

RADIOIMMUNOASSAY OF INFLUENZA A VIRUS
HAEMAGGLUTININ. II.
ANTIGENIC CROSS-REACTIONS OF INFLUENZA A
(H3 SUBTYPE) VIRUSES AS DETERMINED
BY RADIOIMMUNOASSAY AND HAEMAGGLUTINATION
INHIBITION TESTS

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Summary. — Individual rabbits differed greatly in their antibody response to the “strain-specific” and “cross-reactive” antigenic determinants on the haemagglutinin (HA) subunit of influenza virus recombinant MRC11 (H3N2) and influenza virus Dunedin (H3N2), after immunization with whole virus or bromelain-released haemagglutinin (B-HA). Consequently, diverse cross-reactions between these viruses and A/Hong Kong/68 virus were found in the haemagglutination inhibition (HI) test as well as in homologous radioimmunoassay (^{125}I -B-HA from MRC11: anti MRC11 serum, and ^{125}I -B-HA from Dunedin: anti Dunedin serum) when sera from different animals were employed. Radioimmunoassay (RIA), over and above to the HI test, was able to differentiate clearly the respective HAs also with antisera reacting to the same HI titre with both corresponding influenza virus strains. Thus it appeared that antigenic differences could be identified with higher sensitivity by homologous RIA than by the HI test and that multiple antigenic determinants were reactive on the ^{125}I -B-HA in the RIA procedure employed. MRC11 and A/HK/68 viruses were also compared by heterologous RIA (^{125}I -B-HA from MRC11: anti A/HK/68 serum). It was found that preferentially antigenic determinants with a high degree of cross-reactivity could be studied in the heterologous system.

Key words: Influenzavirus A; radioimmunoassay; haemagglutination inhibition test; cross-reaction; antigenic determinant

Introduction

Two groups of antigenic determinants have been detected on the influenza virus haemagglutinin (HA): the “cross-reactive” (CR) determinants which

induce antibodies reacting with the HA subunit of viruses within a whole HA subtype, whereas "strain-specific" (SS) determinants induce antibodies reacting with the HA subunit of the homologous virus strain (Laver *et al.*, 1974; Virelizier *et al.*, 1974). The above findings have been later extended by the use of monoclonal antibodies which allowed the dissection of the CR group into many individuals, with different degrees of cross-reactivity within a HA subtype (Gerhard, 1976).

It is now known that individual animals (and men) differ greatly in their antibody response to the CR and SS determinants of influenza virus HA, after immunization or infection (Laver *et al.*, 1976; Webster *et al.*, 1976). Due to different widely ratios of antibodies to the CR and SS determinants in different sera, diverse data on cross-reaction (e.g., in the HI test) with various influenza virus strains have been obtained. Consequently, the estimation of exact antigenic differences and relationships of influenza virus strains within a given HA subtype with procedures and antisera generally employed (e.g. HI test, immunodiffusion, etc.) is very difficult.

In our preliminary experiments (Russ *et al.*, 1976) we were able to show that double-antibody competitive-inhibition RIA using ^{125}I -B-HA contaminated with neuraminidase was very sensitive in elucidating differences even among closely related influenza A virus strains containing H3 haemagglutinin. Thereafter we prepared very pure B-HA from influenza virus recombinant MRC11 (H3N2) by bromelain treatment and rate zonal gradient centrifugation (Russ *et al.*, 1978a), suitable for RIA experiments. In the present paper we demonstrate that different antigenic determinants are reactive on ^{125}I -B-HA and that antigenic differences could be studied more precisely by RIA using ^{125}I -B-HA than by the HI test.

Materials and Methods

Viruses. The following influenza A virus strains or recombinants were used: A/Hong Kong/1/68 (H3N2) which will be referred to as "HK/68"; A/Dunedin/4/73 (H3N2) — "Dunedin"; MRC11 (H3N2; antigenically identical to A/Port Chalmers/1/73) — "MRC11"; HKe (H3N1) — "HKe"; and X-31 (H3N2; antigenically identical to HK/68 — "X-31". With the exception of KHe and X-31 recombinant supplied by Dr. E. D. Kilbourne, New York, all viruses were obtained from Dr. G. C. Schild, London. The conditions for infection of embryonated hen's eggs and the mode of purification of the viruses have been described (Styk and Blaškovič, 1973; Russ *et al.*, 1974).

