Salmonella-Specific Monoclonal Antibodies against Recombinant Salmonella typhi 36-Kilodalton Porin

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Mouse monoclonal antibodies were raised against recombinant Salmonella typhi 36-kDa porin monomer. Specificities of 16 monoclonal antibodies were analyzed as reactivity patterns in dot immunobinding and Western blot (immunoblot) assays using isolated outer membrane proteins of gram-negative bacteria and cloned purified S. typhi porin monomers and trimers. Four monoclonal antibodies were specific for Salmonella spp.

Porins are surface proteins found in the outer membranes of gram-negative bacteria that form transmembrane channels involved in the transport of solutes (5). Some porins are receptors for bacteriophages, as is the case with the Salmonella typhi 36-kDa porin (1). They appear to be involved in the ability of Salmonella typhimurium to produce disease (7) and are involved in antibiotic susceptibility (1). Porins from Salmonella typhi and other gram-negative bacteria have been used in diagnostic tests (6) and in the development of vaccines (13), as there is experimental evidence suggesting that the host immune response to Salmonella porins generates humoral and cell-mediated protective immunity (4, 21, 23). The porins of S. typhi are conserved among different isolates (8). Nucleotide sequence analysis of the genes encoding different porins has revealed approximately 60% homology in primary structure between them, yet monoclonal antibodies (MAbs) produced against one were unable to recognize epitopes in others (14, 18). This indicates that they express species-specific as well as cross-reacting epitopes (14, 18).

We have developed and described a strain of Escherichia coli, UH302(pST13), that expresses recombinant S. typhi 36-kDa porin as its sole porin (1). Porin purified from this strain was used to develop a panel of MAbs. We have found that some of these MAbs react specifically with Salmonella porins and are candidates for genus-specific diagnostic tools in antigen capture and antibody assays and for studying the role of porins and antibodies to them in the biology of Salmonella infections.

Outer membrane proteins (OMPs) were isolated from E. coli UH302, E. coli UH302(pST13), S. typhi Ty2, S. typhimurium, S. paratyphi A, S. paratyphi C, S. choleraesuis, S. enteritidis, S. newport, S. anatum, and other bacteria grown in L broth at 37°C as previously described (1, 17). Salmonella strains were provided by M. Shayeagani, New York State Department of Health (Albany, N.Y.). OMPs from clinical isolates of Neisseria meningitidis and Haemophilus influenzae grown on chocolate agar under microaerophilic conditions at 37°C were isolated in a similar manner (1, 8).

E. coli UH302(pST13) served as the source of S. typhi 36-kDa porin (1). OMPs were isolated by ultracentrifugation following Triton X-100 solubilization (1). Porin monomers and trimers were then isolated by extraction with β-mercaptoethanol–sodium dodecyl sulfate (SDS) buffer followed by ultracentrifugation at 130,000 × g for 18 h (11, 17). Porin trimer was purified by running this extract lysate through a 5 to 40% sucrose gradient and concentrating appropriate fractions by Amicon filtration. Porin monomer was prepared by boiling one half of the initial isolate in extraction buffer followed by purification on a Sepharose 4B column and concentration by Amicon filtration (11, 17). OMP, porin monomer, and porin trimer concentrations were estimated colorimetrically with the bicinchoninic acid protein assay kit with bovine serum albumin as the standard (Pierce Chemical, Rockford, Ill.). S. typhi smooth lipopolysaccharide (LPS) or E. coli K-12 complete core LPS was obtained from Sigma, St. Louis, Mo., and List Biological Laboratories, Campbell, Calif.

MAbs were produced in 10-week-old BALB/c mice immunized with porin monomer reconstituted in 2% SDS in phosphate-buffered saline as described previously (10, 12). Hybridomas were screened for antibody production by enzyme-linked immunosorbent assay (ELISA) using the porin preparation as the antigen (10, 12). Sixteen hybridomas demonstrating anti-porin activity were subcloned by limiting dilution, isotyped by indirect ELISA using a commercially available kit (Hyclone Laboratories, Logan, Utah), and used in further studies.

Dot blotting was done by a modification of the standard technique (1, 3). SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting of porin monomer, porin trimer, LPS, and extracted OMPs were performed as previously described (3, 4). The binding of anti-porin MAbs to surface epitopes on whole cells and sacculles of E. coli UH302, E. coli UH302 (pST13), S. typhi, and other Salmonella spp. was detected with fluorescein-conjugated rabbit anti-mouse immunoglobulin (Ig) (Jackson ImmunoResearch Laboratories, West Grove, Pa.) and a fluorescent microscope (Leitz Dialux 22EB) (9, 15).

