Use of Monoclonal Antibodies To Type Shigella flexneri in Bangladesh

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A panel of 10 mouse and rat monoclonal antibodies specific for different type- and group-specific O-antigenic determinants of Shigella flexneri lipopolysaccharide was used to serotype 240 isolates of S. flexneri from Bangladesh. Three immunoglobulin M antibodies were used in a direct slide agglutination test; seven immunoglobulin G antibodies were absorbed to Staphylococcus aureus and used in a coagglutination assay. All but 13 of the isolates could be serotyped by using the monoclonal antibodies. The six most common serotypes were (in descending order) 2a, 2b, Y (E1037), 1a, 3a, and 1b and accounted for more than 80% of all isolates. Two of the nontypable strains were found to be of a new provisional serotype of S. flexneri (T. Wehler and N. I. A. Carlin, Eur. J. Biochem. 176:471–476, 1988). The 11 remaining strains were found to be rough and therefore nontypable. The serotyping scheme based on the panel of monoclonal antibodies is specific and holds the potential to be developed into a useful tool for epidemiological investigation. The study also demonstrates that the recently described E1037 antigen is commonly found among at least four serotypes (4a, 6, X, and Y) of S. flexneri.

Diarrheal diseases, including shigellosis, are among the major causes of morbidity and mortality in developing countries. The dysentery caused by shigellae is characterized by frequent passage of blood and mucus in the stools, fever, and abdominal pain. In Bangladesh, the most common Shigella species is Shigella flexneri, although S. dysenteriae type 1 is also common and is frequently found as the cause of epidemics.

Epidemiological studies of the disease require typing systems that allow efficient and accurate designation of the strains isolated. Classification of the shigellae is based on the antigenic specificity that resides in the O antigens (7). Anti sera against the O antigens are traditionally raised by immunization of rabbits with whole heat-killed bacteria. The antisera are then tested and, if needed, absorbed to render them specific. For most Shigella species, e.g., S. dysenteriae, S. boydii, and S. sonnei, serotype-specific antisera are readily obtained by this method. Although the serotyping system for S. flexneri is well established (7), it is not commonly used, largely because high-quality reagents are not routinely available (6). This is because antisera are cross-reactive, and even after repeated absorptions, titers and specificities of the resulting factor sera are often questionable (6, 7). According to the present typing scheme for S. flexneri (7), which is based on the scheme of Boyd (1), S. flexneri serotypes are defined by a series of type and group antigens. Type antigens are designated by Roman numerals I to VI and are found only within one serotype (e.g., serotype 1a and 1b share the type antigen I). Group antigens are designated by Arabic numerals 3, 4, 6, and 7.8. These group antigens can be shared among different serotypes (e.g., group antigen 7,8 is found in bacteria of serotypes 2b, 3a, 5b, and X). The different combinations of type and group antigens result in a total of 14 serotypes and variants of S. flexneri.

We have recently produced a number of monoclonal hybridoma antibodies specific for all S. flexneri type and group antigens (2–4). In addition, a monoclonal antibody (MASF B) has been characterized that recognizes the putative group antigen 1 in S. flexneri, an antigen that is present on all serotypes of S. flexneri (4). Furthermore, a monoclonal antibody has been obtained that has specificity for a new antigenic determinant (E1037), present on some strains of S. flexneri serotype 4a, X, and Y and, as shown in this report, also on some strains of serotype 6 (4).

We report here on the use of these monoclonal antibodies to serotype 240 clinical isolates of S. flexneri from the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh.

MATERIALS AND METHODS

Bacterial strains. Staphylococcus aureus Cowan 1 was available from previous investigations (3). Clinical isolates of S. flexneri. The clinical isolates used were from patients attending the diarrhea treatment center in Matlab, Bangladesh. This treatment center, operated by the International Centre for Diarrhoeal Disease Research, Bang la desh, has treated about 12,000 diarrhea patients annually since 1963. Isolates used in this study represent all of the S. flexneri isolates identified between January 1985 and February 1987 from patients living within the surveillance area.

Bacteria were isolated from fecal specimens by culture on salmonella-shigella (Difco Laboratories, Detroit, Mich.) and MacConkey (Difco) agars. Non-lactose-fermenting colonies were identified as Shigella species by standard biochemical methods and were tested in polyvalent serogroup-specific rabbit antisera (Wellcome Diagnostics, Dartford, England) specific for S. dysenteriae, S. flexneri, S. boydii, and S.
sonneni; i.e., the strains were not typed to the serotype level at this stage. The strains were kept as stab cultures in blood agar base slants for a maximum of 30 months before being subtyped.

