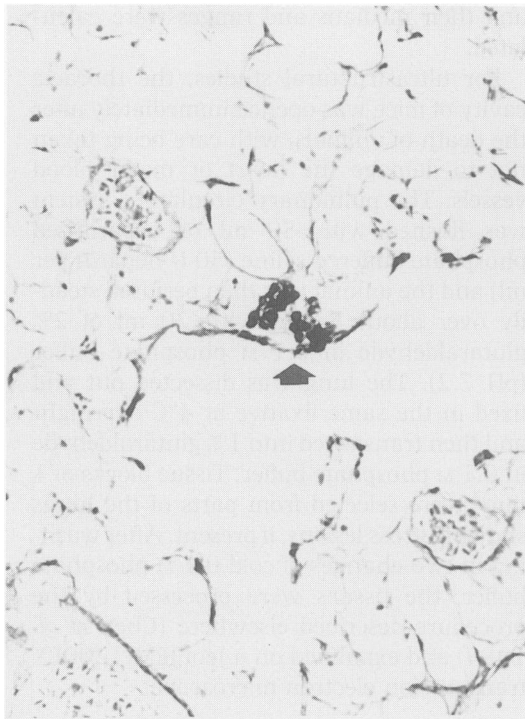

Fig. 2. The lung from a 3-week-old SCID mouse showing the presence of a small cluster of PC (arrow) in the alveoli near the alveolar ducts. GMS. ×240.

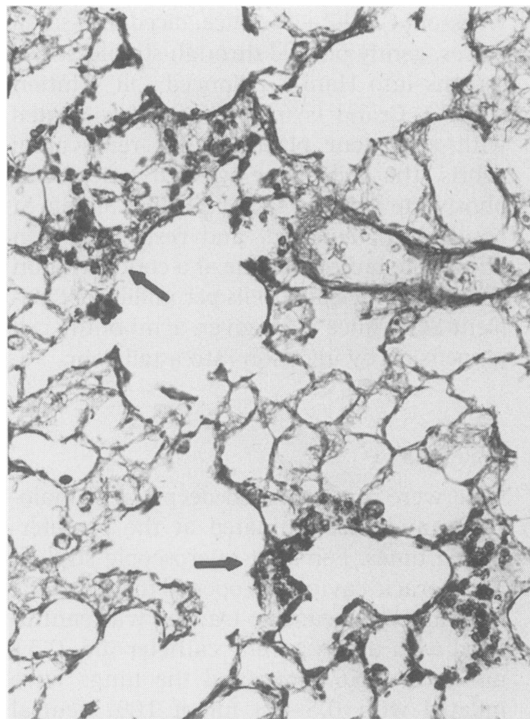

Fig. 3. The lung from a 5-week-old SCID mouse showing the confinement of moderate numbers of PC (arrows) in the alveoli near terminal airways. The infection is absent from the alveoli between terminal airways. GMS. ×200.

histopathologically normal although sections stained with GMS showed the occasional presence of single or small clusters of PC. These clusters of PC were confined within the alveoli near the alveolar ducts and terminal bronchioles (Fig. 2). The intensity of PC infection increased moderately in the lungs of 5–6-week-old mice. Eosinophilic material was first evident within the alveoli in HE-stained sections, and the consecutive sections stained with GMS showed these to be argyrophilic. The alveoli between adjacent terminal airways, however, remained free of signs of infection (Fig. 3). Alveolar macrophages were morphologically normal although their numbers varied between animals from within normal range to slightly increased. A very mild infiltration of neutrophils into the alveolar spaces was seen in one animal only.

Both the intensity of infection and the extent of lung involved had increased markedly in 7–8-week-old mice. More eosinophilic material was evident within the alveoli near terminal airways, and clusters of PC could be found in more distal alveoli. However, pulmonary inflammation remained minimal in most animals.

The outstanding feature in the lungs of 10-week-old mice was the presence of foamy eosinophilic material that is characteristic of PCP in man (Hasleton *et al.* 1981; Weber *et al.* 1977). In addition, all animals killed at this time showed a mild to moderate inflammation in the areas infected with PC (Fig. 4). This consisted mainly of intra-alveolar infiltration of macrophages and neutrophils with a few binuclear and multinuclear giant cells as well as perivascular aggregation of moder-

ate numbers of mononuclear cells (Fig. 4). In addition, margination of leucocytes was seen within some blood vessels that had prominent perivascular aggregation of mononuclear cells.

