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DETERMINATION OF SEROTYPES OF RED CLOVER NECROTIC MOSAIC VIRUS BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary. — The direct double antibody sandwich (DAS) type of enzyme-linked immunosorbent assay (ELISA) was used to determine the degree of serological and antigenic differences among the three serotypes (A, B, C) of red clover necrotic mosaic virus (RCNMV). Homologous and heterologous antibody titres in the used IgGs to isolates TpM34 (serotype A), TpM48 (serotype B) and isolate No. 6 (serotype C) as determined by ELISA were 100- to 200-fold higher than by ring precipitation test. Intensity of homologous and heterologous reactions in ELISA depended on the concentration of antigen, of the IgG used for coating and of the labelled IgG. The IgG preparations used contained 50 to 100 times higher concentration of homologous (serotype-specific) than heterologous (interserotype-specific) antibodies. Such a great difference between the two antibody types accounts for a comparatively high degree of selectivity of the homologous reactions.

Key words: *red clover necrotic mosaic virus*; *serotypes*; *ELISA*

Introduction

Red clover necrotic mosaic virus (RCNMV) of the Diathovirus group is characterized by a relatively great antigenic variability manifested by the occurrence of 4 different serotypes (Rao *et al.*, 1987), 3 of them in Czechoslovakia (Musil and Gallo, 1982; Musil *et al.*, 1982) in all insofar determined virus isolates. In connection with the use of polyclonal and monoclonal antibodies to the representatives of the Dianthovirus group, including the RCNMV isolates, Hiruki *et al.* (1984) found a high degree of specificity (selectivity) of sera containing polyclonal antibodies when tested by the direct double antibody sandwich (DAS) type of enzyme-linked immunosorbent assay (ELISA). Our experiments were aimed at the elucidation of selectivity of ELISA with three serotypes of RCNMV as well as at the use of the direct DAS type of ELISA for determination of serological and antigenic differences among the RCNMV serotypes studied.

Materials and Methods

Virus isolates and antigens. In comparative tests we used the purified suspensions of three RCNMV isolates, namely TpM34 (serotype A), TpM48 (serotype B), and the isolate No. 6 (serotype C) prepared as described (Musil and Gallo, 1982; Musil *et al.*, 1982) and designated further as virus isolates and antigens 34, 48, and 6, respectively. Nucleoprotein concentrations in the purified virus suspensions were determined spectrophotometrically and adjusted in all virus isolates to the same value corresponding to the $OD_{260nm} = 9.0$. The virus suspensions were diluted further in the sampling buffer mostly 1 : 500 to 1 : 5,000 with regard to the purpose of our study, exceptionally they were double-diluted to the 10^{-6} value (to determine the titre of the antigen).

As controls in ELISA the following antigens were used instead of RCNMV antigen: a) suspension of partially purified proteins from a healthy bean plant (*Phaseolus vulgaris* L. cv. Perlička); b) suspension of serologically unrelated virus (e.g. red clover mottle virus); c) the sampling buffer only. The first two (a and b) control preparations were diluted proportionally to the dilutions of RCNMV antigens.

Sera and immunoglobulin (IgG) fractions. In the precipitation and immunodiffusion tests hyperimmune rabbit sera against 34, 48, and 6 RCNMV isolates and the IgG fractions prepared from these sera were employed. Properties and preparation of antisera against isolates 34 and 48 were described elsewhere (Musil, 1969; Musil and Gallo, 1982). Antiserum to the isolate 6 was prepared by repeated intravenous immunization of the rabbit with 2 ml aliquots of the purified virus suspensions on days 1, 14, and 40; the animals were bled on day 14 following the last immunization dose.

The IgG fractions were isolated as proposed by Clark and Adams (1977) with a modification using the IgG separation on DEAE-52 cellulose (SERVA) column. A part of IgG solution was used for coating and the rest was conjugated with alkaline phosphatase (type V11, SIGMA; 1,000 U per ml) as described by Clark and Adams (1977).

