Protective capacity of antibodies to outer-membrane components of *Escherichia coli* in a systemic mouse peritonitis model

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**Summary.** Antibody-mediated protection was studied in an experimental murine model of peritonitis-septicaemia with *Escherichia coli* O18:K1. Protection from lethal intraperitoneal challenge was achieved by passive immunisation with horse anti-K1 capsular antiserum (H46) or rabbit antiserum to the homologous O18 antigen. The maximum increase in LD50 achieved with anti-K1 and anti-O18 antibodies was 10- and 5-fold, respectively. The protective capacity of the anti-O serum was found to be in the IgG fraction. Rabbits were also immunised with various semi-purified or purified outer-membrane-protein preparations (porins and OmpA protein) from rough *E. coli* or *Salmonella* strains or with whole *E. coli* 15 bacteria. Although this immunisation resulted in high antibody titres to homologous and, to a lesser extent, also to heterologous antigens, none of the antisera protected against challenge with the capsulate *E. coli* O18:K1 bacteria.

**Introduction**

*Escherichia coli* strains that possess the K1 capsular antigen are the most frequent cause of meningitis in the newborn (Speer et al., 1985) and of nosocomial gram-negative bacteraemia with gram-negative bacilli in adults (Weinstein et al., 1983). The K polysaccharide is recognised to be a virulence factor responsible for bacterial resistance to phagocytosis and the bactericidal action of serum (Horwitz and Silverstein, 1980).

Antibodies to the capsular polysaccharide are protective in experimental *E. coli* infections (Bor-tolussi and Ferrieri, 1980; Cross et al., 1983), but unfortunately the K1 antigen is a poor immunogen. The O-antigenic polysaccharide part of the *E. coli* lipopolysaccharide (LPS) is, similarly, a target of protective antibodies (Kaijser et al., 1972). Recently Pluschke and Achtman (1985) reported that monoclonal IgM antibodies to the O18 serotype protected infant rats from meningitis caused by capsulate *E. coli* O18:K1. Antibodies to deeper structures of LPS (core glycolipid-lipid A) have been reported to be effective in preventing fatal infections with gram-negative bacilli (Ziegler et al., 1973; Braude et al., 1977) but contradictory results have also been published (Greisman et al., 1978; Welch et al., 1979).

The major outer-membrane (OM) proteins, including porins and the OmpA protein, show antigenic cross-reactivity within the family Enterobacteriaceae (Hofstra and Dankert, 1980), and this has raised the possibility of their use as vaccines (Kuusi et al., 1979). However, van der Ley et al. (1986) have recently shown that anti-porin antibodies could not bind to their target antigen on smooth *E. coli*. Furthermore, Saxén et al. (1986) showed that the protective action of polyclonal anti-porin sera in mouse salmonellosis was due to anti-O rather than anti-protein antibodies.

We have systematically examined the role of antibodies to the major cell-surface antigens (capsule, O antigen, LPS core, and major outer-membrane proteins) in protection from experimental mouse peritonitis-septicaemia caused by capsulate *E. coli* O18:K1.

**Materials and methods**

**Bacteria**

For mouse challenge we used *E. coli* strain IH 3080 (serotype O18ac:K1:H7) which was a clinical isolate from human neonatal meningitis (Nowicki et al., 1986). It was stored at −70°C in small volumes until use. The
inoculum for injection into the mice was prepared by growing the bacteria overnight in Luria Broth (Tryptone, Difco, 5 g/L; Yeast Extract, Difco, 5 g/L; NaCl 5 g/L (pH 7-0) at 37°C on a rotatory shaker then diluting the culture 1 in 10 and growing for a further 2 h in similar conditions.

