Chlamydia trachomatis major outer membrane protein epitopes expressed as fusions with LamB in an attenuated aroA strain of Salmonella typhimurium; their application as potential immunogens

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The major outer-membrane protein (MOMP) of Chlamydia trachomatis is the focus of attention for chlamydial vaccine design, particularly those serovar- and subspecies-specific epitopes which provoke neutralizing immune responses. Selected surface-exposed B-cell epitopes of MOMP, incorporating B-subspecies specificities, were expressed as fusions with LamB, an inducible outer-membrane transport protein of Escherichia coli. These recombinant chlamydial–LamB proteins were correctly transported to the outer membrane of both E. coli and an aroA mutant of Salmonella typhimurium. The immunogenicity of the constructs was investigated in a mouse model of chlamydial salpingitis. After oral immunization, recombinant S. typhimurium were recovered from the livers of mice for up to two weeks, and a serum IgG response was induced both to the Salmonella and to the inserted chlamydial epitopes. By contrast, intravenous inoculation was ineffective. Although these LamB fusions proved only weakly immunogenic, this approach should be useful for investigating the ability of attenuated S. typhimurium vaccines incorporating chlamydial epitopes to stimulate protective mucosal immunity in the mouse model of chlamydial salpingitis.

Introduction

Chlamydia trachomatis serovars A, B and C are the infectious agents of trachoma, a major cause of preventable blindness world-wide. These three serovars belong either to the B complex (serovar B), or to the C complex (serovars A and C), according to which subspecies epitopes they express. Trachoma vaccines which use killed whole chlamydiae afford only short term, serovar-specific immunity associated with local tear antibody, and in some cases, when subjects subsequently become infected with heterologous serovars, increase disease severity (reviewed by Schachter & Dawson, 1978). A subunit vaccine which incorporated protective epitopes towards all three trachoma serovars but excluded antigens which induce damaging hypersensitivity responses (Morrison et al., 1989) might be more successful.

Ideally, such a vaccine should generate neutralizing mucosal antibody to the surface of the infectious EB, as well as systemic immunity, in order to prevent initial eye infection before the organisms become sequestered within host cells.

The most promising subunit vaccine candidate is the major outer membrane protein (MOMP), a porin which maintains structural rigidity of the outer envelope, regulates nutrient influx and which controls early intracellualr differentiation of the chlamydiae (Bavoil et al., 1984; Hatch et al., 1984). Immunodominant serovar- and subspecies-specific epitopes on MOMP are surface-exposed, and antibodies to them are neutralizing in vivo (Zhang et al., 1987). By contrast, most species- and genus-specific epitopes on MOMP are surface-exposed, and antibodies to them are neutralizing in vivo (Zhang et al., 1987). By contrast, most species- and genus-specific epitopes on MOMP are inaccessible to antibody (Batteiger et al., 1986; Kuo & Chi, 1987; Zhang et al., 1987; Conlan et al., 1989) and thus non-neutralizing, although a surface-exposed/species-reactive MOMP epitope has been described (Collett et al., 1989). Two B-complex-specific, surface-exposed linear epitopes (B1 and B2) have been identified within variable segment 4 (VS4) of serovar B MOMP (Conlan et al., 1989). By contrast, a C-complex-specific, surface-exposed epitope is located within VS1 of serovar A.
MOMP (Hayes et al., 1990). Rabbit antisera to synthetic peptide analogues of epitopes B1, B2 and a modified analogue of the C-complex specific epitope react with the surfaces of live chlamydiae of all three trachoma-causing serovars, as demonstrated by immunogold transmission electron microscopy (Conlan et al., 1989 and unpublished results). Thus, a subunit vaccine incorporating these epitopes might protect against trachoma infection, especially if such a vaccine was engineered to elicit local, as well as systemic immunity. The necessary antigenic persistence for stimulating mucosal immunity might be achieved using a suitable live, attenuated vaccine organism.

A mouse model for chlamydial infection is available (Tuffrey et al., 1986). When progesterone-treated C3H mice are inoculated into the uterus or ovarian bursa with a human genital tract isolate of C. trachomatis, they develop salpingitis and infertility. Since successful antigenic priming at any mucosal site, for example the gut, should protect against challenge at any other mucosal site, this model is suitable for preliminary trials of trachoma vaccine candidates.

The LamB protein of E. coli was chosen as a suitable vehicle for expression of chlamydial epitopes in the outer membrane of a live bacterial vaccine. LamB is a 47 kDa outer membrane protein which, like MOMP, is a porin and which regulates maltose transport in E. coli. The lamB gene has been cloned, placed under tac promoter control (Bouges-Bocquet et al., 1984) and engineered to create a unique BamHI site allowing insertion of foreign epitopes at amino acid position 153 of the mature LamB protein (Boulain et al., 1986). Epitopes inserted at this site are located on a surface-exposed loop (Boulain et al., 1986; Charbit et al., 1991). As a vaccine vector for the modified LamB protein, we chose attenuated, non-reverting aroA strains of Salmonella. These organisms colonize the mouse gut but do not multiply, protecting mice against subsequent infection with the virulent parent strains (Hoiseth & Stocker, 1981). AroA strains have been used to elicit immune responses in mice to a variety of foreign antigens expressed both cytoplasmically (Brown et al., 1987; Poirier et al., 1988; Clemets et al., 1986; Fairweather et al., 1990), and at the cell surface (Charbit et al., 1987; Maskell et al., 1987; Wu et al., 1989). This approach is potentially useful in humans since various attenuated strains of Salmonella typhi, including aroA, are undergoing trials as live oral vaccines for use in humans (Tacket et al., 1990).

