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Characterization of the Recognition Site and Diagnostic Potential of an Enterovirus Group-Reactive Monoclonal Antibody

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The epitope for a monoclonal antibody reactive against different enteroviruses was characterized. The epitope could be located to residues 40 to 48 of VP1, and the most-essential residues for monoclonal antibody binding coincided with those conserved among the majority of known enteroviruses, indicating a high diagnostic potential.

The human enterovirus genus includes poliovirus (3 serotypes), coxsackievirus A (23 serotypes), coxsackievirus B (6 serotypes), echovirus (31 serotypes), and enteroviruses 68 to 71. Enteroviruses are often associated with asymptomatic or mild infections but may also cause severe diseases such as myocarditis, meningitis, and encephalitis.

We have recently characterized a major recognition site for human antibodies which are cross-reactive between different types of enteroviruses (7). The antigenic region could be located to residues 37 to 51 of VP1 (positions based on alignment with poliovirus 1 VP1, Mahoney strain, throughout this work).

Enterovirus group-reactive mouse monoclonal antibody (MAb) clone 5-D8/1 (M7064; DAKO A/S, Copenhagen, Denmark), originally described by Yousef et al. (9), has recently become available commercially. Cross-reactive MAbs able to detect the majority of enteroviruses known today will be important tools in the rapid diagnosis of enteroviral infections. We therefore characterized the recognition site for the MAb 5-D8/1 to predict its cross-reactivity and thereby its diagnostic potential.

Peptides were synthesized, cleaved, and deprotected by a "tea-bag" method using 9-fluorenylmethoxycarbonyl-protected amino acids as described previously (2, 6). Fifteen-residue-long peptides (with a 12-amino-acid overlap), covering residues 31 to 96 of poliovirus 1 Mahoney strain, and an additional set of 11 peptides (with a 5-amino-acid overlap), covering residues 31 to 148 of coxsackievirus B1, were produced. A set of 15 substitute peptide analogs covering residues 35 to 51 of poliovirus 1 was produced as described previously (7) and used to characterize the binding of the MAb. Reactivity of the MAb was analyzed against the peptides in enzyme-linked immunosorbent assays (ELISAs) (7). The MAb was incubated on the peptide-coated plates for 90 min at 37°C. The amount of MAb bound was then indicated by horseradish peroxidase-conjugated rabbit antimouse immunoglobulin G (P260; DAKO). The substitute peptide analogs were also analyzed in inhibition tests as described previously (10). In brief, the plates were coated with the original peptide covering residues 35 to 51, and the substitute peptide analogs serially diluted 10-fold from 10,000 to 1 ng were added simultaneously with the MAb. Thereby, the importance of each substituted residue within the peptide can be estimated on the basis of the change in the binding relative to that of the original peptide. The cross-reactivity of the MAb was tested with heat-treated enterovirus antigens of 18 different serotypes in an indirect ELISA described previously (8). The antigens used include the most frequently isolated echo- and coxsackievirus strains, echovirus 4, 6, 9, 11, 18, 22, 25, and 30 and coxsackievirus A9 and B1-5, as well as enterovirus 71 and poliovirus 1 to 3. The coxsackievirus B5 antigen was prepared from prototype strain Faulkner, poliovirus 1 was prepared from prototype strain Brunhilde, and poliovirus 3 was from strain Saukett, while the other antigens were prepared from patient isolates. The MAb was serially diluted fourfold from 1:50 to 1:51,200.

The epitope for MAb 5-D8/1 was located to residues 40 to 48 (Glu-Ile-Pro-Ala-Leu-Thr-Ala-Val-Glu) (Fig. 1). The reactivity was confirmed by inhibition tests in which peptides in solution containing residues 40 to 48 were able to inhibit the peptide binding of MAb 5-D8/1. With the set of 11 overlapping peptides covering residues 31 to 148 of coxsackievirus B1, the epitope was confirmed to reside within positions 41 to 55.

