Spectrum of Monoclonal Antibodies to Coxsackievirus B-3
Includes Type- and Group-Specific Antibodies

SHIGEYO YAGI,1 DAVID SCHNURR,4* AND JIEYAN LIN1,2

Viral and Rickettsial Disease Laboratory, Division of Laboratories, California Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704, and GuangXi Provincial Antiepidemic and Hygiene Center, GuangXi, People’s Republic of China2

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Fifteen monoclonal antibodies (MAbs) made to coxsackievirus B-3 were tested against a panel of enteroviruses by indirect immunofluorescence. The MAbs included seven which reacted with coxsackievirus B-3 only, five which reacted with more than one enterovirus included in the panel, one which reacted with broad enteroviral specificity and did not react with any heterologous virus (group specific), and two which reacted with all enteroviruses tested and at least one heterologous virus. The group-specific MAb identified 44 of 45 clinical isolates as enteroviruses, while the two broadly reactive MAbs reacted with all 45 of the clinical isolates. These MAbs are potentially important diagnostic reagents for grouping and typing enteroviruses by indirect immunofluorescence.

Monoclonal antibodies (MAbs) to enteroviruses have been used for the study of strain variation (2, 6, 11), analysis of mutation rates (1), and identification of neutralization epitopes (2), and they also have diagnostic applications (5, 18). This report describes a number of MAbs made to coxsackievirus B-3 (CBV-3), including one enterovirus group-specific and two other broadly reactive MAbs which have been tested against a panel of enterovirus prototypes, several strains of CBV-3, 45 clinical enterovirus isolates, and heterologous viruses.

Virus isolates can be tentatively identified as enteroviruses by the type of cytopathic effect they cause and the cell types in which they grow (9). However, these methods lack the specificity of an immunologic test. Serotyping by means of the Lim-Benyesh-Melnick immune serum pools by using the neutralization test is the standard method for typing (5), but the supply of the pools is limited and neutralization tests are labor-intensive and require a minimum of several days to complete. An alternative to typing by neutralization is to place an isolate in the enterovirus group by hybridization to (7) or amplification of (4, 8, 19) nucleic acid or by testing with group-specific immunologic reagents (15, 18). Although a polyvalent group-reactive immune serum for enteroviruses (15) and an enterovirus group-reactive MAb (17, 18) have been described, the potential for use of such reagents requires further study.

For the production of MAbs, CBV-3(M), a myocarditic strain obtained from the laboratory of Jack Woodruff (deceased), Cornell University Medical College, New York, N.Y., was used to immunize BALB/c mice. In a series of experiments, a number of mice were immunized with live CBV-3(M) and myeloma cells of the SP 2/0 line were fused with spleen cells. From these fusions there were few hybridomas and none producing CBV-3-specific MAbs. Although no measurements were made, the spleens of immunized mice appeared atrophied and were extremely fibrous. The observation of splenic atrophy following CBV-3 infection was previously reported by Bendinelli et al. (1).

Because of the failure to produce MAbs by using live virus, immunization with inactivated CBV-3 was attempted. CBV-3(M) was grown in Buffalo green monkey kidney (BGMK) cells and concentrated and purified by differential and CsCl gradient centrifugation (3). The virus concentrate was inactivated with UV light, resulting in a reduction of the 50% tissue culture infective dose from 1010 to 10⁷/ml. Mice were immunized with the inactivated virus, first intraperitoneally, followed 12 days later by inoculation into the tail vein and 40 days after that by intrasplenic immunization (14). Three days after the final inoculation, spleens were removed and spleen cells were fused with SP 2/0 myeloma cells. Thirty-three hybridomas secreting antibodies to CBV-3(M) were identified by indirect immunofluorescence (IIF) (2). Hybridomas secreting antibodies to CBV-3 were cloned by the limiting dilution method, and immune ascitic fluids were produced for certain of the hybridomas.

Of 15 MAbs further characterized, none were neutralizing for CBV-3 as determined by the microtiter method (13). Among these 15 MAbs were 9 of the immunoglobulin M (IgM) and 6 of the IgG class (Table 1) as determined by use of MonoAb-ID ELISA kit (Zymed Laboratories, Inc.).

To ascertain the range of reactivity of the MAbs, serial 10-fold dilutions of ascitic fluids starting at 1:100 were tested against a panel of viruses and uninfected cells (Table 1). The coxsackie A viruses (CAVs) were grown in human rhabdomyosarcoma cells, while the other serotypes were grown in BGMK cells. The MAbs included seven which reacted with CBV-3 specificity (i.e., reacted with only CBV-3), such as 2-B-8 and 9-D-1, and eight which reacted with two or more of the enteroviruses. Three MAbs, 2-E-11, 5-F-2, and 9-D-5, with broad enteroviral specificity, were selected as potentially group reactive.