Strain EH 778 was a spontaneous non-capsulate mutant of strain IH 3080 selected on the basis of resistance to the K1 capsule-specific phage, pK1a (Gross et al., 1977). Strain EH 786 was a rough, non-capsulate mutant of strain IH 788 selected on the basis of resistance to the LPS-specific phage, C21 (Wilkinson et al., 1972). Strain J5 (Elbein and Heath, 1965) was a rough, UDP-galactose epimerase-deficient mutant of E. coli O111 received from Dr E. Ziegler, San Diego, CA, USA. Strain EH 219 was strain W 3350, a K-12 rough strain of E. coli (Mayer et al., 1976) received from Dr W. Brammar, University of Leicester. Strain RHE 214 was E. coli strain DM 3219-54 (serotype O18ac:K5:H7) received from Drs F. and I. Orskov, Statens Serum Institute, Copenhagen, Denmark. Strain SL 1909 was a rough (rfaJ) mutant of S. typhimurium LT2 with LPS of chemotype Rb2 (Mäkelä and Stocker, 1984) and lacking the OmpA protein (Saxén et al., 1986). Strain SH 9013 was his-sfl (Nikaido et al., 1967), a his-rfb deletion mutant of S. typhimurium; its LPS was of chemotype Ra. Strain SH 6929 was a leaky rough (rfaJ) derivative of S. typhimurium LT2 (M. Nurminen at this Institute, personal communication).

Triton X treated envelopes. Triton X 100 (TX)-treated lysozyme-ethyleneaminetetra-acetate (EDTA) envelopes were prepared according to Nurminen (1978).

Crude porin. This was prepared by a modification of the method of Kuusi et al. (1979). Briefly, 15 mg wet weight of TX-envelopes were treated with 0.2 mM MgCl2 in 100 ml of sodium dodecyl sulphate (SDS) 4% for 30 min at 50°C. After centrifugation at 13 000 g for 10 min the sediment was treated with 40 ml of TX-100 0.2% in the presence of 0.05 M EDTA and 0.3 M NaCl for 60 min at 45°C. Tris-buffer (pH 8.6) was added to the mixture until the pH was over 8. Thereafter 2.5 mg of trypsin was added and the mixture was incubated overnight at 37°C. The mixture was then concentrated in an Amicon Diaflo apparatus with an XM-50 filter.

Purified porins. These were prepared as previously described (Saxén et al., 1986). TX-envelopes were electrodialysed by keeping the temperature below 30°C, and centrifuged for 10 min at 10 000 g. The pH of the sediment was raised to 8 in the presence of TX-100 2% to dissolve the porins. After centrifugation the supernate was treated overnight with trypsin at 37°C and the porins were precipitated overnight with 0.3 M NaCl, pH 6 at 42°C. After centrifugation they were dissolved in SDS 4% and further purified by gel filtration in an Ultrogel AcA 34 1·6 x 100 cm column (LKB, Bromma, Sweden) previously equilibrated with 10 mM phosphate buffer, pH 7.1 containing SDS 0.25%, mercaptoethanol 0.05%, and 5 mM EDTA. The porin-containing fractions, as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) were collected and used as purified porins. In all preparations the porins were present as high-mol.-wt complexes, as judged by SDS-PAGE of the unheated preparations, which probably represented porin trimers, the major native form of porins (Saxén et al., 1986). The purified preparations were relatively free of contaminating proteins as judged by SDS-PAGE and the LPS contamination of the preparations was estimated to be <0·5% w/w of its protein content by comparison of silver-stained SDS-PAGEs (Tsai and Frasch, 1982) of the porin preparations and LPS standards (Saxén et al., 1986). For immunisation and enzyme immunoassay (EIA) the SDS of the purified porins was exchanged for TX-100 0·02% by extensive dialysis.

Reconstituted porin. This was prepared as described before (Saxén et al., 1986) by mixing 1 mg of purified porin with 0·1 mg of R-form LPS, that had been obtained from S. typhimurium mutant SH 6929, in the SDS-containing phosphate buffer, as above. The excess of the buffer was removed by ultrafiltration (Amicon PM-30 filter) or, in some cases, by dialysis against sterile water. Triton X-100 0·02% was added to the reconstituted preparations to keep the porins in solution.

