

Induction of interferon by bacterial vaccines and allergenic extracts

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Licensed bacterial vaccines with no specific United States standard of potency and allergenic extracts were examined for their ability to induce interferon, an antiviral protein. Samples representative of these products were injected into mice and interferon production was tested by titration of mouse sera with the use of a standard vesicular stomatitis virus yield reduction method in primary mouse embryo fibroblast cell cultures. Antiviral activity was assessed by challenge with a lethal dose of Semliki Forest virus. High levels of circulating interferon were induced by certain of the bacterial vaccines and allergenic extracts. Interferon was detected when the intravenous route of inoculation was used and in certain instances after subcutaneous administration. The time of appearance and the duration of the interferon production were similar to those reported for endotoxin. There was also a second interferon-inducing component in some of these products. Experiments in which mice were challenged with Semliki Forest virus demonstrated that the levels of interferon induced by selected products provided a high degree of protection against the virus challenge. Thus, a new activity, the induction of interferon, has been found for certain of these vaccines and extracts. The data suggest that investigations be performed to explore the possible significance of the results derived in the mouse system for man.

Since the initial report of induction of interferon, an antiviral protein, by a virus in 1957¹ there has been increasing activity and interest in this field. Inducers of interferon are no longer limited to viral agents but include other microorganisms such as Chlamydia, Rickettsia, bacteria, protozoa, and extracts of the above in addition to plant and fungal extracts and synthetic polymers.² Some of the licensed bacterial vaccines for which no specific United States standard of potency has been assigned contain bacteria which have been reported as capable of inducing interferon,² and since exotoxin-like activity has been reported in an allergenic extract of house dust³ and endotoxin is a known potent interferon inducer,⁴⁻⁶ it seemed reasonable to conclude that these products might be capable of inducing interferon. This report presents data which show that representative lots of bacterial vaccines with "No. United States Standard of Potency" and allergenic extracts indeed were capable of inducing interferon in an in vivo mouse system.

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MATERIALS AND METHODS

Bacterial vaccines

Licensed bacterial vaccines with no specific United States standard of potency, which are used for prophylaxis or therapy against infections associated with respiratory disease or for hyposensitization, were purchased from the manufacturers. Table I lists the contents of each of the biological products tested. The vaccines were used as originally packaged except for one product which required dilution in saline in order that the mice could survive the inoculation.

Allergenic extracts

Licensed allergenic extracts of house dust and ragweed pollen were diluted in saline for injection except for lot X₁ which was diluted in pyrogen-free water. The lots of extract of house dust were given intravenously at the lowest dilution which allowed survival of the injected mice. All subcutaneous injections were with undiluted extract. An experimental extract of mites prepared by grinding and extracting 10 mg. of *Dermatophagoides farinae* with 1 ml. of phosphate-buffered saline was kindly provided by Dr. Harold Baer. Table II lists the extracts used for this study.

Induction of interferon

Groups of 10 general purpose female mice, weighing 10 to 15 grams (obtained from Animal Production Section, Laboratory Aids Branch, NIH), were inoculated intravenously into the tail vein or subcutaneously into the right inguinal fold with 0.5 ml. each of the particular product to be tested. Two hours after inoculation (except for the kinetic studies), blood was obtained from the orbital plexus of each mouse in a group and pooled. The sera were stored at -20° C. after separation.

Interferon assay

A virus yield reduction method was used.⁷ Beginning with a 1:8 dilution of the serum pool, subsequent twofold dilutions in Eagle's minimal essential medium with one per cent fetal calf serum were made and incubated on primary mouse embryo fibroblast monolayers (prepared by the Tissue Culture Section, Laboratory of Virology and Rickettsiology, DBS). After 6 hours of incubation the serum was decanted; the cells were washed and then challenged with vesicular stomatitis virus at a multiplicity of infection of 20 to 100. Interferon was considered to be present when virus yields from cells previously incubated with test sera were less than from control cells by one half log₁₀ or more. The interferon titers were expressed as the reciprocals of the highest dilutions of serum causing this decreased virus yield.

Mouse protection studies

Groups of 10 general purpose female mice, 10 to 15 grams, were inoculated either intravenously or subcutaneously with 0.5 ml. of vaccine, allergenic extract, or saline. Six hours later the mice were injected intraperitoneally with 0.5 ml. of Semliki Forest virus (SFV) containing 10³ plaque-forming units. Previous experiments had indicated that this amount of virus caused death of approximately one half of the animals inoculated during a 10 day period of observation.

