Anti-idiotypic Antibody as Potential Serodiagnostic Reagent for Detection of Bluetongue Virus Infection

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Bluetongue virus (BTV), an arthropod-borne orbivirus, infects domestic and wild ruminants (24, 25). At least 24 serotypes of BTV, defined by serum neutralization, are found within the BTV serogroup of orbiviruses (7, 19). The serotypes are determined by two structural proteins, VP2 and VP5. One of the major viral proteins, VP7, is responsible for serogroup specificity as defined by enzyme-linked immunosorbent assay (ELISA) and complement fixation tests (10). Infection with BTV is commonly detected by testing for the presence of serum antibodies to the group-specific antibodies (12) in a competitive ELISA (1, 3, 4). This test, as well as an indirect ELISA, relies on tissue culture-derived or yeast-expressed BTV antigens which contain viral, cellular, and medium components.

An idiotope (Id), defined as an epitope within the variable region of immunoglobulin, is used as a structural and functional marker of the variable region. Anti-idiotypic antibodies (anti-Ids or Ab2s) can be generated upon immunization with an antibody (Ab1) which contains a variety of Ids. One set of Ab2s, referred to as internal-image Ab2s (11), recognizes an Id which is shared by antibodies with the same or similar specificities. This Id is designated a common Id. Anti-Ids that recognize a common Id and represent an internal image of the antigen have been generated in many systems (for reviews, see references 18 and 20). Their antigenic mimicry makes them valuable as substitutes not only for certain hormones, e.g., gonadotropin (22), by binding to a specific cell receptor but also for infectious agents as immunogens by eliciting a specific antibody response (for a review, see reference 23). Development of anti-Ids as diagnostic reagents has not been achieved to the same extent. The approach of using anti-Ids as reagents has several advantages over current immunosassays. Since anti-Ids are immunoglobulins and are not infectious, they (i) overcome the inherent problems of working with dangerous animal and human pathogens, (ii) facilitate continuous production of the reagents by hybridoma technology, (iii) enhance the test reproducibility and standardization, and (iv) improve the test specificity.

In this report, polyclonal Ab2s were generated by the sequential immunization of rabbits with three monoclonal antibodies (MAbs) specific for a major bluetongue virus (BTV) protein, VP7. The anti-Ids, designated RAb2s, recognized idiotopes which were located within or near the antigen-combining sites of the MAbs and were associated with both heavy and light chains of MAbs. RAb2s inhibited the MAbs from binding to BTV antigens, and their interaction with MAbs was inhibited by BTV antigens. By recognizing the common idiotopes, RAb2s detected anti-BTV antibodies from bovine antisera; their interaction was also partially inhibited by BTV antigens. These results indicated that RAb2s recognized the common idiotopes on anti-VP7 antibodies obtained from mice and cattle and that at least a portion of RAb2s were internal-image anti-Ids that functionally mimicked VP7. RAb2s may be used to substitute for the tissue culture-derived viral antigen in currently used serological assays for the detection of antibodies to BTV.

MATERIALS AND METHODS

Radioimmunoprecipitation (RIP). Monolayers of BHK-21 cells were infected with BTV 11 at a multiplicity of infection of 0.1 PFU per cell. After virus adsorption for 45 min at 37°C, the inoculum was replaced by fresh minimum essential medium containing 10% fetal bovine serum. After an additional 12 h of incubation, the cells were rinsed with methionine-free minimum essential medium and incubated for 1 h with the same medium supplemented with 100 μCi of [35S]methionine (Amersham Canada Ltd., Oakville, Ontario, Canada) per ml. After being washed three times with phosphate-buffered saline (PBS), the cells were disrupted in lysis buffer (0.05% Nonidet P-40, 0.04 M Tris-HCl [pH 7], 0.04 M EDTA, and 2 mM phenylmethylsulfonyl fluoride). The mature virions were removed from the cell lysate by centrifugation in sucrose (20%, wt/vol) at 26,000 × g for 1 h at 4°C. [35S]-labelled BTV-infected cell (10^9) lysate (i.e., top layer) was mixed with individual MAbs (5 μg) and incubated for 2 h on ice. Twenty microliters of protein A-Sepharose CL-4B (Sigma) was added to the mixtures and held on ice for another 30 min. The immune complexes were washed five times with lysis buffer (described above), and the pellet was suspended and applied to a 12.5% polyacrylamide–sodium dodecyl sulfate (SDS) gel as described by Laemmli (14). The fixed and dried gels were then autoradiographed as described elsewhere (8). An RIP inhibition assay was performed as described above except 20 μg of purified RAb2s was incubated with individual MAbs for 1 h at 37°C before being added to the [35S]-labelled BTV-infected cell lysate.