Serological tests. The basic direct DAS type of ELISA was carried out by the procedure of Clark and Adams (1977) on microtitration plates (Novogen, ÚMG, Czechoslovakia). Wells of the plates were coated with IgG directed to the individual RCNMV isolates. The initial solutions (1 mg/ml) were diluted according to the purpose of experiments(most frequently 1 : 500, 1 : 1,000). To detect the antigen captured by antibody adsorbed on the wells, anti-34, anti-48, and anti-6 IgG fractions (1 mg/ml) were conjugated with alkaline phosphatase in a required dilution (mostly in the range from 1 : 200 to 1 : 1,600). The antigen, i.e. appropriately diluted suspensions of purified RCNMV isolates, was added in 100 µl volumes. After incubation for 4 hr at 37°C with conjugated IgG, substrate (4-nitrophenylphosphate bis/2-amino-2-ethyl-1,3-propanediol/ salt) was added in 110 µl volumes of diethanolamine buffer (pH 9.8) per well. The reaction was stopped after 30 min at 37°C by the addition of 50 µl of 3 mol/l NaOH per well. The results were evaluated spectrophotometrically by absorbancy measurements at 410 nm (Dynatech MR-250 spectrophotometer). The obtained absorbancy values were within a range from 0.001 to 3.000. As positive reaction was considered that with an absorbancy value higher than 0.01 after subtracting the absorbancy value of the appropriate control sample.

Titres of homologous and heterologous antibodies in the initial hyperimmune sera and their IgG fractions were determined by the ring precipitation test and by the agar-gel double diffusion test (Musil and Gallo, 1982).

Results

Titre of precipitating antibodies

In hyperimmune sera from which the IgG fractions were obtained, the titre of precipitating antibody to the homologous antigens as determined by the ring precipitation test was 640 (anti-6;and anti-34 sera) and 1,280 (anti-48 serum). In the IgG fractions purified from these antisera and adjusted to the same concentration (1 mg/ml), the titre of precipitins reacting with homologous antigen dropped to 80 (anti-6 and anti-48 IgG) and 160 (anti-34 IgG), respectively. In contrast, the antibodies reacting with heterologous antigen

