

Sensitization to ragweed pollen in *Bordetella pertussis*-infected or vaccine-injected mice

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Mice infected by intranasal instillation of Bordetella pertussis then exposed to an aerosol of ragweed pollen became sensitized to the pollen as shown by development of anaphylactic reactions following intravenous injection of pollen extract. Sensitivity could also be demonstrated by intraperitoneal injection of mice with killed pertussis vaccine as well as by intranasal instillation of living culture. Sensitivity toward pollen extract developed when mice were infected with B. pertussis or when injected with pertussis vaccine during the late summer when ragweed pollen was present in the air.

We have shown in earlier experiments¹ that when mice were infected by intranasal instillation of *Bordetella pertussis* and then subjected to the aerosol of an aqueous solution of human serum albumin, they became sensitized to the albumin. This was demonstrated by development of anaphylactic reactions following intravenous injection of albumin. It was also possible to sensitize mice to albumin by subjecting them to an albumin aerosol after injection of pertussis vaccine intraperitoneally.

In this report we describe experiments conducted to determine whether *B. pertussis*-infected or pertussis vaccine-injected mice become sensitized after exposing them to atomized giant ragweed pollen (*Ambrosia trifida*). This material can be given without subjecting the mice to the high humidity inevitable when aqueous albumin aerosols are administered. Experiments were also made to determine whether mice infected with *B. pertussis*, or injected with killed vaccine during the late summer months when ragweed pollen is normally present in the air, develop sensitivity to this allergen.

MATERIALS AND METHODS

Female CFW mice weighing 16 to 20 Gm. were used in the experiments. They were lightly anesthetized with ether; then 0.03 ml. of a suspension of a 20 hr. old culture of *B. pertussis* strain 18323 was instilled into the nares. This dose contained 0.01 opacity units (2 to 5 million colony-forming units). Eighteen per cent of the infected mice died between the time of inoculation and challenge, and are not included in the tabulations.

The crude pollen extracts used for challenging the mice were prepared by defatting the pollen with petroleum ether and extracting in Ungar's solution.² They contained approximately

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1.0 mg. of N per milliliter. The challenge dose was 0.3 ml. of extract, and it was injected into a tail vein. This volume of extract did not elicit anaphylaxis-like symptoms when injected intravenously into normal mice. Anaphylactic reactions following challenge with extract were recorded as: none, severe, or died. Those with severe reactions became excited and then prostrate. Frequently the hind legs were extended backwards. Cyanosis was observed and respiration was labored. The mice that recovered did so within an hour or two. Reactions less than severe were classed with those showing no reactions.

The pertussis vaccine used in the experiments was a thimerosal-killed suspension of organisms. The dose was 0.5 ml. (10 opacity units) and was injected intraperitoneally.

For inducing pollen sensitivity, the mice were placed in a 7 gallon glass jar. The jar, placed on its side, was fitted with a wire mesh floor. The mouth of the jar was almost completely covered, and sufficient pollen was atomized into it throughout the exposure period so that the air contained 80 to 100 thousand pollen grains per cubic yard of air. The atomizer was constructed of a short piece of glass tubing 22 mm. in diameter. One end was fastened into the jar cover. The other end designed to blow pollen into the jar was fitted with a nozzle connected to a compressed air line. A hopper to hold the pollen consisted of a cone-shaped piece of glass tubing fastened at a right angle to the center of the glass cylinder. A spiral piece of wire, turned slowly by a stirring motor, was introduced into the pollen to keep it from packing and to aid its flow through the 0.5 mm. opening in the tip of the hopper. Compressed air was delivered to the atomizer continuously, but pollen flowed into it only when the wire turned. The stirring motor was set to operate for 5 minutes at alternate 5 minute periods. The number of grains was determined by aspirating 2 L. of air from the jar through a Millipore filter (2.8 cm. in diameter, pore size, 0.45μ), counting the grains in one square centimeter and calculating the number per cubic yard.

To determine whether mice become sensitive to ragweed pollen extract during the time of the year when ragweed pollen is present in the air, two rooms were used. In one room the air was filtered through glass fiber filters. This was quite effective because ragweed pollen grains were detected on only 3 of the 12 occasions when tests were made, and these counts were very low (10, 7, and one grain per cubic yard). The other room, supplied with unfiltered air, had on one wall three large windows that were left wide open during the time the experiments were in progress. Cages housing the animals were placed about 6 feet from these windows and were constructed entirely of $\frac{1}{4}$ inch wire mesh to allow free passage of air.