Antisera. Hyperimmune antisera were prepared in rabbits as described (Styk and Blaškovič, 1973; Russ *et al.*, 1978a). They were absorbed with host antigen prepared from chicken chorio-allantoic membranes (Russ *et al.*, 1978a). Absorption of sera by viruses and dissociation of antibodies were done as described by Laver *et al.* (1974, 1976).

Haemagglutination and haemagglutination inhibition (HI) titres were determined by a micro-technique (Russ *et al.*, 1978a). HI titres were expressed per 0.025 ml as reciprocals of the highest initial dilution of serum causing inhibition of 4 to 8 haemagglutination units of the virus used in the form of infected allantoic fluid. Nonspecific inhibitors were removed by RDE by the standard procedure (Palmer *et al.*, 1975; Styk *et al.*, 1977).

Purification of B-HA. B-HA was released from MRC11 and Dunedin viruses by treatment with bromelain (Sigma, grade II) and purified by rate zonal gradient centrifugation (Brand and Skehel, 1972; Russ *et al.*, 1978a, b).

B-HA iodination and competitive-inhibition RIA were performed as described (Russ *et al.*, 1978a) except that in the present experiments labelled and unlabelled antigens simultaneously competed five days for limiting concentration of antibody and only thereafter bound and free antigens

Table 1. Antigenic cross-reactions of influenza A (H3 subtype) viruses as revealed by HI tests

Rabbit antisera to	Hong Kong/68	Viruses MRC11/73	Dunedin/73
MRC11 recombinant			
No. 210	1024 (1/1)	1024	1024
No. 211	16 (1/32)	512	512
B-HA from MRC11			
No. 270	512 (1/1)	512	512
No. 281	8 (1/128)	1024	512
Dunedin virus			
No. 335	1024 (1/2)	2048	2048
No. 336	2048 (1/1)	2048	2048
No. 337	128 (1/4)	512	512
HK/68 virus, No. 1	1024	32 (1/32)	
HKe recombinant, No. 8	512	128 (1/4)	
X-31 recombinant, No. 40	1024	128 (1/8)	

Numbers in parentheses represent indices of cross-reactivity in the HI test (ratios HI titres to which the serum reacted with heterologous and homologous viruses).

were separated by antiglobulin. The competition was examined at a concentration of antibody that precipitated approximately 50 % of labelled antigen, i. e. the ratio of the amount of labelled antigen, bound to the antibodies (B) and of the free antigen (F) in the absence of unlabelled antigen $(B/F)_0$ was approx. 1. Total concentration of labelled antigen T corresponds to the sum of bound and free antigen, i. e. $T = B + F$. The value $(B/T)_0$ obtained in the absence of unlabelled antigen was taken as 100 per cent and the values of (B/T) under various concentrations of unlabelled antigen were calculated to this value. Values $(B/T)/(B/T)_0 \times 100$ were then plotted against the logarithm of the unlabelled antigen concentrations and the 50 per cent inhibitory concentrations were determined graphically. The way of evaluation of experimental data will be published in detail elsewhere.

Neuraminidase activity was determined as described (Russ *et al.*, 1974).

Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Results

Antigenic cross-reactions of influenza A (H3 subtype) viruses as revealed by HI tests with different rabbit sera

Sera from rabbits immunized in the same way with the same preparations of influenza A virus recombinant MRC11 [antigenically identical to the strain A/Port Chalmers/1/73 (H3N2)] or purified B-HA from the same recombinant showed in the HI test diverse cross-reactions between MRC11 and HK/68 viruses. The apparent antigenic relationships and the apparent antigenic differences of corresponding haemagglutinins were thus dependent on the serum used in the HI test. Altogether 11 sera were prepared. Particularly interesting were sera from two rabbits (Nos 210 and 270; see Table 1) which reacted to the same HI titre with MRC11 and HK/68 viruses (suggesting that these rabbits gave a preferential antibody response to the CR determinants with high degree of cross-reactivity) and from another two rabbits

but to a low titre with the HK/68 influenza strain (suggesting preferential antibody response against SS determinants and/or CR determinants with (Nos 211 and 281) which reacted to a high titre with MRC11 recombinant, low degree of cross-reactivity) (Table 1). A further 6 sera were prepared against influenza virus Dunedin and two of them (Nos 335 and 336) reacted with the same HI titre with both Dunedin and HK/68 viruses (Table 1).

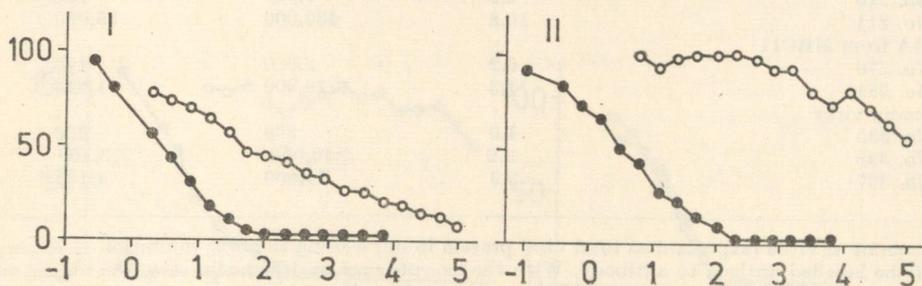


Fig. 1.

Homologous RIA (^{125}I -B-HA from MRC11: anti MRC11). Inhibition of precipitation of ^{125}I -B-HA by competition with MRC11 (●) and HK/68 (○) viruses

I — anti MRC11 serum No. 210

II — anti MRC11 serum No. 211

Abscissa: ng of total viral protein (log); ordinate: per cent of control (B/T)/(B/T)₀

These sera (two against MRC11 virus, two against B-HA from MRC11 and three against Dunedin virus — see Table 1) were used in RIA experiments described below, to investigate the ability of this technique in estimation of antigenic differences and relationships of influenza virus haemagglutinins.

Antigenic cross-reactions of influenza A (H3 subtype) viruses as revealed by RIA with different rabbit sera

1. Characterization of the iodinated B-HA

For the present RIA experiments, very pure B-HA was obtained from influenza A virus recombinant MRC11 or from influenza virus Dunedin as described in Materials and Methods. Estimates of residual neuraminidase activity, immunodiffusion analysis and radioimmunoprecipitation experiments showed that this B-HA was devoid of any enzymatically and antigenically active neuraminidase (for details see Russ *et al.*, 1978a, b; Poláková *et al.*, 1978). B-HA was labelled with ^{125}I by the chloramin T procedure. Analysis of ^{125}I -B-HA by sodium dodecylsulphate polyacrylamide gel electrophoresis revealed essentially the same pattern as described by Poláková *et al.* (1978). The ^{125}I -B-HA was more than 90% precipitable in trichloroacetic acid. Rabbit sera reactive against haemagglutinin, e. g. anti MRC11 sera, regularly precipitated at least 75% of ^{125}I -B-HA in antibody excess. Antibodies against host antigenic determinants (carbohydrate side chains)

Table 2. Antigenic cross-reactions of influenza A (H3 subtype) viruses as revealed by competitive-inhibition RIA

Rabbit antiserum to	Homologous virus (MRC11 or Dunedin) ¹	Hong Kong/68 virus ¹	I.C.R. (RIA) ²
MRC11 recombinant			
No. 210	4.9	1,385	180
No. 211	10.8	430,000	55,000
B-HA from MRC11			
No. 270	6.2	2,800	140
No. 281	11.3	19,900	1,200
Dunedin virus			
No. 335	1.0	320	320
No. 336	3.2	≥10,000	≥3,100
No. 337	3.5	14,500	4,150

1) Amount of virus (expressed as total viral protein in ng) leading to 50 % inhibition of binding of the labelled antigen to antibody. With the exception of anti-Dunedin sera, the values are means from 6–12 independent RIA analyses.