Monoclonal antibodies. Production and immunochemical characterization of the monoclonal antibodies used have been described elsewhere (2–4). Their antigenic specificities and uses in serotyping are presented in Table 1.

Preparation of IgM reagents. Antibodies of the immunoglobulin M (IgM) class (MASF B, II, and Y-5) were used for conventional glass slide agglutination. Ascites fluids (11) were diluted in phosphate-buffered saline, pH 7.4 (PBS), to concentrations (1/5 to 1/20) giving an agglutination visible to the naked eye within 60 s.

Preparation of IgG reagents. To render the IgG antibodies (MASF IV-1, V, VI, 6 and 7, 8) agglutinating, the antibodies were absorbed to sensitized (formaldehyde- and heat-treated) S. aureus Cowan I as described previously (3, 9). Ascites fluid (0.1 ml; specific IgG concentration of approximately 1 to 3 mg/ml) diluted 1/5 to 1/40 in PBS was added to 1.0 ml of a 10% (vol/vol) suspension of sensitized staphylococci in PBS. After incubation and washing, the reagent was diluted to 2% (vol/vol) in the same buffer.

For mouse IgG1 (MASF 1) and rat IgG2c (MASF IV-2) antibodies, a slightly different procedure was used. The sensitized staphylococci were washed once in 1.5 M glycine–3 M NaCl buffer (pH 8.9). Then 0.1 ml of ascites fluid diluted in the same buffer was added to 1 ml of a 10% suspension of staphylococci, and the preparation was incubated as described above. The staphylococci sensitized with mouse IgG1 antibodies were washed and stored in the 1.5 M glycine–3 M NaCl buffer (pH 8.9), whereas the staphylococci sensitized with rat IgG2c antibodies were washed and stored in PBS. Use of the high-ionic-strength buffer for these antibodies increased binding to the staphylococci by a factor of 10 or more (Pharmacia Separation News 13(5):1–3, 1986). All coagglutination and IgM reagents were stored at 4°C with 0.01% (wt/vol) Merthiolate as a preservative agent.

For the serotyping procedure, the strains were inoculated onto MacConkey or tryptic soy broth (Difco) agar from the slant and tested directly for agglutination. Agglutination testing was performed on glass slides, and results were recorded as ++ (if agglutination was apparent to the naked eye within 30 s) or + (if a magnifying glass was needed for observation).

Polyvalent rabbit antisera. Type- and group-antigen-specific rabbit antisera, used only for testing of the nontypable strains, were obtained from Wellcome Diagnostics and from the Department of Bacteriology, National Bacteriological Laboratory, Stockholm, Sweden.

RESULTS

During this study, a total of 560 clinical isolates from the Matlab field station were classified as Shigella species on the basis of agglutination with species-specific rabbit antisera and biochemical data. Of the total, 47.5% were S. flexneri, 38.0% were S. dysenteriae serotype 1, and 14.5% were other Shigella species. A total of 240 strains originally identified as S. flexneri and stored on blood agar base slants were recovered. Upon subculture, 11 strains autoagglutinated when tested with 0.9% NaCl, MASF B, and polyvalent anti-S. flexneri rabbit antiserum, which indicated that they had become rough (i.e., no longer produced O-antigenic polysaccharide chains). Two strains, provisionally labeled Y394 and Y400, did not agglutinate in 0.9% NaCl but were positive with MASF B and the polyvalent anti-S. flexneri group-specific rabbit antiserum. These two strains did not react with any type- or group-antigen-specific antiserum tested, polyvalent or monoclonal.

The remaining 227 strains were serotyped with the panel of monoclonal antibodies according to the typing scheme shown in Table 1. All 227 strains reacted with MASF B (Table 2), in agreement with earlier results (4). Use of the panel of monoclonal antibodies allowed unambiguous typing of all strains to the serotype level; i.e., both type and group antigens could be assigned to each strain. Some strains of serotypes 1a and 4a reacted only with MASF B and the type-antigen-specific antiserum. The isolates belonging to serotypes 3a, 3b, and 3c reacted only with group-antigen-specific antiserum. Since there is no epitope that constitutes the type antigen III (5), these serotypes are defined by group antigens. Use of the MASF IV-1 antibody, detecting the provisional E1037 antigen (4), allowed further grouping of some serotype 4a, 6, X, and Y strains. The serotype 6 strain that reacted with this antibody is the first reported isolate of this serotype with the E1037 antigen.

