

BRIEF COMMUNICATION

The lungs as the site of delayed-type hypersensitivity reactions in guinea pigs

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Guinea pigs immunized by a single intramuscular injection of dry killed tubercle bacilli were exposed to purified protein derivative (PPD). Respiratory frequency and airway resistance showed no obvious changes immediately after inhalation of PPD, but respiratory frequency increased gradually after 6 hours and reached a maximum around 24 to 48 hours. At this time respiratory frequency was 1.5 to 2.5 times more than the control and declined to the control values over the next 48 to 72 hours. The lungs showed gradually increasing hepatization-like changes which reached a maximum around 24 to 48 hours and gradually subsided within 7 days after inhalation of PPD. Histologically, polymorphonuclear cells were predominant initially but mononuclear cells became dominant in 24 to 48 hours. Considerable thickening of the interalveolar septa was observed. Guinea pigs passively sensitized with splenic cells showed identical changes although less marked.

Delayed-type hypersensitivity has been studied most commonly using skin as the site of the reaction, while studies of delayed-type hypersensitivity reactions in the lung have been very few.¹⁻³ Consequently, presented here is a brief report on delayed-type hypersensitivity reactions in the lungs of guinea pigs.

Guinea pigs weighing approximately 300 grams were immunized by a single intramuscular injection of 0.5 mg. of dry killed tubercle bacilli suspended in paraffin oil. Eight weeks after immunization the animals were tested with an intracutaneous injection of 0.1 μ g of purified protein derivative (PPD). Those animals showing a strong skin reaction within 24 hours were chosen for the following studies. The animals were placed in a chamber, and PPD in a concentration of 0.2 mg. per milliliter was aerosolized through a nebulizer of the Vaponephrine type into the chamber for approximately 20 minutes. The animals showed no sign of acute respiratory distress either during the inhalation or immediately thereafter. Respiratory changes were measured by the oscillation method⁴ using a pneumotachograph and a body plethysmograph every 3 hours for the first 24 hours, every 12 hours for the following 24 hours, and then every 3 days for the next 30 days. The principle of the oscillation method involves the use of an imposed pressure or volume change, sine wave in form and at a frequency such that the mechanical resistance is zero. Resistance measurements

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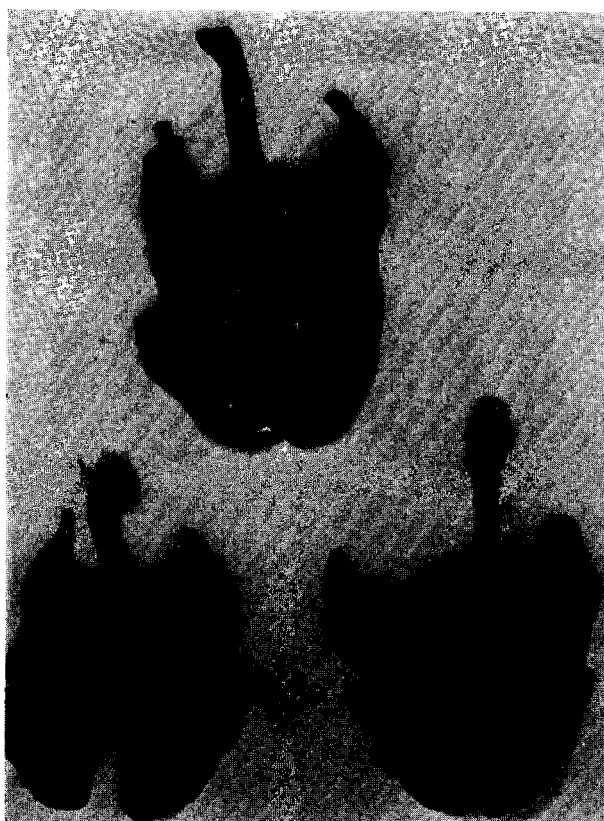


FIG. 1. The macroscopic appearance of the lungs of guinea pigs 24 hours after inhalation of PPD. **Top**, Normal control; **lower right**, actively sensitized; **lower left**, passively sensitized. Lungs of actively and passively sensitized guinea pigs showed hepatization-like changes consisting of an edematous brownish induration.

were obtained from the oscilloscope vectors of flow and pressure. At least two animals were killed after each measurement for pathologic studies of the lungs, totaling 60 guinea pigs. Respiratory frequency and airway resistance showed no obvious changes immediately after inhalation. However, respiratory frequency and body temperature increased gradually after 6 hours and reached a maximum around 24 to 48 hours, at which time the respiratory frequency was 1.5 to 2.5 times control values and the body temperature had increased by 1.5° C. to 3.0° C. These measurements gradually returned to the control values over the next 48 to 72 hours. No significant changes of airway resistance were noted throughout the study, however.

