PAPER NO. 7

APPLICATION OF ANIMAL MODELS FOR IMMEDIATE AND DELAYED PULMONARY HYPERSENSITIVITY: CHARACTERISTICS OF DELAYED REACTIONS TO TUBERCULIN PROTEIN

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INTRODUCTION

A method was described recently for monitoring delayed-onset pulmonary hypersensitivity reactions in guinea pigs (Karol et al., 1981). To induce sensitivity, animals were injected with Freund's complete adjuvant. Pulmonary reactivity was demonstrated three to five weeks later by bronchial provocation challenge with aerosolized tuberculin protein derivative (PPD). Because onset of reaction occurred typically 9-12 hr following challenge, it was necessary to challenge animals in the "heads-only" inhalation chamber, then remove them from the chamber immediately following challenge and place animals in individual whole body plethysmographs for long-term monitoring. In this way, guinea pigs were free to move about during the ensuing 24 hr while respiratory responses were being monitored.

However, the transfer of animals from the "heads-only" to the whole body plethysmographs resulted in loss of measurements for about 1 hr. In order to provide continuous measurements of respiratory rate, beginning prior to challenge and continuing for 24 hr, a system was established based on a recent report (Zelenak et al., 1982).

The current study describes characteristics of the continuous respiratory response to PPD in guinea pigs. In addition, experiments were performed to determine the influence of several factors on the pattern of delayed-onset responses. Such factors included: repeated inhalation challenge with homologous antigen, time interval between challenges, and history of previous pulmonary reactions to heterologous antigens. Results indicated that respiratory responses to PPD were influenced by previous exposure only to homologous antigen.

METHODS

ANIMALS

Male, English smooth-haired guinea pigs (Hilltop Laboratories, Scottdale, Pennsylvania) weighing 250-300 g were used for sensitization.

SENSITIZATION AND ELICITATION OF PULMONARY RESPONSE

Animals were sensitized to Freund's complete adjuvant (FCA, Calbiochem) by injection of 50 µl of FCA-saline emulsion (1:1 v/v) into each of four footpads (200 µl/animal, Karol et al., 1981). Three weeks later, animals were placed in individual whole body plethysmographs for inhalation challenge. Challenge was performed by generating known atmospheres of PPD aerosol into a 2.1 liter primary chamber. Four individual plethysmographs (each 2 liter) were connected to the central primary chamber via small bore teflon tubing (Zelenak et al., 1982). Aerosols were generated into the primary chamber by a Pitt #1 generator (Wong and Alarie, 1982). Aerosol was pulled from the primary chamber into the individual plethysmographs at 2 liter/minute for each chamber. Total airflow through the system was 20 liter/minute.

MEASUREMENT OF THE RESPONSE

A Statham PM 197 differential pressure transducer was attached to each plethysmograph to detect changes in pressure with each respiration (Karol et al., 1981). The signal from the pressure transducer was displayed on a Gould 200 oscillograph and additionally fed into a Gould Biotachometer set in the averaging mode. The output of the Biotachometer was displayed on a recorder to monitor the average breathing frequency of each animal.

EXPERIMENTAL

REPEATED INHALATION CHALLENGE, CONTINUOUS RESPIRATORY MONITORING

Initial inhalation challenge of FCA-injected animals with PPD aerosol resulted in respiratory reactions beginning 9-12 hours following challenge (see Figure 1A). Animals were allowed a 2 week rest period, then rechallenged in an identical manner with PPD aerosol. This pattern of challenge followed by 2-week rest was repeated for a total of 4 challenges. Response to second and subsequent challenges followed a pattern different from that seen after first challenge (Figure 1A). Repeated challenge resulted in a response by 2 hours which was maximal at 4-5 hours. In addition, there was more rapid recovery from response when compared to that observed following first challenge. The typical respiratory reactions to first,

second, and third challenges of guinea pigs are shown in Figure 1A. These responses were indistinguishable from those observed previously in animals challenged using a "heads only" chamber (Stadler and Karol, 1982) rather than the whole body plethysmograph. The mechanism underlying the respiratory pattern was investigated in experiments described below.