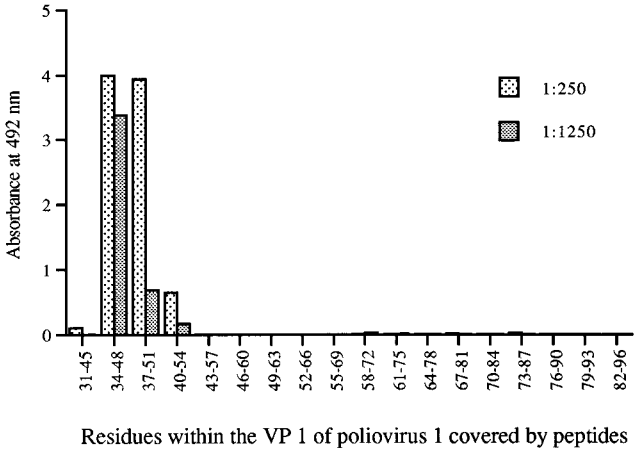

FIG. 1. Reactivity to peptides covering residues 31 to 96 of poliovirus 1 VP1 of the MAb. Reactivity is given as A_{492}. Two different dilutions of the MAb are depicted.

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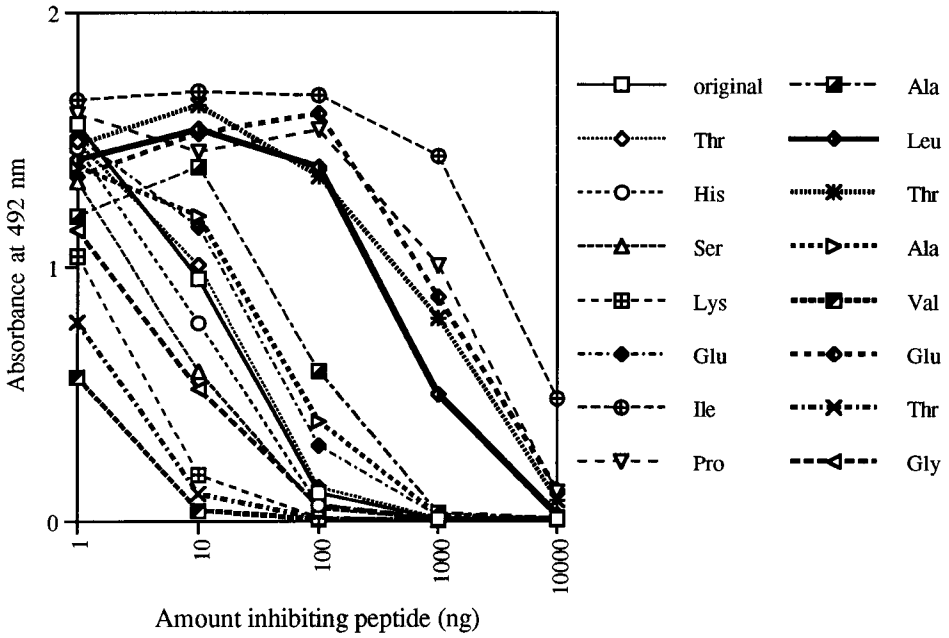

FIG. 2. Recognition pattern of the MAb 5-D8/1 analyzed with substitute peptide analogs of residues 35 to 51 (Pro-Thr-His-Ser-Lys-Glu-Ile-Pro-Ala-Leu-Thr-Ala-Val-Glu-Thr-Gly-Ala). Amino acids were replaced by Ala (or Gly when the original residue was Ala) and are represented by the symbols given in the figure. The reactivity for the MAb (diluted 1:1,000) is given as A_{492} and shows the change in relative peptide binding induced by the respective substitute peptide analog.

