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The relative immunogenicity in mice of whole and split influenza virus

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SUMMARY

The relative immunogenicity in mice of whole influenza virus, and virus split with different disrupting agents, was compared. Using the single radial immunodiffusion test to estimate the haemagglutinin antigen concentration in different virus preparations, it was found that, in general, split virus preparations induced substantially lower titres of HI antibody in mice than whole virus after one or two injections of the antigen.

INTRODUCTION

Although influenza virus vaccines prepared by disrupting influenza virus with ether (Davenport *et al.* 1964), sodium deoxycholate (DOC) (Webster & Laver, 1966), or tri(*n*-butyl) phosphate (TNBP) (Neurath *et al.* 1970) have been shown to be less pyrogenic than whole virus vaccines, conflicting claims have been made about the immunogenicity of such preparations when compared with whole virus. Thus, whereas influenza viruses disrupted with ether (Davenport *et al.* 1964) or TNBP (Neurath *et al.* 1970) were claimed to be as immunogenic as whole virus, similarly disrupted influenza viruses were reported to induce significantly lower primary antibody responses in mice than whole virus (Barry, Staton & Mayner, 1974). Also, vaccines containing TNBP-disrupted influenza virus were claimed to be significantly less immunogenic in man than whole virus vaccines (Barry *et al.* 1976). Similarly, DOC-disrupted influenza viruses were not as immunogenic as whole virus (Webster & Laver, 1966; Rubin, Pierzchala & Neurath, 1967).

This apparent conflict could be due to the methods used for the estimation of the haemagglutinin antigen in disrupted virus preparations. In the studies reported above, the haemagglutinin antigen concentrations were measured by the haemagglutination or chicken cell agglutination methods which rely on the ability of the haemagglutinin antigen to bridge and agglutinate erythrocytes. Since disrupted influenza virus preparations may contain a variety of particles with differing abilities to bridge and agglutinate erythrocytes, such methods are considered unsuitable for estimating the haemagglutinin antigen concentrations of disrupted virus preparations (Perkins & Regamey, 1973). A single radial immunodiffusion test (SRD) which measures the ability of the haemagglutinin antigen to combine with specific antibody and hence is independent of problems associated with those methods which rely on bridging and agglutination of erythrocytes has been proposed (Schild, Wood & Newman, 1975).

Using the SRD test to estimate the haemagglutinin antigen concentrations, we have compared the relative immunogenicity in mice of whole virus and virus split with disrupting agents. In our study, we have used three vaccine strains of influenza virus (H3N2) and three disrupting agents currently employed in commercial production of influenza vaccines.

MATERIALS AND METHODS

Viruses

Three recombinant strains of influenza virus, MRC 2, MRC 11 and IVR 2, were kindly supplied by the Director, Commonwealth Serum Laboratories, Parkville, Australia. MRC 2, MRC 11 and IVR 2 are recombinants between A/Mt Sinai/PR8/34 and A/England/42/72, A/Port Chalmers/1/73 and A/Papua New Guinea/1/75 (A/Victoria/3/75 type) respectively.

Concentration and purification of virus

Virus was grown in the allantoic cavities of 10-day-old embryonated hens' eggs and purified by one cycle of rate zonal centrifugation in 15–60% (w/v) sucrose.

Inactivation of virus

To prevent antibody responses due to infection of mice by live virus in antigen preparations, purified virus was inactivated by ultraviolet light. The absence of live virus in irradiated preparations was confirmed by tests in embryonated hens' eggs.

Preparation of antigens

Preparation of ether-split virus. The purified inactivated virus was disrupted with Tween 80 and ether essentially by the method described (Davenport *et al.* 1964).

Preparation of DOC-split virus. The purified inactivated virus was disrupted with DOC essentially by the method described by Webster & Laver (1966).

Preparation of TNBP-split virus. The purified inactivated virus was disrupted with Tween 80 and TNBP essentially by the method described by Neurath *et al.* (1970).

Whole virus. This was a purified inactivated whole virus preparation.