OmpA. Two separate methods were used to prepare OmpA. Firstly, the TX envelopes (5 mg of protein) were treated with trypsin for 2 h at 37°C and electrophoresed in 15% preparative SDS-acrylamide gel (Kallio et al., 1986). The OmpA of mol-wt 24 x 103 was eluted from the gel with SDS 0.05% at 37°C overnight. SDS was removed by dialysis and the protein was concentrated by vacuum dialysis. By the second method, the TX envelopes were first electrodialysed. After centrifugation the pH of the sediment was raised to 8·6 in the presence of TX-100 2% followed by sonication and centrifugation. The supernate was precipitated with 0·3 M NaCl at pH 4·5. The mixture was then centrifuged and the pellet was dissolved in TX-100 2% and 0·1 mM Tris buffer. The insoluble precipitate was discarded and the NaCl precipitation repeated. After centrifugation, SDS(4%)-buffer was added to the sediment followed by ultrafiltration. The OmpA-containing peak, as determined by SDS-PAGE, was collected and run in SDS(10%)-PAGE. The OmpA-containing band was eluted from the gel with SDS(4%)-buffer followed by vacuum concentration.

Lipoprotein. Lipoprotein was extracted from the K-12 strain EH 219 as described by Braun et al. (1976).

Lipopolysaccharide. Two methods were used to isolate LPS. R-form LPS was isolated from SH 6929 according to the method of Galanos et al. (1969) and O18-LPS from EH 778 according to the method of Westphal and Jann (1965).

Antisera. Hyperimmune rabbit sera were obtained after subcutaneous immunisation of White New Zealand rabbits by four injections over a period of 6 weeks of 100-500 µg of protein or 108 heat-killed bacteria/injection (Saxén and Mäkelä, 1982) without adjuvant, or by three injections into the popliteal lymphnodes (Leinonen, 1985) of 30 µg of protein/injection over a period of 4 weeks.
The rabbits were bledd 10 days after the last injection. The K1 capsule-specific hyperimmune serum of a horse (H46) immunised with Neisseria meningitidis type B was a gift from Dr J. Robbins, National Institute of Health, Bethesda, MD, USA.

Separation of rabbit immunoglobulin classes. Separation of IgG and IgM fractions was performed as described previously (Reif, 1969; Saxén et al., 1986). One ml of antiserum was diluted with 3 ml of water at 4°C and 10 g of wet DEAE cellulose equilibrated to pH 8-0 with 0-01 M phosphate buffer was added. After incubation for 60 min at 4°C, the DEAE cellulose was removed, and the supernate was called the IgG fraction. Another 1 ml of serum was added to 6 ml of 0-1 M phosphate buffer at pH 8-0; then 7 ml of wet protein A-Sepharose was added and the mixture was incubated at 4°C with occasional stirring. The sorbent was removed by filtration and the filtrate was called the IgM fraction.

Antibody assays

Antibody titres were determined by enzyme immunoassay (EIA) (Engvall and Perlmann, 1972), essentially as described earlier (Saxén and Mäkelä, 1982). Method (a): 96-well plates (Flat Bottom Microelisa plates; Immulon, Dynatech, London) were coated with 100 µl of antigen diluted in PBS, pH 7-4. The optimum antigen concentration for coating the microtitration wells was 5 µg/ml for the purified E. coli porins, 1 µg/ml for purified salmonella porin, 1 µg/ml for E. coli OmpA-protein and 1 µg/ml for purified lipoprotein. Purified O18-LPS (1 µg/ml) was diluted in PBS pH 7-4, containing FCS 10% (PBS-FCS). A negative control well containing FCS 10% only was included in each assay. Thereafter the plates were washed three times with PBS containing Tween 0-05%. Antiserum diluted in PBS-FCS was added to the plates (100 µl/well) for 1 h at 20°C followed by washing as above. Peroxidase-conjugated goat anti-rabbit IgG and IgM (Cappel Laboratory, Cochranville, PA 19330, USA) diluted 1 in 2000 in PBS-FCS were added to the wells for 1 h at 20°C. After washing as above, 100 µl of the substrate (4 mg of 1,2-phenyldiaminedihydrochloride, Fluka AG, Buchs SG, Switzerland, and 4 µl of 30% H2O2 in 10 ml of phosphate-citrate-buffer, pH 5-5) was added to each well. The enzyme reaction was allowed to proceed for 15 min at 20°C and then stopped with 50 µl of 4 N H2SO4. The absorbance was measured in a Titertek Multiskan spectrophotometer (Lab systems, Helsinki, Finland) at 495 nm. The titres were expressed as log 10.

