Production of an Interferon-Like Agent Following Inoculation with Bacterial Vaccine (35551)

MIKLOS DEGRE AND HELEN DAHL
(Introduced by H. E. Morgan)
Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, University of Oslo, Rikshospitalet, Oslo, Norway

Bacterial vaccines have been employed in the treatment of recurrent respiratory infections, chronic respiratory infections, asthma and other related illnesses for many years. The effect of this treatment has been subject of discussion ever since its initiation. Several authors reported significant improvement in different vaccine treated groups (1, 2). Others found no difference between patients treated with vaccine and those given placebos (3, 4).

The probability that the effect is due to immunological mechanisms is small, as the majority of respiratory tract infections is due to viral infections. Several studies showed that bacterial infections (5–7), injections of live or killed bacteria (8–11) or bacterial extracts, such as endotoxin (12–15) induce interferon (IF) *in vivo*. The effect of IF and IF inducers in the prevention of viral respiratory infections is well documented (16). In the light of this information we decided to investigate whether bacterial vaccines induce production of IF or IF-like agents, and whether the vaccine is able to protect against experimental viral infection.

Materials and Methods. Bacterial vaccine. Standard bacterial vaccine (SBV) was purchased from the National Institute of Public Health, Oslo. The vaccine is composed of formalin-killed bacteria suspended in NaCl solution, and contains (per ml):

Staphylococcus aureus	90×10^6
Streptococcus (pyogenes and viridans)	60×10^6
Streptococcus pneumoniae	300×10^6
Haemophilus influenzae	1250×10^6
Klebsiella pneumoniae	20×10^6
Neisseriae	80×10^6
Total bacteria per ml	1800×10^6

Virus. Vesicular Stomatitis Virus (VSV) Indiana strain, was used as challenge virus. It was passed 2 times in the allantoic cavity of embryonated hens egg in our laboratory, and the allantoic fluids were pooled and stored at —20°. Infectious titer was estimated in L-929 cells by the end point titration method.

Cells. Mouse embryo fibroblasts, L-929 strain, were obtained from Dr. A. Zetterlund, Karolinska Institutet, Stockholm. The cells were grown in Eagle's minimum essential medium (MEM) supplemented with 5% inactivated calf serum and maintained with 2% calf serum. The Vero line of African green monkey cells was obtained from Dr. J. C. Ulstrup, Ulleval Hospital, Oslo. These cells were grown in 199 medium supplemented with 5 and 2% calf serum as for L-cells.

Mice. HaM/ICR/CSE/Bom albino mice of either sex were originally obtained from the National Institute of Public Health, Oslo. For IF production, young adults; and, for mortality studies, 2–3-week-old mice were used.

Interferon production. Mice were inoculated intraperitoneally with a total volume of 0.1 to 0.3 ml of vaccine. Blood was obtained from the axillary vein at various times after injection. Serum was separated after clotting at room temperature for 1 hr and overnight at +4°. If not otherwise indicated, sera were adjusted to pH 2 with 1 *N* HCl and placed at +4°. After 2 days the samples were neutralized with 1 *N* NaOH.

Interferon assay. Sera were tested for interferon activity by means of the infectivity inhibition test. Tubes were seeded with 10^5 L-929 cells/tube and grown for 2 days. Sera were diluted in maintenance medium 1:10, 1:20 . . . 1:320; and 0.5 ml was added to each tube. The tubes were incubated at 37° in a roller apparatus. After 24-hr incubation

the serum dilutions were poured off and 10 $TCID_{50}$ of VSV was added in a total volume of 1 ml. After 2 more days incubation, the tubes were examined for presence of cytopathogenic effect. The interferon titer was calculated as the dilution which inhibited the cytopathogenic effect in 50% of the tubes.

Results. Induction of virus inhibitor by SBV. Preliminary experiments indicated that following intraperitoneal injection of undiluted standard bacterial vaccine (SBV), the serum of mice posessed antiviral activity against VSV in tissue culture.

The kinetics of the production of inhibitor was determined after intraperitoneal injection of 0.2 ml of undiluted SBV (Fig. 1). Three mice were bled each time and their serum was pooled. Antiviral activity could be detected from 2 to 24 hr after SBV injection. Maximal titer was obtained 4 hr past injection.

Dose response. Groups of mice were injected with increasing concentrations of SBV. Four hr later, all mice were bled and the antiviral capacity of their serum was determined. The production of virus inhibitor was dose dependent. The smallest amount which induced detectable activity was 3.5×10^5 bacteria, increasing to 80 IF units following 3.5×10^7 bacteria.

Characterization of the inhibitory agent. The properties of the inhibitor were identical with that of interferon; it was insensitive to pH 2 treatment for 48 hr, for heating at 56° for 1 hr, dialysis against 0.9% NaCl and it was not sedimented by centrifugation at 30, 000 rpm for 2 hr. The activity was destroyed by 0.01% trypsin treatment at 37° for 1 hr. Species specificity was shown by the lack of activity in Vero monkey cells.

