The structural polypeptide VP3 of infectious bursal disease virus carries group- and serotype-specific epitopes

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Two independent non-overlapping epitopes could be demonstrated on the structural protein VP3 of infectious bursal disease virus by non-neutralizing monoclonal antibodies produced against serotypes I and II.

Both serotypes have one epitope in common, whereas the second epitope is distinct for serotype I and serotype II.

The aetiological agent of infectious bursal disease, an economically important infection of chickens, is a member of the Birnaviridae family (Dobos et al., 1979). The main structural characteristic of the non-enveloped virus particle, which has a diameter of about 60 nm, is the bisegmented dsRNA genome (Nick et al., 1976; Müller et al., 1979). The larger RNA segment (M, 2-25 × 10^3) codes for three structural proteins (Azad et al., 1985), two of which (VP2, M, 40K and VP3, M, 32K) represent the major structural elements of the virus particle. Besides this pathogenic prototype of the infectious bursal disease virus (IBDV), which replicates preferentially in the bursa of Fabricius of the chicken (Becht, 1980; Müller, 1986), a second serotype (McFerran et al., 1980) has been isolated from turkeys (McNulty et al., 1979; Jackwood et al., 1982). This serotype II does not have a selective tropism for the bursa and is not pathogenic for chickens. VP2 of serotype II is markedly larger (M, 48K) than the corresponding component of serotype I, whereas VP3 of serotype II has only a very slightly retarded migration rate in SDS-PAGE (Becht et al., 1988) when compared to that of serotype I.

The two serotypes can be distinguished by neutralization. Neutralizing antibodies are induced by a conformation-dependent antigenic domain, located on VP2 (Azad et al., 1987; Becht et al., 1988). It is composed of at least three independent epitopes as recently demonstrated by the study of escape mutants (Öppling et al., 1991). A further epitope located on VP2, which is sequence-specific, is common to both serotypes (Becht et al., 1988).

No difference in the antigenic structure of VP3 of the two serotypes has been noted so far. All VP3-specific monoclonal antibodies (MAbs) generated in a previous study were bound efficiently by both serotypes (Becht et al., 1988). In the course of a more detailed antigenic analysis of VP3, MAbs which reacted with the VP3 of only one serotype became apparent. Differentiation of the two serotypes of IBDV by use of these VP3-specific antibodies is consequently the objective of this communication.

IBDV strain Cu-1 isolated from chickens (Nick et al., 1976) represents serotype I. The turkey isolate 23/82 (Chettle et al., 1985) belongs to serotype II and was obtained from N. E. Reed, Central Veterinary Laboratory, Weybridge, U.K. Both strains were propagated in chicken embryo cells (CEC) at 39 °C. MAbs were prepared essentially as described (Becht et al., 1988). Briefly, BALB/c mice were injected intraperitoneally three times at intervals of about 3 weeks with a suspension of whole purified virus particles in PBS, which had been mixed with an equal volume of aluminum hydroxide (Alu-Gel S; Serva). Four days after the last injection, fusion of SP2/0 myeloma cells was carried out according to standard procedures (Fazekas de St. Groth & Scheidegger, 1980; Lane, 1985). Hybridomas secreting virus-specific antibodies were detected by ELISA using whole purified virus as the coating antigen. Culture media of hybridomas, which had been cloned twice, were tested for their reactivity patterns against viral proteins by radioimmunoprecipitation assays, by immunoblotting and by virus neutralization assays essentially as previously described (Becht et al., 1988; Öppling et al., 1991).

The preparation and specific reactivity of the MAbs employed in this study are summarized in Table 1; none of the VP3-specific antibodies had any neutralizing capacity. MAbs I/A10 (anti-Cu-1) and S/D52 (anti-23/82) belong to the group of antibodies previously defined as being VP3-specific and cross-reactive (Becht

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Table 1. Summary of IBDV VP3-specific MAbs

<table>
<thead>
<tr>
<th>MAb designation</th>
<th>I/G4</th>
<th>I/A10</th>
<th>30/13</th>
<th>S/D52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepared against serotype*</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>Serotype reactivity with VP3</td>
<td>I</td>
<td>I + II</td>
<td>II</td>
<td>II + I</td>
</tr>
<tr>
<td>Neutralizing activity</td>
<td>-†</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Serotype I, IBDV strain Cu-1 (Nick et al., 1976); serotype II, IBDV strain 23/82 (Chettle et al., 1985).
† - , No neutralizing activity.

The results of radioimmunoprecipitation assays presented in Fig. 1 reveal common and type-specific reaction patterns. As shown in Fig. 1(a), lanes 5 and 6, precipitates formed after the addition of MAb I/A10, raised against the serotype I strain Cu-1, to extracts prepared from CEC infected with viruses belonging to serotypes I or II. Corresponding results were obtained when MAb S/D52, raised against the serotype II strain 23/82, was employed (Fig. 1(b), lanes 5 and 6). This reciprocal reactivity supports previous conclusions that the VP3s of both serotypes carry a common epitope. In agreement with previous observations, VP3 appears to be slightly larger in the serotype II strain. MAb I/G4, on the other hand, formed a precipitate only when it was in contact with extracts prepared from cells infected with strain Cu-1, against which this antibody had been produced; no reaction took place after addition of this antibody to serotype II antigens (Fig. 1(a), lanes 3 and 4). Converse results were obtained with MAb 30/13, directed against strain 23/82. Once again, only the homologous antigen was recognized, and no reaction was observed when this antibody was in contact with serotype I antigens (Fig. 1(b), lanes 3 and 4).