The lungs of 12-week-old mice showed higher intensity scores of PC infection and more severe inflammation compared with those of 10-week-old mice. Many alveoli were densely packed with eosinophilic honeycomb material and sometimes with macrophages and very few neutrophils (Fig. 5). The sections stained with GMS showed that these alveoli were partially or completely packed with large clumps of PC (Fig. 5). The organisms were also observed in the interalveolar septa but were found only very occasionally in the lumen of small airways. Some macrophages were large and had a moderate foamy cytoplasm. The presence of argyrophilic material within phagocytes was seen only occasionally. The pulmonary inflammation seen in 14-week-old mice was slightly more extensive but similar in nature to that seen in 12-week-old mice, but the intensity scores of PC infection were similar in these two groups.

Resolution of PCP in reconstituted SCID mice

The intensity scores of PC infection in SCID mice killed at different times after reconstitution are presented in Fig. 6. Reconstitution of SCID mice resulted in complete clearance of PC from their lungs by DPR 21. Clearance did

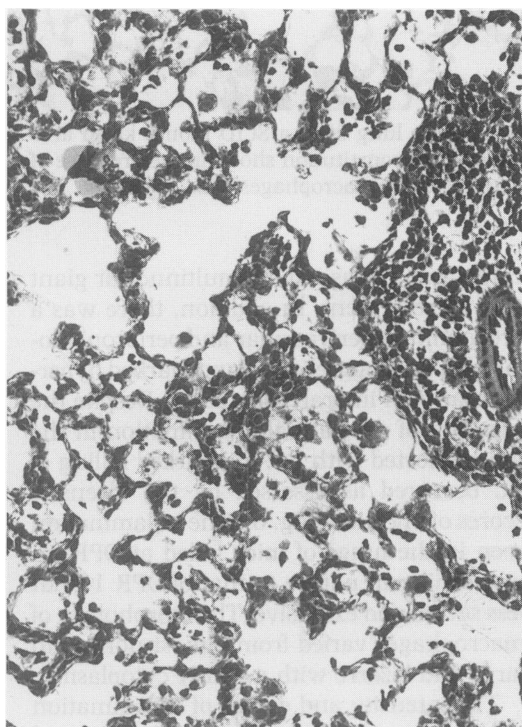

Fig. 4. The lung from a 10-week-old SCID mouse showing perivascular aggregation of moderate numbers of mononuclear cells and the infiltration of mixed inflammatory cells into the alveolar spaces. HE. ×160.

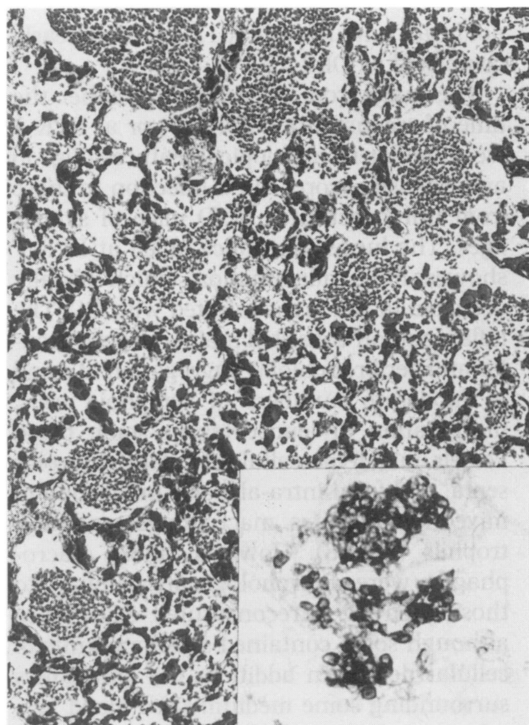

Fig. 5. The lung from a 12-week-old SCID mouse showing that most alveoli are fully filled with eosinophilic honeycomb material and some inflammatory cells (HE, ×160). Inset: the presence of densely packed argyrophilic organisms within the alveoli (GMS, ×330).

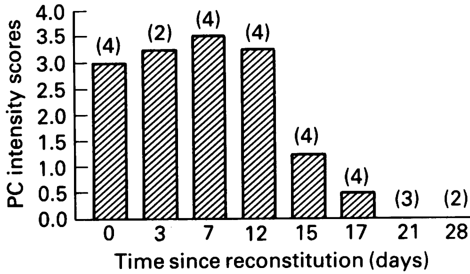

Fig. 6. Intensity scores of PC infection in the lungs of SCID mice reconstituted with spleen cells from immunocompetent mice. Data are presented as medians with numbers of mice (compiled from two experiments) in parentheses. Ranges are omitted for clarity and are within 1 score ± medians in all groups except the group killed on 15 days post-reconstitution that has a range of 0.5–3.

not begin until after DPR 12 and was nearly completed by DPR 17.