Effect of serially repeated SBV injection of the interferon production. A large group of mice was injected with 0.2 ml of undiluted SBV. Three mice were bled 4 hr later. The remainders were given a new SBV dose 2 days later. Four hr after the second injection, a new group of 3 mice was bled. The process was repeated 3 times. Interferon activity was determined in the sera of mice given 1 to 5 doses of SBV. The IF titer was 20 in the mice given 2 and 5 doses and 40 after 3 and 4 doses, compared with the initial titer of 80 following 1 dose of SBV.

Attempt to produce interferon in vitro *by SBV.* No IF was detected in the supernatants of L-929 cell or primary mouse embryo cultures following inoculation with 0.3 ml of undiluted SBV.

Preventive effect of SBV on the VSV caused mortality. Infant mice were injected intraperitoneally with 0.1 ml of undiluted SBV. Three hr later they were inoculated intranasally with 0.015 ml of VSV dilutions containing 1 and 10 LD_{50} respectively. Control groups received the same intranasal inoculation but no SBV. From the third day after inoculation, the mice began to develop the characteristic picture of VSV infection and to die. All groups were observed for 10 days (Table I). More mice died and they died earlier in the control groups compared with the groups given SBV. The difference in the length of survival is significant ($p < 0.05$) for both groups (Wilcoxon's two sample test).

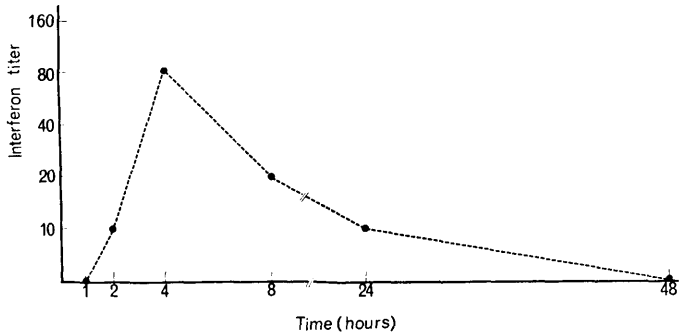

FIG. 1. Kinetics of the interferon production following intraperitoneal injection of standard bacterial vaccine. Serum from 3 mice was pooled for each determination.

TABLE I. Preventive Effect of Standard Bacterial Vaccine on the Mortality of Mice Following Infection with Vesicular Stomatitis Virus.

	(LD_{50}) VSV			
	1		10	
	With vaccine	Without vaccine	With vaccine	Without vaccine
Total no. of mice	13	13	13	15
No. of mice dead	1[a]	5	9	13
Mortality (%)	7.7	38.5	69	86
Survival (days) total	122	96[b]	103	89[b]
mean	9.4	7.4	7.9	5.9

[a] After 10-days observation.
[b] Significant on the 5% level.

Discussion. The data indicate, that parenterally administered bacterial vaccine induces production of an antiviral agent. The properties of this agent are comparable with that of IF. At the present stage of the study we cannot define the relative role of the different components in the vaccine. The vaccine contains gram-negative rods, *H. influenzae* and *K. pneumoniae*, both of them shown to be stimulators of IF production (9, 13).

The characteristics of the vaccine stimulated IF do not classify it clearly according to the inducer. The early start and maximum of production is comparable with the endotoxin induced IF (9, 14, 17). Also the failure to stimulate production of IF in tissue cultures is similar to that of endotoxin-induced IF (18, 19). However, endotoxin-induced IF was reported to be sensitive to heating and to dialysis (5). The vaccine-stimulated IF was resistant to both.

The initial release of IF is followed by a refractory period during which no new IF can be detected. In our experiments the reduction of titer after repeated injections of SBV was surprisingly small, if any. A possible explanation for this finding is that the refractory period in our experimental model might be shorter than 48 hr. The refractoriness of rabbit kidney cells to a second induction by synthetic double-stranded RNA lasted for 48 hr (20). This period can be interpreted as the time necessary to restore the IF precursor and might be of different duration in different experimental systems. An alternative explanation might be, that the dose of SBV employed is insufficient to stimulate all competent cells to produce IF. The cells not stimulated by the primary injection are able to produce IF following the secondary stimulation.

A single intraperitoneal injection of SBV gave some protection against VSV challange 3 hr later, mainly by extending the survival. Although bacterial vaccines initiate a complex of immunological response it is doubtful whether the course of viral infection is significantly influenced by the relatively slow immunological process. Different IF stimulating agents gave protection of varying extent against different viral infections (8, 13, 15, 21), and this may be the case in the present experiments too. More investigation is needed to determine whether these findings have any bearing on the vaccine treatment of human respiratory infections.

Summary. Bacterial vaccine, inoculated intraperitoneally into mice, stimulated a virus inhibitor in the serum with the characteristics of interferon. The inhibitor could be detected from 2 to 24 hr after inoculation. Serial inoculations on alternating days resulted in slightly lower, but still significant, interferon titers compared with that after the initial inoculation. One dose of vaccine was protective against intranasal inoculation with vesicular stomatitis virus, shown by extension of the survival time.

The excellent technical assistance of Miss Signe K. Ystenaes is gratefully acknowledged.

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Received Jan. 4, 1971. P.S.E.B.M., 1971, Vol. 137.