RESULTS

Bacterial vaccines

The vaccines which were studied for their capacity to induce an antiviral substance consisted of three general types: (1) those containing whole organisms, (2) those consisting of acellular extracts of the organisms, or (3) those

TABLE 1. Interferon induction by bacterial vaccines with no United States standard of potency

Product code	Contents	Quantity (millions/c.c.)	Interferon titer
A ₁ *	Staphylococci	1,200	A ₁ -1,024
A ₂	Streptococci	200	A ₂ -256
	<i>Diplococcus pneumoniae</i>	150	
	<i>Neisseria catarrhalis</i>	150	
	<i>Klebsiella pneumoniae</i>	150	
	<i>Haemophilus influenzae</i>	150	
	Preservative: Thiomersol	1:10,000	
B	<i>Neisseria catarrhalis</i>	200	1,024
	<i>Klebsiella pneumoniae</i>	200	
	<i>Diplococcus pneumoniae</i>	100	
	Streptococci	100	
	Staphylococci	600	
	Preservative: Thiomersol	1:10,000	
C (diluted 1:4)	Acellular material made from:		64
	Streptococci		
	<i>Diplococcus pneumoniae</i>		
	Staphylococci		
	<i>Escherichia coli</i>		
	<i>Neisseria catarrhalis</i>		
	<i>Haemophilus influenzae</i>		
	Preservative: Thiomersol	1:10,000	
D	Bacterial antigen from:		64
	Pneumococci		
	Streptococci		
	<i>Haemophilus influenzae</i>		
	<i>Neisseria catarrhalis</i>		
	Staphylococci		
	Preservative: Thiomersol	1:10,000	
E	<i>Neisseria catarrhalis</i>	200	64
	Staphylococci	200	
	Streptococci	500	
	<i>Haemophilus influenzae</i>	500	
	<i>Diplococcus pneumoniae</i>	500	
	<i>Klebsiella pneumoniae</i>	100	
	Preservative: Thiomersol	1:10,000	
F	Killed bacteria and soluble antigens from:		64
	<i>Klebsiella pneumoniae</i>		
	<i>Diplococcus pneumoniae</i>		
	<i>Neisseria catarrhalis</i>		
	Streptococci		
	Staphylococci		
	<i>Corynebacterium diphtheroid</i>		
	<i>Haemophilus influenzae</i>		
	Preservative: 0.2% Creosol		
G	Lysate of <i>Staphylococcus aureus</i>		< 8
Control	Pyrogen-free saline or water		< 8
Control	Thiomersol	1:10,000 in saline	< 8
Control	<i>Salmonella typhosa</i> lipopolysaccharide (100 µg)		256

*A₁ and A₂ designate two lots of the same product from one manufacturer.

TABLE II. Interferon induction by allergenic extracts

Product code	Contents	Interferon titer
X ₁ * (diluted to 0.312 mg./ml.)	House dust extract	2,048
X ₂ (diluted to 0.312 mg./ml.)		64
X ₃ (diluted to 6.25 mg./ml.)		256
Y	House dust extract (50,000 PNU/ml.)	256
Z	House dust extract (10,000 PNU/ml.)	32
M	Mite extract	< 8
R	Ragweed pollen extract (20,000 PNU/ml.)	32

*X₁, X₂, and X₃ designate different lots of the same product from one manufacturer.

containing a combination of whole organisms and extracts of the organisms. All three types contained active inducers of an antiviral substance which, for reasons outlined below, was considered to be interferon. As shown in Table I, all but one of the seven products tested induced measurable amounts of circulating interferon at 2 hours after intravenous injection of mice. The titers ranged from 1:64 to 1:1,024. The one vaccine which was inactive was a phage lysate of *Staphylococcus aureus*. Each of the active vaccines contained either whole cells or extracts of at least two or more gram-negative organisms.

Interferon was not detected following the injection of pyrogen-free water, saline for injection, or saline plus thiomersol (1:10,000), the preservative used in most of these products. A control preparation of endotoxin consisting of 100 μ g of *Salmonella typhosa* lipopolysaccharide induced a titer of interferon of 1:256.