Estimation of the haemagglutinin (HA) antigen

The HA antigen concentration in each of the preparations was measured by the SRD test as described (Schild *et al.* 1975). Based on their values of annulus areas of zones produced in the test, each of the preparations was diluted so that they all contained the same amount of HA antigen. This was confirmed by reassaying the diluted samples by the SRD test. For the whole virus preparation, this was equivalent to a haemagglutination titre of approximately 10000/0·25 ml.

The monospecific HA antiserum for each strain used in the SRD tests was prepared by immunizing rabbits with purified HA prepared by the bromelain method as described by Brand & Skehel (1972). Double immunodiffusion tests

were performed on these sera to establish their specificity as judged by the absence of antibodies to other viral antigens.

Immunization of mice

For each strain of virus, all four antigen preparations were injected at two dose levels, undiluted and diluted 1/10. Each dilution of antigen was injected intraperitoneally in a volume of 0·25 ml into each of sixteen 8-week-old inbred Quackenbush mice. Three weeks after the first injection half the mice were bled. The others received a second injection identical to the first injection, and were bled 12 days later. Sera were separated and stored at −20 °C until tested.

Haemagglutination-inhibition assay for HI antibodies

The sera were treated with *Vibrio cholerae* receptor-destroying enzyme (Wellcome Reagents Ltd, Beckenham, England) to destroy non-specific inhibitors of haemagglutination (Isaacs & Bozzo, 1951). The sera were titrated in microtitre plates as described by Palmer *et al.* (1975).

RESULTS

The data presented in Fig. 1 show, for each virus strain, titres of HI antibody induced by one or two injections of different antigen preparations at two dose levels.

The titres of HI antibody induced by one injection of DOC-, ether- or TNBP-split virus preparations at both dose levels were significantly lower than those induced by the corresponding whole virus preparations. (The levels of significance of the differences are indicated in Fig. 1.) Also, in no instances were the geometric mean HI titres induced by the higher doses of the split virus preparations greater than those induced by the lower doses of the corresponding whole virus preparations.

The titres of HI antibody induced by two injections of most of the split virus preparations were also significantly lower than those induced by the corresponding whole virus preparations (see Fig. 1). The only exceptions were the ether-split preparation of the MRC 11 strain of virus at both dose levels and the TNBP-split preparation of the same strain of virus at the lower dose level. Here, the titres of HI antibody induced by these preparations, although lower than, were not significantly different ($P > 0·05$) from those induced by the corresponding whole virus preparation.

In most instances, the geometric mean HI titres induced by the higher dose of the split virus preparations were not greater than those induced by the lower dose of the corresponding whole virus preparations. Also, in most instances, the geometric mean HI titres induced after two injections of the split virus preparations at both dose levels were not greater than those induced after one injection of the corresponding whole virus preparations.