After washing by filtration, 150 µl of peroxidase-conjugated antibody (as in method (a)) was added to the wells for 30 min at 20°C. The washing step was repeated. After incubation for 15 min at 20°C with 150 µl of the substrate, the reaction was stopped as before. The coloured substrate was then transferred to a standard microtitration plate and the absorbance was measured.

Mouse protection assay

(CBA x C57Bl/6) F1 hybrid mice bred at this Institute (5–10 weeks old) were used in all assays. The mice were passively immunised by an intraperitoneal or an intravenous injection of 0-2 ml of different dilutions of antisera or their fractions inactivated at 56°C for 30 min. Control animals were similarly given NaCl 0-9%. The bacterial challenge (10-fold dilutions of exponentially-growing bacteria of strain IH 3080 in 0-2 ml of NaCl 0-9%) was injected intraperitoneally 2 h after the serum injection. The LD50 was determined from the 5-day survival data (Reed and Muench, 1938) of groups of 6 mice each.

Protein assays

The protein content was determined by the method of Lowry et al. (1951). The SDS-PAGE of proteins was performed in slabs by the method of Laemmli (1970).

Results

The experimental infection model

The mortality-dose response curve with the capsulate E. coli, serotype O18:K1:H7 (strain IH 3080) was steep (figure). The LD50 for the strain

![Figure](image-url)

**Figure.** The mortality dose-response curve in mice given injections of 0-2 µl of anti-K1 (●), 20 µl of anti-O18 (○) antibodies or saline (■), and, 2 h later, given graded doses of E. coli O18:K1 bacteria by ip injection.
was estimated from several experiments to be $4 \times 10^5$ bacteria. The course of the infection was rapid; most deaths occurred 24–48 h after challenge. With inocula below the LD50, the numbers of live bacteria in the peritoneal cavity and blood began to decrease by 12 h and were completely cleared by 24 h.

**Protective effects of anti-K and anti-O sera.**

Antiserum to the K1 capsular polysaccharide protected the mice from lethal infection, and the maximum increase in LD50 achieved was 10-fold (figure). When the challenge dose was c. 2.5 LD50, 0.02 μl of the antiserum per mouse (corresponding to c. 28 ng of anti-capsular antibodies; Allen et al., 1982) was sufficient to give 50% protection.

Protection was also provided with anti-O antiserum obtained by immunising rabbits with whole heat-killed *E. coli* O18. A dose of 9 μl of the antiserum per mouse was needed to give 50% protection from c. 2.5 LD50 (table I). The maximum increase in LD50 obtained with a larger dose of the antiserum (20 μl/mouse) was 5-fold (figure).

Neither antiserum changed the median time to death of the mice. The protective effect of both antisera remained the same irrespective of whether the antiserum was administered intravenously or intraperitoneally (data not shown).

**Ig class of the protective antibodies**

The horse anti-K1 serum used and proved to be protective contained predominantly IgM-class antibodies to the K1 polysaccharide (Allen et al., 1982). However, when the rabbit anti-O18 serum was separated into IgG and IgM fractions, only IgG was found to be protective (table I). A dose of 8 μl of this fraction per mouse afforded 50% protection compared to 9 μl of the unfractioned antiserum. The IgM fraction was not protective at the maximum dose of 67 μl used. The unfractionated serum had a high titre of anti-O antibodies of both the IgG and IgM classes as measured by Ig class-specific EIA with the O18-LPS as antigen (table I). The EIA titres of the fractions showed that the fractionation had been successful, because the IgG fraction showed no reactivity with anti-IgM, and *vice versa*.

**Antibodies to other outer-membrane components**

Rabbits immunised with purified or semi-purified outer-membrane proteins, porins or OmpA developed high titres of IgG antibodies measurable by EIA (table II). By contrast, none of the sera gave measurable IgM titres. The highest antibody titre was usually obtained with the homologous antigen, but moderate titres to LPS and protein antigens were also detected, probably indicating the presence of impurities in the immunising preparations.

