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Induction of Interferon in Man by Vaccines[1] (38785)

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A number of vaccines have been described in the literature as having the potential of interferon induction in man (1–4). In view of the fact that no other safe and effective interferon inducers are currently available, these vaccines remain the only readily available agents which could be employed for possible prophylactic or therapeutic interferon induction. The purpose of this investigation was to attempt to extend the spectrum of vaccines capable of interferon induction in man. Two vaccines were studied. One was a commercially available live, oral poliomyelitis vaccine, type 2, and the other a new live, attenuated influenza A/England/42/72 (H3N2) vaccine which is currently undergoing field trials.

Materials and Methods. Poliomyelitis vaccine. Poliovirus vaccine, live, oral, type 2 Sabin strain, Wyeth, Lot No. 17702 was given by mouth in a dose of approximately $10^{4.7}$ $TCID_{50}$. The type 2 vaccine was chosen because of the three monovalent types available, it has been associated with the fewest complications (product information, Wyeth). Blood samples were obtained on days 0, 2, 5, 10, 15, 20 and 25 from five volunteers. Rectal swabs were also obtained on day 5, since in previous studies this had been determined to be the time of maximal fecal excretion of the vaccine strain. Neutralizing antibody titers were performed in the standard manner employing 100 $TCID_{50}$ of the type 2 vaccine strain of poliovirus. Serial serum dilutions in 2% E-MEM (Microbiological Associates [MBA], Bethesda, MD) were incubated with the viral inoculum for 1 hr at room temperature and then 0.2 ml of the mixture was added to WI-38 tubes (MBA). The end point was taken as the highest dilution giving 50% suppression of cytopathogenic effect. Viral isolation was carried out in both primary rhesus monkey kidney cells and in WI-38 cells.

Influenza vaccine. "Alice" strain of A/England/42/72 (H3N2) vaccine (Smith, Kline and French Co., Philadelphia, PA) was given in a dose of approximately $10^{7.5}$ $TCID_{50}$ by nasal drops on day 0 and 14 of the study. Blood was obtained for interferon and hemagglutination inhibition (HI) antibody determinations on days 0, 3, 7, 16, 23 and 30. Nasal washes were obtained at the same time utilizing Ringer's lactate solution. Viral isolation from nasal washings was performed in primary rhesus monkey kidney tubes (MBA) which were incubated on a roller drum at 33° and 12 rev/hr. Hemadsorption was performed with 0.5% guinea pig erythrocytes following incubation for 7 and 14 days and after one blind passage. HI antibody titers were done according to standard techniques, employing 4 hemagglutinating units of influenza A/England/42/72 (H3N2) antigen (Smith, Kline and French). All sera were treated with receptor destroying enzyme (RDE) of *Vibrio cholerae* (MBA). Following overnight incubation at 37°, RDE and complement were inactivated by 2.5% sodium citrate and heating at 56° for 30 min. Microtiter "V"-plates were used in the procedure (MBA).

Interferon assay. Titration of interferon was performed as previously described employing vesicular stomatitis virus and human foreskin fibroblasts (5). Employing this assay system, British human research interferon standard A 69/19 was found to have a titer of 5990 units. GMT was computed on interferon positive sera only.

Results. Results of the attempted induction of interferon with the polio vaccine are summarized in Table I. Serum samples were obtained on days 0, 2, 5, 10, 15, 20 and 25 following immunization. These times

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TABLE I. INDUCTION OF INTERFERON IN MAN BY LIVE POLIOVIRUS TYPE 2 VACCINE[a].

No.	Subject	Previous polio vaccine history	Neutralizing antibody response[b] Day	Titer	Interferon response[c]
1	R. M.	Live, trivalent	0	48	None
		1 yr prior	25	32	
2	D. D.	Live, trivalent	0	12	None
		10 yr prior	25	>256	
3	M. N.	Live, trivalent	0	32	None
		7 yr prior	25	26	
4	D. D.[d]	Never received oral	0	<4	None
			25	256	
5	S. R.	Unknown	0	54	None
			25	44	

[a] Given by mouth in a dose of approximately $10^{4.7}$ $TCID_{50}$ on day 0 (polio vaccine monovalent, type 2, Sabin strain, Wyeth, Lot. No. 17702).

[b] Against 100 $TCID_{50}$ of homologous strain, in WI-38 cells.

[c] Sera obtained on days 0, 2, 5, 10, 15, 20 and 25 following immunization.

[d] Virus recovered from rectal swab on day 5.

of sampling were chosen based on the reports in the Russian literature on interferon induction following oral poliovirus vaccine (6). Even though one subject had an undetectable neutralizing antibody titer and experienced a very marked rise in neutralizing antibodies, and another individual who had been immunized 10 yr previously also exhibited a significant antibody rise, no interferon response was observed in their sera. The results were similarly negative in the other three volunteers.