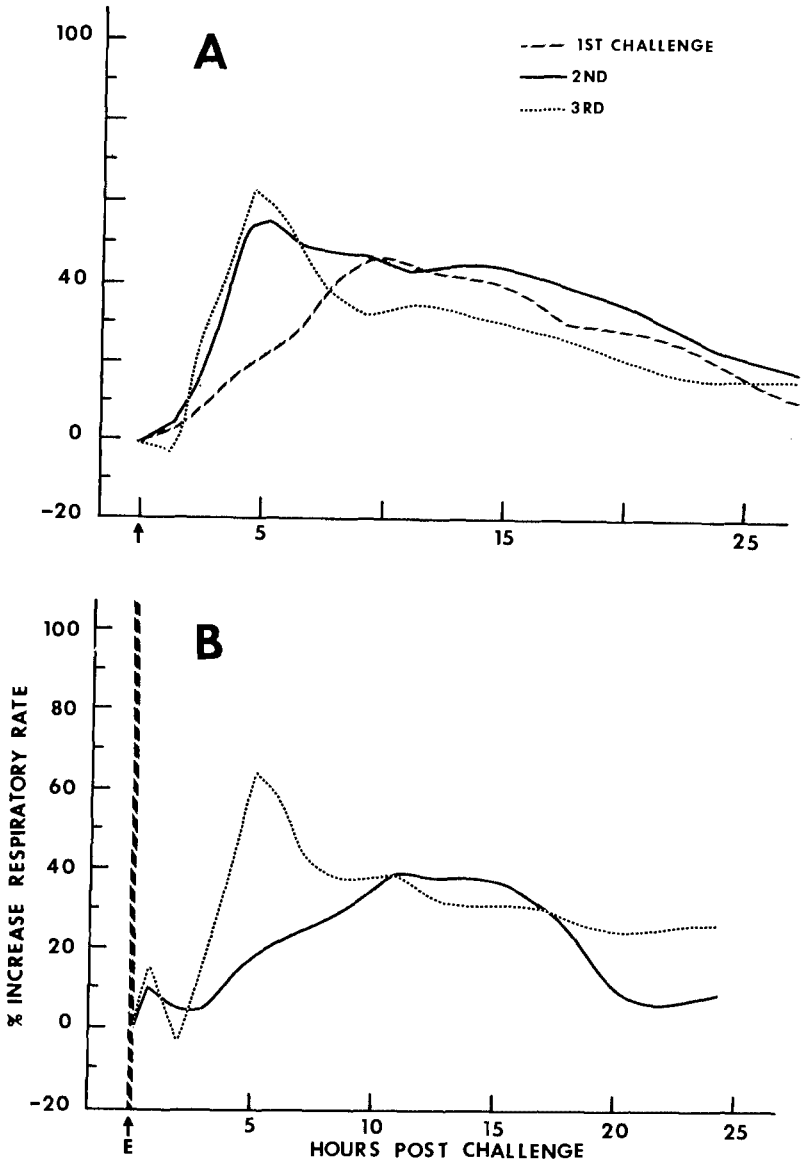

Figure 1. Respiratory reactions to repeated bronchial provocation challenge.

A. Average response of 6 guinea pigs to each of 3 bimonthly inhalation challenges with PPD aerosol.

B. Average response of 6 guinea pigs to OA aerosol (first challenge) followed by bimonthly challenges with PPD aerosol (second and third challenges). E: time of exposure.

EFFECT OF REST PERIOD BETWEEN CHALLENGES

Two groups of guinea pigs were sensitized to PPD by footpad injection with FCA. Three weeks later, all animals were challenged by inhalation of PPD aerosol and displayed the typical delayed-onset pulmonary reactions to first and second challenges (Table 1). Guinea pigs were then divided into two groups. Group A was again challenged with PPD aerosol two weeks following second challenge. This group responded to third challenge with an increase in respiratory rate having maximum intensity 5-6 hours post-challenge. The second group (Group B) was allowed a 6 week rest, rather than the 2 week rest, between second and third challenge. Response of Group B to third challenge was maximal 9-10 hours post challenge. In this regard, it resembled a typical "first challenge" response. Moreover, recovery from third challenge in Group B was prolonged and typical of that seen after first challenge of animals (Figure 1A) rather than typical of third challenge response. The intensity of respiratory responses to each challenge was essentially unchanged and typically averaged 50% (see also Karol et al., 1981; Stadler and Karol, 1982).