The lungs showed scattered, gradually increasing, hepatization-like changes consisting of uniform edematous brownish induration with irregular limits or a nodular appearance on macroscopic examination. These changes became apparent in 6 hours, reached a maximum around 24 to 48 hours, and gradually subsided within 7 days after inhalation of PPD (Fig. 1). Histologically, polymorphonuclear cells were predominant initially after antigen exposure, but mono-



FIG. 2. Histologic appearance of the lung of an actively sensitized guinea pig 24 hours after inhalation of PPD. Predominantly small and large mononuclear cells infiltrated into the interstitial spaces in the lungs, producing a considerable thickening of the interalveolar septa. Macrophages and polymorphonuclear cells were also seen.

nuclear cells became gradually dominant, and at the maximum infiltrative changes in 24 to 48 hours mononuclear cells were playing the major role. At that time there were large collections of mononuclear cells, mostly lymphocytes, and some polymorphonuclear cells in peribronchial and perivascular areas. Predominantly small and large mononuclear cells also infiltrated into the interstitial spaces in large areas of the lungs, producing a considerable thickening of the interalveolar septa. Eosinophils were occasionally observed. A marked dilatation of the capillaries was also seen in the thickened alveolar septa. Small hemorrhagic areas were also observed occasionally. Inflammatory edematous changes were noted in the interstitial and the peribronchial tissues in the heavily damaged areas. The histological changes, although minimal, were already

seen in 3 hours, became more noticeable around 6 hours after antigen exposure, and reached a maximum in 24 to 48 hours. Seven to 10 days later such histologic changes were no longer marked. These histological findings are quite compatible with the cutaneous changes in sensitized guinea pigs after intracutaneous injection of PPD.⁵

To confirm that these respiratory changes definitely represent delayed-type hypersensitivity reactions, passive sensitization studies were carried out. One milligram of living BCG suspended in saline was injected intravenously into guinea pigs previously sensitized with dry killed tubercle bacilli. Two weeks after injection the spleen, which was enlarged granulomatously, was excised, and splenic cells were separated by passage through a 200 mesh filter and suspended in medium 199.⁶ Four to 8×10^8 cells were injected intravenously into 20 normal guinea pigs. These passively sensitized normal guinea pigs were exposed to aerosolized PPD immediately after injection and were subjected to studies identical to those described previously. Identical results both physiologically and histologically, were obtained in all as with actively sensitized guinea pigs. Although much less marked in some, they indicated that these described changes were due to delayed-type hypersensitivity reactions (Fig. 2). No such reaction was observed in the lungs of unsensitized control guinea pigs exposed to the inhalation of PPD or in the lungs of sensitized animals not exposed to the inhalation of PPD. Thus it appears that a delayed-type hypersensitivity reaction analogous to the tuberculin skin reaction can be produced in the lung by the inhalation of antigen.

One, 10, 100 or 2,000 μ g of PPD was intravenously injected into 2 of 16 guinea pigs which were either actively or passively sensitized. Surprisingly enough, no obvious respiratory changes were observed in any case. Body temperature increased 0.2° C. to 0.8° C. by the fifth hour but fell to the initial values in 24 hours. One to 2 ml. of sera obtained from sensitized guinea pigs were intravenously injected into 6 normal guinea pigs and PPD was aerosolized, but no respiratory changes were noted.

Previously, a 1:1 concentration of old tuberculin was inhaled by sensitized guinea pigs.³ Some of the animals presented labored breathing during and immediately after inhalation. Respiration was slowed with prolongation of the expiratory phase and an increased airway resistance. Four of 30 guinea pigs died apparently with anaphylactic shock during inhalation. Their lungs showed hyperinflation. The bronchi presented marked spastic changes. Guinea pigs surviving such acute respiratory distress apparently returned to normal within one hour after cessation of inhalation. Respiratory frequency, however, gradually increased after 6 to 12 hours and reached a maximum in 24 to 48 hours. Physiologic and pathologic changes were identical with PPD inhalation. Histologic changes were more striking, and in addition there was an exudate in alveolar spaces. Since old tuberculin is a crude preparation and a mixture of polysaccharides and proteins, immediate and delayed-type hypersensitivity reactions appeared to be produced sequentially after inhalation of old tuberculin. Some of the guinea pigs receiving sera from sensitized guinea pigs showed temporary respiratory distress immediately after inhalation of old tuberculin.

Recently similar studies were carried out with *Candida albicans* and identical results were obtained.⁷

It is concluded from these studies that delayed-type hypersensitivity reactions can be produced in the lungs after inhalation of antigen, and it appears that the protein fraction is more related to delayed-type hypersensitivity reactions caused by tubercle bacilli and *Candida albicans*. The clinical implications are under study.

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