Allergenic extracts

Table II shows the interferon titers in sera of mice following intravenous injection of allergenic extracts. The highest titer of interferon (1:2,048) was found following the inoculation of lot X₁ of house dust extract which previously had been shown to be lethal for mice at concentrations greater than 1:3,200 (0.312 mg. per milliliter) (80 times more dilute than the reconstituted initial dilution). Lot X₂ of this product, which was empirically diluted the same as lot X₁ without prior toxicity testing, induced an interferon titer of 1:64. Lot X₃ of this product was much less toxic for mice than was X₁, requiring a dilution of only 1:160 (6.25 mg. per milliliter) (4 times more dilute than the reconstituted initial dilution). An interferon titer of 1:256 was induced by this lot.

The injection of a lot of freeze-dried extract of house dust from a second manufacturer, reconstituted in saline to contain 50,000 protein nitrogen units (PNU) per milliliter, induced an interferon titer of 1:256, while that from a third manufacturer, containing 10,000 PNU per milliliter, induced a titer of 1:32.

Recent reports^{8, 9} have suggested that the primary allergenic component of house dust is a species of mites in the genus *Dermatophagoides*. An extract of

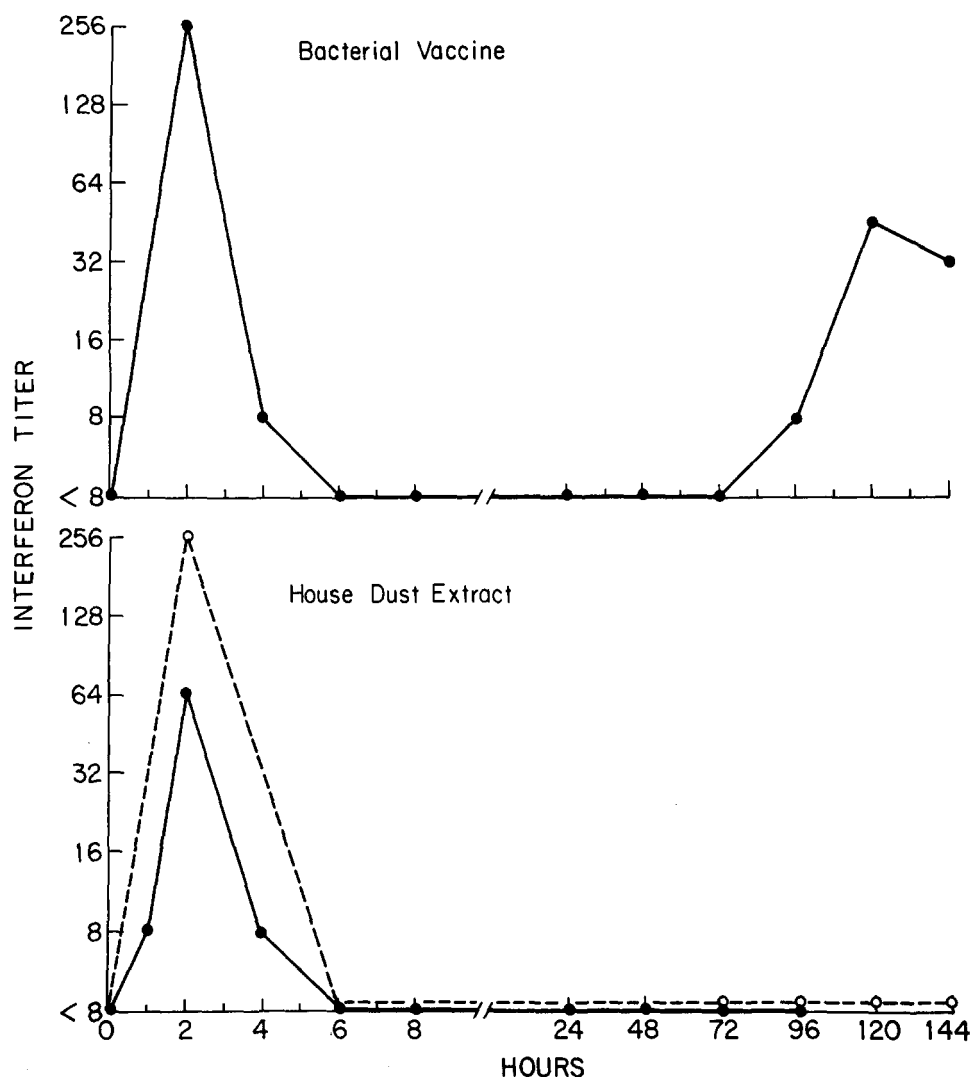


Fig. 1. Kinetics of interferon production following intravenous inoculation of bacterial vaccine lot A₂ and house dust extract X₂ (—●—) and X₃ (---○---).

mites failed to induce detectable circulating interferon in our test system. This indicated that the mite was not the agent responsible for inducing interferon.

