

The effect of nitrogen mustard on anaphylaxis in the pertussis-vaccinated mouse

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The addition of 60 or 70 γ of nitrogen mustard (HN2) to a mixture of antigen and as little as 6×10^4 pertussis organisms enhanced the anaphylactic sensitization of mice. The optimum dose of vaccine without HN2 is 6×10^9 cells. When 50 γ of HN2 was injected on the day before the antigen-pertussis mixture, there was no alteration of anaphylactic death rates. However, if given after the immunizing mixture, HN2 caused a decrease in the death rate of about 50 per cent. The administration of HN2 either before or after antigen alone did not produce a sensitization. A dose of 20 to 50 γ of HN2 in combination with antigen and 6×10^8 or 10^9 Bordetella pertussis cells caused the elaboration of antibody-containing ascitic fluid; given one day prior to the pertussis, HN2 produced an increased accumulation. The inoculation of 6×10^9 pertussis organisms had no effect on toxicity when given before or after the HN2. A decrease in toxicity was noted when 50 to 70 γ of HN2 was given together with 6×10^4 to 10^8 B. pertussis organisms.

Bordetella pertussis Phase I vaccine greatly enhances the susceptibility of inherently resistant mice to death from histamine,¹ serotonin,² and bradykinin³ administration. Mice inoculated with a mixture of a sensitizing antigen and pertussis organisms consistently respond to anaphylactic challenge with a high death rate, whereas antigen alone is relatively ineffective.⁴ Release of mediators such as histamine, serotonin, and/or bradykinin has been implicated in the observed responses to anaphylactic challenge in this species.⁵ However, antihistamines which protect mice from exogenous histamine do not prevent anaphylaxis, except at much higher dosages⁶; nor do serotonin antagonists protect mice from anaphylaxis although they are effective against the administration of serotonin.⁷

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Nitrogen mustard (HN2), as well as being an antitumor agent,⁸ acts as an immunosuppressor—probably by inhibition of antibody formation.⁹ A lessening of HN2 toxicity by administration of antihistamines or a serotonin antagonist (KB-95) has been reported by Field and associates.^{10, 11}

These studies were undertaken to determine what effect *B. pertussis* might have on the toxicity of HN2 and subsequently what effect the combination of *B. pertussis* and HN2 might have on anaphylaxis in the mouse.

MATERIALS AND METHODS

Animals

Female CFW mice, weighing about 20 grams at the start of the injections, were used in all of the experiments.

Toxicity

Mice were injected intraperitoneally on Day 0 with HN2 (Mechlorethamine HCl, Merek Sharp & Dohme) in concentrations of 50, 100, 200, or 400 γ as a single dose contained in 0.5 ml. of physiological saline. On Day -4, -1, or +1, the mice were injected intraperitoneally with 6×10^9 killed *B. pertussis* Phase I organisms in 0.5 ml. of saline. Control mice received no pertussis vaccine.

Sensitization

Mice, in groups of 10 or 20, were sensitized by a single intraperitoneal injection of a mixture of 0.03 ml. of horse serum (HS) and varying concentrations of pertussis vaccine and HN2. All intraperitoneal injections were contained in a

Table I. Toxicity

HN2 (Day 0)	6×10^9 <i>B.</i> <i>pertussis</i> (Days +1,* -1,† -4‡)	Number of				
		Day 0	Day 1	Day 2	Day 3	Day 4
400 γ per mouse	None given§	5	5	5	4	2
	+1	5	5	5	4	0
	-1	8	0			
	-4	10	2	0		
200 γ per mouse	None given§	5	5	5	5	3
	+1	5	5	5	5	2
	-1	8	7	7	6	5
	-4	10	8	8	8	8
100 γ per mouse	None given§	5	5	5	5	4
	+1	5	5	5	5	3
	-1	8	8	8	8	8
	-4	10	10	10	10	10
50 γ per mouse	None given§	35	35	35	35	35
	+1	15	15	16	14	13
	-1	16	16	10	16	16
	-4	10	10	14	10	10

*1 day after HN2.

†1 day before HN2.

‡4 days before HN2.

§None given on Day 0.

total volume of 0.5 ml. Challenge consisted of an intravenous dose of 0.1 ml. of HS injected into the tail vein 11 days later. Deaths were recorded at 24 hours. The experiment was terminated after an additional 24 hours.

In other groups, 50 γ of HN2 was given on Day 0 and HS or *B. pertussis* plus HS was given on Day -1 or +1. The mice were challenged on Day 11.