The anti-O18 titres in all of these sera were more than 10-fold lower than the corresponding titre of the protective anti-O18 whole bacterial serum. Titres against the O18 LPS antigen were also found in the sera of rabbits immunised with preparations derived from heterologous bacteria, the rough *E. coli* J5 or salmonellae. These titres probably indicate antibodies directed to antigenic determinants of the common LPS core structures (table II).

Antiserum raised to the porin preparations that were derived from the rough non-capssulate *E. coli* reacted most strongly with the homologous porin, whereas antiserum to porins prepared from *E. coli* J5 or *S. typhimurium* elicited equally high titres to at least one of the heterologous porin preparations. The anti-OmpA sera gave very high anti-OmpA titres and also variable titres to the porin preparations and lipoprotein. By contrast, serum from the rabbit immunised with whole J5 bacteria gave relatively low titres to all the protein antigens tested, similar to those of the antiserum to whole O18 bacteria (table II).

When the sera were tested for their binding to whole intact bacteria (table III), only the anti-O18 serum bound to the parental O18:K1 bacteria. Its non-capssulate (O18:K1−) mutant bound antibodies from the anti-O18 serum and also to a lesser extent from the anti-J5 serum. By contrast, only the rough non-capssulate organisms (O18−:K1− or J5) bound

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**Table I. Protective capacity of rabbit anti-O18 antiserum and its immunoglobulin fractions and anti-O18-LPS titres following *E. coli* O18:K1 challenge (2.5 × LD50)**

<table>
<thead>
<tr>
<th>Antibody preparation†</th>
<th>50% protective dose‡ (μl of serum/mouse)</th>
<th>EIA titre§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Whole serum</td>
<td>9</td>
<td>4.7</td>
</tr>
<tr>
<td>IgG fraction</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td>IgM fraction</td>
<td>&gt;67</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

* Given intraperitoneally in a volume of 0.2 ml 2 h after serum injection
† Given intraperitoneally in a total volume of 0.2 ml
‡ 5 days observation; volume of antibody fraction was calculated as compared to the original serum, the IgG fraction was diluted to 1/4 and IgM fraction to 1/2 compared with the unfractioned serum during the separation
§ Log 10; measured with O18-LPS as antigen.
Table II. EIA titres and protective capacity against challenge with *E. coli* O18:K1 of sera from rabbits immunised with various outer membrane component preparations.

<table>
<thead>
<tr>
<th>Type of antiserum</th>
<th>Antigen derived from</th>
<th>IgG titres (log) to</th>
<th>59% protective dose of serum/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R-E. coli</td>
<td>J5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O18-LPS</td>
<td>porin</td>
</tr>
<tr>
<td>anti-O18 bacteria</td>
<td>RHE 214</td>
<td>4.7</td>
<td>3.9</td>
</tr>
<tr>
<td>anti-crude <em>E. coli</em> porin</td>
<td>EH 786</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>anti-purified <em>E. coli</em> porin</td>
<td>EH 786</td>
<td>3.4</td>
<td>4.4</td>
</tr>
<tr>
<td>anti-purified J5 porin</td>
<td>J5</td>
<td>3.6</td>
<td>4.8</td>
</tr>
<tr>
<td>anti-purified <em>Salm.</em> porin</td>
<td>SL 1909</td>
<td>3.2</td>
<td>4.9</td>
</tr>
<tr>
<td>anti-<em>Salm.</em> OmpA2</td>
<td>SH 9013</td>
<td>n.t.</td>
<td>4.4</td>
</tr>
<tr>
<td>anti-J5 OmpA3</td>
<td>J5</td>
<td>n.t.</td>
<td>&gt;4</td>
</tr>
<tr>
<td>anti-J5 bacteria</td>
<td>J5</td>
<td>&lt;3</td>
<td>3.6</td>
</tr>
<tr>
<td>pre-immune</td>
<td>—</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

n.t., not tested

1 Reconstituted with *Salmonella* R form-LPS as described in Methods.
2 Prepared by Method 2 (see OmpA, in Methods).
3 Prepared by Method 1 (see OmpA, in Methods).
* The figures are log 10 of the reciprocal of the dilution giving the absorbance of 0.2 at 495 nm. The IgM titres (not shown) were <2 for each serum and antigen except for anti-O18 LPS antiserum shown in table I.