2) Index of cross-reactivity in RIA: mean ratios of HK/68 and homologous viruses (MRC11 or Dunedin) causing 50 % inhibition of binding of the labelled antigen to antibody. About 1 ng of ¹²⁵I-B-HA (from MRC11 recombinant in experiments with anti MRC11 sera, and from Dunedin virus in experiments with anti-Dunedin sera) with a specific activity of 10,000 count/min per ng of protein was used.

revealed only a low binding activity. Nevertheless, for all RIA experiments, these antibodies were removed by absorption with host antigen. Sera from non-immunized animals, even with a high level of nonspecific HI inhibitors did not precipitate a significant amount of labelled antigen (1.5 % or less). ¹²⁵I-B-HA was used for up to 6 weeks after labelling. No significant loss in immunoreactivity of the labelled antigen was observed during this period.

2. Homologous RIA

Unlabelled detergent-disrupted viruses were tested for their ability to compete with ¹²⁵I-B-HA (from MRC11 in experiments with anti MRC11 sera and from Dunedin in experiments with anti Dunedin sera) for binding limiting amount of antibody. As expected, homologous virus (MRC11 or Dunedin) competed precipitation of ¹²⁵I-B-HA more effectively than HK/68 virus. No competition was observed with viruses belonging to H0, H1 and H2 subtypes even at very high concentrations. Typical inhibitory curves for two rabbit sera obtained after immunization with whole virus particles of MRC11 recombinant, expressing widely different cross-reactivity in the HI test (see Table 1), are illustrated in Fig. 1. Concentrations of viruses resulting in a 50 % decrease of ¹²⁵I-B-HA binding to antibodies as well as indices of cross-reactivity in RIA are presented in Table 2. It is evident from these data and Fig. 1 that diverse cross-reactions between MRC11 (or Dunedin) and HK/68 viruses were found in RIA, like in the HI test (Table 1), when sera from different animals were used. In the case of anti

MRC11 sera, two haemagglutinins (MRC11 and HK/68) were more effectively differentiated in homologous RIA with sera from rabbits Nos 211 and 281 (exhibiting a very low degree of cross-reactivity in the HI test) than with sera from rabbits Nos. 210 and 270 (expressing complete cross-reactions in the HI test). Nevertheless, it is clear from Tables 1 and 2 that indices of cross-reactivity from RIA and HI tests were not related by a constant factor.

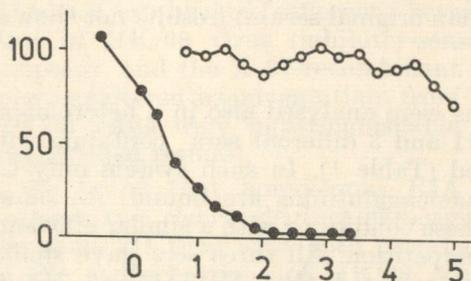


Fig. 2.

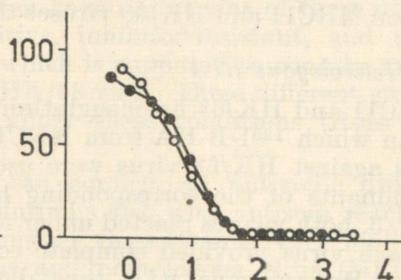


Fig. 3.

Fig. 2. Homologous RIA (¹²⁵I-B-HA from MRC11: anti MRC11 No. 210 absorbed with HK/68 virus). Inhibition of precipitation of ¹²⁵I-B-HA by competition with MRC11 (●) and HK/68 (○) viruses.

Abscissa and ordinate as in Fig. 1.