There were 3 MAbs (M-13, M-18, and M-19) specific for the porin monomers of Salmonella spp. among 16 MAbs raised against the 36-kDa S. typhi monomer. These reacted with a 36-kDa porin band in the extracted Salmonella OMPs but reacted only weakly with a 30.5-kDa band in the OMPs of S.
choleraesuis (Table 1, Fig. 1A). A fourth antibody, M-26, reacted with a 36-kDa porin band in each of the extracted Salmonella OMPs but did not react with any band in the OMPs of S. choleraesuis. These four Salmonella-specific MAbs did not react with any of the OMPs from E. coli, Shigella sonnei, Enterobacter cloacae, Serratia marcescens, Proteus mirabilis, Klebsiella pneumoniae, Citrobacter freundii, N. meningitidis, or H. influenzae (Fig. 1B). They also reacted with purified porin monomer and trimer in immunodots (data not shown). In SDS-PAGE immunoblots, however, they reacted only with porin monomer and with a 36-kDa band present in the heated OMPs of E. coli UH302(pST13) (Fig. 1); they did not react with trimer. They also failed to react with the trimer complex in the OMPs of S. typhi (data not shown). The isotype of M-13 and M-18 is IgG2b, and that of M-19 and M-26 is IgG1. None of these four anti-S. typhi 36-kDa porin MAbs reacted with S. typhi or E. coli K-12 LPS in immunodots. We conclude that these MAbs are specific for Salmonella sp. porins.

MAbs M-13, M-19, and M-26 were used in immunofluorescence studies with whole cells and detergent-generated saccules of E. coli UH302, E. coli UH302(pST13), S. typhi Ty2, S. typhimurium, S. paratyphi A, S. paratyphi C, S. choleraesuis, S. enteritidis, S. newport, and S. anatum in order to determine whether any of these MAbs could recognize the porins in the native state on the surface of the bacterial cell. Whole cells of E. coli UH302 and UH302(pST13) and of S. typhi Ty2 and the other seven Salmonella spp. failed to fluoresce after being reacted with the MAbs. Fluorescence was also not observed when whole cells of the S. typhi rough mutant MMW 001 were used (15). Saccules prepared from E. coli UH302(pST13), expressing the S. typhi 36-kDa porin, showed slight to strong fluorescence with MAB M-13. S. typhi saccules did not show fluorescence when incubated with any of the three MAbs, nor did saccules of S. newport or S. paratyphi A show fluorescence. This suggests that these MAbs do not recognize porin epitopes on the surface of Salmonella sp. cells but are able to recognize epitopes in the saccules of some of these bacteria.

MAbs M-13, M-18, M-19, and M-26 were Salmonella sp. specific and did not recognize epitopes in the denatured OMPs from other enterics, from N. meningitidis, or from H. influenzae.

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* Light band seen at 30.5 kDa.
Salmonella infections (1, 2, 6, 16). It would also be of interest whether synthetic polynucleotides coding for them could be buried in the bacterial membrane and consequently are not exposed on the bacterial surface or the periplasmic space (4, 9). These anti-S. typhi porin MAbs are more species specific than MAbs raised to OmpD and OmpC porins of S. typhimurium (18). The latter MAbs reacted with porins of many other species of the family Enterobacteriaceae (18). These Salmonella-specific MAbs could perhaps be used in capture assays to detect the presence of Salmonella porin and in sandwich ELISA assays to detect anti-porin antibodies in Salmonella infections (3, 4, 16, 20). The fact that these Salmonella-specific MAbs do not appear to react with whole cells does not rule out their use to detect porins as the bacterial cells are lysed by host defenses and porins are resistant to proteases (16). Alternatively, samples could be enriched and heat treated to expose porin epitopes recognized by these antibodies (22). The use of these porin-specific MAbs and the purified cloned 36-kDa porin of S. typhi should increase the specificity of these antigen- and antibody-detecting assays in Salmonella infections (1, 2, 6, 16). It would also be of interest to characterize these apparently Salmonella-specific unexposed epitopes, investigate whether they induce a protective immune response in the host infected with a Salmonella sp., and explore whether synthetic polynucleotides coding for them could be used in PCR assays to detect Salmonella spp. (19). In summary, we have generated four anti-S. typhi porin MAbs which are specific for Salmonella spp. and another MAb that permitted the identification of S. choleraesuis. More studies will be needed to confirm their usefulness in detecting Salmonella spp. and antibodies in clinical samples.

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REFERENCES