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Antigenicity in hamsters of inactivated vaccines prepared from recombinant influenza viruses

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SUMMARY

Inactivated vaccines prepared from influenza virus strains obtained by the recombination of A/PR/8/34 (H1N1) or A/FM/1/47 (H1N1) viruses with A/Victoria/3/75 (H3N2) virus, were tested for their antigenicity in hamsters. The parental origin of the genes of each cloned recombinant virus was determined by polyacrylamide gel electrophoresis, and vaccines prepared from each strain by concentration, purification on sucrose density gradients and inactivation with formalin. All the recombinant strains used in these studies possessed surface haemagglutinin and neuraminidase antigens derived from the A/Victoria/75 parent strain.

On inoculation into hamsters, at equivalent concentrations, these vaccines varied in their ability to induce haemagglutination-inhibiting (HI) antibodies in the serum. This variation was not dependent on concentration and was observed using neutralization and single radial haemolysis, as well as HI. The possible reasons for the findings are discussed.

INTRODUCTION

Immunization of man against influenza is usually carried out using inactivated whole or subunit virus vaccines (see reviews Davenport, 1979; Potter, 1979). Whole virus vaccines have been used for many years for the protection of humans against influenza (Davenport, 1961), while subunit vaccines, containing only the haemagglutinin (H) and neuraminidase (N) antigens of the influenza virus particle, have only recently become available (Bachmayer, 1975). To obtain these vaccines seed virus strains with good growth capacity and bearing relevant surface antigens are grown in embryonated eggs, inactivated by treatment with formalin or β-propiolactone and concentrated and purified by zonal centrifugation in sucrose density gradients (Brady & Furminger, 1976). These materials are then used either as whole virus preparations, split to produce a disrupted virus vaccine or split to give preparations from which purified, subunit vaccines can be prepared (Laver & Webster, 1966; Bachmayer, 1975; Brady & Furminger, 1976). The seed viruses used for vaccine production are obtained by recombination (Kilbourne, 1963) of wild-type influenza viruses bearing the current haemagglutinin (H) and neuraminidase (N) antigens with strains such as A/PR/8/34, a virus with high-yielding

growth characteristics (Kilbourne, 1969; McCahon & Schild, 1972; McCahon, Stealey & Beare, 1976). Those viruses bearing surface antigens derived from the wild-type parent virus and showing high-yielding growth characteristics are used for vaccine production.

In this approach to influenza virus vaccine production the assumption is made that the serum haemagglutination-inhibiting (HI) antibody titres induced by the vaccine are directly related to the virus antigen content of the vaccine, and that vaccines prepared from recombinant strains with high growth capacity and bearing the relevant surface antigens will constitute the best vaccines (Baez, Palese & Kilbourne, 1980). This is not necessarily true, since previous studies from this laboratory suggest that different recombinant viruses bearing the same surface antigens may vary significantly in their capacity to induce serum antibody in hamsters (Jennings & Potter, 1973; Jennings, Potter & McLaren, 1974).

In the present study, we report differences in the ability of inactivated vaccines prepared from recombinant influenza viruses, bearing the same surface antigens and containing the same concentration of haemagglutinin, to induce serum HI antibody in hamsters. The recombinants were all derived from A/Victoria/75 virus with either A/PR/8/34 (H1N1) or A/FM/1/47 (H1N1) virus as the second parent. The HA content of vaccines prepared from these strains were all standardized by rocket immunoelectrophoresis.

MATERIALS AND METHODS

Virus strains

Influenza viruses A/PR/8/34 (H1N1), A/FM/1/47 (H1N1), A/Victoria/3/75 (H3N2) and X47 (H3N2), a recombinant of A/PR/8/34 and A/Victoria/75 viruses, were obtained from Dr J. J. Skehel, National Institute for Medical Research, Mill Hill, London. A group of A/PR/8/34 and A/Victoria/75 recombinants were obtained from Dr C. Huygelen, Recherche et Industrie Therapeutique, Rixensaart, Belgium. Virus pools were prepared by the allantoic inoculation of embryonated hens' eggs, as described previously (Jennings, Denton & Potter, 1976). Virus infectivity titres were determined using the allantois-on-shell method (Fazekas de St Groth, Witchell & Lafferty, 1958) and the haemagglutinin titres by standard methods (Expert Committee on Respiratory Virus Vaccines, 1959).