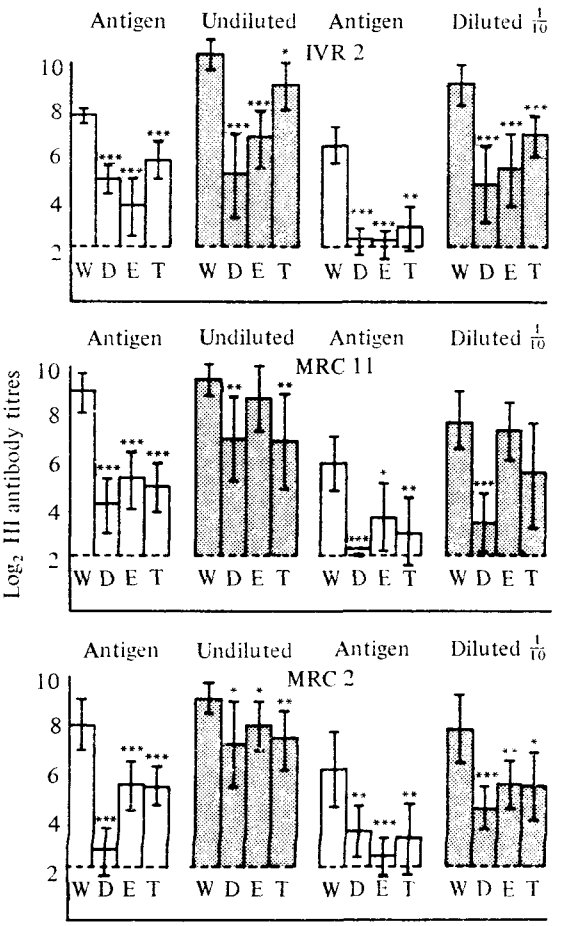

Fig. 1. Titres of HI antibody induced by different antigen preparations of MRC 2, MRC 11 and IVR 2 strains of influenza virus (bars indicate 95 % confidence limits of the mean). □, After one injection; ▨, after two injections; W, whole virus; D, DOC-split virus; E, Ether-split virus; T, TNBP-split virus. Student's *t* test analyses were carried out between HI titres of each split virus preparation and the corresponding whole virus preparation in each group and the levels of significance of the differences are represented by the asterisks. *** $P < 0{\cdot}001$, ** $0{\cdot}01 > P > 0{\cdot}001$, * $0{\cdot}05 > P > 0{\cdot}01$.

DISCUSSION

For split virus vaccines to be acceptable as alternatives to whole virus vaccines it would be desirable that they are not only less pyrogenic than but also as immunogenic as whole virus vaccines.

An apparent conflict in the reported studies on the relative immunogenicity of split virus and whole virus vaccines has been referred to in the Introduction and, as pointed out, a comparison of antibody responses to such preparations is difficult to interpret because of the improper methods used for estimating the antigenic dose of such preparations.

Using the SRD method of estimating the HA antigen (Schild *et al.* 1975) which relies on the ability of the HA antigen to bind specific antibody, we have com-

pared the relative immunogenicity of whole virus and virus split with different disrupting agents. The results presented in this paper show that, following one injection of whole virus or variously split virus preparations, whose antigenic dose had been validly estimated, the titres of HI antibody induced by the split virus preparations were significantly lower than those induced by the corresponding whole virus preparations. Also, the geometric mean HI titres induced by ten times higher doses of split virus preparations were no greater than those induced by the lower doses of whole virus preparations.

This reduced immunogenicity of split virus preparations with respect to their whole virus preparations was also obvious, in most instances, after two injections of these preparations.

Also, in most instances, at comparable dose levels, the geometric mean HI titres induced by two injections of the split virus preparations at both dose levels were not greater than those induced by one injection of the corresponding whole virus preparations.

There is no obvious explanation for reduced immunogenicity of the viral subunits, present in split virus preparations, although it has been suggested (Davenport, 1968) that this may be due to the smaller size of the antigen and the consequent failure of the effectors of the immune system to process it optimally. A direct relationship between the size of the immunizing antigen and its immunogenicity has been demonstrated (Neurath & Rubin, 1971; Howard *et al.* 1971).

If these findings in immunologically unprimed mice can be extended to humans then it seems possible that split virus vaccines would be less effective than whole virus vaccines in an essentially immunologically virgin population, e.g. very young children or during the emergence of a totally new strain of influenza virus to which the majority of the population would not have been exposed. This was demonstrated during the recent vaccination campaign in the U.S.A. against a swine influenza virus where split virus vaccines were found to be considerably less immunogenic than whole virus vaccines in people with no previous exposure to the antigens in the vaccines (Parkman *et al.* 1976). However, whole virus vaccines were found to be moderately reactive.

Our results would suggest that if split virus vaccines were to be used in an immunologically virgin population, at least a two-dose schedule would be essential. Although the HI titres induced by two doses of the split virus preparations were lower than those induced by the whole virus, they were, generally, substantially higher than those induced by a single dose. Alternatively, a two-dose schedule of a much lower dose of whole virus vaccines would be at least as effective.

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