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Brief Note

Experimental Model of Extrinsic Allergic Alveolitis in Rats

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Extrinsic allergic alveolitis (EAA), also referred to as hypersensitivity pneumonitis and microgranulomatous hypersensitivity reaction, is defined as an inflammatory and immunologically-mediated disease of the peripheral airway and gas-exchanging portion of the lung caused by the inhalation of a variety of organic dusts. In human cases, the histology of EAA is characterized by the constellation of the findings; (1) chronic inflammatory infiltrates along small airways, (2) diffuse interstitial infiltrates of lymphocytes and (3) scattered small non-necrotizing granulomas. Scattered giant cells often containing cholesterol clefts, foci of organizing pneumonia and bronchiolitis obliterans, and features of obstructive pneumonia with foamy histiocytes in airspaces are also seen. Pathogenetically, this type of hypersensitivity reaction was thought to be mediated by immunoglobulins, lymphokines, and immune effector cells. More recent evidence, however, suggested an implication of cell-mediated immune reaction. At any rate, the pathogenetic mechanism of EAA still remains to be clarified.

Several experimental models of EAA[1,2] have been reported using different animal species and different antigens with different methods and time sequence of exposure. Previously, one of the investigators in our laboratory[2,3] has clearly shown that similar reactions could be induced by subcutaneous sensitization and subsequent inhalational exposure, using bacterial α amylase as an antigen in guinea pigs and mice. Considering that subsets of T lymphocytes can be identified by commercially available monoclonal antibodies in rats, and that horseradish peroxidase which is sufficiently purified is easily obtained, we decided to use rats as an animal and horseradish peroxidase as an antigen. In our model, granuloma formation is prominent and histological changes are quite similar to those of the human diseases. Herein, we describe our method of producing an experimental model of EAA.

Eighteen female Wistar rats (Clea Japan, Inc.), 10-12 weeks old and weighing between 180 and 200 gm, were utilized in this study. They received food and water ad libitum. The sensitizing antigen consisted of 4 mg horseradish peroxidase (HRP, Sigma, type IV) dissolved in 1.0 ml of 0.9% normal saline solution, and was mixed with 1.0 ml of complete Freund's adjuvant (CFA), in a standard mixing syringe until a creamy emulsion is obtained. Twelve rats were sensitized by subcutaneous injection of this emulsion into two toe pads with a total volume of 50 μl, five times every other day. Three weeks after the last subcutaneous sensitization, 50 μl of HRP in the concentration of 4 mg/ml dissolved in saline solution was given intraperitoneally as a booster. One week

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after the booster injection, four rats were exposed simultaneously to 4 ml of aerosolized HRP saline solution in a concentration of 1 mg/ml for approximately 30 minutes, twice a day. This inhalational challenges were repeated every other day for the total exposure of 3 (group 1), 5 (group 2), and 10 (group 3). Rats were sacrificed at 24 hours, 1, and 2 weeks after the last inhalational challenge. Controls included animals unsensitized and unchallenged, and those sensitized with CFA but unchallenged. Lungs were fixed by intratracheal infusion of 10% buffered formalin, and sliced to obtain the largest cut surface in the mid-saggital plane. Tissue blocks were processed routinely and 4 μm thick sections were stained with hematoxylin-eosin, and elastica van Gieson (EVG) stain, respectively.

In group 1, bronchial epithelium were hyperplastic at 24 hours. Lymphocytic aggregates with occasional lymph follicles were already present in bronchial walls with surface epithelial change of bronchus-associated lymphoid tissue (BALT). There was a patchy thickening of respiratory bronchioles and alveolar septae and infiltration of lymphocytes, histiocytes and occasional neutrophils, while cell infiltration was seldom noticed around terminal bronchioles. In adjacent alveolar spaces, histiocytic infiltration and occasional discrete granulomas as well as Masson bodies were present. In EVG-stained sections, elastic fibers along alveolar walls were revealed to be distorted and fragmentary. These lesions were diffusely and evenly distributed. Type II cells in adjacent parenchyma were plump. Around small vessels, interstitial edema and many lymphocytes were present. At 1 and 2 weeks after the last exposure, the lesions produced were similar to those of 24 hours. Sometimes, alveolar spaces were still filled with foamy histiocytes. In the group 2 and 3, similar changes had been induced and were much severer than group 1. In places, airways, from respiratory bronchioles through alveolar ducts, were occluded by spindle shaped cells and were associated with a mild degree of interstitial fibrosis (Fig. 1). This change was reminiscent of bronchiolitis obliterans and organizing pneumonia. At 1 week, small, non-necrotizing granulomas were scattered only in the alveolar parenchymal portions, but not in peribronchial or peribronchiolar