Animals

The source of hamsters and the methods used for virus infection of hamsters together with the preparation and titration of lung homogenates is described elsewhere (Abou-Donia, Jennings & Potter, 1980).

Preparation of recombinant viruses

(*a*) *A/PR8–A/Victoria/75 recombinants*

Recombinants were prepared according to the methods described by Lubeck, Palese & Schulman (1979). Confluent MDCK cell monolayers on plastic dishes were infected with 10 and 100 PFU per cell of A/PR/8/34 and A/Victoria/3/75 viruses respectively, in the presence of 0·25 u./ml of TPCK trypsin (Worthington Biochemical Company, New Jersey, U.S.A.). After 30 min at room temperature

(RT) the monolayers were washed with PBS, and Eagles medium containing 2% foetal calf serum added. After incubation at 33 °C for 18 h in atmosphere of 5% CO_2 in air, the mixed yields were assayed for plaques at 33 °C in MDCK cell monolayers in the presence of trypsin and A/PR8 antiserum. Plaques with morphology similar to those produced by A/PR8 or untypical of those produced by A/Victoria/75 virus were cloned at least twice by plaque-to-plaque passage in the presence of A/PR8 antiserum prior to the preparation of virus pools in embryonated eggs.

(b) A/FM1–A/Victoria/75 recombinants

Recombinant virus strains were prepared by the double infection of either 10-day embryonated eggs (McCahon & Schild, 1972) or MDCK cell monolayers (Lubeck *et al.* 1979). In embryonated eggs, double infections were carried out by the allantoic inoculation of $10^{3\cdot0}$ and $10^{4\cdot0}$ $EBID_{50}$/ml of A/FM1 and A/Victoria/75 parent strains respectively. After incubation for 18 h at 33 °C the allantoic fluids were collected, and 0·1 ml samples of diluted fluids mixed with A/FM1 antiserum and left overnight at 4 °C. The virus-antiserum mixtures were then inoculated into eggs or egg-bit pieces, incubated at 33 °C for 48 h and fluids from individual cultures passaged twice at high limit dilution in eggs or egg-bits in the presence of A/FM1 antiserum, and virus pools prepared in embryonated eggs.

All virus-containing allantoic fluids were tested by HI and neuraminidase inhibition (NI) tests and strains bearing N3N2 surface antigens used for further study.

Isotopic labelling of virus-specific polypeptides in infected cells

Monolayers of MDCK cells in Petri dishes were inoculated with 10 to 100 PFU/cell of various putative recombinant viruses, and incubated for 30 min at room temperature. Maintenance medium was added and the dishes incubated at 33 °C for 6 h in an atmosphere of 5% CO_2 in air. The medium was then removed and 1 ml of MEM deficient in methionine and containing 20 μCi/ml of (^{35}S) methionine (The Radiochemical Centre, Amersham, Bucks) was added to the cells. After 15 min, the label was removed, each monolayer was washed with cold PBS and immediately lysed with 0·2 ml of a solution containing 2% SDS, 1% mercaptoethanol, 6 M urea, 0·005% bromophenol blue and 0·0625 M Tris hydrochloride (pH 6·8).

SDS-polyacrylamide gel electrophoresis

Virus-specific polypeptides from infected cells were subjected to SDS-polyacrylamide gel electrophoresis using the methods of Laemmli (1970) as applied by Ritchey, Palese & Schulman (1977). Thus, the lysates from cells infected with putative recombinant viruses were boiled for 2 min and analysed for polypeptides in SDS-polyacrylamide gradient and single-strength gels. A constant 200 V was applied to the gels for 4 to 8 h and tris-glycine buffer circulated using a peristaltic pump. After electrophoresis, gels were dried and exposed to X-ray film for 1 to 7 days to localize the labelled proteins. The parental origin of the recombinant virus polypeptides was determined by direct reference to the migration of polypeptides from the parent viruses tested in the same gel.