The lungs from mice killed at either the time of reconstitution (DPR 0) or at DPR 3 were similar in histological features (Fig. 7) and intensity scores of PC infection to those from unreconstituted SCID mice of similar ages. The lungs from mice killed at DPR 7 showed mild inflammation which usually occurred in the areas infected with PC. The inflammation consisted mainly of perivascular and peribronchiolar aggregation of small to moderate numbers of lymphoid cells, margination of leucocytes in some blood vessels, mild hypercellularity of interalveolar septa, and focal intra-alveolar infiltration of mixed lymphocytes, macrophages and neutrophils (Fig. 8). However, most macrophages were morphologically similar to those seen in unreconstituted SCID mice although some contained small amounts of cellular debris. In addition, the lymphatics surrounding some medium-sized blood vessels were often markedly dilated.

Inflammation was most severe and extensive in the lungs of mice killed at DPR 12, at which time almost the entire lung was involved (Fig. 9). Many macrophages seen at this time were large and had a foamy

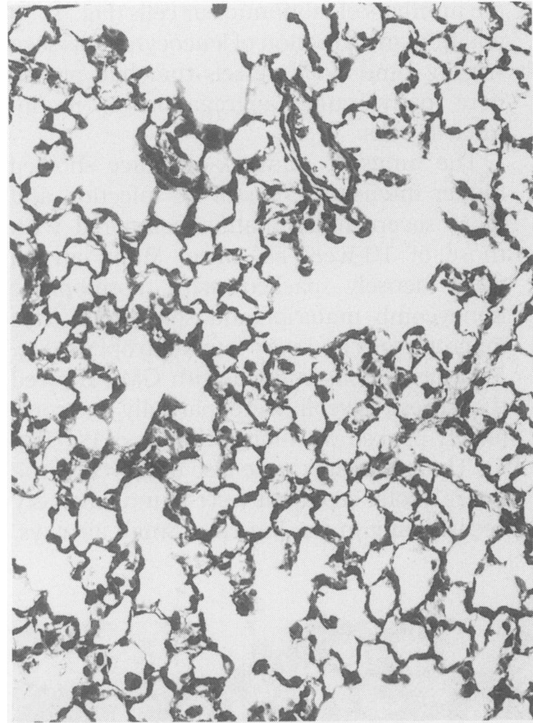

Fig. 7. The lung from a SCID mouse killed at 3 days post-reconstitution showing the presence of a few alveolar macrophages. HE. × 160.

cytoplasm. Occasionally, multinuclear giant cells were present. In addition, there was a predominant perivascular and peribronchiolar lymphoid aggregation and marked hypercellularity of interalveolar septa. Despite the presence of substantial inflammation in the areas infected with PC, no marked killing of PC occurred as assessed by the intensity scores of infection (Fig. 6). The inflammation seen in the lungs of mice killed at DPR 15 was similar in nature to that of DPR 12 but less severe and extensive. The morphology of macrophages varied from almost normal to large and bizarre with a foamy cytoplasm.

The intensity and extent of inflammation in the lungs of mice killed at DPR 17 varied between animals. In general, it became much milder than that seen at DPR 12 or DPR 15. Macrophages were morphologically less active and had a dense cytoplasm (Fig.

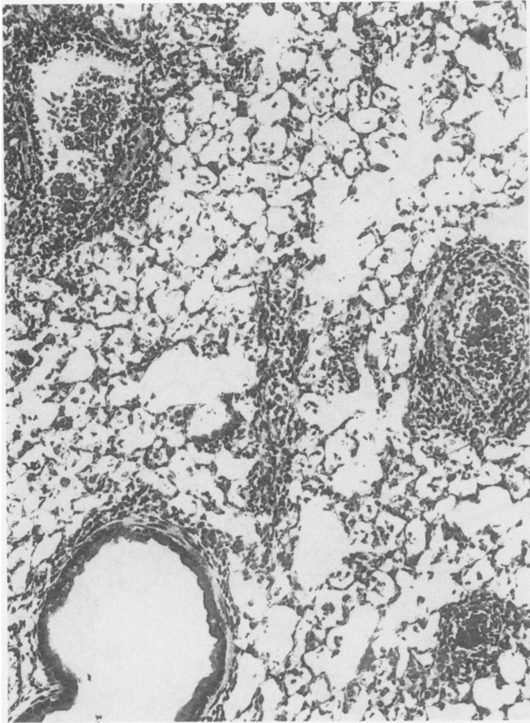

Fig. 8. The lung from a SCID mouse killed at 7 days post-reconstitution showing the infiltration of mixed inflammatory cells into the alveolar spaces and mild to moderate perivascular and peribronchiolar accumulation of lymphoid cells. HE. ×80.