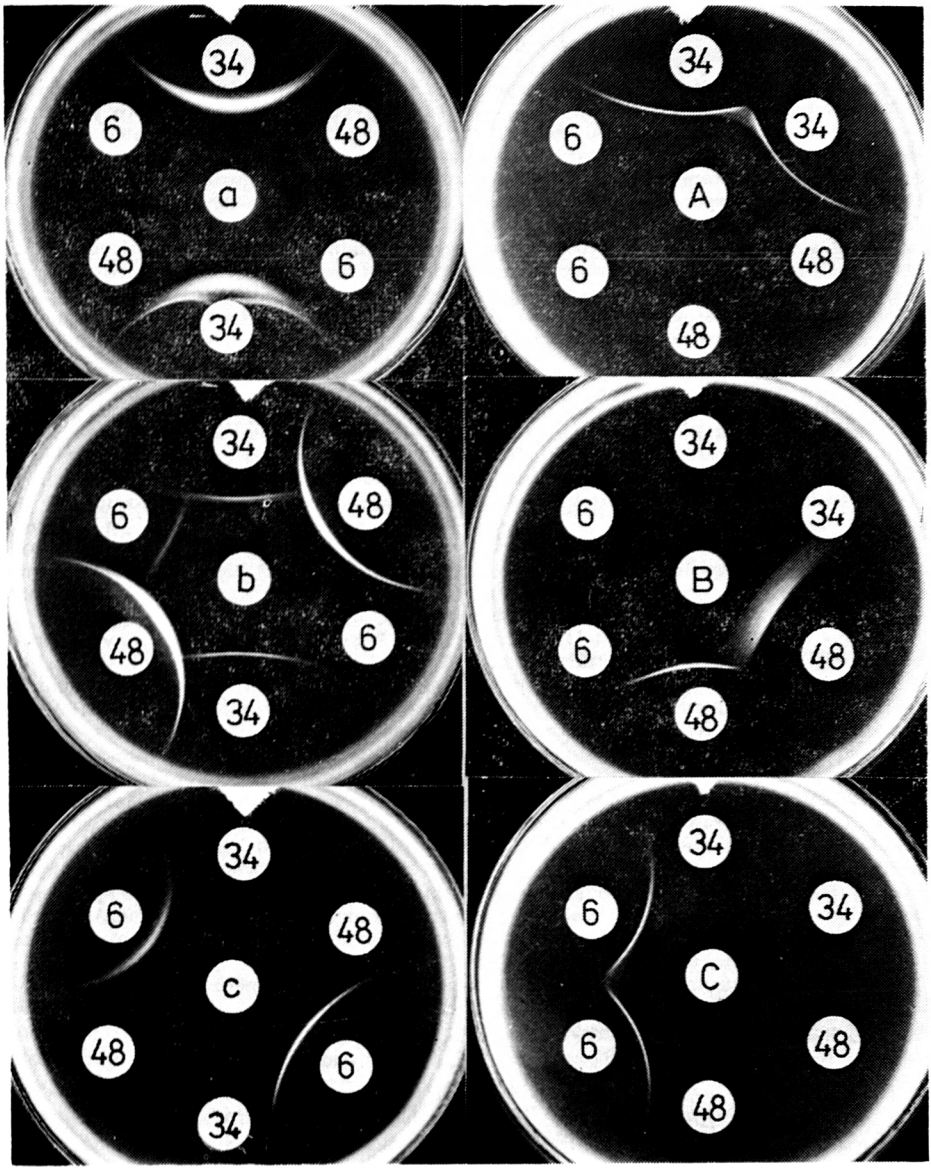

Fig. 1 **Fig. 2**

Fig. 1. Reaction of the three RCNMV isolates (No. 34, 48, and 6) with antisera against serotypes A, B, and C in immunodiffusion test.
Central wells: antisera a — TpM_{34}, b — TpM_{48}, c — 6
Peripheral wells: antigens 34, 48, and 6

Fig. 2. Reactions of RCNMV isolates (34, 48, and 6) with anti-34, 48, and 6 IgG in immunodiffusion test.
Central wells: IgG anti-34 (A), anti-48 (B), anti-6 (C)
Peripheral wells: antigens 34, 48, and 6

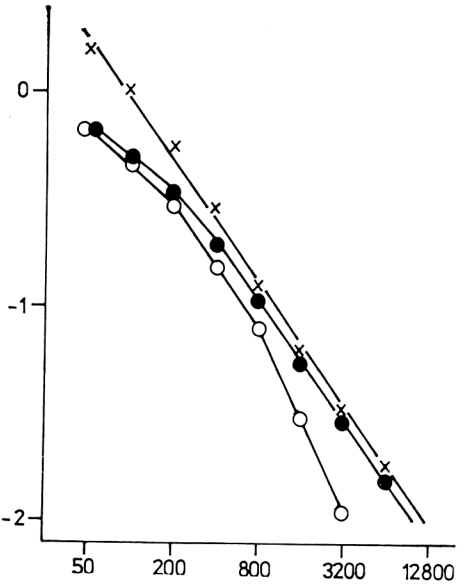

Fig. 3
ELISA of gradual dilutions of 34,48, and 6 antigens with homologous IgG (coat IgG diluted 1 : 500, conjugate diluted 1 : 1600)
Antigen 34 (×), 48 (●), 6 (○)
Abscissa: reciprocals of antigen dilution × 10^2
Ordinate: absorbance at 410 nm ($\log_{10}$ values)

titred in anti-34 serum 1 : 20 to antigen No. 6 and 1 : 8 to antigen 48; in anti-6 serum the antibody titre to antigen 48 reached 1 : 10 to 1 : 20, but no antibody levels to antigen 34 could be detected. Finally, in anti-48 serum antibodies to antigens 6 and 34 were present in titres 1 : 80 and 1 : 160, respectively. The presence of these antibody groups in the examined sera was confirmed also by agar-gel immunodiffusion test (Fig. 1). On the other hand, the tests used did not detect any antibody activity to heterologous