Identical results were obtained when these MAbs were tested by immunoblotting after the structural proteins of purified virus particles had been transferred to membranes following electrophoresis on 12.5% SDS-polyacrylamide gels (Fig. 2a, b). Type-specific reactivities were visible at the VP3 position of the homologous virus, whereas no antibody was bound by the heterologous virus strain (lanes 1 and 2). Here again the common epitope was delineated reciprocally by antibodies directed against both serotypes (lanes 3 and 4). This means that all antibodies reacting with VP3, reciprocally or in a type-specific manner, recognize sequence-dependent epitopes. The immunoblots shown in Fig. 2 also confirm that VP3 of strain 23/82 is slightly larger than the corresponding structure of serotype I (lanes 3, 4).

Competitive ELISAs confirmed that the type-specific and the cross-reacting MAbs recognize different epitopes on VP3. For the analysis depicted in Fig. 3(a), a plate was coated with cross-reactive MAb I/A10. All

Fig. 1. Demonstration of MAb specificity by radioimmunoprecipitation. Chicken embryo cells were infected with serotype I strain Cu-1 (lanes 2, 3 and 5), serotype II strain 23/82 (lanes 4, 6 and 8 in a; lanes 4, 6 and 7 in b), or were mock-infected (lanes 1 and 7 in a; lanes 1 and 8 in b). (a) MAbs prepared against serotype I: I/G4, lanes 3 and 4; I/A10, lanes 5 and 6. (b) MAbs prepared against serotype II: 30/13, lanes 3 and 4; S/D52, lanes 5 and 6. Serotype I- or II-specific rabbit hyperimmune sera were employed in (a) lanes 1 and 2, and (b) lanes 7 and 8. Precipitated 35S-labelled polypeptides were analysed by SDS-PAGE and autoradiography. The arrows on the left indicate, from the top, IBDV intracellular polypeptides VP1, VP2a, VP3 and VP4.
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Fig. 2. Demonstration of MAb specificity by immunoblotting. (a) MAbs prepared against serotype I (I/G4, lanes 1 and 2; I/A10, lanes 3 and 4) were reacted with structural polypeptides of serotype I strain Cu-I (lanes 1 and 3) or serotype II strain 23/82 (lanes 2 and 4). (b) MAbs prepared against serotype II (30/13, lanes 1 and 2; 5/D52, lanes 3 and 4) were reacted with structural polypeptides of serotype II in lanes 1 and 3, and serotype I in lanes 2 and 4. The arrows on the left indicate, from the top, the positions of IBDV structural polypeptides VP1, VP2, VP3 and VP4.

Reagents of this assay system were employed in concentrations which yielded an A_{492} of 1.5 to 2.2. Beginning with 100 μg/ml of antibody, serial dilutions of the cross-reactive MAbs I/A10 or 5/D52, or the serotype I-specific MAb I/G4 were mixed with Cu-I virus. After a reaction period of 1 h at room temperature, the virus/antibody mixtures were added to the blocked and washed wells of the antibody-coated plate. The amount of virus that could be bound by the capturing antibody was determined by the addition of rabbit anti-Cu-I hyperimmune serum after 1 h, which in turn was visualized by anti-rabbit IgG conjugates. The results presented in Fig. 3(a) indicate that MAb I/A10, which had been defined as cross-reactive, competed efficiently with the capturing antibody for binding sites on the viral antigen. This was also true for MAb 5/D52. Although the blocking effect exerted by this anti-serotype II antibody was less pronounced than that exerted by I/A10, the results presented in Fig. 3(a) show that the homologous and heterologous antibodies were competing for the same antigenic determinant. No interference of virus binding to the capturing antibody occurred when anti-VP3 type-specific MAb I/G4 was added to the virus. Analogous results were obtained when, in turn, serotype I-specific MAb I/G4 was employed as the capturing antibody (Fig. 3(b)).

It can be concluded from these results that one group of MAbs directed against IBDV serotypes I and II is reciprocally cross-reactive with a closely related or identical epitope on the structural protein VP3. A second group of VP3-specific MAbs however, only recognizes an epitope on the homologous virus strain against which it had been prepared. Obviously, the epitopes recognized by these two groups of MAbs are not overlapping. It will be necessary to confirm more differentiated antigenic structures of VP3 by more detailed analysis of the genomic organization of segment A. From a diagnostic point of view, these antibodies may be useful for the differentiation of serotypes I and II by immunoblotting, particularly in those cases where field isolates cannot be grown in vitro and are therefore not available for neutralization assays.

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References


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