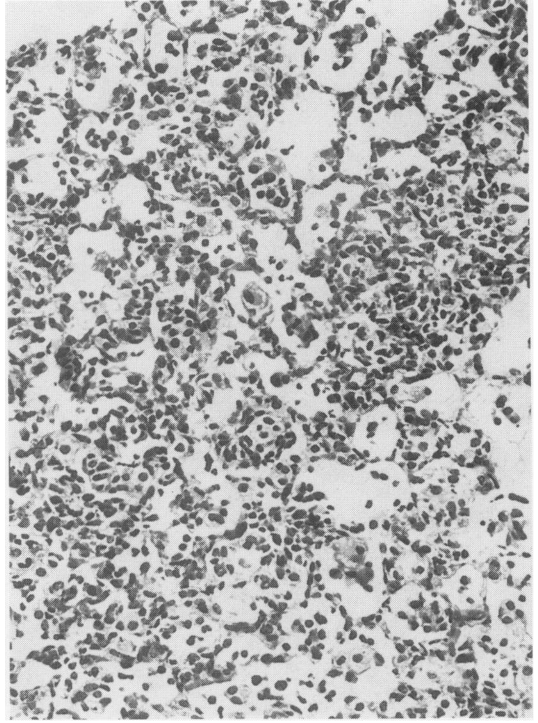

Fig. 9. Extensive and severe pulmonary inflammation seen in a SCID mouse killed at 12 days post-reconstitution. HE. ×160.

10). The lungs of most animals killed at DPR 21 had entered a resolution phase. Although about 70% of alveoli still contained some inflammatory exudate, the amount had decreased substantially. The cellular components of the exudate consisted almost exclusively of macrophages with very occasional multinuclear giant cells. Many macrophages were normal in appearance, but focal intra-alveolar accumulations of foamy macrophages could occasionally be found. In addition, small to moderate numbers of lymphocytes were still present in the perivascular and peribronchiolar regions (Fig. 11).

Most alveoli in the lungs of mice killed at DPR 28 were fully aerated and free of inflammatory exudate (Fig. 12). However, the alveoli in some areas contained slightly more than the normal number of macrophages. Mild perivascular lymphoid aggregation and focal cellular infiltration of the interalveolar septa remained in some areas until the end of the experiment.

Interaction between inflammatory cells and PC

Electron microscopic examination of the lungs of mice killed at or between DPR 10 and 17 confirmed the histopathological findings as described above. There were deep layers of densely packed thin-walled PC trophozoites over type I pneumocytes in animals killed at DPR 10 or 13 (Fig. 13). Intact cysts containing several internal bodies (sporozoites) and collapsed cysts appearing as crescents but with thickened walls were also occasionally seen. Alveolar

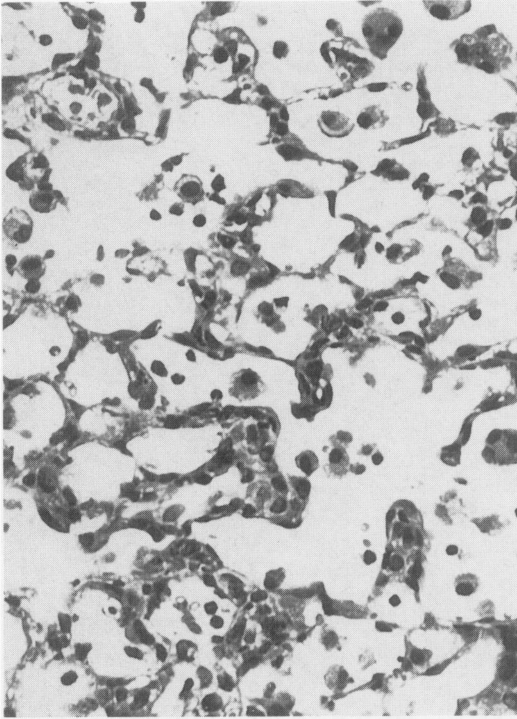

Fig. 10. The lung of a SCID mouse killed at 17 days post-reconstitution showing the subsiding inflammation consisting mainly of macrophages with a dense cytoplasm. HE. × 260.

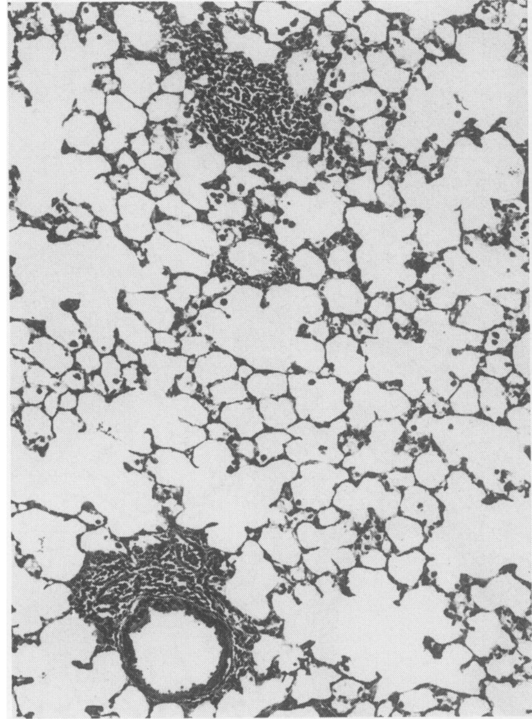

Fig. 11. The lung from a SCID mouse killed at 21 days post-reconstitution showing partial resolution of cellular inflammatory exudate. Mild perivascular and peribronchiolar aggregation of lymphoid cells and the intra-alveolar infiltration of a few inflammatory cells remain evident. HE. × 80.