The residues found to be essential for MAb binding were Ile-41, Pro-42, Leu-44, Thr-45, and Glu-48, as determined by the inhibition assay (Fig. 2). When the MAb was tested against the substitute peptide analogs with which the plates had been coated, residues Ala-43 and Ala-46 were also found to be essential (data not shown). All of these residues except for the divergent echovirus 22 (GenBank release 97) are well conserved among different enteroviruses. As a confirmation of this prediction, we found that MAb 5-D8/1 reacted with 16 of the heat-treated enteroviral antigens. In enterovirus 70, there is a substitution for Thr-45 by Asn-45 (5), and in coxsackievirus A2 and A16, the Thr-45 is substituted by Gln-45 (4). Unfortunately, it was not possible to analyze whether the MAb was reactive with these viral antigens. In the original description of MAb 5-D8/1, the MAb was found to be reactive to echovirus 22; we were unable to confirm this (9). Enterovirus 71 was also not recognized by the MAb.

We have recently shown that positions 42 to 50 contain a highly conserved sequence recognized by a majority of serum antibodies of enterovirus-infected humans (7). In this report, we show that MAb 5-D8/1 recognizes an epitope overlapping this region. On the basis of the residues essential for MAb binding, it was predicted that MAb 5-D8/1 should be highly cross-reactive. This was confirmed when testing the MAb 5-D8/1 against heat-treated antigens. All antigens which are presently known to be conserved within the majority of the essential residues for antibody binding, i.e., poliovirus 1 to 3 and coxsackievirus A9, B1, and B3-5, were identified correctly. The lack of recognition of echovirus 22 is not surprising since this virus is known to differ from the main enterovirus group in molecular (3) and clinical (1, 3) aspects. Why enterovirus 71 was nonreactive is unclear, but it can be speculated that enterovirus 71, like echovirus 22, shows major amino acid variation within this epitopic region. Future sequence analysis of enterovirus 71 will resolve this question. In conclusion, this study shows that the MAb fulfills the recognition pattern. The cross-reactivity may be useful in the laboratory diagnosis of a majority of the clinically most common enteroviral serotypes.

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REFERENCES

1. **Ehrnst, A., and M. Eriksson.** 1993. Epidemiological features of type 22 echovirus infection. Scand. J. Infect. Dis. **25:**275–281.
2. **Houghten, R. A.** 1985. General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA **82:**5131–5133.
3. **Hyypiä, T., C. Horsnell, M. Maarone, M. Khan, N. Kalkkinen, P. Auvinen, L. Kinnunen, and G. Stanway.** 1992. A distinct picornavirus group identified by sequence analysis. Proc. Natl. Acad. Sci. USA **89:**8847–8851.
4. **Pöyry, T., T. Hyypiä, C. Horsnell, L. Kinnunen, T. Hovi, and G. Stanway.** 1994. Molecular analysis of coxsackievirus A16 reveals a new genetic group of enteroviruses. Virology **202:**982–987.
5. **Ryan, M. D., O. Jenkins, P. J. Hughes, A. Brown, N. J. Knowles, D. Booth, P. D. Minor, and J. W. Almond.** 1990. The complete nucleotide sequence of enterovirus type 70: relationships with other members of the picornaviridae. J. Gen. Virol. **71:**2291–2299.
6. **Sällberg, M., U. Rudén, L. O. Magnius, E. Norrby, and B. Wahren.** 1991. Rapid "tea-bag" peptide synthesis using 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acids applied for antigenic mapping of viral proteins. Immunol. Lett. **30:**59–68.
7. **Samuelson, A., M. Forsgren, B. Johansson, B. Wahren, and M. Sällberg.** 1994. Molecular basis for serological cross-reactivity between enteroviruses. Clin. Diagn. Lab. Immunol. **1:**336–341.
8. **Samuelson, A., E. Skoog, and M. Forsgren.** 1990. Aspects on the serodiagnosis of enterovirus infections by ELISA. Serodiagn. Immunother. Infect. Dis. **4:**395–406.
9. **Yousef, G. E., I. N. Brown, and J. F. Mowbray.** 1987. Derivation and biochemical characterization of an enterovirus group-specific monoclonal antibody. Intervirology **28:**163–170.
10. **Zhang, Z. X., M. Chen, K. Wallhagen, J. Tronjar, L. O. Magnius, B. Wahren, and M. Sällberg.** 1994. Molecular basis for antibody cross-reactivity between the hepatitis C virus core protein and the host-derived GOR protein. Clin. Exp. Immunol. **96:**404–409.