TABLE 1. EFFECT OF EXTENDED REST PERIOD ON THE PATTERN OF RESPIRATORY RESPONSE TO PPD INHALATION CHALLENGE

Guinea Pig Group	Time of Response				
	Challenge #1		Challenge #2		Challenge #3
A (N=6)	9-10 hours*	2 week →	5-6 hours	2 week →	5-6 hours
B (N=5)	9-10 hours	2 week →	5-6 hours	6 week →	9-10 hours

* Time of maximal respiratory reaction following challenge.

EFFECT OF ANIMAL AGE ON RESPIRATORY RESPONSE

The possible effect of the age of the animal on the onset of the respiratory response was explored. At the time of initial immunization, guinea pigs weighed 250-300 g. At first challenge they typically weighed 500 g (Table 2). One month later, at third challenge, they were 600 g. In order to determine if maturity (assessed by weight) of the animals at the time of second and third challenge contributed to the earlier response to challenge, the following experiment was performed. A group of guinea pigs was immunized by injection with FCA, and three weeks later, each animal was skin tested with PPD (Karol et al., 1981) to assess sensitivity. Eight animals displaying the most severe skin responses were then divided into 2 groups. Group C was challenged shortly thereafter with PPD aerosol. These animals weighed approximately 500 g at the time of this first inhalation challenge. All animals in

Group C responded to PPD challenge with respiratory reactions 9-10 hours following challenge. Upon second challenge two weeks later, response in these animals occurred 5-6 hours following challenge. Two weeks later, response to third challenge occurred 4-5 hours post-challenge (Table 2).

TABLE 2. EFFECT OF AGE ON ONSET OF RESPIRATORY RESPONSE TO PPD

Weight at Sensitization	Time of Response			
	Challenge #1	Challenge #2	Challenge #3	Challenge #4
Group C				
250-300 g (N=4)	9-10 hours* (500 g)	5-6 hours	4-5 hours (600 g)	4-5 hours
Group D				
250-300 g (N=4)	---	---	Challenge #1	Challenge #2
			9-10 hours (600 g)	4-5 hours

* Time of maximum response following inhalation challenge.

The second group of animals (Group D) received first inhalation challenge 6 weeks after the skin test. (This was the period of third inhalation challenge for Group C animals). Guinea pigs in Group D were 4 weeks older than those in Group C at the time of first challenge. Group D animals responded to first inhalation challenge with an increase in respiratory rate 9-10 hours following challenge (Table 2). Second inhalation challenge, performed two weeks later, resulted in respiratory reactions 4-5 hours following challenge. It was concluded from these experiments that the earlier response typically seen following second and third inhalation challenges was not caused by maturation of animals but rather was a retest response of the lung. Experiments were then performed to further define the retest response.

EFFECT OF A PREVIOUS RESPIRATORY REACTION TO HETEROLOGOUS ANTIGEN

The possibility existed that as a result of a first hypersensitivity reaction in the lung, a state of "bronchial hyperreactivity" was established which, in turn, affected subsequent pulmonary responses to heterologous inhaled antigens. To evaluate this supposition, the following experiment was performed (Table 3).

TABLE 3. PROTOCOL TO EVALUATE EFFECT OF A PRIOR HETEROLOGOUS PULMONARY SENSITIVITY REACTION ON ONSET OF PULMONARY RESPONSE TO PPD

Day 1	Immunization with Freund's Complete Adjuvant (FCA)
Day 8	Injection with ovalbumin (OA)
Day 22	Inhalation challenge with OA
Day 36	Inhalation challenge with PPD
Day 48	Inhalation challenge with PPD

A set of 6 guinea pigs was immunized by injection with FCA. One week later, animals were injected intraperitoneally with 1 mg ovalbumin (OA) to establish sensitivity to a second antigen. Two weeks following this injection a bronchial response to OA was elicited by bronchial provocation challenge with OA aerosol (Karol et al., 1978). Each of the animals responded within 2-3 minute of challenge with a severe anaphylactic reaction. Animals developed cyanosis and respiratory rates increased by more than 100% (see Figure 1B). This response was similar to that previously observed in guinea pigs sensitized as a result of inhalation exposure to OA (Karol et al., 1978).