macrophages, lymphocytes and occasionally neutrophils were often present in the alveoli (Fig. 13) and the interalveolar septa, but direct contact between those cells and PC was infrequently observed (Fig. 13). Phagocytosis of PC by macrophages or neutrophils was not commonly observed although some phagocytes had many cytoplasmic vacuoles containing myelin-like materials and other cellular debris (Fig. 14). The lungs of mice killed at DPR 15 showed the presence of many apparently activated macrophages (numerous pseudopodia on their surface and abundant phagosomes in their cytoplasm) in the areas infected with many trophozoites, but, as at earlier times, they were rarely in direct contact with the organisms. Although a few neutrophils were seen near the organisms at this and earlier stages, no ingested organisms were observed.

Discussion

Histopathological findings of naturally acquired PCP in our SCID mice shared most characteristic features of typical PCP as described in immunocompromised patients (Hasleton *et al.* 1981; Saldana *et al.* 1991; Weber *et al.* 1977). These findings are consistent with those described previously by Roths *et al.* (1990), further supporting that the SCID mouse is an ideal model for studying the immunopathogenesis of PCP in immunocompromised hosts (Harmsen & Stankiewicz 1990; Roths *et al.* 1990).

One aim of this study was to examine histopathologically the development of naturally acquired PCP in SCID mice. The infection started with focal alveolar coloniza-

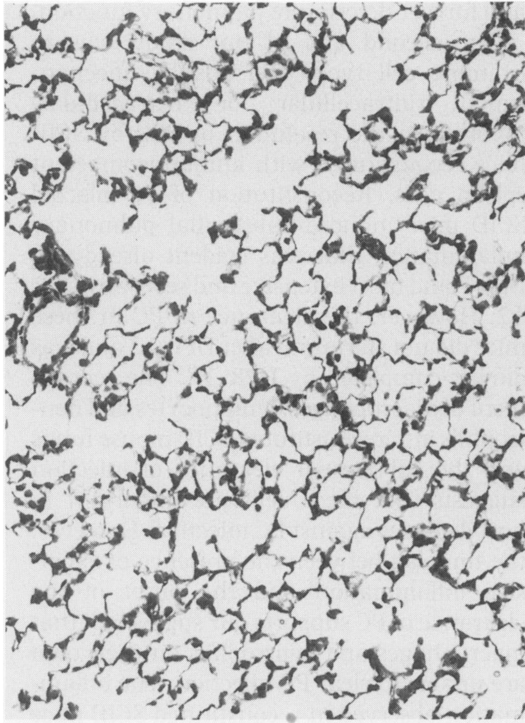

Fig. 12. The lung from a SCID mouse killed at 28 days post-reconstitution showing the fully aerated alveoli and the disappearance of inflammatory exudate in most areas. HE. ×80.

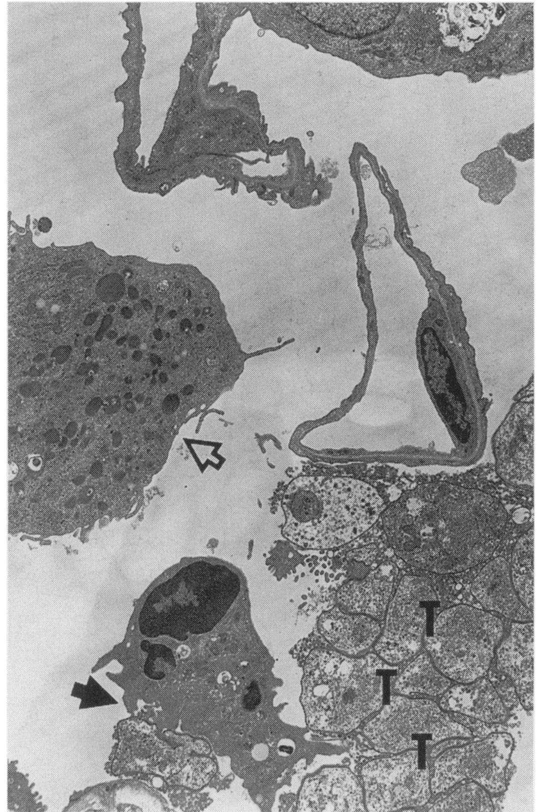

Fig. 13. An alveolus from a SCID mouse killed on 13 days post-reconstitution showing the presence of multiple layers of densely packed PC trophozoites (T) along the cytoplasmic extensions of type I pneumocytes and intra-alveolar infiltration of macrophage and neutrophil. Note a neutrophil (solid arrow) in close contact with the trophozoites. Macrophage (open arrow). TEM. ×3375.