With the live attenuated influenza virus vaccine, interferon was detected in three of 15 volunteers (20%); all three of them exhibited a fourfold HI antibody response (Table II). This corresponds to an overall interferon response of 33.3% in the nine subjects who showed an equal to or greater than fourfold rise in HI antibodies. The interferon geometric mean titer (GMT) was low: 2.5 units. No interferon was detected, however, in sera or nasal washings of a subject (B.T.) who had shed the virus 3 days following the first dose of the vaccine, nor in another subject (L. A.) from whom virus was also recovered. No interferon was

TABLE II. INDUCTION OF INTERFERON IN MAN BY LIVE INFLUENZA A/ENGLAND/42/72 VACCINE[a].

No.	Subject	HI antibody response[b] Day	Titer	Interferon response[c] (units/2 ml) Serum (day detected)	Nasal wash
1	B. T.[d]	0	8	None	None
		30	32		
2	D. W.	0	16	None	None
		30	16		
3	D. B.	0	<8	2 units	None
		30	16	(day 3)	
4	C. S.	0	256	None	None
		30	128		
5	L. A.[d]	0	<8	None	None
		30	16		
6	L. L.	0	8	None	None
		30	128		
7	H. M.	0	8	None	None
		30	16		
8	P. J.	0	8	None	None
		30	8		
9	P. C.	0	<8	4 units	None
		30	32	(day 3)	
10	H. P.	0	32	None	None
		30	32		
11	T. M.	0	<8	2 units	None
		30	16	(day 3)	
12	D. D.	0	64	None	None
		30	64		
13	B. D.	0	8	None	None
		30	32		
14	K. G.	0	8	None	None
		30	32		
15	P. G.	0	<8	None	None
		30	16		

[a] Given intranasally by drops in dose of approximately $10^{7.5}$ $TCID_{50}$ on day 0 and 14 (Influenza A/England/42/72, Alice Strain, Smith, Kline and French).

[b] Against 4 hemagglutinating units of homologous strain.

[c] Sera obtained on days 3, 7, 16 and 23 following immunization. No response unless otherwise stated.

[d] Virus recovered in nasal wash on day 3 and 16 respectively.

detected in the nasal washings of any of the other subjects in the study, nor in the sera of subjects other than those alluded to above.

Discussion. Interferon induction has been reported following administration of a

TABLE III. INTERFERON INDUCING POTENTIAL OF VARIOUS VACCINES IN MAN.

Vaccine	Interferon induction		Reference
	Systemic	Local	
Vaccinia	N.D.[a]	+ (dermal crusts)	Wheelock, Proc. Soc. Exp. Biol. Med. **117**, 650 (1964).
Yellow fever (17-D)	+ (serum)	N.D.	Wheelock and Sibley, New Engl. J. Med. **273**, 194 (1965).
Measles (Edmonston)	+ (serum)	N.D.	Petralli *et al.*, New Engl. J. Med. **273**, 198 (1965).
Measles (Schwarz)	+ (serum)	N.D.	Desmyter *et al.*, J. Immunol. **99**, 771 (1967).
Live enteroviral vaccines	+ (serum) (urine)	+ (nasal wash)	Voroshilova, PAHO Scientific Publ. #226, p. 133 (1970).
Polio (type 2)	+ (serum)	N.D.	Smorodinstsev *et al.*, Ann. N.Y. Acad. Sci. **173**, 811 (1970).
Mumps	+ (serum)	+ (nasal wash)	Ibid.
Influenza (A_0, A_2 and B)	+ (serum)	+ (nasal wash)	Ibid.
Influenza (A_2)	+ (serum)	+ (nasal wash)	Jao *et al.*, J. Infect. Dis. **121**, 419 (1970).
Influenza (A_2)	+ (serum)	+ (nasal wash)	Murphy *et al.*, J. Infect. Dis. **128**, 988 (1973).
Mixed bacterial vaccine	0	N.D.	Rytel *et al.*, Amer. J. Epidemiol. **99**, 347 (1974).

[a] N.D. = not done.

number of currently available vaccines (1–4, 6, 7). These are summarized in Table III. In general, most of the vaccines which have been found to be good interferon inducers have been comprised of myxoviruses or paramyxoviruses such as influenza, measles, and mumps. It is well recognized that interferon response is more marked following infection with these groups of viruses than most other viral groups (8). For example, in our previous studies, Newcastle disease virus produced high serum interferon titers in mice, whereas coxsackievirus B_3 infection was associated with but a meager interferon response (9). It is thus perhaps not surprising that in this study, interferon was detected in 33.3% of subjects who developed a significant HI antibody response following intranasal administration of live attenuated influenza A/England/42/72 (H3N2) vaccine, but in none of the subjects following poliovirus vaccine administration.