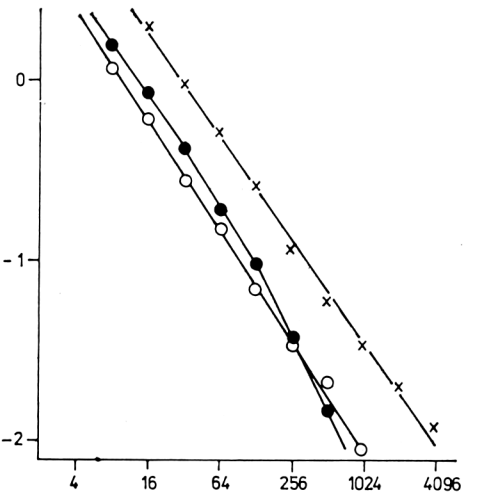

Fig. 4
ELISA of gradual dilutions of anti-34, anti-48, and anti-6 conjugates with homologous antigens captured by homologous IgG (coat IgG diluted 1 : 500, antigen diluted 1 : 1000) anti-34 conjugated IgG (x), anti-48 (●), anti-6 (○)
Ahscissa: reciprocals of conjugate dilution x 10^2
Ordinate: absorbance at 410 nm ($\log_{10}$ value)

Table 1. Reactions in ELISA of anti-6, anti-34, and anti-48 conjugates (diluted 1 : 800 (I) and 1 : 1,600 (II) with homologous RCNMV antigen (diluted 1 : 2,000) bound to different concentrations of coating IgG

Dilution of coating IgG	Absorbance at 410 nm with conjugates					
	anti-6		anti-34		anti-48	
	I	II	I	II	I	II
1 : 500	1.427	0.719	3.000	2.171	1.379	0.730
1 : 1.000	1.290	0.697	3.000	2.011	1.252	0.710
1 : 2.000	1.140	0.628	3.000	1.843	0.992	0.565
1 : 4.000	0.806	0.464	3.000	1.705	0.703	0.444

antigens in the IgG fractions except of the anti-48 IgG which showed a titre of 20 to antigen 34; no antibodies to antigen 6 were detected by the tests in question. In the concentrated IgG preparations, however, antibodies responsible for cross-reactions with heterologous antigens could be found (Figs. 1 and 2).

ELISA of antigens 6, 34, and 48 with homologous IgG

Antigens 6, 34, and 48 captured in the wells coated with homologous antibody reacted till a dilution of 2×10^{-6} (antigen 6) or 10^{-6} (antigen 34 and 48) using homologous conjugates diluted 1 : 800 and 1 : 1,600, respectively. The antigen reaction values ranged from dilutions 2×10^{-4} to 2×10^{-6} decreasing proportionally with the dilution coefficient (Fig. 3).

The titres of labelled homologous antibody in the conjugates (as tested with antigens diluted 10^{-3} and with 2×10^{-3} dilution of homologous coating IgG) reached the values 10^{-5} in anti-6 and anti-48 IgG, and 5×10^{-6} in anti-34 IgG. The titre of labelled antibody was about two-fold lower when tested with conjugates absorbed to the extract from the healthy bean plant.

Table 2. Reactions in ELISA of anti-6, anti-34, and anti-48 conjugates (diluted from 1 : 800 to 1 : 3,200) with different dilutions of homologous antigen (1 : 5,000—I, 1 : 10,000—II, 1 : 20,000—III) and with a 1 : 500 dilution of coating IgG

Conjugate dilution	Absorbance at 410 nm with conjugates								
	anti-6			anti-34			anti-48		
	I	II	III	I	II	III	I	II	III
1 : 800	1.304	0.715	0.435	3.000	1.49	0.72	1.50	0.93	0.60
1 : 1.600	0.66	0.47	0.29	1.52	1.05	0.57	0.66	0.49	0.33
1 : 3.200	0.36	0.25	0.19	0.84	0.62	0.40	0.35	0.24	0.17

Table 3. Reactions in ELISA of anti-6, anti-34, and anti-48 conjugates with homologous antigen bound to the heterologous coating IgG (dilution of coating IgG 1 : 500, of antigens 1 : 500—I and 1 : 1,000—II, of conjugates 1 : 200—1 : 800)

Coat—Ag—conjug.	Absorbance at 410 nm with conjug. diluted 1 : 200		1 : 400		1 : 800	
	I	II	I	II	I	II
anti-6—No.34—anti-34	0.26	0.07	0.15	0.09	0.13	0.05
anti-6—No.48—anti-48	0.67	0.43	0.56	0.36	0.43	0.34
anti-34—No.6—anti-6	0.19	0.10	0.14	0.09	0.11	0.07
anti-48—No.6—anti-6	0.87	0.52	0.55	0.42	0.50	0.34
anti-48—No.34—anti-34	3.00	1.96	3.00	1.51	3.00	1.33
anti-34—No.48—anti-48	0.10	0.05	0.08	0.04	0.06	0.03

In reactions of gradually diluted IgG conjugates with homologous antigen the absorbance values were proportional to the degree of conjugate dilutions except those higher than 10^{-5} or lower than 10^{-3}, when the absorbance values did not always show a linear dependence or they were higher than 3.000 (Fig. 4).