Serial 10-fold dilutions of eight of the MAbs (five which were CBV-3 specific one which reacted with CBV-2, CBV-3, and CBV-5; and two of the broadly reactive MAbs) were also tested with CBV-3(Nancy), CBV-3(M), and eight strains representing CBV-3 isolates made from 1950 until 1989. Group-reactive MAb 2-E-11 reacted with all strains tested, while 5-F-2 reacted with all but one of the strains. Type-specific MAb 2-B-8 reacted with all of the CBV-3 strains, while the other MAbs failed to react with one or more of the strains tested (Table 2). The MAbs all reacted

* Corresponding author.
with CBV-3(M), the immunizing strain, and with CBV-3(Nancy), the strain from which the M strain originated (16). However, individual MAbs were less likely to react with a given strain, the greater the time between the isolation of the immunizing strain and the isolation of the strain being tested. This indicated a drift away from the expression of certain epitopes over time.

Further testing with broadly reactive MAbs 2-E-11, 9-D-5, and 5-F-2 was undertaken with a series of 45 clinical isolates typed by this laboratory. The clinical isolates included 5 polioviruses, 2 CAVs, 26 CBVs, 11 echoviruses, and 1 isolate of enterovirus 71. Uninfected control cells were always tested at the same time. The isolates were tested in the cell types in which they had been isolated, primary monkey kidney (PMK) or human fetal diploid kidney (HFDK), or were passaged in BGMK prior to testing. Heterologous viruses influenza A, vesicular stomatitis virus, herpes simplex virus type 1, rhinoviruses 2 and 30, and reovirus type 3 were also tested. Nonspecific staining of HFDK and PMK cells was blocked by incubation of cell spots with 1% casein for 30 min at 37°C prior to IIF staining. Subsequently, the blocking step was included for all staining with these MAbs. Staining with group-reactive MAbs was at 37°C for 1 h.

The broadly reactive MAbs were tested with the clinical isolates at dilutions determined by titration of the MAbs with CBV-3(M). A range of dilutions were tested with the field isolates in order to determine the relative expression of the reactive determinant detected during infection with other enterovirus serotypes. Group-specific MAb 5-F-2 reacted with all the clinical strains except for an isolate of echovirus 14, while 2-E-11 and 9-D-5 reacted with all the clinical isolates tested (Table 3). In every case there was no staining of uninfected cells and the MAbs stained the field isolates with the cytoplasmic pattern typical of picornaviruses. Figure 1 shows typical staining of uninfected and enterovirus-infected cells with MAb 5-F-2.

There was no staining of cells infected with heterologous viruses by MAb 5-F-2. However, MAbs 9-D-5 reacted with rhinovirus types 2 and 30 and MAb 2-E-11 and 9-D-5 both reacted with reovirus type 3. Since rhinoviruses and enteroviruses belong to the same family, cross-reactivity with broadly reactive MAbs is not unreasonable. However, the cross-reactivity of two of the MAbs with reovirus-infected cells was unexpected and compromises the use of these two MAbs as enterovirus grouping reagents.

The broad range of reactivity of certain of these MAbs may have resulted from the use of inactivated virus as immunogen and acetone-fixed virus as the target for detection, as it is known that both these procedures result in an

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**TABLE 1. Class and IIF Reactivity of CBV-3 MAbs**

<table>
<thead>
<tr>
<th>MAb</th>
<th>Class</th>
<th>Reactivity* with:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CBV-1</td>
</tr>
<tr>
<td>2B8</td>
<td>IgG</td>
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<tr>
<td>2E10</td>
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<td>IgG</td>
<td>—</td>
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<tr>
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<td>≥3</td>
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<tr>
<td>5D2</td>
<td>IgM</td>
<td>—</td>
</tr>
<tr>
<td>7G7</td>
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<tr>
<td>9E10</td>
<td>IgM</td>
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a Log<sub>10</sub> of highest reactive dilution. —, not reactive at 1:100 dilution. Polio, poliovirus; Echo, echovirus; Rhino, rhinovirus.

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**TABLE 2. Reaction of MAbs with field strains of CBV-3 by IIF**

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<tr>
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</table>

a Log<sub>10</sub> of highest dilution of ascitic fluid. —, not reactive at 1:100 dilution.

* Substrain of Nancy.

NA, not available.
increased broadening in the antigenicity of enteroviruses (5). Two of the three broadly reactive MAb s were of the IgM class. The IgM response to enterovirus infection is known to be more widely reactive than the IgG, and there have been several studies using the cross-reactive IgM response as a serological marker for enterovirus infection in humans (12). The group-reactive MAb described by Yousef et al. (17, 18) was of the IgG2A subclass. This was a nonneutralizing antibody which reacted with the VP1 peptide and was reactive in several other assays. The broadly reactive MAb s reported in this study failed to react with CBV-3 in both radioimmunoprecipitation and enzyme-linked immunosor-