The number of grains per cubic yard of air was determined by exposing glass microscope slides, counting the pollen grains, and calculating the pollen content as described by Durham.³

The human serum albumin was a 25 per cent solution routinely distributed by the Michigan Department of Public Health.

RESULTS

The sensitivity to ragweed pollen extract developed after subjecting pertussis-infected mice to an aerosol of giant ragweed pollen for 4 hours is given in Table I, group 1. The mice were exposed to the pollen 8 or 9 days after infection and then challenged by injecting 0.3 ml. of pollen extract intravenously 14 days later. In group 2 the infected mice received three 4 hour exposures to pollen at 3 day intervals. The first exposure was given 9 days after infection, and the mice were challenged 5 days after the last exposure. In these experiments, from one-third to two-thirds of the mice died or developed severe anaphylactic shock after injection of the challenging antigen. The number of animals having anaphylactic reactions was not materially different in groups 1 and 2 even though those in the latter group received 3 exposures to pollen.

The animals in group 3 received 0.5 ml. of pertussis vaccine (10 opacity units per milliliter) intraperitoneally followed by a 4 hour exposure to pollen a day

Table I. Development of sensitivity toward ragweed pollen extract in *B. pertussis*-infected

Group	Test No.*	Treatment with <i>B. pertussis</i>	Sensitization with pollen	
			Method	Days after treatment
1	1	Infected intranasally	Inhalation (one 4 hr. exposure)	9
	2			9
	3			8
2	3	Infected intranasally	Inhalation (three 4 hr. exposures)	9
3	1	Vaccine injected intraperitoneally	Inhalation (one 4 hr. exposure)	1
4	2	Infected intranasally	Inhalation (one 4 hr. exposure)	1
	2			9
5	1	Infected intranasally (control)	None	
	2			
	3			
6	1	Not infected (control)	Inhalation (one 4 hr. exposure)	9
	2			9
7	1	Not infected (control)	Not sensitized	
	2			
	3			

*Tests with the same number were made concurrently.

Table II. Development of sensitivity toward ragweed pollen in *B. pertussis*-infected or pertussis vaccine-injected mice during the late summer when the air normally contains ragweed pollen

Group	Air supplied to rooms housing mice and length of time they remained there	Experiment No.	Treatment of mice	No. of mice	Severity of anaphylaxis		
					Died	Severe	None
1	Room supplied with filtered air for 9 days after infection or one day after injection of vaccine; then transferred to room with windows wide open for 14 days	1	Infected i.n.	58	1	24	33
		2	Vaccine i.p.	18	3	7	8
		3	None (control)	0			
2	Room supplied with unfiltered air and with windows wide open between time of treatment and challenge	1	Infected i.n.	{ 51 12	1 1	21 5	29 6
		2	Vaccine i.p.	{ 24 14	7 2	7 4	10 8
		3	None (control)	20	0	0	20
3	Room supplied with filtered air between time of treatment and challenge	1	Infected i.n.	21	0	0	21
		2	Vaccine i.p.	10	0	0	10
		3	None (control)	10	0	0	10
4	Inhalation of pollen—three 4 hr. exposure (control)	1	Infected i.n.	13	7	2	4
		2	Vaccine i.p.	13	2	6	5
		3	None (control)	19	0	0	19
				10	0	0	10

later. These mice were challenged with extract 13 to 14 days after exposure. The data in the table show that some of these mice also became sensitive to pollen extract and that the number with symptoms is approximately the same as in the groups of mice that were infected.

In group 4 the mice were infected intranasally, treated with a pollen aerosol

or pertussis vaccine-injected mice after exposure to ragweed pollen

Days between sensitization and challenge with pollen extract	Total number of mice	Severity of anaphylaxis		
		Died	Severe	No reaction
14	6	0	2	4
14	23	9	5	9
14	29	6	9	14
14	32	11	9	12
(after first exposure to pollen)				
14	8	1	1	6
13	17	2	6	9
14	12	0	0	12
(challenged with human serum albumin)				
Challenged with pollen extract 23 days after infection	10	0	0	10
	10	0	0	10
	40	0	0	40
14	10	0	0	10
13	12	0	0	12
Challenged with pollen extract 23 days after beginning of exposure	10	0	0	10
	10	0	0	10
	13	0	0	13

9 days later, then challenged by injecting 0.05 ml. of 25 per cent human serum albumin intravenously. No reactions were elicited, which suggests that the sensitivity developed is not a nonspecific nature.