Reactions of antigens with homologous labelled antibody were influenced to a lesser extent also by the degree of dilution of the used coating IgG, e.g. the reaction values of antigens diluted 5×10^{-4} when captured by the coating

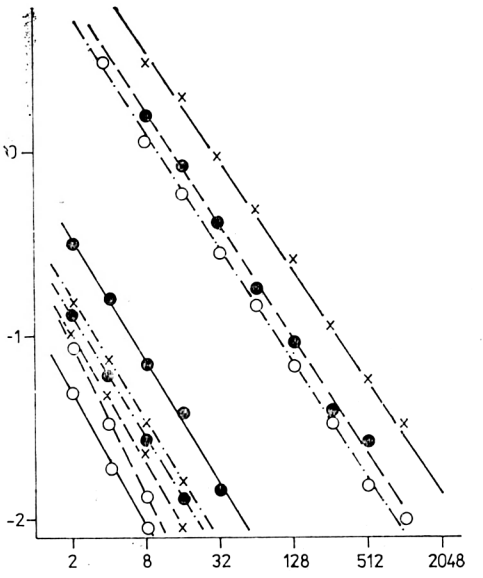

Fig. 5

ELISA of anti-34, anti-48, and anti-6 conjugates with homologous and heterologous antigens captured by homologous IgG coat (coat IgG diluted 1 : 500, antigen diluted 1 : 1000)

Combinations: 34-34-34 (×———×)
48-48-48 (●— — —●)
6- 6- 6 (○—.—.—○)
34-34-48 (●———●)
34-34- 6 (○———○)
48-48-34 (×— — —×)
48-48- 6 (○— — —○)
6- 6-34 (×—.—.—×)
6- 6-48 ●—.—.—●)

Abscissa: reciprocals of conjugate dilution × 10^2

Ordinate: absorbance at 410 nm ($\log_{10}$ value)

Table 4. Reactions in ELISA of gradually diluted anti-6, anti-34, and anti-48 conjugates with homologous and heterologous RCNMV antigen bound to the homologous coating IgG diluted 1 : 500 (antigen dilution 1 : 1000)

Coat—Ag—conjug.	Absorbance at 410 nm of conjug. diluted to							
	200	400	800	1600	3200	6400	12 800	25 600
anti-6—No.6—anti-6	3.00	3.00	1.37	0.81	0.40	0.19	0.08	0.03
anti-6—No.6—anti-34	0.15	0.07	0.02	0.01	0.01	0.00	0.00	0.00
anti-6—No.6—anti-48	0.13	0.05	0.02	0.01	0.01	0.00	0.00	0.00
anti-34—No.34—anti-34	3.00	3.00	3.00	1.52	0.83	0.33	0.09	0.05
anti-34—No.34—anti-48	0.32	0.13	0.06	0.02	0.01	0.00	0.00	0.00
anti-34—No.34—anti-6	0.05	0.03	0.01	0.00	0.00	0.00	0.00	0.00
anti-48—No.48—anti-48	3.00	3.00	2.21	0.99	0.51	0.23	0.12	0.04
anti-48—No.48—anti-34	0.05	0.02	0.01	0.00	0.00	0.00	0.00	0.00
anti-48—No.48—anti-6	0.09	0.05	0.01	0.01	0.00	0.00	0.00	0.00

IgG diluted from 2×10^{-3} to 2.5×10^{-4} were not directly proportional to the dilution of coating IgG, because instead of the expected two-fold only 1.1—1.4-fold decrease of the absorbance values was demonstrated (Table 1).

Reactions of antigens 6 and 48 with the homologous conjugate did not differ substantially within a certain range of dilution of the antigens as well as of the coating and conjugated IgG. Only antigen 34 gave higher absorbance values than gave antigens 6 and 48 against the homologous conjugate in all experiments carried out under the same conditions (Table 2).