The extract of freeze-dried low ragweed pollen, reconstituted to contain 20,000 PNU per milliliter, was also found to be capable of inducing an interferon response (titer of 1:32) in mice.

Characterization of the viral inhibitor

The inhibitors induced by a bacterial vaccine, lot A₁, and an allergenic extract, lot X₁, were characterized as mouse interferon on the basis of their species specificity (preparations causing greater than one log₁₀ inhibition of

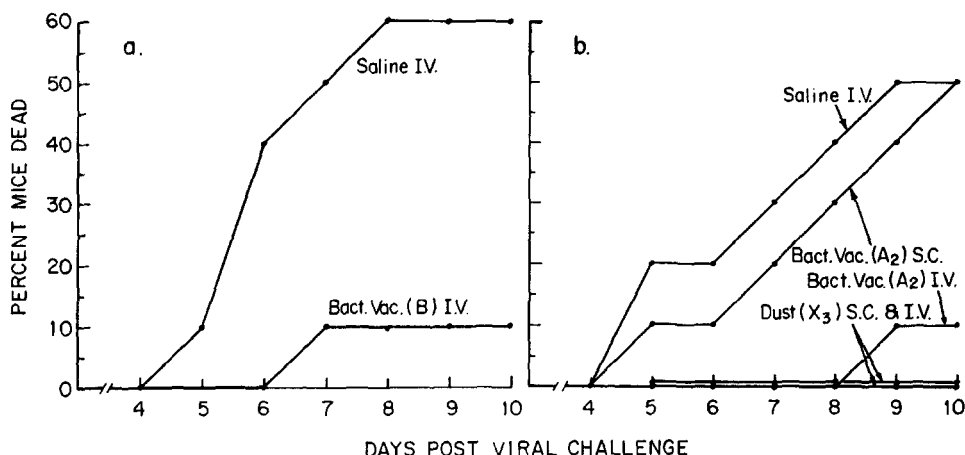


Fig. 2. Protection of mice against a virus challenge with Semliki Forest virus by: **a**, bacterial vaccine B; and **b**, bacterial vaccine lot A₂ and house dust extract lot X₃.

viral growth in mouse cells had no activity in chick cells), broad viral spectrum (antiviral activity in mouse cells was present against vesicular stomatitis virus, Semliki Forest virus, and Sindbis virus), resistance to acidity (stable at pH 2 for 24 hours), and heat stability (loss of activity at 56° C. for one hour but stable at 37° C. for one hour).

Effect of route of administration

The results reported above reflect data obtained when these materials were inoculated intravenously. In an attempt to simulate more closely the method of administration of these products to man, mice were inoculated subcutaneously with 0.5 ml. of bacterial vaccine lot A₂ or allergenic extract of house dust lot X₃. Both of these products induced circulating interferon at a titer of 1:16, 2 hours after inoculation.

Kinetics of interferon production

As shown in Fig. 1, peak titers of interferon were obtained with the bacterial vaccine lot A₂ and the house dust extract lots X₂ and X₃, 2 hours after intravenous inoculation. Beginning at Day 4 and continuing through Days 5 and 6, a second peak of antiviral activity appeared with lot A₂ in some experiments. This second peak was not observed with either of two lots of house dust extract X₂ or X₃.

In vivo protection

Mice injected intravenously with bacterial vaccine lot A₂ or allergenic extract of house dust lot X₃ were afforded a significant degree of protection against a Semliki Forest virus challenge. Fig. 2, b shows the percentage of mice surviving during the period of observation. A second bacterial vaccine B (Figure 2, a) also gave similar protection. The subcutaneous administration of house dust extract (25 mg. per milliliter also provided significant protection, al-

though this was not seen after subcutaneous administration of the bacterial vaccine. However, the circulating interferon titers were the same at 2 hours for both products.

DISCUSSION

Interferon production following the administration of viral vaccines has been reported.¹⁰⁻¹³ In addition, typhoid vaccine has been reported to act as an inducer,¹⁴ presumably due to the presence of endotoxin. Our data indicate that additional currently licensed biologics have the ability to induce interferon.