Fig. 3. Heterologous RIA (¹²⁵I-B-HA from MRC11: anti HK/68 serum). Inhibition of precipitation of ¹²⁵I-B-HA by competition with MRC11 (●) and HK/68 (○) viruses

Abscissa and ordinate as in Fig. 1.

The most important aspect of these experiments was the finding that homologous RIA was able to distinguish clearly two haemagglutinins also with antisera (Nos 210, 270, 335, 336) which reacted to the same HI titre with both corresponding influenza virus strains (Tables 1 and 2; Fig. 1-I).

Dose response curves for HK/68 virus revealed a lesser slope than curves corresponding to MRC11 recombinant (a reduction in the slope was more distinct with sera from rabbits Nos 211 and 281) and HK/68 virus competed precipitation of ¹²⁵I-B-HA either completely or to a limited extent (Fig. 1). Under the conditions employed, saturation was not reached and no clear plateau appeared on the experimental curves, even at high doses of HK/68 virus. Accordingly, the existence of strictly SS determinants on the MRC11 haemagglutinin was not clearly demonstrated. A limited number of experiments with anti Dunedin sera yielded similar results. For further RIA analysis we attempted, therefore, to separate antibodies against SS and CR determinants by an absorption procedure. Antiserum to MRC11 recombinant (No. 210) was absorbed with HK/68 virus particles and the CR antibodies were removed completely, leaving behind antibodies to the SS determinant. In an experiment with antiserum prepared against the SS determinant, binding of ¹²⁵I-B-HA from MRC11 decreased to 50 % of control with 5.3 ng of

MRC11 whereas HK/68 produced slight inhibition (probably non-specific) only at very high doses of virus (Fig. 2). This experiment thus strongly suggests that ^{125}I -B-HA contained at least one SS determinant reactive in RIA. It was reported (e. g., Drescher and Verhagen, 1978) that antibodies against CR determinants could be completely recovered after absorption from virus particles. Paradoxically, we observed that antibodies recovered from HK/68 virus particles, under various dissociation conditions, always revealed a higher specificity in RIA (i. e. a lower degree of cross-reactivity between MRC11 and HK/68 viruses than original serum) (results not shown).

3. Heterologous RIA

MRC11 and HK/68 haemagglutinins were analysed also in a heterologous RIA in which ^{125}I -B-HA from MRC11 and 3 different sera containing antibodies against HK/68 virus were used (Table 1). In such system only CR determinants of the corresponding haemagglutinins are bound. As shown in Fig. 3, both viruses reacted under these conditions with a similar efficiency and each virus provided complete competition. All three sera gave similar results. Heterologous RIA thus demonstrated that MRC11 and HK/68 haemagglutinins contained comparable amounts of CR determinants with a high degree of cross-reactivity.

Discussion

We compared haemagglutinins from distinct influenza A virus strains by the HI test as well as by RIA. Evidence obtained with different immune rabbit sera clearly indicates that RIA can provide more precise and more complete information about antigenic differences and relationships of influenza virus haemagglutinin than the HI test.

It is now well established that influenza virus haemagglutinin possesses several antigenic determinants with different degrees of cross-reactivity within a given HA subtype, and that individual animals (and men) differ greatly in their antibody response to these antigenic determinants after immunization or infection (Laver *et al.*, 1974; Virelizier *et al.*, 1974; Gerhard *et al.*, 1976; Laver, 1976; Webster *et al.*, 1976). This explains why our sera from rabbits immunized with influenza virus recombinant MRC11, B-HA from MRC11 recombinant and influenza virus Dunedin showed diverse cross-reactions, between these viruses and HK/68 virus in the HI test. From a number of rabbit anti MRC11 sera we selected four for examination in RIA experiments. Two of these sera (210 and 270) reacted to the same HI titre with MRC11 and HK/68 viruses, suggesting so incorrectly that the corresponding haemagglutinins were either closely related or identical. But in homologous RIA both sera mentioned above (210 and 270) clearly differentiated haemagglutinins of MRC11 and HK/68 viruses (the concentrations of MRC11 and HK/68 viruses causing 50 % inhibition of binding of labelled antigen to antibody differed more than 100 times). Similarly, homologous RIA clearly identified the antigenic difference between haemagglutinins of