Serological tests

(*a*) *Haemagglutination-inhibition*

HI tests were carried out using a modification of the microtitre method (Sever, 1962). Before testing, serum samples were treated with cholera filtrate for 18 h at 37 °C and subsequently heated for 60 min at 56 °C. The serum dilutions in PBS were mixed with equal volumes of buffer containing eight (50 %) haemagglutination (HA) units of RIT 4050 virus antigen. A further volume of 0·5 % fowl erythrocytes in PBS was added to each well and the cells allowed to settle at RT. Titres were expressed as the highest serum dilution causing a 50 % reduction in HA. The sera used for identification of the HA antigens of the recombinant virus strains were mono-specific sera raised against A/Victoria/75, A/PR8 and A/FM1 viruses in ferrets.

(*b*) *Neutralization tests*

Sera were tested for neutralizing antibody to RIT 4050 virus by the allantois-on-shell (A-O-S) method (Fazekas de St Groth *et al.* 1958), as described elsewhere (Potter *et al.* 1975). In the present study RIT 4050 virus was used as antigen at a concentration of eight haemagglutinating units. Neutralizing antibody titres were calculated by the method of Reed & Muench (1938).

(*c*) *Single radial haemolysis* (*SRH*)

SRH was carried out to determine the antibody response of hamsters to immunization, using the methods of Oxford *et al.* (1979). Immunoplates containing virus-sensitized cells and guinea-pig complement in agarose gels were prepared according to standard methods (Schild, Oxford & Virelizier, 1976). The strain of virus used in all SRH tests was RIT 4050 virus.

(*d*) *Neuraminidase-inhibition tests*

NI tests were carried out using standard World Health Organisation methods (Aymard-Henry *et al.* 1973), using the X42 (HEqui–1/N2) recombinant strain as antigen. The N2 antigen of this virus was derived from A/Port Chalmers/73 (H3N2), and hyperimmune rabbit serum to X42 virus was used for the identification of the neuraminidase antigens of recombinant virus strains.

Rocket immunoelectrophoresis

The concentration of HA in the inactivated virus vaccines prepared from different recombinant viruses was standardized by rocket immunoelectrophoresis (Laurell, 1965; Oxford, *et al.* 1979), using monospecific antiserum to purified influenza virus RIT 4050 HA. This antiserum was prepared according to the methods of Brand & Skehel (1972).

After electrophoresis the rocket-shaped precipitates were stained with 0·3 % Kenacid blue (British Drug Houses, Poole, Dorset) in 50 % methanol containing 7 % (w/v) glacial acetic acid, and the rockets' heights measured using a micrometer eye-piece (AMT Marketing and Sales, Shalford, Surrey). HA concentrations in each preparation were determined by comparison with a standard preparation of A/Victoria/75 virus of known HA concentration, kindly supplied by Dr J. S. Oxford, National Institute for Biological Standardisation and Control, Holly Hill, London.

Preparation of inactivated influenza virus vaccines

Pools of parental A/Victoria/75 virus and recombinant virus strains prepared from A/Victoria/75 and A/PR8 or A/FM1 viruses were made in 10-day embryonated eggs, concentrated and purified by differential and rate-zonal centrifugation in 10–40 % linear sucrose density gradients and inactivated using 0·04 % formaldehyde solution. Prior to inoculation into hamsters the concentration of HA in each vaccine was adjusted to give an inoculum of 20 μg/HA per ml.

RESULTS

The surface antigens of the influenza virus strains

The identity of the HA antigens in the three parental viruses and ten recombinant virus strains used in the present study was determined by HI tests using monospecific ferret antisera. In addition, all the viruses were tested for 50 % inhibition of neuraminidase activity using antiserum prepared against X42 virus. The results are shown in Table 1. All the recombinant virus strains possessed surface antigens derived from the A/Victoria/75 parent strain and showed H3N2 specificity, although the dilution of standard serum required to inhibit the HA or NA activity of the various recombinant strains was variable, particularly for the NA antigen. Thus, a 1 in 20000 dilution of N2 antiserum inhibited the NA activity of strain VFE-2, but dilutions of 1 : 1000 were required for strains VP1 and VP2.