Reactions of antigens 6, 34, and 48 captured by the antibody in heterologous coating IgG with their homologous conjugates

Intensity of the reaction of conjugated anti-6, anti-34, and anti-48 IgG with homologous antigen captured, however, by the antibody present in the heterologous coating IgG was lower than that observed at the completely homologous reaction. The highest reactivity was found with antigen 34 which bound to the antibody in the coating anti-48 IgG in such an extent that the resulting value of 3.000 in its reaction with homologous antigen was similar to that observed with the completely homologous antigen. However, in the reaction of the same conjugate with antigen 34 bound to the antibody in coating anti-6 IgG, the absorbance value was 0.26 only, i.e. it represented approximately 1/40 of that of the complete homologous reaction. Similarly, lower absorbance values from 0.11 to 0.19 and from 0.06 to 0.10, respectively, were demonstrated when anti-6 and anti-48 IgG conjugates were added to the homologous antigens bound to the heterologous coating anti-34 IgG. Higher intensity of reaction was observed in the combination of homologous IgG conjugates with antigen No. 48 bound to the anti-6 IgG coated antibody (absorbance values from 0.43 to 0.67) and with antigen No. 6 bound to the coated anti-48 IgG antibody (absorbance values from 0.50 to 0.87). Differences in the intensity of homologous reactions of the conjugates with the antigens bound to the antibody in heterologous coating IgG were preserved also when using dilutions of antigens, conjugates and coating IgG other than shown in Table 3. The values of reactions of the same dilutions of antigens with conjugates diluted from 1 : 200 to 1 : 800 were not proportional to the degree of dilution of the conjugates used, because instead of two-fold only 1.1 to 1.4-fold decrease of absorbance values was noticed (Table 3).

Reactions of antigens 6, 34, and 48 with the heterologous conjugate

Anti-6, anti-34, and anti-48 IgG conjugates reacted less intensively with heterologous antigens captured by homologous coating IgG as compared with homologous antigens. In reaction of anti-6 conjugate with antigen 48 and 34, respectively, the absorbance value was 50- and 100-fold lower than that obtained in reaction with homologous antigen. Similarly, 40- to 100-fold lower absorbance values were found in reaction of anti-34 conjugate with antigen 6 and 48 than with homologous antigen 34. The anti-48 conjugate reacted also 30- to 60-fold less intensively with antigen 34 and antigen 6 than in the completely homologous reaction with antigen 48 (Table 4; Fig. 5).

Table 5. Reactions in ELISA of anti-6, anti-34, and anti-48 conjugates with heterologous RCNMV antigens bound to the heterologous antibodes of the same (A) or different (B) type as the used conjugate

Coat—Ag—conjug.	Absorbance at 410 nm with conjug. diluted 1 : 200	1 : 400	1 : 800	1 : 1600
A: anti-6—No.34—anti-6	0.03	0.01	0.00	0.00
anti-6—No.49—anti-6	0.03	0.01	0.00	0.00
anti-34—No.48—anti-34	0.02	0.01	0.01	0.00
anti-34—No.6—anti-34	0.17	0.05	0.03	0.01
anti-48—No.6—anti-48	0.03	0.02	0.01	0.01
anti-48—No.34—anti-48	0.15	0.05	0.02	0.01
B: anti-48—No.34—anti-6	0.00	0.00	0.00	0.00
anti-34—No.49—anti-6	0.02	0.01	0.00	0.00
anti-48—No.6—anti-34	0.02	0.01	0.01	0.00
anti-6—No.48—anti-34	0.15	0.08	0.03	0.01
anti-5—No.34—anti-48	0.02	0.01	0.01	0.00
anti-34—No.6—anti-48	0.03	0.01	0.01	0.00

Very low absorbance values were observed in reactions of individual antigens with heterologous IgGs when the same animal species was used as source of the antibody both for coating and for the conjugate. In such cases values from 0.02 to 0.03 in reaction of antigen 48 with anti-34 IgG and of antigen 6 with anti-48 IgG were found; somewhat higher values from 0.15 to 0.17 were detected in the reaction of antigen 6 in combination with anti-34 IgG and of antigen 34 in combination with anti-48 IgG. In contrast with these results obtained with conjugates diluted 1 : 200, in reactions using more diluted (from 1 : 400 to 1 : 1,600) conjugates in some cases either no reactivity was found or it could be hardly detected, i.e. the absorbance value was 0.01 only (Table 5).