Dunedin and HK/68 strains when two rabbit sera reacting to the same HI titre with both virus strains were employed. All these experiments thus convincingly demonstrated that RIA had a markedly greater potential to reveal antigenic differences of influenza virus haemagglutinin than the HI test. We assume that homologous RIA practically eliminates the risk that a distinct difference would be overlooked, especially in those cases when only a limited number of randomly chosen sera is available. An interesting example of this high resolution ability could be the slight, but in homologous RIA well established, difference between haemagglutinins of three different lines of HK/68 virus (inhibitor-sensitive, inhibitor-resistant, and mouse-adapted) and the X-31 recombinant which is supposed to contain antigenically unaltered haemagglutinin from HK/68 virus. Three different stocks of HK/68 virus were indistinguishable under these conditions (Russ *et al.*, unpublished results).

We found that homologous RIA can confirm an antigenic difference between two clearly distinct haemagglutinins also with antisera reacting to the same HI titre with both corresponding virus strains. Very high ratios of the concentrations of HK/68 virus and homologous virus (MRC11 or Dunedin) causing 50 % inhibition of binding (as compared with the indices of cross-reactivity in the HI test) strongly suggest that RIA will also allow to detect smaller antigenic differences than the HI test.

MRC11 and HK/68 viruses were more effectively differentiated in homologous RIA with sera expressing a considerably lower degree of cross-reactivity in the HI test (211 and 281) than with sera exhibiting complete cross-reactivity in the HI test (210 and 270). In spite of that, cross-reference data from RIA and the HI test were not related by a constant factor. Consequently, it does not seem reasonable to expect that two haemagglutinins will always be more effectively differentiated in homologous RIA with sera showing a low degree of cross-reactivity in the HI test and vice versa. RIA measures total antigen binding capacity and thus may recognize also antibodies which do not inhibit haemagglutination or which considerably vary in their efficiency to inhibit viral haemagglutination. Six and Kasel (1978) interpreted in the same way their findings that the antibody level determined by radioimmunoprecipitation assay could not be related to HI or virus neutralization titres by a constant factor.

The preparation of ^{125}I -B-HA for RIA involves release of HA from virus by bromelain, purification and iodination. It was reported (Laver *et al.*, 1974) that B-HA from HK/68 virus has lost its SS antigenic determinant(s) while in the case of Mem/72 virus one of the SS as well as the CR determinants survived bromelain digestion. Immunization of rabbits with B-HA from MRC11 resulted in vastly different antibody response to different antigenic determinants as indicated by examination of these sera in the HI test. Individual rabbits differed greatly in their antibody response also after immunization with B-HA from Dunedin virus (Russ *et al.*, unpublished results). Therefore we believe that practically all antigenic determinants on B-HA from MRC11 as well as from Dunedin viruses survived bromelain

treatment. Some antigenic determinants could be eventually destroyed during the labelling procedure when B-HA is exposed to oxidizing and later reducing conditions (Hunter, 1978; Russ *et al.*, 1978a). We found the diverse cross-reactions between either MRC11 recombinant or Dunedin virus and the HK/68 virus strain not only in the HI test but also in homologous RIA, when sera from different animals were used. Since different ratios of antibodies to different antigenic determinants are apparently responsible for the diverse degree of cross-reaction in the HI test, the diverse cross-reactions found also in homologous RIA should be considered good evidence that different antigenic determinants were reactive on ^{125}I -B-HA in the RIA procedure employed. Further evidence comes from RIA experiments with antiserum prepared for the SS antigenic determinant(s), discussed below. The present results, of course, did not demonstrate directly that all antigenic determinants of MRC11 and Dunedin haemagglutinins (their exact number is unknown) survived bromelain treatment and iodination without any change and that they were still reactive on ^{125}I -B-HA in homologous RIA.