Polypeptide analysis of influenza viruses

The gene composition of each virus was determined by polypeptide analysis, and it was found that virus strains VFEB-82, VFEB-91 and VFE-1 possessed both matrix (M) and non-structural (NSl) proteins coded for by genes derived from A/FM1. The M proteins of three other strains, VP1, VP2, and VP7 migrated similarly to the M protein of A/PR8 and these viruses thus derive the gene coding for this protein from A/PR8. Similarly, the gene's coding for the NS proteins of strains VP1 and VP2 were derived from A/PR8 and also the NP genes of VP1 and VP7.

The gene composition of RIT 4050 was reported by Florent *et al.* (1977), while the gene constellation of X47 virus is identical to that of RIT 4050. Thus both RIT 4050 and X47 derive P2, P3, M and NS genes from A/PR8. RIT 1454 virus has obtained all its genes, with the exception of P3, and possibly P2, from A/Victoria/75.

Serum HI antibody responses to recombinant virus vaccines

To test the capacity of the recombinants to induce serum HI antibody, each strain was prepared as an inactivated, whole virus vaccine in PBS, standardized to contain 20 μg of HA/ml and inoculated in 1 ml amounts into hamsters. Groups of 5–12 hamsters were inoculated intramuscularly with each vaccine, and blood samples collected at 21 days post-immunization were tested for HI antibodies against RIT 4050 virus together with serum obtained prior to immunization. In four separate experiments the serum HI antibody responses of hamsters were relatively uniform for a given vaccine. Thus, the geometric mean titre (g.m.t.) of

Table 1. *Serological specificity of the surface antigens of parent and recombinant influenza virus strains*

Virus strain	Derivation of virus strain	Inibition of virus HA activity with		Inhibition of virus neuraminidase activity with N2 antiserum
		H3 antiserum	H1 antiserum	
Parent virus				
A/Victoria/3/75	Wild-type virus	2560*	< 20	2500
A/FM/1/47	Laboratory virus strain	< 20	1280	< 50
A/PR/8/34	Laboratory virus strain	< 20	2560	< 50
Recombinant viruses				
RIT 4050	A/PR8 × A/Victoria/75	2560	< 20	10000
X47	A/PR8 × A/Victoria/75	1960	< 20	10000
RIT 1454	A/PR8 × A/Victoria/75	2560	< 20	8400
VP1	A/PR8 × A/Victoria/75	2560	< 20	1000
VP2	A/PR8 × A/Victoria/75	2560	< 20	960
VP7	A/PR8 × A/Victoria/75	2560	< 20	10000
VFEB-82	A/FM1 × A/Victoria/75	2560	< 20	13300
VFEB-91	A/FM1 × A/Victoria/75	640	< 20	10600
VFE-1	A/FM1 × A/Victoria/75	2560	< 20	9500
VFE-2	A/FM1 × A/Victoria/75	640	< 20	20000

* Titres expressed as the reciprocal of the serum dilution inhibiting either HA or NA activity of the virus by 50%.