The latter finding is at variance with reports in the Russian literature where interferon response had been reported following both polio type 2 and "live enteroviral vaccine" (LEV) comprising ECHO viruses (6, 7). The explanation for this is not readily apparent, but is unlikely to be due to the insensitivity of our interferon assay system because the British human interferon standard A 69/19 which contains approximately 5000 units was found to have 5990 units of interferon in our assay. Admittedly, our findings and conclusions are based on a limited number of study subjects, only two of whom had evidence of infection with the vaccine strain of polio type 2. However, it is relevant to point out that D. A. J. Tyrrell and T. Matthews (personal communication)* were similarly unable to confirm the interferon-inducing potential of a live attenuated enteroviral vaccine, LEV-4 (ECHO 1) of Russian origin. These negative results were obtained despite the fact that the subjects studied by Tyrrell and Matthews developed a fourfold or greater neutralizing antibody response, their blood was sampled for interferon at frequent time intervals, and the interferon assay employed was a sensitive one.

The interferon response following immunization with the Alice strain of influenza virus A/England/42/72 (H3N2) occurred in fewer subjects, and the levels were lower

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than in a study reported by Jao *et al.* in which Bethesda 10/63 strain of influenza A_2 was employed (10). In their study, 10 of 15 subjects (66.7%) who became infected had demonstrable interferon levels in nasal washings and/or sera. The serum interferon GMT was 6.0 compared with 2.5 in the present study. Their strain, however, was less attenuated than ours. In a recent report by Murphy *et al.* where interferon response was studied following administration of two influenza virus strains, one a wild type, influenza A/Bethesda/1968 (H3N2) and the other a temperature-sensitive recombinant virus, influenza A (H3N2)-ts-1-[E], a consistently lower and less prolonged interferon response was found with the attenuated strain (11). Interferon was detected in nasopharyngeal washings of 8 of 17 subjects (47.1%) infected with the attenuated strain, and of six of seven subjects (85.7%) given the wild type. The GMT's also differed significantly: 2.3 vs. 78, respectively. The peak of interferonemia occurred on day 2 with the attenuated, and day 3 with the wild strain. We did not commence our sampling until day 3 following immunization (based on the previously reported results of Jao *et al.* (10) who found maximal interferon response on day 4), and thus, we may have missed the time of peak response. Our data also suggests that the attenuation of the vaccine employed in the present study was not due to its high interferon-inducing potential.

What then are the prospects for the possible use of vaccines as interferon inducers in man? Their role will probably be quite limited. Some vaccines (influenza A) appear to lose their interferon-inducing potential with attenuation. Enteroviral vaccines (including polio) do not appear to be good interferon inducers, which correlates with the poor interferon response elicited by this group of viruses in experimental infections in mice (9). Finally, vaccines containing bacterial antigens do not seem to induce interferon in man, even though various bacteria, and their products, are capable of interferon induction in experimental animals when given in doses disproportionately larger than those which could be safely administered in man (5).

Summary. The purpose of this study was to extend the spectrum of vaccines with interferon-inducing potential in man. The vaccines selected for study were the commercially available attenuated poliomyelitis vaccine type 2 (Sabin strain) and the new live attenuated influenza A/England/42/72 (H3N2) vaccine ("Alice" strain). Five subjects, two of whom had low or undetectable polio type 2 neutralizing antibody levels were given the type 2 vaccine ($10^{4.7}$ $TCID_{50}$) in the standard manner orally. Even though the two individuals with low titers experienced a fourfold or greater antibody rise and one of them shed the virus in his stool, neither they nor the remaining three volunteers developed detectable levels of interferon in their sera obtained at very closely spaced intervals from day 0 to day 25 following immunization. Fifteen subjects were given approximately $10^{7.5}$ $TCID_{50}$ of influenza A/England/42/72 (H3N2) by nasal drops. Specimens consisting of sera and nasal washings were obtained at closely timed intervals for 23 days, starting with day 3 following immunization. Interferon could be detected in three of nine (33.3%) subjects who had fourfold or greater HI antibody rises. No interferon was detected in nasal washings, however. It is concluded that poliomyelitis is not a good interferon inducer in man. Live attenuated influenza vaccine does induce an interferon response in subjects with low initial serum antibody titers. This response is at best modest. This latter finding also suggests that the attenuation of the Alice strain of influenza A vaccine is not dependent on its interferon inducing potential.

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