RESULTS

No apparent difference in survival rates was observed between control mice and mice treated with *B. pertussis*, whether the vaccine or the HN2 was administered first. The survival times are shown in Table I. Since the HN2 at 100 γ or higher was uniformly lethal, lower doses were used for the sensitization experiments.

In the groups of mice receiving a combined injection of pertussis vaccine, HN2, and HS, many deaths, thought to be a result of toxicity, occurred at doses of 50 γ of HN2 or higher. The death rate before challenge may be seen in Graph I. The peak in the death rate occurred between 5 and 8 days after the initial injection. In the high HN2 dose levels, the animals showed symptoms of toxicity, such as diarrhea, ruffled and dirty fur, hunched posture, and loss of weight. The weight loss averaged from 1 to 4 Gm. per mouse. In the groups subsequently showing a high anaphylactic death rate, the mice gained weight normally. There was a reduction in prechallenge deaths when 50 to 70 γ of HN2 were combined with from 6×10^4 to 6×10^8 *B. pertussis* cells. Some deaths before time of challenge resulted from pertussis alone at a concentration of 6×10^{10} organisms.

<i>mice alive</i>						
<i>Day 5</i>	<i>Day 6</i>	<i>Day 7</i>	<i>Day 8</i>	<i>Day 9</i>	<i>Day 10</i>	<i>Day 11</i>
0						
0						
2	0					
0						
4	3	2	2	2	2	2
1	0					
6	6	5	4	3	3	1
9	7	6	4	4	4	4
34	33	30	30	29	28	28
11	11	11	11	11	11	11
16	14	14	14	14	14	14
10	10	10	10	10	10	10

γ HN2	100	3/5										
	90		10/10									
	80			8/10								
	70				3/10	0/10	0/10	0/10	0/10	0/10	2/10	
	60					1/10	1/10	0/10	1/20	0/20	3/10	
	50	7/35					2/10	1/10	0/10	0/10	8/20	
	40							0/10	0/10	0/10	0/10	
	30								0/10	0/10	0/10	
	20									0/10	0/10	
	10										0/10	
	0		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10
n =		0	1	2	3	4	5	6	7	8	9	10
$6 \times 10^9 B. pertussis$												

Graph I

Decrease of HN2 toxicity by simultaneous administration of *B. pertussis*. Fraction indicates the number of mice dead of the number injected. $6 \times 10^9 B. pertussis$ is the regularly used dose for sensitization.

γ HN2	100	0										
	90											
	80			0								
	70				0	0	0	0	0	0	0	
	60					0	0	0	1	0	0.5	
	50	0					0	0.5	0	4	6	
	40							0	0	5	14	
	30								0	1	11	
	20									6	4	
	10										0.5	
	0		0	0	0	0	0	0	0	0		0
n =		0	1	2	3	4	5	6	7	8	9	10
$6 \times 10^9 B. pertussis$												

Graph II

Amounts of ascitic fluid produced within a narrow range of HN2 and pertussis dosage. Fluid collected from mice is designated in milliliters.

An incidental but interesting finding was the accumulation of various amounts of ascitic fluid within a narrow range of the HN2 and pertussis dosage. Mice having ascitic fluid were not included in the weight averages. The amounts of fluid produced are shown in Graph II; when pertussis at a concentration of 6×10^8 or 6×10^9 cells was given in combination with HN2, there was an appreciable amount of fluid. If the HN2 was given one day prior to the vaccine, the quantity of fluid was considerably increased. Production of large amounts of ascitic fluid has been utilized as a source of antibody in mice. Repeated injections of Freund's adjuvant¹² or pertussis¹³ have been used to cause fluid accumulation.

γ HN2	100											
	90											
	80			1/2								
	70			0/7	10/10	6/10	8/10	9/10	10/10	7/8		
	60				0/9	8/9	7/10	17/19	17/20	7/7		
	50	0/7				1/8	1/9	1/10	8/10	10/12		
	40						3/10	2/10	5/10	10/10		
	30							1/10	3/10	9/10		
	20								5/10	6/10		
	10									9/10		
	0		0/10	0/10	0/10	0/10	0/10	1/10	0/10	3/10		5/6
n =		0	1	2	3	4	5	6	7	8	9	10
6×10^8 <i>B. pertussis</i>												

Graph III

Anaphylactic death in mice sensitized with *B. pertussis* plus HN2 plus 0.03 ml. horse serum (HS) intraperitoneally, and challenged with 0.1 ml. HS, intravenously, on Day 11. Fraction indicates the number of mice dead of those challenged.