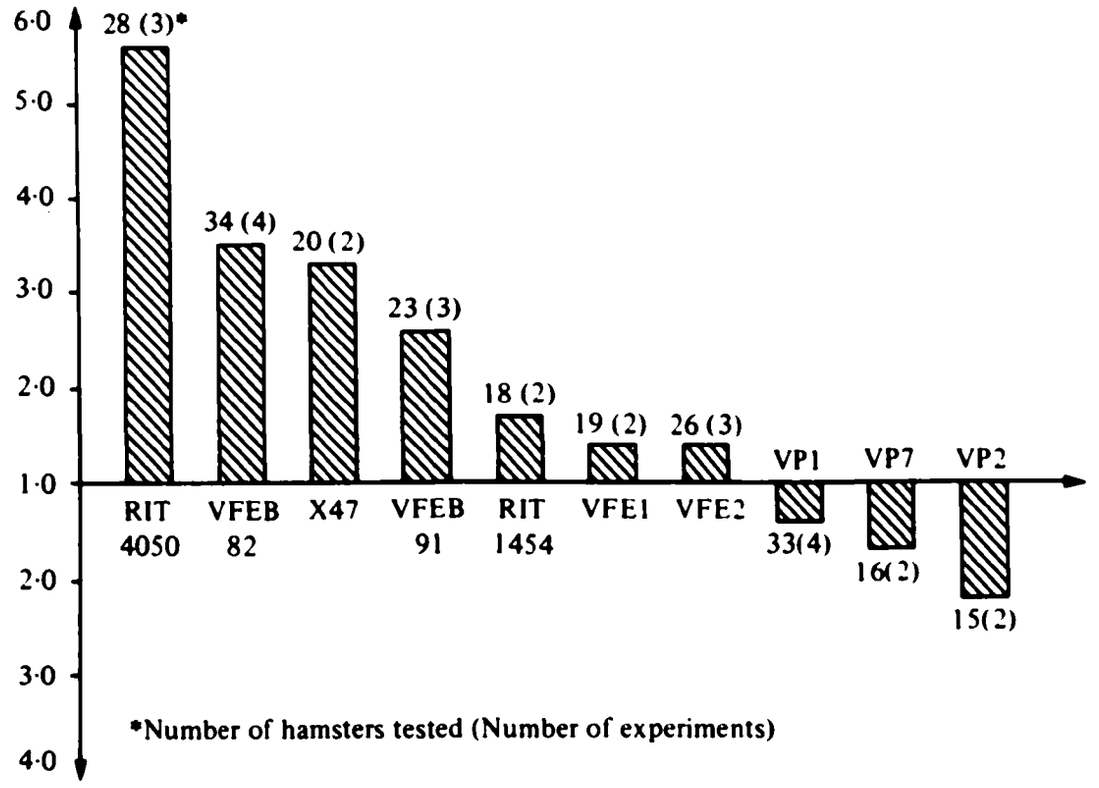

Fig. 1. Serum HI Antibody Response of Hamsters to A/PR/8/34 or A/FM/1/47 Recombinant viruses in comparison to their response to the wild-Type A/Victoria/3/75 parent virus. The figures on the ordinate represent the fold-difference in mean antibody titre of the virus shown, to that of A/Victoria/75 virus represented by the abscissa.

HI antibody in hamsters recieving 20 μg/HA of A/Victoria/75 vaccine varied from 16 to 39 with an overall g.m.t. for all 30 animals of 25·1. In contrast, hamsters immunized with RIT 4050 or VFEB-82 recombinant virus vaccines developed relatively high titres of serum HI antibody. Thus, in three separate experiments, the g.m.t. of hamsters given VFEB-82 vaccine were 160, 105 and 156, with an overall mean titre for all hamsters of 131. Similarly high antibody responses were observed in animals given 20 μg/HA of RIT 4050 virus vaccine (individual g.m.ts, 165, 170, 134; overall g.m.t., 157·4). However, the A/PR8–A/Victoria/75 recombinant virus vaccine, VP-1, was similar to the A/Victoria/75 parent virus in inducing a relatively poor serum HI antibody response, and in three separate experiments g.m.t. values of 24 to 41 were observed, with an overall mean titre of 29·8.

Figure 1 shows the serum HI antibody responses of hamsters to ten different recombinant virus vaccines. The results are expressed as the fold-differences in serum HI antibody elicited by the recombinant virus vaccines relative to that observed for A/Victoria/75 vaccine and indicate that the different recombinants, each bearing HA antigen coded for by a gene derived from A/Victoria/75, and inoculated into hamsters at the same dose, induced significantly different titres of serum HI antibody. RIT 4050 vaccine for instance, induced 5·6-fold greater antibody titres than A/Victoria/75 vaccine ($T = 7{\cdot}82$, $P = < 0{\cdot}01$); VFEB-82 induced 3·5-fold greater antibody titres than A/Victoria/75 vaccine ($T = 6{\cdot}16$, $P = < 0{\cdot}01$), whilst VP-2 vaccine induced 1·2-fold lower antibody titres than A/Victoria/75 vaccine, although this difference was not significant.

