IMMUNOLOGICAL INVOLVEMENT IN PULMONARY FIBROSIS INDUCED BY PEPLOMYCIN

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Pulmonary fibrosis in mice induced by peplomycin (PEP) was suppressed by administration of anti-inflammatory agents such as prednisolone and D-penicillamine during or after the administration of PEP. Pulmonary fibrosis was also suppressed by administration of cyclophosphamide, an immunosuppressive antitumor agent before, during or after the administration of PEP. The pulmonary fibrosis in athymic nude mice induced by PEP was less than that in normal mice. The low response in the nude mice was enhanced by transfer of thymocytes to the same level as that in the normal mice. This suggests that the immune system, especially thymus-dependent immunity, is involved in the pulmonary fibrosis induced by PEP.

Peplomycin (PEP) which is one of several new biosynthetic bleomycins[1] has been used clinically in Japan since 1981. PEP shows less pulmonary toxicity[2,3] and a more potent antitumor activity[4] than does bleomycin. Its clinical use, however, is still limited by pulmonary fibrosis. The pathogenesis of pulmonary fibrosis induced by bleomycin is not fully understood, but there is some evidence which suggests the involvement of the immune or inflammatory systems[5~7]. Therefore, we have examined the effects of anti-inflammatory or immunosuppressive agents on the pulmonary fibrosis in mice induced by PEP.

Materials and Methods

Chemicals and Animals

PEP was prepared at Nippon Kayaku Co., Ltd. (Tokyo, Japan). Prednisolone acetate and cyclophosphamide were purchased from Shionogi Pharmaceutical Co., (Osaka, Japan), and D-penicillamine was from Sigma Chemical Co., (St. Louis, USA). Male 14-weeks-old ICR mice (SPF) were purchased from Shizuoka Agricultural Association for Laboratory Animals (Hamamatsu, Japan), and male 7-weeks-old ICR-*nu*/*nu* and −*nu*/+ mice were from Charles River Japan Inc., (Atsugi, Japan). They were used in experiments at ages of 15 or 8 weeks, respectively. They were fed sterilized CRF-1 diet made by Oriental Yeast Co., (Tokyo, Japan) and sterilized water *ad libitum* under filtered-air conditions. Each experimental group consisted of 10 animals.

Experimental Schedule for Combination with Anti-inflammatory or Immunosuppressive Agents

PEP was administered intravenously (iv) once a day for 7 days (day 0 to 6) to ICR mice. Prednisolone and D-penicillamine were injected intraperitoneally (ip) at doses of 2, 0.5 and 0.125 mg/kg, and at doses of 25 and 5 mg/kg, respectively before, during or after PEP administration. Cyclophosphamide was injected iv at doses of 25, 12.5 and 6.25 mg/kg before, during or after the PEP administration. In the case of the post-treatment, 2 schedules were performed: once a day for 7 successive days (day 7 to

13); or twice a week for 5 weeks (from day 7). These doses did not produce systemic toxicity in mice.

Experimental Schedule for *nu/nu* Mice

In order to test the involvement of cellular immune processes in the pathogenesis of pulmonary fibrosis induced by PEP, the response of *nu/nu* mice to intratracheal instillation of PEP was compared with that of *nu/+* mice. Eight or 16 μg of PEP in 50 μl of saline was instilled intratracheally in mice to cause pulmonary fibrosis. Control mice were instilled with saline. To understand the role of thymocytes in the development of the pulmonary fibrosis, thymocytes from *nu/+* mice were transferred to *nu/nu* mice and the response to pulmonary fibrosis was examined. Thymocytes obtained by chopping the thymus of *nu/+* mice were washed with Hanks' balanced salt solution 3 times and counted undzr light microscopy using a hemocytometer after treatment with trypan blue. A given number of thymocytes were transferred iv to *nu/nu* mice 6 days before PEP administration. During the experimental period, the *nu/nu* mice carring thymocytes rejected allografts.

Morphological Evaluation of Pulmonary Fibrosis

In the case of iv administration of PEP, the mice were sacrificed 5 weeks after the last administration, and in the case of intratracheal instillation, the mice were sacrificed 4 weeks after instillation. Lungs were fixed with 10% formalin solution, embedded in paraffin, processed and stained with both hematoxylin and eosin, and azan and mallory. The pulmonary fibrosis was evaluated by a procedure previously reported[8]. To establish significance between PEP alone and experimental animals, U-test was used.

Results

Effects of Anti-inflammatory or Immunosuppressive Agents on Pulmonary Fibrosis

Prednisolone distinctly suppressed pulmonary fibrosis at a high dose of 2 mg/kg. Fibrosis was suppressed more effectively by simultaneous- and post-treatment of prednisolone (Fig. 1). D-Penicillamine suppressed pulmonary fibrosis at doses of 25 and 5 mg/kg/day in a similar manner as prednisolone (Fig.

Fig. 1. Effect of prednisolone on the pulmonary fibrosis caused by peplomycin.
* $P<0.01$ from peplomycin alone, ** $P<0.05$ from peplomycin alone.

