

Some Studies on the Selection and Efficiency of Live Influenza Vaccine Viruses

D. A. J. TYRRELL¹ & A. S. BEARE¹

Live influenza virus vaccination has potential advantages and disadvantages compared with killed virus vaccine given by intramuscular injection.

Attempts have been made to achieve complete attenuation of influenza A2 and B strains by up to 30 serial passages in the allantoic cavity of eggs at 33°C. No attenuation occurred. The method of passing viruses in the presence of an increasing concentration of horse serum, thus selecting inhibitor-resistant variants, has also been tested. It was shown that serial passage induced an increasing degree of inhibitor resistance in the virus and an increasing degree of attenuation when tested in volunteers. This was observed with both an Asian strain and a Hong Kong strain. The Hong Kong virus was also passed serially at 25°C in eggs but did not seem much further attenuated as a result; however, a virus strain supplied by H. F. Maassab with a much higher degree of adaptation to low temperature is being tested.

Partly or completely attenuated strains have been used to induce or detect protection. Influenza B strains gave partial protection after 1 dose and almost complete protection after 2 doses and protection persisted for 7 months. A comparative study showed that there was protection after vaccination with killed virus by the parenteral route, and with live virus by the nasal route.

Live influenza vaccines have been under study for many years, but our group has been working in this field for only about 10 years, and then only on a relatively small scale. We shall limit ourselves to pointing out some of the potential advantages and disadvantages of this method of immunization, as opposed to that of parenterally administered killed virus vaccine, and to summarizing some of the work carried out by the Common Cold Research Unit.

ADVANTAGES AND DISADVANTAGES OF LIVE VACCINES

Advantages

A virus that multiplies can be expected to induce an antigenic response with a small inoculum, whereas with killed virus a relatively large inoculum

is needed. Nevertheless, this advantage might be lost if over-attenuated viruses—which could multiply only a little—were used, or if much virus were lost during distribution (e.g., during freeze-drying or when praying the virus at the time of administration). The vaccinee would find live virus more pleasant to take, since no needle is required, but the absence of local pain might be outweighed later by coryza or other symptoms of infection. Finally, live virus might induce a more satisfactory form of immunity than killed parenteral vaccine; in particular, it might stimulate the production of local secretory (IgA) antibody to a greater and more uniform extent.

Disadvantages

In order to prepare live vaccine it is necessary not only to grow the virus in eggs, in the same way as for killed vaccine, but also to produce a suitable degree of attenuation so that the virus multiplies and

¹ Common Cold Research Unit, Salisbury, Wilts., England.

induces immunity with little or no symptoms or signs of infection. Providing a virus grows well, it can be harvested and inactivated and used for the preparation of a killed vaccine. However, there are no agreed methods for attenuating viruses for man, and no agreed laboratory "markers" of an attenuated strain. Nevertheless, if a vaccine were to be used against a rapidly advancing pandemic such as that of the Hong Kong strain, it would be necessary to have a well worked-out and reliable technique in order to have any hope of getting the vaccine to the population before the first epidemic wave occurred. Although fears have been expressed that attenuated vaccines might, in fact, initiate an epidemic, there is at present little or no evidence that vaccine strains spread.

EXPERIMENTS ON ATTENUATION

In general, the viruses used at the Common Cold Research Unit have been recovered from clinical specimens by inoculation of leucosis-free eggs. They have then been passed and titrated in the allantoic cavity of eggs incubated at 33°C. Volunteers have been inoculated in isolation and tested for virus infection and observed clinically by methods described elsewhere.

It has been reported on several occasions that viruses have been attenuated by serial passage in eggs. For example, Isaacs & Roden (1956) could not infect volunteers with over 100 000 EID₅₀ of an influenza A/1953 strain passed briefly in eggs, and Ždanov (1967) reported complete loss of infectivity for man after further passages of an A2 strain in eggs. We found some evidence of a loss in virulence of an influenza B strain, B/England/101/62, as it became adapted to eggs, but thereafter it and other strains retained their capacity to infect and produce mild reactions after up to 30 passages *in ovo* (Beare et al., 1968). A proportion of volunteers in factories to whom it was administered experienced unpleasant symptoms (Table 1), and such viruses would probably not be acceptable for repeated or general use. About 10⁵ EID₅₀ (representing 1 ml of a dilution of 1/100 000 of a typical allantoic fluid) infected a substantial proportion of volunteers. On the other hand, we found the inhibitor-resistant Iksha strain well attenuated for man after 6 passages in eggs and it became over-attenuated after 1 further human and egg passage, and would no longer infect (McDonald et al., 1962; Beare et al., 1967).

TABLE 1
RESULTS OF INOCULATING VOLUNTEERS WITH EARLY-
AND LATE-PASSAGED INFLUENZA B/England/13/65
VIRUS VACCINE

No. of egg passages	No. of volunteers	No. of virus isolations	No. of antibody rises	No. of clinical reactions
5	15	6	4	7
30	9	7	6	5

More recently attempts were made to attenuate viruses by selecting inhibitor-resistant (non-avid or " - ") strains by serial passage in the presence of increasing concentrations of normal horse serum,¹ and the viruses acquired resistance to inhibitors and became substantially attenuated. We are therefore able to support the suggestion of earlier workers (e.g., Solov'ev et al., 1961) that inhibitor-resistant strains are attenuated.