In completely heterologous reactions, i.e. when antigen captured by heterologous IgG coat was allowed to react with heterologous IgG conjugate other than that used for coating, low absorbance values (0.01—0.04) or absence of reactivity was observed even with conjugates diluted 1 : 200. The use of higher conjugate dilution in several cases gave no positive reaction with the absorbance value less than 0.01 (Table 5).

Characterization of the antibody in the antisera and their IgG fractions

Immunodiffusion tests and ELISA allow to differentiate the type-specific antibody from antibody reacting with the heterologous antigen. Type-specific antibodies (aA in anti-34, bB in anti-48, and cC in anti-6 IgG) in antisera and their IgG fractions are in the highest concentration corresponding to the antibody titre found in the reaction with homologous antigen. By

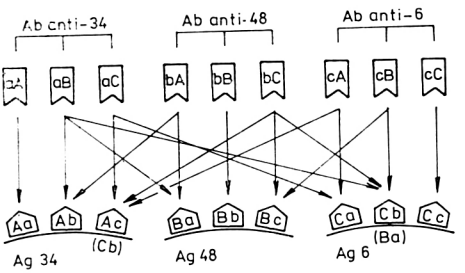

Fig. 6
Scheme of representation of antibody groups in anti-6, anti-34, and anti-48 IgG and their interaction with corresponding antigenic determinants of the RCNMV isolates No. 6, 34, and 49

contrast, further antibodies responsible for the reactivity with heterologous antigens were in a substantially lower concentration in all antisera and IgG fractions in question. Of these minor antibodies, 2 groups were proved in the anti-34 IgG, namely group aB reacting with antigen 48 (serotype B), the group aC reactive with antigen 6 (serotype C); the aB antibody group participated also in the latter reaction. Similarly, in anti-48 IgG the bA antibody group reacted specifically with antigen 34 (serotype A), whereas the group bC reacted with antigen 6 but participated also in the reaction with antigen 34. In anti-6 IgG, the group cA reacted with antigen 34 and the group cB with antigen 48, though either antibody group was hardly detectable by both immunodiffusion test and ELISA.

Representation of antigenic determinants in the RCNMV isolates

Based on the representation of antibody groups in individual antisera and their IgG fractions as well as on their reactivity in immunodiffusion test and ELISA, the antigenic determinants in individual RCNMV isolates can be arranged as follows:

In the isolate No. 34 three antigenic determinants were found, the major being the Aa determinant reacting with serotype-specific aA antibody group present only in the homologous antiserum. Further two determinants, namely Ab and Ac, are of minor importance corresponding to the aB and aC antibody groups in homologous anti-34 serum and enabling reactivity with bA and cA groups in anti-48 and anti-6 sera. Also in the isolate 48 apart from the major serotype-specific Bb antigenic determinant reacting only with bB antibody group present in the homologous antiserum, two minor antigenic determinants can be distinguished, namely Ba and Bc, corresponding to the bA and bC antibody groups in the homologous antiserum or to the aB and cB antibody groups in the anti-34 and anti-6 serum, respectively. In the isolate 6, the major type-specific Cc antigenic determinant reacts with corresponding serotype-specific antibody group cC present only in the homologous antiserum; the two minor antigenic determinants Ca and Cb correspond to minor cA and cB antibody groups in the homologous antiserum, and/or to bC and aC antibody groups in the anti-48 and anti-34 sera, respectively.

Representation of individual antigenic determinants in RCNMV isolates 34, 48, and 6 as well as their interaction to the found antibody group is schematically presented in Fig. 6.

Discussion

As follows from the presented results, the direct DAS ELISA appeared suitable not only to differentiate between individual RCNMV serotypes, but also to determine the degree of their serological and antigenic difference, respectively. In three RCNMV isolates representing A, B, C serotypes, the above mentioned technique proved apart from homologous also heterologous reactions which were adequate to the individual groups of antibodies in the IgG fractions used and to the representation of corresponding antigenic determinants on the virions of isolates under study.

