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BRIEF COMMUNICATION

Animal model of sensitization by inhalation

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SUMMARY

Groups of rats exposed to daily inhalation challenge with aerosolized pigeon serum developed precipitating antibody within 2 weeks and definitive granulomatous inflammatory changes in the lung after 7 weeks of exposure. The dissociation of the two responses to an inhalation challenge indicate that the rat model may serve for screening of the various inhalant antigens for their sensitizing potential, and for investigation of the contributory role of some of the factors involved in the pathogenesis of hypersensitivity pneumonitis.

INTRODUCTION

The inhalation route of sensitization is increasingly being associated with the development of hypersensitivity pulmonary reactions in man. In addition to bronchial asthma, hypersensitivity pneumonitides in their varied forms have been shown to develop after repeated inhalation of organic materials (Pepys, 1969; Fink *et al.*, 1968). The immunologic processes involved in the pathogenesis of the hypersensitivity pneumonitides are not entirely clarified, but both the cellular and humoral components of the immune system are believed to be activated (Moore *et al.*, 1974).

Animal models are needed to study the antigenic potential of the growing number of suspected inhalant antigens and to assess the possible associated impairment of the pulmonary structures. Previous findings with rats have shown that the antigenicity of inhaled allergens, as represented by development of serum precipitins, differs from their antigenic potency after subcutaneous or intramuscular sensitization. It was found, for instance, that inhalation exposure to an extract of pigeon droppings sensitized the animals quite effectively while purified bovine serum albumin failed to do so (Fink, Hensley & Barboriak, 1970). Since it has been suggested that pigeon droppings contain an immunoglobulin derived from serum (Edwards, Barboriak & Fink, 1970), we decided to study whether whole pigeon serum (PS) could also sensitize experimental animals by the inhalation route. The use of PS rather than a crude extract of pigeon droppings would reduce the food-induced variability in antigenic composition of the sensitizing material and eliminate the possible effect of microbial contamination usually found in the fecal matter. The length of time needed for the development of a demonstrable humoral response, the possible enhancement of such a response by pretreatment with complete Freund's adjuvant, as well as the extent of pulmonary damage produced by repeated inhalation exposures were also investigated.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 200–250 g each were housed in stainless steel cages, six to eight animals to a cage, in an animal room separate from either the preparation or insufflation of antigens. Pigeon blood was obtained by exsanguination of commercially obtained pigeons of either sex using cardiac puncture. The pooled serum was stored in 50-ml aliquots at −20°C until needed for insufflation.

Three different series of similar experiments were carried out. Each series consisted of fifteen to twenty-four animals. Ten to sixteen animals in each series were pretreated with weekly intramuscular injections of 0·25 ml of an emulsion prepared

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using equal volumes of Freund's complete adjuvant (FCA) (Difco Labs, Detroit, Michigan) and saline. The remaining rats received an intramuscular injection of 0·5 ml saline following the same weekly injection schedule.

After 4 weeks, one half of the animals in the adjuvant-pretreated groups and the animals injected with saline were insufflated with PS. The rats were placed in airtight box and exposed to aerosolized serum produced by a DeVilbiss Model 65 ultrasonic nebulizer (DeVilbiss Corporation, Somerset, Pennsylvania). The exhaust was connected to a flask of water to prevent contamination of the outside air (Barboriak, Sosman & Reed, 1965). Between 25 and 30 ml of the serum was insufflated into the box over a 1 h period each day for 23 days (Series I), 35 days (Series II) or 49 (Series III). Small blood samples were taken from the tail at weekly or bi-weekly intervals and the serum tested for the presence of precipitins to the pigeon serum using a modification of the Wadsworth technique (Barboriak *et al.*, 1965).

At the termination of the individual series the animals were anaesthetized with sodium pentobarbital (Diamond Laboratories, Des Moines, Iowa), exsanguinated by cardiac puncture and the lungs removed. The lungs were inflated by delivering 6% glutaraldehyde under 40 cm of water pressure through a tracheal cannula and were fixed in the same solution. The fixed lungs were examined after sectioning and staining with haematoxylin and eosin.