The sera examined contained a very heterogeneous population of antibodies and the predominant kind (type) of antibody pool was against CR determinants. To demonstrate SS antigenic determinant(s) it was therefore necessary to prepare an antiserum for this determinant(s) with a restricted heterogeneity of antibody population. Antiserum to MRC11 recombinant (210) was absorbed with HK/68 virus particles and antibodies reactive with the CR determinants were removed completely, leaving behind antibodies to the SS determinant(s). According to an about 50-fold decrease of the RIA titre after absorption, SS antibodies represented about 2 % or more of the total antibody population. Antiserum prepared in this way for the SS determinant showed in homologous RIA good competition with MRC11 recombinant but no competition with HK/68 virus. We can conclude, therefore, that ^{125}I -B-HA from MRC11 contained at least one SS antigenic determinant reactive in RIA.

It was reported (Laver *et al.*, 1974; Drescher and Verhagen, 1978) that antibodies to CR antigenic determinants could be completely recovered after absorption from virus particles. Our antibodies recovered under various dissociation conditions from HK/68 virus particles, surprisingly, revealed in homologous RIA a higher specificity than original serum, i. e. differentiated MRC11 and HK/68 more effectively than original serum. This discrepancy can be explained by a failure of antibodies to dissociate completely from virus particles, namely those with a high degree of cross-reactivity. Indeed, in experiments with ^{125}I -labelled IgG we observed (unpublished results) that a significant portion of antibodies remained associated with virus particles. This assumption is in agreement with the higher avidity of antibodies against CR antigenic determinants than against SS determinants (Laver *et al.*, 1974; Drescher and Verhagen, 1978) and with the finding of Lecomte and Tyrrel (1978) that antibodies against CR determinants were more tightly bound to virus-immunoadsorbent than antibodies against SS determinants. It is noteworthy that Laver *et al.*, (1974) in one out of two cases, and Drescher and Verhagen (1978) in all cases also obtained antisera prepared against CR

determinants which reacted with the corresponding viruses with more different binding constants than original antisera.

Heterologous RIA confirmed that MRC11 and HK/68 viruses contained comparable amounts of CR determinants with a high degree of cross-reactivity. Since in heterologous RIA preferentially antigenic determinants with a high degree of cross-reactivity could be studied, heterologous RIA would be suitable for rapid detection of related virus strains and corresponding antibodies as well as for estimation of very low concentration of viruses (e. g. for use in competitive-inhibition RIA) if the latter are not available in purified form.

Any comparison of our results with the published RIA for influenza virus haemagglutinin (Nath *et al.*, 1975; Schieble and Cottam, 1977; Russel and Jackson, 1978; Six and Kasel, 1978) can be made only with caution because these authors used various tracers, different sera, different viruses and different conditions for RIA. The most similar RIA to ours were those of Berezina *et al.* (1978) and Yamnikova *et al.* (1978). However, these authors allowed to compete labelled and unlabelled antigens for limiting concentration of antibody for only 13 hours, while in our experiments competition proceeded for not less than 4 days, which we found as the shortest incubation time necessary to reach equilibrium (manuscript in preparation).

The present results clearly indicate that RIA will allow the examination of antigenic differences and relationships of influenza virus haemagglutinins with a higher sensitivity than the HI test. Furthermore, RIA will allow investigation of different groups of antigenic determinants (e. g. using homologous and heterologous RIA) without absorption of antisera requiring large amounts of purified viruses as well as without dissociation of antibodies from virus particles which yields incomplete recovery. Finally, RIA experiments with influenza virus haemagglutinins are not affected by non-specific inhibitors (Russ *et al.*, 1978a) which is a very important advantage because non-specific inhibitors often cannot be removed completely without affecting specific antibodies. The RIA technique thus becomes, at least, a useful addition to currently available methods (HI test, immunodiffusion, etc. (for virus research and diagnostic laboratories, provided that all reagents and procedures will be strictly standardized.

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