tion that was confined to the alveoli near terminal airways. These findings together with the fact that the size of PC organisms (4–8 μm) is within the range of respirable particles are consistent with the possibility that mice acquire natural infection of PC through inhalation. As the extent of infection increased, interalveolar spread occurred and the alveoli more distal to terminal airways became involved.

Pulmonary inflammation in SCID mice with PCP was absent in the early stage of infection and minimal in the intermediate stage. Moderate to extensive inflammation occurred only in the advanced stage of infection (in mice of 10 weeks or older). At that time, many macrophages and a few neutrophils and multinuclear giant cells had infiltrated into the infected areas. These findings agree with the results reported previously from studies of advanced PCP in immunocompromised animal models and human patients (Baskeville *et al.* 1991; Hasleton *et al.* 1981; Lanken *et al.* 1980; Millard *et al.* 1990; Stokes *et al.* 1987; Walzer *et al.* 1989). Despite the presence of macrophages and neutrophils in the advanced stage of infection PC burdens remained at a high level, suggesting that these cells alone are not adequate to control PC infection in the lungs of SCID mice.

The mechanism involved in the induction

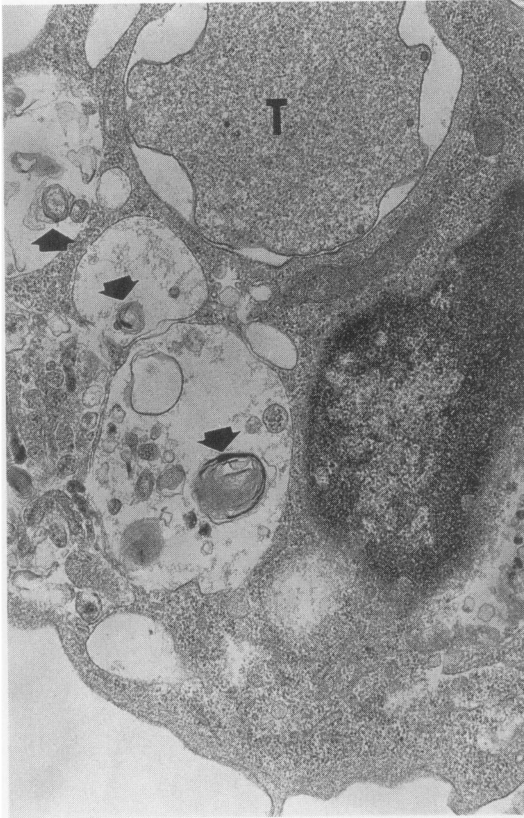

Fig. 14. An alveolar macrophage from a SCID mouse killed on 13 days post-reconstitution containing an ingested trophozoite (T) and some myelin-like materials (arrows) and other cellular debris in its phagosomes. TEM. ×15120.

of extensive pulmonary inflammation during the advanced stage of PC infection in SCID mice is not clear. One possibility is that only heavy burdens of PC are able to generate sufficient antigen to initiate inflammation. Alternatively, this inflammation may merely represent a response to local tissue damage caused by the replication of large numbers of organisms. Indeed, damage to host pulmonary tissues by PC occurs in a very late stage of infection (Hasleton *et al.* 1981; Lanken *et al.* 1980; Millard *et al.* 1990; Roths *et al.* 1990; Weber *et al.* 1977). Extensive cellular infiltration into the alveoli of hosts at this stage can exacerbate the degree of hypoxaemia and further deteriorate pulmonary function.

The second aim of our study was to examine cell types and possible mechanism(s) (intracellular or extracellular) involved in the resolution of PCP by SCID mice reconstituted with immunocompetent spleen cells. Reconstitution of PC-infected SCID mice induced substantial pulmonary inflammation that was evident already by DPR 7 and most extensive and severe by DPR 12. However, the clearance of PC in these mice did not start until after DPR 12 and was almost completed by DPR 17. The recruitment of macrophages, lymphocytes and neutrophils into reconstituted SCID mouse lungs and the subsequent clearance of infection suggests that these cells are important in host defences against PC infection. However, the time-lag between the presence of extensive inflammation and the onset of the clearance of PC supports our suggestion that macrophages and neutrophils on their own are unable to clear PC infection. The inflammation observed in reconstituted SCID mice is not due to the reconstitution *per se* because inflammation was not induced in PC-free SCID mice with the same protocol (Harmsen & Stankiewicz 1990).