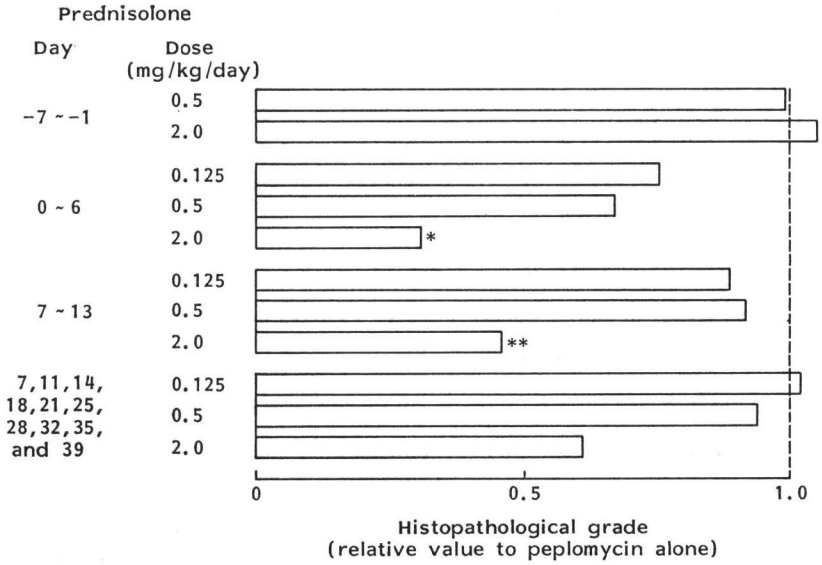


Fig. 2. Effect of D-penicillamine on the pulmonary fibrosis caused by peplomycin.
* $P<0.05$ from peplomycin alone, ** $P<0.01$ from peplomycin alone.

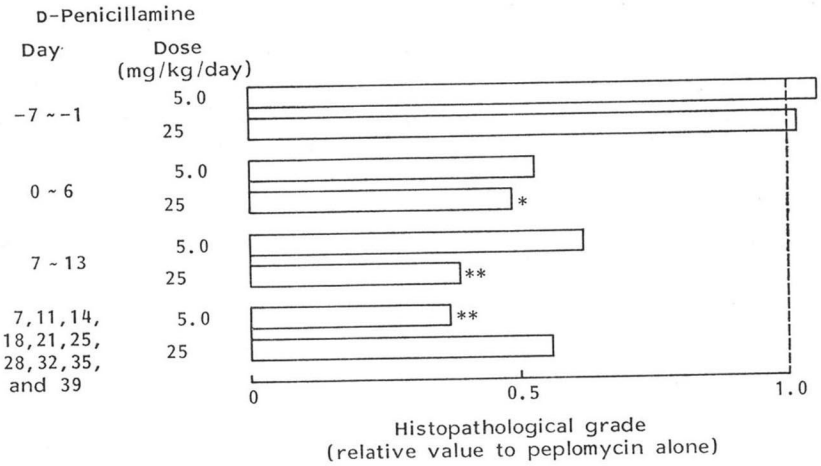
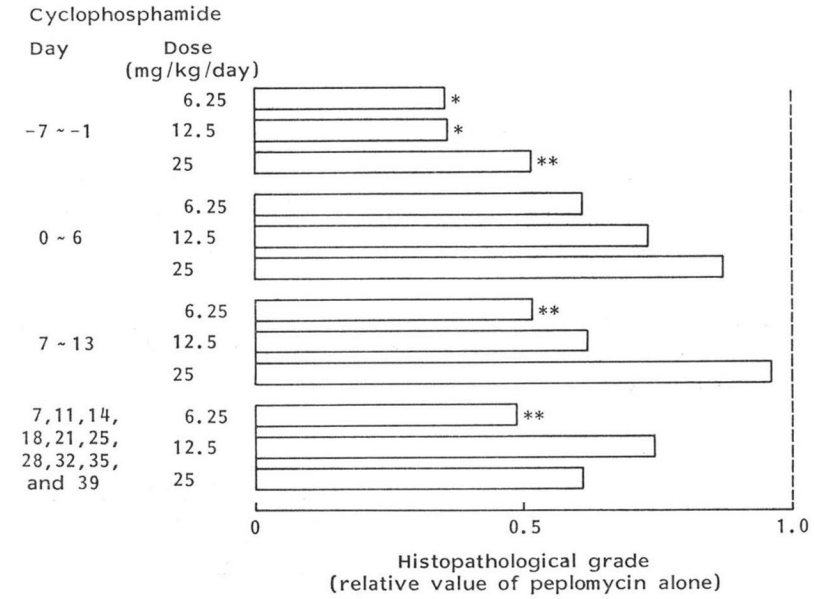


Fig. 3. Effect of cyclophosphamide on the pulmonary fibrosis caused by peplomycin.
* $P<0.01$ from peplomycin alone, ** $P<0.05$ from peplomycin alone.