(i) Serum was given intraperitoneally in a total volume of 0.2 ml. (ii) The bacterial challenge (2.5 × LD50) was given intraperitoneally in a volume of 0.2 ml 2 hrs after the intraperitoneal serum injection; 5 days observation.

Table III. EIA titres (IgG) of rabbit antisera against different *E. coli* strains

<table>
<thead>
<tr>
<th>Type of antiserum</th>
<th>EIA titres* against whole bacteria:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O18:K1</td>
</tr>
<tr>
<td>anti-O18</td>
<td>4.3</td>
</tr>
<tr>
<td>anti-J5</td>
<td>&lt;2.7</td>
</tr>
<tr>
<td>anti-<em>E. coli</em> porin</td>
<td>&lt;2.7</td>
</tr>
<tr>
<td>anti-<em>Salm.</em> OmpA</td>
<td>&lt;2.7</td>
</tr>
<tr>
<td>preimmune</td>
<td>&lt;2.7</td>
</tr>
</tbody>
</table>

n.t., not tested.

* log 10; the IgM titres (not shown) were <2 for each serum.

Discussion

Our *E. coli* peritonitis-septicaemia model bears similarities to infections with gram-negative bacilli in man, making it suitable to study the role of antibodies in host defence against *E. coli*. Its advantages are that it does not require adjuvants or a specially sensitised host, that the LD50 is low and the challenge *E. coli* causes natural infection in man. We found that antibodies to the capsular polysaccharide and to the O antigen were protective whereas those directed against several other outer-membrane structures were not.

The role of anti-capsular antibodies in protection against capsulate bacteria, including *E. coli* K1, is well documented (Robbins 1978). Recently even monoclonal anti-K1 antibodies have been shown to protect against experimental *E. coli* K1 infection (Kim et al., 1985). Our results with the hyperimmune antiserum to *N. meningitidis* group B capsular polysaccharide, which is immunologically identical to the K1 *E. coli* capsule (Kasper et al., 1973), are in agreement with these findings.

The role of anti-LPS antibodies in protection against capsulate organisms is less clear. In some
studies, antibodies to the outermost part of LPS, the O antigen, have been protective (Kaijser et al., 1972; Pluschke and Achtman, 1985), but in others they have not been (Welch et al., 1979). In this study we observed definite protection with a hyperimmune rabbit anti-O18 antiserum against challenge with capsule E. coli bacteria. The O18 antiserum also contained antibodies to several outer-membrane proteins, but the highest titre was found with the homologous O18-LPS. An immunoblotting analysis revealed that the anti-O18 antiserum reacted with the polysaccharide chain and with deeper LPS structures of the core region (data not shown) which explains why these antibodies were able to bind to rough as well as to smooth capsule E. coli bacteria in EIA (table III). Although the protective effect of anti-O serum was weaker than that of the anti-K1 serum, the protection obtained shows that O-antigenic LPS was exposed enough on the surface of the capsule bacteria to react with antibodies. In the study in which anti-O antibodies were not protective, different E. coli serotypes were used (Welch et al., 1979). The exposure of O-antigenic LPS may vary with the strain and the O serotype as well as with the capsular type of the bacteria. It also seems relevant to consider the fact that anti-O antibodies do not agglutinate capsule E. coli unless the capsule is destroyed by heating (Kaufmann, 1966); however, agglutinability may require more numerous or more closely spaced antigenic sites to be exposed on the bacterial surface than is needed in vivo for the protection that is probably mediated by phagocytosis following opsonisation. The protective effect of the homologous anti-O18 antiserum (or anti-K1) is unlikely to be due to in-situ agglutination of the challenge O18:K1 bacteria in the peritoneal cavity, as the route of antiserum administration had no effect on the protective capacity.