The fluid from the HN2-pertussis mice does contain antibody to the antigen as detected by the tanned-cell-hemagglutination technique.

HN2 in a narrow range from 60 to 70 γ per mouse enhanced the anaphylactic sensitization of mixtures containing as little as 6×10^4 pertussis organisms. These results are depicted in Graph III. The critical amount of pertussis organisms for sensitization resulting in a 95 to 100 per cent death rate without HN2 is 6×10^8 cells. When 50 γ of HN2 was injected on the day before or the day after HS, no deaths resulted from challenge with HS. When *B. pertussis* plus HS was given the day after HN2, the mortality rate due to HS challenge was the same as though no HN2 had been administered. However, if *B. pertussis* plus HS was given the day before HN2, deaths due to anaphylaxis were cut approximately in half (Table II).

DISCUSSION

Cytotoxic agents (usually used as immunosuppressors) have been found to enhance antibody synthesis under appropriate conditions of dose and time relationship. This list includes FUDR (5-fluoro-2-deoxyuridine), uracil mustard, cyclophosphamide, cortisone, 6-mercaptopurine, penicillamine, colchicine, and certain endotoxins,¹⁴ as well as the lipopolysaccharide of *B. pertussis*.¹⁵ Merritt and Johnson¹⁶ reported that a single large dose of FUDR given one hour before, with, 1 hour after, or 4 days after antigen resulted in increased antibody formation. In general, to effect this enhancement, the cytotoxic agent must be given before or with the antigen. The proposed mechanism is that cellular destruction by the drugs releases nucleic acids which in turn enhance antibody production.¹⁴ This suggestion may not be tenable with respect to HN2, for its cytotoxic effect is actually decreased when given together with pertussis.

When given 24 to 48 hours after the antigen, FUDR was found to inhibit

Table II. *Anaphylactic death, relationship to day of administration of HN2*

Day -1	Day 0	Day +1	Challenge day	
			10	11
HS	HN2			0/9*
<i>B. pertussis</i> + HS	HN2			6/14
	HN2	HS		0/12
	HN2	<i>B. pertussis</i> + HS		10/11
	<i>B. pertussis</i> + HS		17/18	
	HS		0/10	

*No. dead/no. challenged. The mice were sensitized with 6×10^9 *B. pertussis* + 0.03 ml. of HS intraperitoneally, and challenged by 0.1 ml. of HS intravenously. The HN2 dose was 50 γ per mouse intraperitoneally.

antibody production.^{16, 17} Although less striking, this inhibition was observed also when the immunosuppressive agent was given 32 hours after an antigen-endotoxin mixture.¹⁷ Our results with HN2 on a pertussis-antigen mixture are similar. Nathan and associates¹⁸ used a dose of HN2 equivalent to 20 γ per mouse per day and observed a significant reduction in antibody titer. This dose is quite toxic, however, as 50 per cent of the mice died. At half that dose there was no significant reduction in titer. Small doses of the mustard, 2-chloropropyl-bis-(2-chloroethyl)-amine, were found to have an enhancing effect,¹⁹ and Legezshinskii²⁰ reported an increase in agglutinins, precipitins, and hemolysins with doses of 0.05 to 0.1 mg. per kilogram of HN2 or HN3, tris-(2-chloroethyl)-amine. Thus, there appears to be no constancy in the action of the cytotoxic agents as immunosuppressors or enhancers except that there seems to be a necessary temporal relationship between the administration of the drug and the antigen.

The apparent reduction of toxicity resulting from the combination of 50 to 70 γ of HN2 with doses of *B. pertussis* ranging from 6×10^4 to 10^8 was quite surprising in view of the reported protective effect of antihistamine and anti-serotonin compounds and the well-known increase of mediator sensitivity in pertussis-inoculated mice. Preliminary studies¹³ indicate that HN2, in the dose ranges used to demonstrate enhanced anaphylactic sensitization, does not increase sensitivity to histamine, either at Day 4 after HN2, at which time histamine sensitivity is at a peak following pertussis treatment, or at Day 10 when anaphylactic sensitivity is at a peak in the mouse. Hence, the effectiveness of HN2 is probably not produced by enhancing sensitization to the mediators. The combined effect of HN2 and pertussis may be regarded as a synergism.

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