Alveolar macrophages are believed to be important in host resistance to PCP in that alveolar macrophages from rats (Ezekowitz *et al.* 1991; Masur & Jones 1978; Pesanti 1991; von Behren & Pesanti 1978) and man (Forte *et al.* 1991) can readily phagocytize and kill PC under optimal culture condition. However, there is no direct in-vivo evidence to implicate macrophages in control of naturally acquired PC infection. We examined the lungs of SCID mice between DPR 13 and 17 by electron microscopy. During these 5 days the intensity scores of PC infection decreased 2–3 grades (Fig. 6) which represents a clearance of about 99% of PC from the lungs (results not shown). It was reasoned that if clearance of PC occurs mainly by phagocytosis, then the frequency of phagocytized PC found in macrophages should be very high during these 5 days. However, just as on DPR 10, macrophages at DPR 13,

15 and 17 rarely contained phagocytized PC even though most alveolar macrophages seen during the later period had an ultrastructurally activated appearance (large size, foamy or vacuolated cytoplasm and many pseudopodia) and contained many myelin-like inclusions in their phagosomes. The above findings are consistent with most studies of naturally acquired or experimentally induced PCP in that intact or degenerated organisms have been found only incidentally within phagocytes (Baskerville *et al.* 1991; Lanken *et al.* 1980; Millard *et al.* 1990). However, a high frequency of phagocytosis of PC by macrophages has been reported by Roths *et al.* (1990) in SCID mice reconstituted with murine bone marrow cells. It is not certain why our results contradict those of Roths *et al.* (1990). However, it is worth noting that we used spleen cells, instead of bone marrow cells as used by Roths *et al.* (1990), to reconstitute SCID mice. In addition, their conclusion about the role of macrophages was based on histological examinations and unpublished results of an ultrastructural study.

That phagocytosis and intracellular killing by macrophages may not be the major mechanism in host defences against PC in reconstituted SCID mice does not necessarily exclude the role of macrophages in the control of PC infection. Alternatively, macrophages could function as antigen-presenting cells for $CD4^+$ cells, participate in antibody-mediated extracellular killing of PC, regulate the local production of cytokines, and/or augment the functions of other immune cells. Indeed, Pesanti (1991) has recently shown that cultured alveolar macrophages can kill PC without direct contact between effector and target cells, supporting the possibility of extracellular killing of PC by alveolar macrophages. In addition, during the preparation of this manuscript, Hidalgo *et al.* (1992) reported that both PC cysts and trophozoites can elicit an oxidative burst from normal rat alveolar macrophages and NR8383 cells, a macrophage cell line. This oxidative burst need not be triggered by phagocytosis because soluble mediators or the binding of microorganisms to surface receptors without phagocytosis can also induce an oxidative burst (Johnston 1978). Such an oxidative burst could in turn result in the release of toxic metabolites that could kill PC extracellularly. Future studies should, therefore, be designed to examine the importance of other microbicidal mechanisms of macrophages, including extracellular mechanisms in host defences against PC *in vivo*.

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References

BASKERVILLE A., DOWSETT A.B., COOK R.W., DENNIS M.J., CRANAGE M.P. & GREENAWAY P.J. (1991) *Pneumocystis carinii* pneumonia in simian immunodeficiency virus infection: Immunohistological and scanning and transmission electron microscopical studies. *J. Pathol.* **164**, 175–184.

BOSMA G.C., CLUSTER R.P. & BOSMA M.J. (1983) A severe combined immunodeficiency mutation in the mouse. *Nature* **301**, 527–530.

CHEN W., ALLEY M.R. & MANKTELOW B.W. (1989) Experimental induction of pneumonia in mice with *Bordetella parapertussis* isolated from sheep *J. Comp. Pathol.* **100**, 77–89.

EZEKOWITZ R.A.B., WILLIAMS D.J., KOZIEL H., ARMSTRONG M.Y.K., WARNER A., RICHARDS F.F. & ROSE R.M. (1991) Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor. *Nature* **351**, 155–158.

FORTE M., RAHELU M., STUBBERFIELD C., TOMKINS L., PITHIE A. & KUMARARATNE D. (1991) In-vitro interaction of human macrophages with *Pneumocystis carinii. Int. J. Exp. Pathol.* **72**, 589–598.

GIGLIOTTI F. & HUGHES W.T. (1988) Passive immunoprophylaxis with specific monoclonal antibody confers partial protection against *Pneumocystis carinii* pneumonitis in animal models. *J. Clin. Invest.* **81**, 1666–1668.