TABLE 1. Key for S. flexneri serotyping

<table>
<thead>
<tr>
<th>Reaction* with given MASF antibody</th>
<th>Type antigen specific</th>
<th>Group antigen specific</th>
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<tbody>
<tr>
<td></td>
<td>I (E−)</td>
<td>II (E+)</td>
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<tr>
<td>1a</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>1b</td>
<td>+++</td>
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<td>+</td>
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<td>Y</td>
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* +++, Positive reaction; +++, variable reaction (antigen may or may not be detectable).

a Shown in parentheses is the antigen recognized.

For mouse IgG1 (MASF 1) and rat IgG2c (MASF IV-2) antibodies, a slightly different procedure was used. The sensitized staphylococci were washed once in 1.5 M glycine–3 M NaCl buffer (pH 8.9). Then 0.1 ml of ascites fluid diluted in the same buffer was added to 1 ml of a 10% suspension of staphylococci, and the preparation was incubated as described above. The staphylococci sensitized with mouse IgG1 antibodies were washed and stored in the 1.5 M glycine–3 M NaCl buffer (pH 8.9), whereas the staphylococci sensitized with rat IgG2c antibodies were washed and stored in PBS. Use of the high-ionic-strength buffer for these antibodies increased binding to the staphylococci by a factor of 10 or more (Pharmacia Separation News 13(5):1–3, 1986). All coagglutination and IgM reagents were stored at 4°C with 0.01% (wt/vol) Merthiolate as a preservative agent.
The prevailing serotypes were 2a, 2b, Y (E1037), 1a, 3a, and 1b, which together accounted for more than 80% of all of the S. flexneri isolates. Serotype 2a was the predominant serotype, as was also found in a previous study in Bangladesh (K. A. Talukder, Q. S. Ahmad, K. Haider, and M. I. Huq, Abstr. Annu. Meet. Bangladesh Soc. Microbiol. 1987, p. 2).

**DISCUSSION**

The use of immunochemically well characterized monoclonal antibodies for typing of S. flexneri proved to be successful. All 227 smooth S. flexneri strains could unambiguously be assigned to a serotype. Two strains (Y394 and Y400) agglutinated only with the MASF B antibody. In a separate study, lipopolysaccharides from these two strains were extracted and subjected to chemical investigation. Both strains were shown to be of a new, as yet undescribed serotype of S. flexneri (12).

The fact that several 1a and 4a strains failed to react with MASF Y-5 (group antigen 3,4-specific monoclonal antibody) probably resulted from variability in the expression of the group antigen 3,4 (4, 7).

S. flexneri is known to exhibit considerable variability in O antigens. Lysogenic conversion by bacteriophages (8), a change from smooth to rough phenotype (13), and form variation (1) can contribute to the difficulties encountered in preparing specific polyvalent typing antisera for S. flexneri. In a recent study (6), Evins and co-workers tested several commercially available polyvalent Shigella typing antisera and concluded that more than 50% of all tested antisera were inadequate for reliable serotyping of shigelae. As demonstrated here, monoclonal antibodies, which have unique, defined, and constant specificities, are ideal tools for typing of this antigenically variable organism.

Use of the antigen E1037-specific antibody (MASF IV-1) has expanded the antigenic markers of S. flexneri, which could be of important epidemiological value. Interestingly, most of the E1037-positive strains seen in Bangladesh were Y strains, whereas in Sweden the E1037 antigen is found mostly in 4a and X strains (4). The unique specificity of the MASF B antibody, which binds to all S. flexneri and S. dysenteriae serotype 1 isolates (4), suggests that it may be useful for antigen detection in stool samples. Furthermore, use of the panel of monoclonal antibodies made it possible to identify two strains (Y394 and Y400) as putative members of a new provisional serotype of S. flexneri (12).

Epidemiological surveillance of areas endemic for shigellosis could also be of value for the production of new attenuated live Shigella vaccines (10). If the protective features of such vaccines are serotype specific, then careful epidemiological studies are needed so that vaccine candidates with relevant O-antigenic side chains can be developed.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**