RESULTS

The data on development of precipitating antibodies against pigeon serum are summarized in Table 1. The precipitin pattern was graded on the basis of line, intensity and their number as described previously (Barboriak *et al.*, 1973). A weak precipitin reaction against PS was first detected in two animals pretreated with CFA and insufflated with PS for 7 days. Multiple precipitin lines were found at 14 days for both the adjuvant-pretreated and non-pretreated groups. However, those pretreated with the adjuvant developed more numerous and stronger lines. The animals pretreated with adjuvant but not insufflated failed to develop any precipitating antibodies to PS. Following 21 days of insufflation, the immunodiffusion patterns tended to stabilize and additional insufflation with PS did not result in production of additional precipitin lines or an increase in the intensity of the precipitin lines. No precipitins against pigeon serum developed in animals pretreated with CFA only and tested at weekly intervals.

TABLE 1. Development of precipitins in sera of animals exposed to pigeon serum by repeated inhalation

	Duration of inhalation exposure (days)				
	7	14	21	35	49
PS	(5) *0	40% W (5) 60% M	25% M (12) 66% S	(5) 100% S	8% W (12) 25% M 66% S
CFA and PS	(6) 30% W	30% W (6) 16% M	14% M (14) 78% S	(5) 100% S	19% M (16) 81% S

PS = Pigeon serum. Precipitin reaction: W = weak, M = medium, S = strong.
* Number of sera tested at each interval is shown in parentheses.

Histological examination of the lungs of animals exposed to PS for 49 days showed mild to severe histiocytic granulomatous inflammation (Fig. 1) with occasional giant cells. In animals receiving the adjuvant only, the histologic changes were limited to rare minute foci of chronic interstitial pneumonitis with few perivenous eosinophils. In the animals pretreated with adjuvant and exposed to PS the foci of interstitial pneumonitis were more numerous; and perivascular infiltration of lymphocytes and plasma cells were also observed (Fig. 2). Similar but less differentiated changes were seen in the lungs of animals killed after shorter exposures to pigeon serum. However, some acute focal alveolitis was observed in animals pretreated with complete Freund's adjuvant and exposed to PS for 21 days.

DISCUSSION

The repeated inhalation exposure of rats to pigeon serum reproduced several features of hypersensitivity

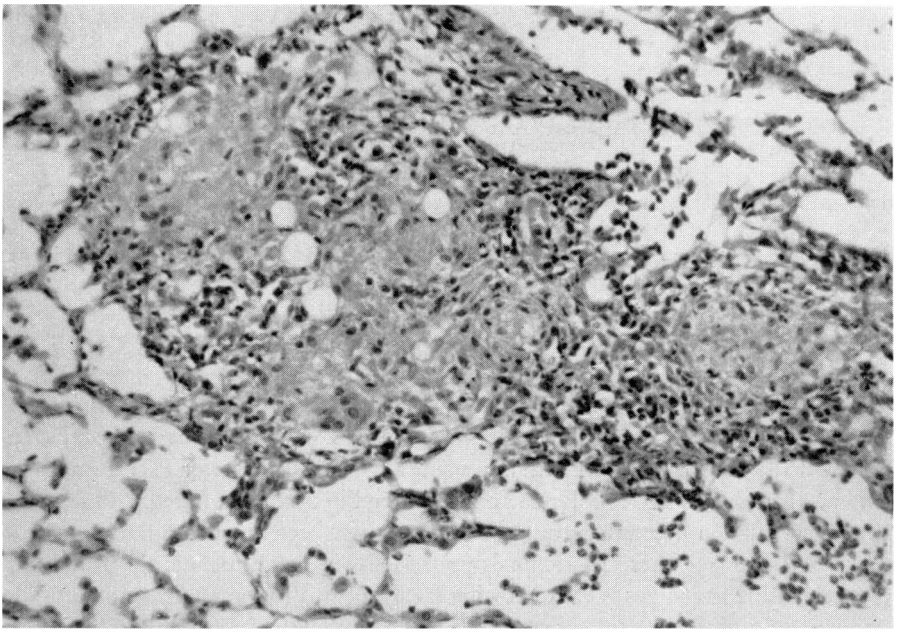

FIG. 1. Histiocytic granulomas in the lung of the rat exposed to pigeon serum by inhalation. (Haematoxylin and eosin; magnification × 112.)