It has been stated that vaccine viruses that have been adapted to growth at 25°C became further attenuated, so that they could be used in children (Smorodincev et al., 1965); furthermore, Maassab (1969) has shown that "cold" variants selected from mouse-virulent strains may be attenuated and immunogenic for that species. In order to eliminate the mild colds produced by the partially inhibitor-resistant strain of A2/Hong Kong/68 virus the virus was further passed, with some difficulty, at 25°C. In addition to studies at Salisbury,¹ this virus was given to a small group of volunteers in a factory. No trouble with symptoms was reported but only 4 out of 20 showed serological evidence of infection and it seems that the virus had become over-attenuated.

Recently Dr H. F. Maassab kindly supplied us with viruses that he had selected by picking plaques in chick embryo kidney cell monolayers. These viruses were better able to grow in chick embryos at 25°C than the viruses we had selected. The studies are incomplete but it seems clear that their infectivity for man has been greatly reduced; only 1 out of 8 antibody-free volunteers who were given 10⁴ EID₅₀ of A2/Aichi/2/68 virus vaccine showed an antibody response, and it seems that the strain may be over-attenuated.

It is possible to produce "cold" or temperature-sensitive mutants of influenza viruses by treatment

¹ See the paper by A. S. Beare on p. 595 of this issue.

TABLE 2
RESULTS OF CHALLENGING PREVIOUSLY VACCINATED VOLUNTEERS WITH
PARTLY ATTENUATED B/England/13/65 VIRUS VACCINES

Vaccine given	Route	Proportion of subjects	
		Infected	With moderate or more symptoms
Live	Nasal	1/40	1/41
Killed	Intramuscular	6/41	5/39
Much diluted, heat-killed	Nasal	6/23	9/24
Killed influenza A2 control	Intramuscular	10/18	12/22
		16/41	21/46

with various mutagens, but we have no experience of their effects in man.

RESISTANCE TO REINFECTION

It is possible to assess the relative effectiveness of killed and live viruses by measurement of circulating antibody, and on this basis the response to intranasal live vaccine is not as good as that to killed parenteral vaccine. Early studies showed that volunteers were resistant to reinfection with the A2/Jksha strain, although some of them developed little antibody that could be detected in the circulation. Challenge with live vaccine or with partly attenuated virus strains seems to us a useful means of detecting immunity in a direct way, and one that may help to predict immunity in an epidemic. Experiments with influenza B virus showed that it was possible, by challenge with a live attenuated virus, to detect resistance to reinfection both in volunteers given killed virus parenterally and in those given an attenuated live vaccine (Table 2). Other studies of this type showed that there was more resistance after 2 doses than after 1 dose of live vaccine, and that resistance persisted 7 months after the initial dose (Beare et al., 1969). Since the Hong Kong virus appeared, we have used challenge with attenuated virus to look for the potential protective effect of killed virus vaccines. The results obtained were inconclusive because the strain used was not sufficiently infectious, but we propose to make further attempts.

FURTHER NEEDS

There seems to be good evidence that Soviet workers have provided protection against epidemic influenza with live influenza vaccines (e.g., Slepüşkin et al., 1967) and our own evidence indicates that this should be possible. We therefore feel that there is need to make careful studies of the comparative effectiveness, practicability, and acceptability to the public of vaccines of this type and of the killed parenteral vaccines, prepared from intact or split viruses and given both with and without adjuvants.

At the same time more information is needed about the relationship between the *in vitro* properties of live vaccine strains and their behaviour in man. We do not know the type or grade of temperature-sensitivity that would give a reliable guide to the degree of attenuation desired. It is possible that there would be better attenuation if temperature-sensitivity were introduced into one part of the genome rather than into another. It might also be possible by recombination to transfer the antigenic character of a new strain of a virus to one that has already attenuated, thus reducing the need for selecting mutants. However, if the attenuated strain were avirulent because of a *ts* step or some other character closely connected with the synthesis of haemagglutinin, this might be impossible. By whatever means it may be done, it seems likely that we shall have to walk a narrow path between choosing viruses that are good antigens but produce unacceptable symptoms and those that produce no symptoms but incomplete immunity.

REFERENCES

- Beare, A. S., Bynoe, M. L. & Tyrrell, D. A. J. (1968) *Brit. med. J.*, **4**, 482
- Beare, A. S., Tyrrell, D. A. J., Hobson, D., Howells, C. H. L., Pereira, M. S., Pollock, T. M. & Tyler, L. E. (1969) *J. Hyg. (Lond.)*, **67**, 1
- Beare, A. S., Tyrrell, D. A. J., McDonald, J. C., Pollock, T. M., Taylor, C. E. D., Howells, C. H. L. & Tyler, L. E. (1967) *J. Hyg. (Lond.)*, **65**, 245
- Isaacs, A. & Roden, A. T. (1956) *Lancet*, **2**, 697
- Maassab, H. F. (1969) *J. Immunol.*, **102**, 728
- McDonald, J. C., Zuckerman, A. J., Beare, A. S. & Tyrrell, D. A. J. (1962) *Brit. med. J.*, **1**, 1036
- Slepuškin, A. N., Bobyleva, T. K., Russina, A. E., Vitkina, B. S., Èllengorn, N. S. & Ždanov, V. M. (1968) *Bull. Wld Hlth Org.*, **36**, 385
- Smorodincev, A. A., Alexandrova, G. A., Čalkina, O. M. & Selivanov, A. A. (1965) In: *First Annual Symposium on Applied Virology, Boca Raton, Fla., 1964*, p. 142
- Solov'ev, V. D., Orlova, T. G., Porubel', L. A. & Vasil'eva, I. N. (1961) *Vop. Virus*, **6**, 684
- Ždanov, V. M. (1967) In: *First International Conference on Vaccines against Viral and Rickettsial Diseases of Man, Washington, D.C., 1966*, Washington, D.C., PAHO/WHO, p. 9
-