When HI tests were carried out using A/Victoria/75 or VFEB-82 as antigens,

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sera from animals given RIT 4050 or VFEB-82 vaccines showed relatively high HI titres compared to those found in hamsters given VP1 or VP2 vaccines, although the differences were not so marked.

Neutralization and single radial haemolysis

Sera from hamsters inoculated with five of the vaccines were also tested for antibody by neutralization and SRH, in order to show that the differences in serological response elicited by the various recombinant virus vaccines were not detectable only in the HI test. The results in Table 2 show that differences in antigenicity between the recombinant virus vaccines were also observed using the neutralization and SRH tests. Thus, by neutralization test, sera from hamsters inoculated with vaccine prepared from strain VFEB-82, showed titres ranging from 1 in 300 to 1 in 1200, with a mean of 1 in 423 in one experiment and a mean of 1 in 363 in a second experiment. Conversely, sera from hamsters immunized with strain VP1, showed considerably lower titres, with means of 56 and 50 obtained in sera from different experiments.

Similarly, when tested by SRH, sera from hamsters inoculated with VFEB-82 and RIT 4050 showed larger areas of haemolysis than those seen with sera from animals inoculated with VP1 vaccine. Indeed, areas of haemolysis were not detectable on SRH of sera from hamsters given the latter vaccine. The A/Victoria/75 parent virus also induced relatively low antibody titres as determined by neutralization or SRH (Table 2).

Protection of hamsters immunized with recombinant influenza virus vaccines

To determine if immunization with recombinant influenza virus vaccines provides protection against challenge infection with A/Victoria/75 virus, groups of hamsters were inoculated intramuscularly with various doses of VP1, VFEB-82 and A/Victoria/75 vaccines. Blood samples were collected 2 and 22 weeks post-immunization, and after the latter blood sampling, each hamster received $10^{6\cdot0}$ $EBID_{50}$ of A/Victoria/75 virus intranasally. Three days later the animals were killed, 40 % lung suspensions prepared and titrated for virus in A-O-S cultures. The results (Table 3) show that at both 2 and 22 weeks higher HI antibody levels were present in sera from hamsters immunized with VFEB-82 vaccine, compared to the levels seen in animals receiving VP1 or A/Victoria/75 vaccines, although the differences were considerably less marked after the longer time interval. At dosages of 2 or 0·2 μg HA neither VFEB-82 nor VP1 vaccines elicited protection against challenge with A/Victoria/75 virus, but protection was evident in hamsters immunized with 20 μg HA of VFEB-82 or A/Victoria/75 vaccines. In contrast, hamsters receiving an equivalent dose of VP1 vaccine showed no protection against challenge infection.

DISCUSSION

In earlier studies of the immune response of hamsters to inactivated influenza A virus vaccine, we have reported that the concentration of virus antigen required to induce a detectable serum HI antibody response varied according to the virus strain used in the vaccine (Jennings & Potter, 1973; Jennings *et al.* 1974). However

Table 2. *Serum antibody responses of hamsters to influenza virus vaccines determined by various serological tests*

Virus vaccine	Vaccine dose (μgHA/ hamster)	Exp. no.	Mean serum antibody titres as determined using: HI — No. sera tested	HI — Range of titres (g.m.t.)	Neutralization — No. sera tested	Neutralization — Range of titres (g.m.t.)	SRH — No. sera tested	SRH — Range of zone area mm² (mean)
A/Victoria/75	20	1	5	40–160 (60)	5	< 100–100 (57)	—*	—
	20	2	5	< 20–20 (11)	5	< 100 (50)	12	7·0–201 (12·5)
RIT 4050	20	1	4	80–320 (190)	4	100–400 (234)	12	452·5 (452·5)
VP1	20	1	5	< 20–80 (26)	5	< 100 (50)	—	—
	20	2	6	20–40 (31)	6	< 100–100 (56)	12	7·0 (7·0)
VFEB-82	20	1	6	320–640 (384)	6	300–1200 (423)	—	—
	20	2	6	320–640 (384)	6	300–400 (363)	12	380–531 (445)

* Sera from this experiment not tested.
g.m.t. = geometric mean titre.