2). Cyclophosphamide suppressed pulmonary fibrosis at a low dose of 6.25 mg/kg/day rather than at a high dose of 25 mg/kg/day in all the schedules used. Fibrosis was suppressed most effectively by pre-treatment of cyclophosphamide (Fig. 3).

Pulmonary Fibrosis Induced by PEP in *nu/nu* and *nu/+* Mice

As shown in Fig. 4, the pulmonary fibrosis in *nu/nu* mice was distinctly less than that observed in *nu/+* mice. In order to understand the role of T-cells, thymocytes obtained from *nu/+* mice were transferred to *nu/nu* mice and PEP was instilled 6 days after the transfer. The severity of pulmonary fibrosis in the *nu/nu* mice given 10^7 thymocytes was almost the same as that in *nu/+* mice (Fig. 5).

Fig. 4. The pulmonary fibrosis caused by peplomycin.
* $P<0.01$ from *nu*/+ mice at each dose.

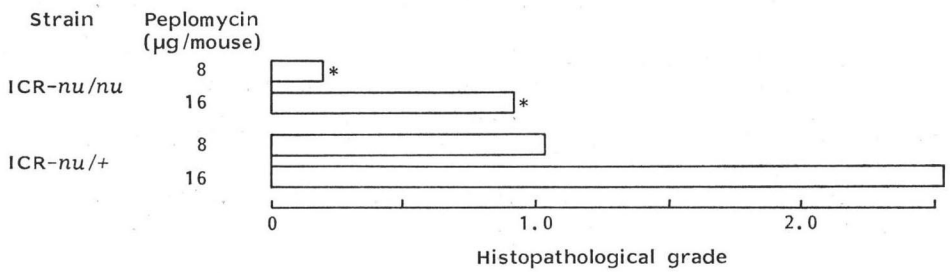
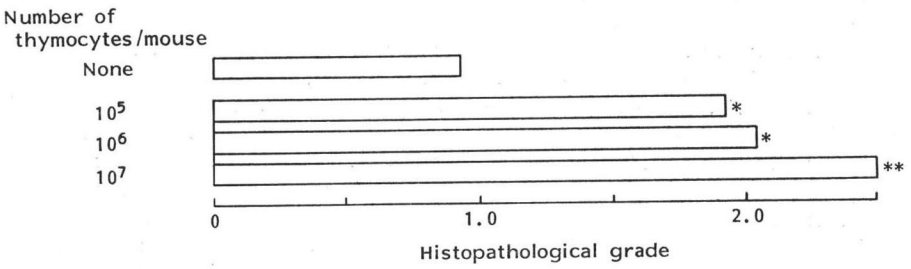


Fig. 5. Effect of thymocyte transfer on the pulmonary fibrosis caused by peplomycin in ICR-*nu*/*nu* mice (16 μg of PEP/mouse).
* $P<0.05$ from *nu*/*nu* mice (none), ** $P<0.01$ from *nu*/*nu* mice (none).



Discussion

Although the pulmonary toxicity of PEP is weaker than bleomycin[2,3], clinical use of PEP is still limited by pulmonary toxicity. It is important to understand mechanisms of occurrence of pulmonary toxicity for the prevention and detection of this toxicity. Involvement of two factors in the pulmonary toxicity has been suggested. One is oxygen toxicity since the morphological changes in the lung caused by bleomycin were similar to those caused by exposure to a higher concentration of oxygen[9~11], the other is the involvement of the immune systems. In patients with idiopathic pulmonary fibrosis, the immunologic response was more sensitive[7,12]. CHANDLER *et al.* reported that when bleomycin was intratracheally instilled in hamsters, the process from pulmonary alveolitis to fibrosis was characterized by changes in populations of infiltrating cells such as eosinophiles, neutrophiles, alveolar macrophages and lymphocytes in the bronchoalveoli[13]. THRALL *et al.* observed similar results using rats[14]. We found that alveolar macrophages accumulated around the fibrotic area and lymphocytes aggregated in subpleural nodules with development of pulmonary fibrosis when PEP was injected iv in mice (unpublished results).

In the present study, it was found that anti-inflammatory agents such as prednisolone and D-penicillamine, as well as, cyclophosphamide, an immunosuppressive agent, decreased the pulmonary fibrosis caused by PEP (Figs. 1, 2 and 3). Recently, SCHRIER *et al.* reported that the level of pulmonary fibrosis induced by bleomycin in *nu*/*nu* mice was less than that in normal mice[15]. Since the sensitivity to pulmonary toxicity is different among strains of mice[8,16], it was not clear whether the low response in *nu*/*nu* mice was due to strain variation or effect of thymocytes. We found that the pulmonary fibrosis in *nu*/*nu* mice was distinctly less as compared to that in *nu*/+ mice (Fig. 4), and that the low pulmonary fibrotic response in *nu*/*nu* mice could be enhanced by transfer of thymocytes (10^7) to the same level as that of *nu*/+ mice (Fig. 5). These results suggest the involvement of cell-mediated immune systems in the development of pulmonary fibrosis induced by PEP.

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