Antibodies to BTV antigens. Three MAbs specific for VP7, designated 1875, 1877, and 1886, originally produced by Appleton and Letchworth (5) were purchased from American Type Culture Collection (Rockville, Md.). The isotypes of these MAbs are immunoglobulin G2a (IgG2a), IgG2b, and IgG2a, respectively. Another MAb, M100 (IgG2a), specific for pseudorabies virus was used as a control MAb (26). Bovine antisera specific for BTV VP7 detected by ELISA as described by Ashraf et al. (3) were used in this study. They were collected 20 days after the infection of cattle with BTV South Africa serotypes 3 (animal 1), 6 (animal 2), and 8 (animal 3) and U.S. serotypes 10 (animal 4), 11

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characteristic of RAb2s. (i) Recognition of IDs on intact MAb1 molecules. SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot (immunoblot) experiments were used to detect ID locations. Intact immunoglobulin molecules (under nonreducing conditions) or separated H and L chains (under reducing conditions) (1 μg) were separated on a 10% polyacrylamide–SDS gel according to the method of Laemmli (14). Separated immunoglobulins were either stained with Coomassie blue or electrophoretically transferred onto polyvinylidene difluoride membrane. Unbound sites on the membrane were blocked with 5% skim milk in PBS containing 0.02% PBSTween-20 for 3 h. The strips of membrane were incubated with 30 ng of RAb2s per ml in 1% skim milk powder in PBST for 1 h. The strips were washed three times with PBS and incubated with horseradish peroxidase–goat anti-rabbit IgG diluted 1/2,000 in 1% skim milk in PBST for 1 h. The remaining color reaction was developed by using diaminobenzidine as recommended by the manufacturer (Sigma). The test was performed at room temperature.

(ii) Inhibition of MAb1-antigen interaction. A competitive ELISA (26) was employed to determine whether the RAb2s could bind MAb1s and inhibit their binding to BTV antigens. Various concentrations of RAb2s were mixed with equal volume of individual MAb1s (1 μg/ml), and the mixture (100 μl) was allowed to interact with the solid-phase BTV antigens (1 μg/ml) purified by the methods described by Mertens et al. (16). The remainder of the assay was performed as described above.

(iii) Inhibition of MAb1-RAb2 interaction. To test the ability of BTV antigen to inhibit the Ab1-Ab2 interaction, an inhibition ELISA similar to that described above was employed with the following modifications. RAb2s (100 μl at 4 μg/ml), along with normal rabbit IgG preparation, were adsorbed on the solid phase, and BTV antigens were used to inhibit the individual MAb1s (500 ng/ml) from binding to the RAb2s. The degree of inhibition was calculated from the following formula: percent inhibition = 100 × (1 − ODsample/ODcontrol) without inhibitor. All assays were performed in triplicate.

(iv) Recognition of bovine anti-BTV antibodies. To determine whether RAb2s recognized common IDs present on bovine antibodies against BTV, an indirect ELISA was performed as described above except that the bovine antisera (diluted 1/25) and horseradish peroxidase–rabbit anti-bovine IgG (Organon Teknika Inc., Scarborough, Ont., Canada) were used to detect the binding of RAb2s to solid-phase RAb2s. Bovine sera were used as controls. To confirm the binding specificity, an inhibition ELISA in which purified BTV antigens (25 μg) were employed to inhibit the interaction between RAb2s (400 ng per well) and bovine antisera (1/25 dilution) was used. The remainder of the assay was performed as described above.

RESULTS

Generation of RAb2s. To determine whether RAb2s could be used in an ELISA for testing antibodies to BTV, an indirect ELISA was employed by coating the solid phase of ELISA plates with RAb2 IgG preparation. RAb2s recognized all three MAb1s but not the anti-pseudorabies virus MAb, M100 (Fig. 1). A normal rabbit IgG preparation had no ability to bind these MAb1s (data not shown). The interaction between RAb2s and MAb1s was dose dependent. RAb2s could detect concentrations of MAb1s as low as 20 ng/ml (2 ng per well).

Characterization of RAb2s. (i) RAb2s recognized MAb1 intact molecules. MAb1s, either intact molecules (under nonreducing conditions) or separated H and L chains (under reducing conditions), were separated by SDS-PAGE. After electrophoretic transfer, MAb1 intact molecules or separated H or L chains were detected by RAb2s. RAb2s recognized intact molecules of three MAb1s (Fig. 2) but did not recognize separated H or L chains of the MAb1s (data not shown). RAb2s did not recognize, in both cases, an unrelated control MAb, M100. These results indicated that (i) RAb2s were indeed generated in the rabbit and were specific for the anti-BTV MAb1s and (ii) the IDs recognized by RAb2s were associated with both H and L chains of the MAb1s.