HARMSEN A.G. & STANKIEWICZ M. (1990) Requirement for $CD4^+$ cells in resistance to *Pneumocystis carinii* pneumonia in mice. *J. Exp. Med.* **172**, 937–945.

HASLETON P.S., CURRY A. & RANKIN E.M. (1981) *Pneumocystis carinii* pneumonia: a light microscopical and ultrastructural study. *J. Clin. Pathol.* **34**, 1138–1146.

HIDALGO H.A., HELMKE R.J., GERMAN V.F. & MANGOS J.A. (1992) *Pneumocystis carinii* induces an oxidative burst in alveolar macrophages. *Infect. Immun.* **60**, 1–7.

JOHNSTON B.R. JR. (1978) Oxygen metabolism and the microbicidal activity of macrophages. *Fed. Proc.* **37**, 2759–2764.

LANKEN P.N., MINDA M., PIETRA G.G. & FISHMAN A.P. (1980) Alveolar response to experimental *Pneumocystis carinii* pneumonia in the rat. *Am. J. Pathol.* **99**, 561–588.

LONG E.G., SMITH J.S. & MEIER J.L. (1986) Attachment of *Pneumocystis carinii* to rat pneumocytes. *Lab. Invest.* **54**, 609–615.

MASUR H. & JONES T.C. (1978) The interaction *in vitro* of *Pneumocystis carinii* with macrophages and L-cells. *J. Exp. Med.* **147**, 157–170.

MILLARD P.R., WAKEFIELD A.E. & HOPKIN J.M. (1990) A sequential ultrastructural study of rat lungs infected with *Pneumocystis carinii* to investigate the appearances of the organism, its relationships and its effects on pneumocytes. *Int. J. Exp. Pathol.* **71**, 895–904.

PESANTI E.L. (1991). Interaction of cytokines and alveolar cells with *Pneumocystis carinii in vitro*. *J. Infect. Dis.* **163**, 611–616.

ROTHS J.B., MARSHALL J.D., ALLEN R.D., CARLSON G.A. & SIDMAN C.L. (1990) Spontaneous *Pneumocystis carinii* pneumonia in immunodeficient mutant *scid* mice. Natural history and pathobiology. *Am. J. Pathol.* **136**, 1173–1186.

SALDANA M.J., MONES J.M. & MARTINEZ G.R. (1989) The pathology of treated *Pneumocystis carinii* pneumonia. *Semin. Diagn. Pathol.* **6**, 300–312.

SHELLITO J., SUZARA V.V., BLUMENFELD W., BECK J.M., STEGER H.J. & ERMAK T.H. (1990) A new model of *Pneumocystis carinii* infection in mice selectively depleted of helper T lymphocytes. *J. Clin. Invest.* **85**, 1686–1693.

STOKES D.C., GIGLIOTTI F., REHG J.E., SNELLGROVE R.L. & HUGHES W.T. (1987) Experimental *Pneumocystis carinii* pneumonia in the ferret. *Br. J. Exp. Pathol.* **68**, 267–276.

VON BEHREN L.A. & PESANTI E.L. (1978) Uptake and degradation of *Pneumocystis carinii* by macrophages *in vitro*. *Am. Rev. Respir. Dis.* **118**, 1051–1059.

WALZER P.D., KIM C.K., LINKE M.J., POGUE C.L., HUERKAMP M.J., CHRISP C.E., LERRO A.V., WIXSON S.K., HALL E. & SHULTZ L.D. (1989) Outbreaks of *Pneumocystis carinii* pneumonia in colonies of immunodeficient mice. *Infect. Immun.* **57**, 62–70.

WALZER P.D., POWELL R.D. JR., YONEDA K., RUTLEDGE M.E. & MILDER J.E. (1980) Growth characteristics and pathogenesis of experimental *Pneumocystis carinii* pneumonia. *Infect. Immun.* **27**, 928–937.

WALZER P.D., STANFORTH D., LINKE M.J. & CUSHION M.T. (1987) *Pneumocystis carinii*: immunoblotting and immunofluorescent analysis of serum antibodies during experimental rat infection and recovery. *Exp. Parasitol.* **63**, 319–328.

WEBER, W.R., ASKIN F.B. & DEHNER L.P. (1977) Lung biopsy in *Pneumocystis carinii* pneumonia. A histopathologic study of typical and atypical features. *Am. J. Clin. Pathol.* **67**, 11–19.

YONEDA K. & WALZER P.D. (1980) Interaction of *Pneumocystis carinii* with host lungs: an ultrastructural study. *Infect. Immun.* **29**, 692–703.

YONEDA K. & WALZER P.D. (1981) Mechanism of pulmonary alveolar injury in experimental *Pneumocystis carinii* pneumonia in the rat. *Br. J. Exp. Pathol.* **62**, 339–346.