Control groups are in the last three listed in the table. The mice in group 5 were infected but not treated with pollen. Animals in group 6 were not infected but exposed to pollen for 4 hours, while those in group 7 were normal uninfected, untreated mice. In none of these control mice were reactions of an anaphylactic nature elicited after challenge with pollen extracts. All of these experiments were performed during times of the year when no ragweed pollen was normally found in the air.

Table II shows the results of sensitivity tests made in mice infected with *B. pertussis* or injected with pertussis vaccine during the late summer when ragweed pollen is present in the air. Some of these mice were housed in a room supplied with filtered air between the time of infection or injection of vaccine and challenge; others were housed in a room supplied with filtered air for 9 days after infection or injection of vaccine and then were moved to a room supplied with unfiltered air for the entire period until the time of challenge.

In the room supplied with unfiltered air, approximately 50 pollen grains per cubic yard of air were found at the beginning of the experiment. At the peak of the ragweed pollen season, 200 to 300 grains were present. The number of grains inhaled over a 24 hour period by each mouse was about one grain for each 25 grains per cubic yard of air. This quantity was calculated on the basis that a 20 Gm. mouse inhales approximately 28 L. of air per day, a number determined experimentally by Guyton.⁴

The mice in the control groups (3 and 4) were maintained in a room that supplied filtered air for the entire period between infection or injection of vaccine and challenge. A group (3) of control mice that were not artificially ex-

posed to pollen showed no evidence of anaphylaxis. A number of mice in control group 4, as expected, that were treated with pollen after infection or injection of vaccine became sensitized to ragweed pollen.

DISCUSSION

In these experiments not all of the infected mice subjected to ragweed pollen became sensitized. This is not surprising. In previous experiments,¹ it was shown that pertussis organisms could not always be isolated or pathological changes demonstrated in the lungs of mice that had received an intranasal dose of *B. pertussis*. It could be surmised that these might be the ones that did not develop sensitivity to the inhaled antigen. This is probably not the entire explanation, because only 25 to 47 per cent of the mice that received pertussis vaccine intraperitoneally previous to a 4 hour exposure to pollen became sensitized. A more probable explanation is that the capacity to develop sensitivity is under genetic control and, since the mice were random-bred, a uniformly high percentage of sensitized animals should not be expected.

Kind and Richardson⁵ found that injection of pertussis vaccine would sensitize mice to rye grass pollen extract and the number of mice sensitized in any single experiment varied widely between tests. We found no evidence of sensitivity toward our crude ragweed pollen extract if the pertussis-infected or vaccine-injected mice had no contact with ragweed pollen. It should be noted, however, that based on nitrogen content our challenge dose was not as high as that administered by Kind and Richardson.

A much greater number of pollen grains were administered to the mice in the experiments where the pollen was given artificially than in the experiments where the mice were exposed to the pollen in the air late in the summer. The period of exposure was much longer in the latter groups of animals, which may account for the sensitivity developed in these mice. The experiments suggest that when using pertussis organisms as an adjuvant for sensitizing mice, precautions may sometimes be necessary to avoid producing antibodies against inadvertently administered airborne antigens.

Stevenet⁶ has suggested that there is an association between whooping cough and hay fever and asthma in children, and that this disease exaggerates or brings out weak familial tendencies toward these allergic diseases. These experiments may add credence to his suggested association.

REFERENCES

- 1 Chang, I. C., and Gottshall, R. Y.: Sensitization of mice by inhalation of antigen after infection with *Bordetella pertussis*, *Infect. Immun.* 6: 92, 1972.
- 2 Abramson, H. A.: *Treatment of asthma*, Baltimore, 1951, The Williams & Wilkins Company, p. 295.
- 3 Durham, O. C.: The volumetric incidence of atmospheric allergens. IV. A proposed standard method of gravity sampling, counting, and volumetric interpolation of results, *J. ALLERGY* 17: 79, 1946.
- 4 Guyton, A. C.: Measurement of the respiratory volumes of laboratory animals, *Am. J. Physiol.* 150: 78, 1947.
- 5 Kind, L. S., and Richardson, L.: Enhanced susceptibility of pertussis inoculated mice to pollen extract, *Proc. Soc. Exp. Biol. Med.* 100: 808, 1959.
- 6 Stevenet, P.: Whooping cough and respiratory allergy in children. Significance of the anti-whooping cough vaccination, *Rev. Hyg. Med. Soc.* 9: 147, 1961.