Several previous studies have reported protection afforded by anti-porin antibodies in infections with gram-negative bacilli (Kuusi et al., 1979 and 1981; Gilleland et al., 1984). In these studies the possible role of contaminating anti-O antibodies has not been ruled out, and in a recent study the protective effect of anti-porin antisera in experimental salmonellosis was, nevertheless, found to be due to the small amounts of anti-O antibodies produced (Saxén et al., 1986). Therefore, we set out to examine the protective potential of different OM structures in the E. coli infection model. Rabbits were immunised with crude and purified preparations of OM components from rough O-antigen deficient E. coli and S. typhimurium. All the preparations were immunogenic in rabbits as determined by EIA. High antibody titres to the homologous antigens, but also rather high titres to heterologous antigens (porin, OmpA protein or lipoprotein) were observed, in keeping with the reported cross-reactions between enterobacterial OM proteins (Hofstra and Dankert, 1980). All the detected responses were of IgG class as expected for protein antigens. The low titres of anti-LPS antibodies produced were most probably directed to the core of LPS. In contrast to the protective action of IgG anti-O antibodies, none of the anti-protein sera afforded any protection from challenge with the capsule E. coli. We believe that this difference is due to the different accessibility of the target antigens to their respective antibodies. Thus, the capsule and smooth type LPS are exposed on the surface of the bacteria and can prevent binding of other antibodies to deeper cell-surface components. Indeed, when van der Ley et al. (1986) studied the binding of monoclonal anti-porin (PhoE) antibodies to E. coli they found that these bound efficiently to the rough K-12 strain but not to smooth clinical isolates of E. coli. Similarly, when using the intact capsule O18:K1 or non-capsulate O18 bacteria as EIA antigens we could not detect binding of the anti-protein antibodies although these bound effectively to rough non-capsulate E. coli. This shows that the anti-protein sera used did contain antibodies directed against native protein structures exposed on the bacterial surface. However, the O antigen, and probably also the capsule, prevent these antibodies from reaching their target on the surface of the OM of the smooth capsule bacteria that cause infections.

The role of LPS core-glycolipid and lipid A in protection against infection with Enterobacteriaceae is also a subject of controversy. Whereas protection against enterobacterial infections in normal and immunosuppressed hosts has been achieved with anti-"core-glycolipid" antisera raised against E. coli J5 or rough S. minnesota, or even with a monoclonal antibody to the J5 LPS (Ziegler et al., 1973 and 1982; Braude et al., 1977; Teng et al., 1985), others have not been able to show protection with similar preparations (Greisman et al., 1978; Welch et al., 1979; Siber et al., 1985). We could not show any protection with anti-J5 serum against challenge with the capsule O18:K1 E. coli, nor could we detect binding of anti-J5 antibodies to the intact O18:K1 bacteria. Although immunisation with a core glycolipid vaccine leads to elevated antibody titres to the homologous J5 bacteria or their LPS, these antibodies show little or no cross-reaction with the core
region of other strains of Enterobacteriaceae (de Jongh-Leuvenink et al., 1985; Siber et al., 1985). To explain these conflicting results it has been suggested that the protective effects seen with the anti-core glycolipid antisera are the result of neutralisation of endotoxin rather than of opsonisation (Ziegler et al., 1982). Our mouse infection model is very susceptible to the size of the challenge inoculum; thus, the protective antibodies probably also act by preventing bacterial growth. Therefore, this model would probably not be very sensitive to anti-endotoxin effects alone.

Our finding that antibodies to deeper outer-

membrane components are not protective in an experimental infection caused by capsulate smooth E. coli O18:K1, suggests that OM proteins are unlikely to be effective as a cross-reactive gram-negative bacillary vaccine. The only anti-protein antibodies shown to afford protection in our model are those directed to the S-fimbriae on the cell surface of these bacteria (Nowicki et al., 1986).

We thank Tapio Linturi and Eila Pelkonen for assistance in separation of Ig fractions and P. H. Mäkelä for constructive criticism. This study was supported by SITRA, the Finnish National Fund for Research and Development.

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