Several reasons for the phenomenon of induction of a viral inhibitor by these products are suggested by a review of known interferon inducers. First, the time of appearance and duration of the interferon response in the present study are similar to those reported for endotoxin induction, with peak titers appearing at 2 hours and then rapidly diminishing.¹⁵ Secondly, some of these bacterial vaccines or antigens are, in part, derived from gram-negative organisms which, no doubt, contain endotoxin or other polysaccharide cell wall components. Virus-inhibiting activities have been associated with extracts of some of the bacteria found in one or another of these products such as *Klebsiella pneumoniae*,¹⁶ *Escherichia coli*,^{5, 6} and *Haemophilus influenzae*.¹⁷ Bacterial vaccines and allergenic extracts are not required to be tested for pyrogenicity. All vaccines containing gram-negative bacteria would contain pyrogenic endotoxin. Experimentally, the house dust lot X₁ was shown to be pyrogenic in the rabbit at a 1:20,000 dilution of the reconstituted material (1.25 micrograms/kg.) (information kindly provided by Dr. Edward Seligmann). Thus, endotoxins or endotoxin-like substances present in these products could be one reason for interferon production. These data confirm those of Peterson and associates³ which showed that some lots of house dust extract contained endotoxin-like activity. Whether this activity is due to gram-negative endotoxin, some other polysaccharide, as indicated by Vannier and Campbell,¹⁸ or other biologic activity associated with dust³ is not known. Double-stranded RNA also has been shown to be an early interferon inducer,¹⁹ and its presence has not been excluded from these products.

Another possibility for interferon induction is based on the observation by DeClercq and Merigan¹⁷ that an extract prepared from *H. influenzae* type b contained an interferon inducer. The activity of this extract differed from that of endotoxin and was unusual in that it induced a second peak of interferon 4 to 6 days after the primary, early-appearing peak. This type of biphasic response was seen only with the bacterial vaccine A₂ which contained *H. influenzae*. Hence, it is reasonable to suggest that there is a second interferon-inducing agent active in those vaccines which contain this organism. However, we found, as did DeClercq and Merigan that this second peak was irregularly observed.

There are conflicting reports in the literature as to the effectiveness of bacterial vaccines in reducing the incidence or severity of upper respiratory infections or complications such as asthma.²⁰⁻²⁹ This is not unexpected in a situation in which the mechanism of action of these products is not completely known and in which potency has not been standardized. Our data indicate that these

biologics contain complex materials capable of causing potent biological activity. However, there was marked variation in such activity not only between similarly formulated products but also between different lots of the same product. With such variation of response as evidenced by the interferon-inducing capacity of these products or the toxicity of the dust, it is not difficult to see how very divergent clinical responsiveness could result from otherwise carefully controlled studies, especially when composition of vaccine, dosage, and frequency of injection are so variable.

Endotoxin-induced interferon has been reported after intravenous^{5, 6} administration of this agent. No reference was found to indicate whether other routes of inoculation had been tried or whether activity was seen if different routes were tried. Assuming that endotoxin is responsible for the early-appearing interferon in the materials tested, we found that it also induced circulating interferon with the use of the subcutaneous route of inoculation. The levels were lower than those obtained by the intravenous route but consistently measurable. In the case of lot X₃, significant protection against viral challenge was present after subcutaneous administration of the material. Lot A₂ did not provide protection by this route although it induced the same levels of interferon in our test system, suggesting a possible additional mechanism of antiviral resistance induced by the house dust extract (X₃).

The results of this study show that additional types of biologics, already widely used in man, are capable of inducing interferon in mice. In attempting to answer the question of whether bacterial vaccines and allergenic extracts induce interferon clinically, it should be noted that the concentration or dosage of the biologic products given mice in our study was often far in excess of that usually recommended in a single human dose. Indeed with some products there was evidence of gross toxicity at the concentrations used. The minimum dose of either type of product which leads to the induction of interferon or the protection of mice against a viral challenge has not been established. Except for some experiments, the intravenous rather than the subcutaneous or intramuscular route was utilized. Also, the viral challenge was given under set laboratory conditions at a time when optimal protection afforded by interferon would be expected. For these reasons, these data derived in mice may have no significance for man, and one must be extremely cautious in making extrapolations to the clinical situation. However, it is intriguing to speculate that one of the mechanisms of action of these biologics is to induce interferon and thereby provide protection against viral assault, especially respiratory infection.

Further studies on these problems, including the effect of repeated administration, are in progress.

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