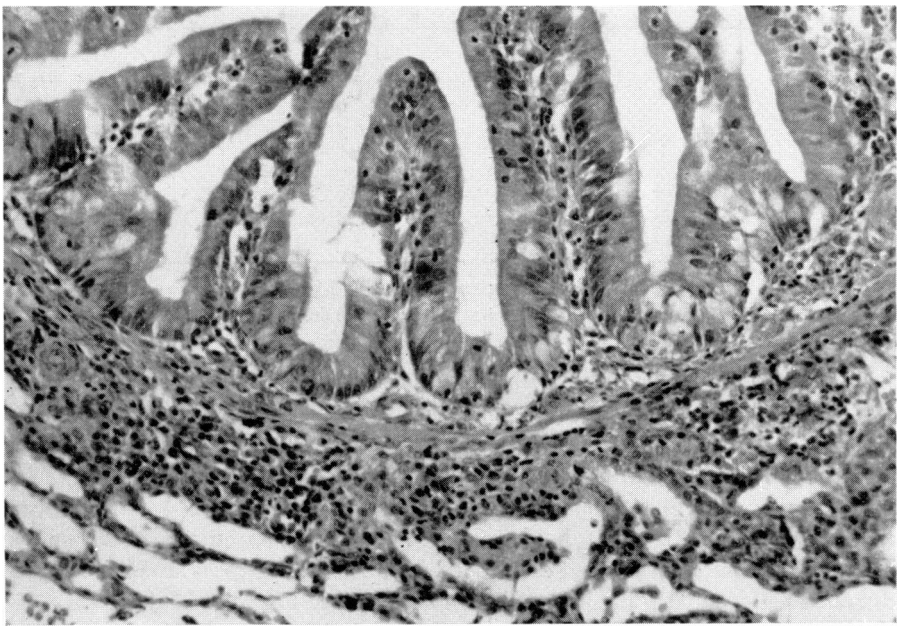

FIG. 2. Perivascular lymphocytic and plasma cell infiltration in the lung of the rat pretreated with complete Freund adjuvant and exposed to pigeon serum. (Haematoxylin and eosin; magnification × 112.)

pneumonitis found in the human counterpart of this disease. Precipitating antibody to the inhaled material was reproducibly detected within 14 days, suggesting that the rat may serve as an inexpensive animal model for testing the immunogenicity of the various inhaled organic materials.

Some of the histologic features of hypersensitivity pneumonitis as seen in the human lung, i.e. development of granulomatous lesions, interstitial pneumonitis and lymphocytic infiltration of perivascular spaces (Hensley *et al.*, 1969) were also observed in the present rat model. Furthermore, these lesions were produced without the use of complete Freund's adjuvant, which occasionally was reported to produce pulmonary damage on its own (Richerson, Cheng & Bauserman, 1971) thus complicating the interpretation of the pulmonary lesions. Interestingly, the granulomatous lesions in the lung developed after a long-term exposure to water-soluble antigens, suggesting that such lesions do not always require the presence of unabsorbable particulate matter (Reed, 1974).

The findings of the present study do not allow any definite conclusions on the possible causal relationship between the production of precipitating antibody to pigeon serum and development of pulmonary lesions. Our previous findings have shown that a repeated exposure to an extract of pigeon droppings led to pulmonary lesions prior to or concommitant with the development of precipitins (Fink *et al.*, 1970). In the present study there was considerable time lag between the appearance of precipitins (2 weeks) and the lung damage (7 weeks). It would seem therefore that the association between the two responses to inhalation challenge may be an indirect one; and that a proper selection of inhalation antigens may help in clarifying the role of the individual factors involved in the pathogenesis of hypersensitivity pneumonitis.

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$$Z = \frac{p}{1-p},$$

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$$Z = \log \frac{p}{1-p}.$$