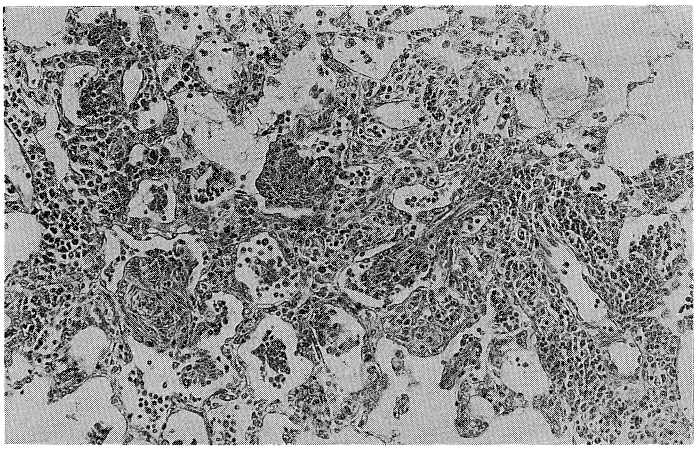

Fig. 1. Pulmonary changes of the group 3 rat at 2 weeks after the last inhalation. Interstitium is markedly thickened with lymphocytes. Masson bodies are prominent.

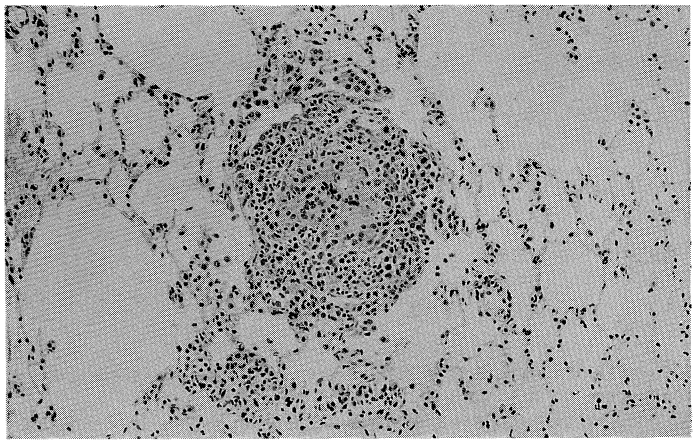

Fig. 2. Granuloma formation in the group 3 rat at one week after the exposure.

regions (Fig. 2). They were both interstitial and intraalveolar in location, and were composed of epithelioid histiocytes, Langerhans or foreign body type giant cells, and lymphocytes. At 2 weeks, granulomas were increased in number and multinucleated giant cell became predominant. On occasion, areas of interstitial fibrosis were associated with dilatation of adjacent alveolar spaces. Lungs of any control groups were essentially normal.

To date, many animal models were produced to study the pathogenesis of EAA. Mice, rats, dogs, rabbits, guinea pigs and monkeys were utilized in combination with various antigens such as fungi, serum proteins, albumin, bacterial α amylase, and horseradish peroxidase. As stated above, we were successful to produce EAA in the rat using horseradish peroxidase. Animals with ten exposures were most suitable in producing granulomatous lesions. In our model of EAA, there are some advantages over other models produced by some authors. Since horseradish peroxidase was used, the antigen could be traced directly by a reaction with 3-3'-diaminobenzidine-tetrahydrochloride (DAB). Histology of this model is much closer to that of human EAA. Additionally, monoclonal antibodies to some subsets of T and B rat lymphocytes are commercially available. For these reasons we concluded that our procedures provide a good animal model for elucidating the pathogenesis of EAA. Immunohistochemical study using this model will be pursued in the future.

Lastly, we must address about so-called adjuvant granulomas. Animals receiving toe pad injections of CFA may develop a few scattered granulomas (called adjuvant granulomas) in lung parenchyma, often peripheral in distribution. The liver, kidneys, and the other organs may have similar granulomas. Granulomatous lesions of the lungs in our model which were located at centriacinar zone differ from and deny the possible implication of adjuvant granulomas, because granulomas were absent in other organs such as liver, kidneys, or spleen, and because no granulomas were seen in any organs of control animals with adjuvant injection but no challenge exposure. The molecular weight of HRP is so small that it may reach the periphery of the lung. This may explain why we succeeded in producing granulomatous lesions in the centriacinar zone.

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