Table 3. *Protection of hamsters immunized with recombinant influenza virus vaccines against A/Victoria/75 challenge infection*

Virus strain	Dose (μgHA)	No. of hamsters	Serum HI titre g.m.t. at: 2 weeks post-immunization	Serum HI titre g.m.t. at: 22 weeks post-immunization	Virus recovery from lung suspensions ($EBID_{50}$/ml) at 22 weeks
VP1	20	10	19	22	$10^{4.4}$*
(A/PR8-A/Victoria/75)	2	10	15	16	$10^{3.0}$
	0·2	10	11	13	$10^{4.1}$
VFEB-82	20	10	174	56	$< 10^{1.8}$
(A/FM1-A/Victoria/75)	2	10	40	30	$10^{3.4}$
	0·2	10	24	13	$10^{4.5}$
A/Victoria/75	20	10	69	45	$< 10^{1.8}$
Nil	—	5	< 10	< 10	$10^{4.8}$

* $EBID_{50}$ titres of pooled lung suspensions.

the vaccines were standardized using chick embryo cell agglutinating (CCA) units. In recent years this method of standardization has been superseded by more accurate techniques for estimating vaccine potency such as single radial diffusion and rocket immunoelectrophoresis (Schild, Wood & Newman, 1975; Oxford *et al.* 1977), which give more reliable quantitation of the haemagglutinin present in the vaccines.

In the present study we have shown that the antigenicity in hamsters, of inactivated, whole influenza virus vaccines prepared from A/PR8–A/Victoria or A/FM1–A/Victoria recombinant virus strains and bearing surface antigens derived from the A/Victoria/75 parent virus, shows wide variation. In addition, the serum HI antibody levels induced by three of the vaccines can be related to resistance against A/Victoria/75 challenge infection, and both VFEB-82 and A/Victoria/75 vaccines induced antibody levels high enough to protect the animals at 22 weeks post-immunization. The antibody levels induced by VP1 vaccines were not protective. No challenge studies were carried out at 2 weeks post-immunization, but it seems probable that similar results would have been obtained.

These findings may have considerable implications for the commercial production of inactivated influenza virus vaccines since the recombinant virus strains used are selected on the basis of their high and rapid growth capacity (McCahon & Schild, 1972; McCahon *et al.* 1976) and not on their ability to induce protective antibody. The antigenicity of inactivated, whole type A influenza virus vaccines for use in humans is determined by studies in animals (Schulman & Kilbourne, 1971; Fenton, Jennings & Potter, 1977), and by clinical trials (Potter *et al.* 1980); however, these studies are directed towards standardizing dosage, and not the comparison of antigenicity of different virus strains. Virus strains of high antigenicity could be used at relatively low concentrations and this could offset the possibly lower titres of virus obtained from eggs and also result in a lower incidence of reactions following immunization.

The reasons for the difference in antigenicity of the various inactivated whole virus vaccines studied in the present report are unknown, but could relate to the physical form of the virus particles in the vaccine, and the form in which antigens are presented to the immune system is known to influence the immune response (Unanue, 1972). Electron microscope studies of the gross appearance of the recombinant virus vaccines showed them to consist of intact spherical particles with few aberrant or filamentous forms, but studies of their ultrastructure were not undertaken. However, differences in the quantity and the arrangement of these antigens on the virion surface and the physical presentation of the antigenic sites, may be important and in this respect differences in the nature of the packing of the internal proteins may affect the packing and configuration of the surface antigens.

Alternatively, the differences in antigenicity may be due to differences in the surface antigens themselves, and although all the vaccines carried HA and NA antigens derived from A/Victoria/75, differences in various phenotypic properties of the HA of influenza virus strains, indistinguishable by normal serological tests, have been reported (Kilbourne, 1978; Erickson & Kilbourne, 1980). Recent studies have shown that influenza A virus variants can readily emerge on passage (Brand & Palese, 1980), and such variants could differ in their antigenicity.

Although the results presented here do not allow us to differentiate between these possibilities, the identification of influenza strains with relatively high antigenicity may have considerable potential value in influenza vaccine preparation.

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