Homologous reactions differed in their intensity from heterologous ones in such an extent that the used optimal conjugate dilutions which gave absorbance values from 1.000 to 3.000 in the homologous reaction, did not react with heterologous antigen at all or reacted with it in a very low intensity only (absorbance values of 0.010—0.020). Intensive positive reactions of such diluted conjugates (i.e. more than 1 : 1,000) with the homologous antigen indicate the high specificity of the IgG fraction isolated from the polyclonal RCNMV antisera as demonstrated by direct DAS ELISA also with other RCNMV isolates and other members of the Dianthovirus group (Hiruki *et al.*, 1984). These authors, however, did not observe in the direct DAS ELISA any reaction with heterologous antigen whether between the members of the Dianthovirus group or between the RCNMV isolates used, which, to a certain extent, corresponded to the findings of Koenig (1978) using some other virus species. In contrast, in our experiments the heterologous reactions were demonstrated by ELISA in all three investigated RCNMV isolates (serotypes A, B, C). The heterologous reactions were limited though by substantially lower representation of "interserotype-specific" than "serotype-specific" antibody not only in the IgG fractions used, but also in the initial antisera.

In anti-34, anti-48, and anti-6 IgG, the direct DAS ELISA confirmed the presence of at least three different antibody groups. Of them, in individual serotypes one group was represented by mutually differing "serotype-specific antibodies" reacting with homologous antigen only and reaching substantially higher titres than other groups of virus-specific antibodies. Further two minor antibody groups present in individual IgG fractions ("interserotype-specific antibodies") were responsible for the reaction with heterologous antigens. It can be assumed that apart from these antibody groups, in the IgG fractions could be present also further minor antibody groups corresponding to the serotypes different from those used in our experiments.

Based on the different intensity of homologous and heterologous reactions, one can suggest the proportional representation of individual antibody groups in the IgG fractions and the proportional representation of antigenic determinants corresponding to the individual antibody groups in the isolates representing three RCNMV serotypes. More objective evaluation of the ELISA results can be achieved by their comparison with those of precipitation and immunodiffusion tests which better discriminate whether one or more antibody groups with corresponding antigenic determinants participate in the resulting reaction. A detailed analysis of qualitative differences between

three RCNMV isolates as found by immunodiffusion test and of quantitative differences as determined by direct DAS ELISA enabled a more complex and adequate analysis of differences observed, which were not possible with the use of a single serological technique only.

The results of homologous and heterologous reactions of three different serotypes in the direct DAS ELISA revealed that individual RCNMV serotypes showed a greater degree of serological difference than that found, e.g. between the strains of maize chlorotic mottle virus (Uyemoto, 1980) or between the representatives of the Comovirus group (Gallo and Musil, 1988). The great degree of selectivity (specificity) of reactions of individual RCNMV serotypes in the direct DAS ELISA is considerably similar to that of reactions with other viruses, e.g. the strains of "Andean potato latent virus", barley yellow dwarf virus, broad bean wilt virus, prunus necrotic ringspot virus, and tobacco mosaic virus (Koenig, 1978; Barbara *et al.*, 1978; Rochow and Carmichael, 1979; Bar-Joseph and Salamon, 1980; Van Regenmortel, 1982; D'Arcy and Hewings, 1986). The results of our experiments with the three RCNMV serotypes indicate that under certain conditions and certain mode of performance of the direct DAS ELISA, the resulting reaction may appear as entirely selective-specific disproving any serological relation with other serotypes. Of the factors limiting heterologous reaction, mainly low concentration of antibody groups responsible for interserotype reactions in the antisera and IgG fractions used, but also minor representation of adequate antigenic determinants on the virions of individual serotypes should be taken into consideration. In addition, the resulting ELISA reaction can be affected by different avidity of individual antibody groups participating in both homologous and heterologous reactions.

From the practical point of view, the obtained data indicate that in spontaneously infected host plants RCNMV antigen should be tested in parallel for all serotypes found in a given locality of the region studied, or on the contrary, for testing the samples, a mixture of IgG containing antibody to all expected serotypes should be used to ensure a sufficiently high reliability of RCNMV detection, regardless of whether it belongs to the individual serotypes.

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