Following this first respiratory response, animals were allowed a 2 week rest period prior to second bronchial challenge. For second challenge, however, a heterologous antigen was used, PPD. In this way, distinction was made between second bronchial response to the same antigen (retest response) and second bronchial response where different antigens provoked first and second response.

Results of these repeated challenges are shown in Figure 1. Bimonthly bronchial provocation challenge of guinea pigs with PPD aerosol produced the typical pattern of retest reaction (Figure 1A). First challenge elicited a response 9-12 hours following exposure; second and third challenges with the same antigen resulted in response with both more rapid onset and more rapid recovery.

The response in animals challenged with the set of heterologous antigens is shown in Figure 1B. Initial response to OA was immediate and severe. Second bronchial challenge performed two weeks later elicited respiratory reactions typical of those observed upon first challenge of animals with PPD. Two weeks later, inhalation rechallenge with PPD resulted in a retest response characterized by earlier onset of reaction and earlier wane of response. Results of these experiments clearly indicated that a theory of "bronchial hyperreactivity" could not be implicated as a causative factor for more rapid reaction and recovery observed following repeated challenges with homologous antigen in the lung.

DISCUSSION

Delayed-onset pulmonary reactions have been reported following exposure to a wide variety of environmental and industrial allergens. Reactions are characterized by onset more than one hour after exposure. Frequently reactions occur from 4-24 hours following exposure. Pathogenesis of such reactions remains unclear.

In the animal model described here, repeated inhalation challenge of guinea pigs with PPD aerosol resulted in a pattern of respiratory response different from that seen upon first bronchial provocation challenge. Second and third challenges elicited reactions with both earlier onset and earlier wane of response. This response pattern was produced consistently when animals were rechallenged with PPD antigen at 2-week intervals. However, by extending from 2 to 6 weeks the time betwen PPD challenges, the respiratory response to challenge resembled that seen upon first challenge. These experiments indicated that time between respiratory response affected onset of delayed pulmonary reactions.

Other factors were evaluated for possible effect on time of respiratory response. Varying the age or maturity of animals did not alter the response pattern. Regardless of the age of the guinea pigs, response to first challenge always occurred several hours later than response to subsequent challenges (Table 2).

Several case reports have indicated that with repeated bronchial reactions, onset of response occurred earlier following exposure. The possible development of lung hyperreactivity as a result of repeated pulmonary reactions was evaluated by inducing in guinea pigs a severe allergic pulmonary reaction to ovalbumin. Two weeks following the severe reaction, a pulmonary response to PPD was induced and found to be identical to the response routinely observed upon first PPD challenge of FCA-sensitized animals. Moreover, second challenge with PPD produced the typical PPD-retest response. Together, these experiments led to the conclusion that previous challenge of animals with OA had no effect on the pattern of reactions elicited by PPD.

In summary, earlier onset of pulmonary sensitivity reactions was observed when guinea pigs were challenged bimonthly with homologous antigen. Recognition of factors influencing onset of delayed lung reactions may help elucidate mechanisms underlying delayed-onset reactions observed following exposure to many industrial and environmental agents.

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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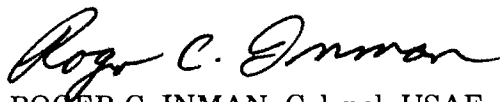

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Papers were presented covering current methods in immunotoxicology, immune mediated effects of specific chemicals, comparative effects of inhaled toxicants in different species, approaches to the mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related chemicals, and toxicity of hydrocarbons and hydrocarbon metabolites.

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PREFACE

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