(ii) RAb2s recognized IDs within the antigen-combining sites. To determine whether RAb2s recognize and bind the ID which is located within or near the antigen-combining sites of MAb1s, various inhibition assays were performed. In an inhibition of antigen-MAb1 interaction, RAb2s effectively inhibited the three MAb1s from binding to the solid-phase BTV antigens (Fig. 3A) even though there was a 10-fold difference in magnitude of the inhibition between the three MAb1s (i.e., 1, 5, and 10 μg of RAb2s per ml were required to achieve greater than 50% inhibition of 1875, 1886, and 1877, respectively). As a control, normal rabbit IgG had no effect on the bindings (data not shown). The inhibitory activity of RAb2s on...
antigen-MAb1 interaction was further tested by RIP assay. When preincubation of MAb1s with RAb2s (20 μg) was carried out, individual MAb1s were completely prevented from precipitating the BTV antigens (Fig. 3B).

Similarly, the ability of BTV antigens to inhibit MAb1-RAb2 interaction was evaluated. As shown in Fig. 4, in the presence of BTV antigens, various degrees of inhibition were observed. BTV antigens at 25 μg/ml inhibited the binding of RAb2s to 1875 by 80% and that to 1877 and 1886 by 50%. No inhibition was demonstrable by using BHK-21 cell control antigens (data not shown). These inhibition results collectively indicated that RAb2s recognized Ids which are located within or near the antigen-combining sites of the MAb1s.

(iii) RAb2s recognized bovine anti-BTV antibodies. To determine whether RAb2s, generated to murine MAb1s, could also recognize bovine antibodies to BTV, an indirect ELISA was employed to test the binding capacity of RAb2s, along with BTV antigens. Six bovine antiserum samples collected 20 days after the infection with various BTV serotypes were used to interact with the solid-phase RAb2s or BTV antigens. These antisera, at a 1:25 dilution, bound not only to the BTV antigens (OD 410 of 0.68 to 1.0) but also to the RAb2s (OD 410 of 0.56 to 0.98; Fig. 5). The presera showed background binding to both BTV antigens and RAb2s (OD 410 of 0.12 to 0.34 and 0.16 to 0.35, respectively).

The specificity of the binding between RAb2s and bovine anti-BTV antibodies was confirmed in an inhibition assay, in which BTV antigens were used to inhibit the binding. Purified BTV antigens partially inhibited the binding (38 to 60%). Specificity of the interaction was also confirmed by the observation that both cell control antigen and bovine anti-EHD-1 and anti-EHD-2 antibodies (2) had no effect on the interaction (data not shown).

DISCUSSION

The experiments described in this report demonstrate the feasibility of using RAb2s as alternative serodiagnostic re-
agents for testing BTV infection. RAb2s were generated by sequential immunization of rabbits with three MAbs to VP7 of BTV. The specificity of RAb2s has been shown to recognize the Id that was associated with anti-VP7 antibodies. The serological interaction of RAb2s with the MAbs required the intact immunoglobulin molecule; i.e., after separation of H and L chains, the Id could not be recognized by RAb2s (Fig. 2).

Three MAbs used in this study were originally generated by Appleton and Letchworth (5). Because of their diverse origins (i.e., from different fusion events), the affinities of their binding to RAb2s would not be expected to be the same. It was apparent that 1875 had the highest binding capacity to RAb2s, compared with those of the other two MAbs (Fig. 1). Consistent with this, RAb2s strongly inhibited 1875 from binding to BTV antigens and their interaction with 1875 was also easily inhibited by BTV antigens (Fig. 3A and 4). Whether the Id location has any direct role on the binding capacity and specificity between the MAbs and RAb2s cannot be concluded at the present. However, the fact that 1875 was inhibited by the antigen from binding to RAb2s more efficiently than 1877 and 1886 implies that the common Id on 1875 is more closely associated with the antigen-combining sites of this MAb1.

Internal-image Ab2 has been shown in many systems to cross genetic barriers in recognizing common Ids shared by antibodies with similar (6, 17, 28) or distinct (13, 28) specificities. This property has been one of the major criteria in defining Ab2s as internal-image antibodies and prompted studies investigating Ab2s as potential vaccine candidates for many infectious organisms (for a review, see reference 27) as well as serodiagnostic reagents (for a review, see reference 15). The use of anti-Id as a diagnostic reagent was first reported in 1982 by Potoczajak et al. for Plasmodium berghei (21), a parasite causing malaria, using an inhibition test in which the radiola-

In the present report, RAb2s generated against MAbs to VP7 of BTV possessed the capacity to recognize bovine antibodies to BTV (by recognizing the common Id). It is surprising that RAb2s had almost the same binding capacity to bovine anti-BTV antibodies as BTV antigens. Bovine antibodies were generated against various serotypes of BTV, and they were expected to contain antibodies to various antigens and epitopes of BTV, which was confirmed by an RIP test (data not shown). In contrast, RAb2s that were generated against mu-

The similar background binding of RAb2s and BTV anti-

clonal anti-Ids. They will be tested against a large number of bovine and other anti-BTV serum samples in order to confirm their usefulness as safe and efficient reagents in indirect as well as competitive ELISAs.

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REFERENCES