In this study, oligonucleotides encoding two B-subspecies epitopes of C. trachomatis were synthesized and cloned into the lamB gene. Both epitopes were incorporated into recombinant LamB proteins expressed in E. coli and S. typhimurium, and were recognized in immunoblots by polyclonal antibodies raised against synthetic peptide analogues. When administered orally to the strain of mouse used to study chlamydial salpingitis, serum IgG responses to S. typhimurium and C. trachomatis were induced but neither mucosal response nor serum IgA was detected.

Methods

Bacterial strains and vectors. Escherichia coli JM109 (Yanisch-Perron et al., 1985) Epicurean competent cells were purchased from Northumbria Biologicals Limited. Salmonella typhimurium strains LB5010 (gale', r', m'; Bullas & Ryo, 1983) and SL3261 (aroA; Hoiseth & Stocker, 1981) and bacteriophage P22 (Schmierer, 1972) were kind gifts of Dr G. Dougan (Wellcome Research Laboratories, Beckenham, UK). The lamB vector, pAJC264 (Boulain et al., 1986) was a kind gift of Dr M. Hofnung (Institut Pasteur, Paris, France).

Enzymes. Restriction endonucleases, T4 DNA ligase and calf intestinal phosphatase were obtained from Boehringer Mannheim. T4 polynucleotide kinase was obtained from Gibco-BRL. All enzymes were used as recommended by the manufacturer.

Oligonucleotides. Complementary oligonucleotide pairs encoding epitopes B1 and B2 were synthesized which utilized E. coli high expression codon preferences (Grosjean & Fiers, 1982). The oligonucleotide pairs contained BamHI-compatible ends which recreated a BamHI site only at the 3' end of the insert when cloned into BamHI-cleaved vector. Positioning of cloning sites on the oligonucleotides resulted in the addition of a proline to the N-terminus and an asparagine to the C-terminus of each epitope. Epitopes B1, B2 and the corresponding oligonucleotide sequences are shown in Fig. 1. Oligonucleotides were synthesized using β-cyanoethyl phosphoramidite chemistry on an automated DNA synthesizer (Applied Biosystems Inc., Foster City, CA).

DNA manipulations. Complementary oligonucleotide pairs were simultaneously phosphorylated with T4 polynucleotide kinase and hybridized together in 70 mM-Tris/HCl (pH 7.6), 10 mM-MgCl2, 5 mM-DTT and 500 μM-ATP overnight at 37°C. Oligonucleotide hybrids were ligated with BamHI-cut, dephosphorylated pAJC264 in 66 mM-Tris/HCl (pH 7.5), 5 mM-MgCl2, 5 mM-DTT and 1 mM-ATP overnight at 4°C. Epicurean JM109 was transformed with the recombinant plasmids according to the supplier's instructions. The constructs made are summarized in Table 1. Recombinant plasmids were prepared by alkaline lysis (Birnboim & Doly, 1979) and purified through NACS Prepac cartridges (Gibco-BRL). S. typhimurium LB5010, a galactose epimerase mutant, was transformed with recombinant plasmids as follows: S. typhimurium LB5010, grown in LB-broth to an OD660 of 0.5, was pelleted, resuspended in 0.5 vols of 100 mM-MgCl2, incubated on ice for 20 min, pelleted again, then resuspended in one-fifth of the original volume of 100 mM-CaCl2. Competent cells (200 μl) were added to 20°C of plasmid and kept on ice for 20 min, at room temperature for 20 min, and on ice for 40 min. The cells were heat-shocked for 2 min at 42°C, then incubated for one hour at 37°C with 800 μl LB-broth, and plated onto LB-agar containing 100 μg ampicillin ml⁻¹. Overnight cultures (200 μl samples) of recombinant S. typhimurium were incubated with dilutions of bacteriophage P22 and plated onto LB-agar containing ampicillin (100 μg ml⁻¹). Recombinant bacteriophage P22 were harvested from plates of confluent plaques. S. typhimurium SL3261 (aroA) was transduced with recombinant φP22 as follows: 200 μl of S. typhimurium SL3261 grown overnight in LB-broth
supplemented with 1 mm-CaCl₂ was incubated with 1 µl of the recombinant phage stock for one hour at 37 °C. The mixture was spread onto LB-agar containing ampicillin (75 µg ml⁻¹) and 5 mm-EGTA. In each of the three bacterial hosts, ampicillin resistant colonies were screened by immunoblotting for expression of the required epitopes. Inserts were checked for correct frame and orientation by DNA sequencing.

Antiserum. Rabbit polyclonal antiserum was raised against synthetic peptide analogues of epitopes B₁ and B₂ as previously described (Conlan et al., 1989).

Immunoblotting. E. coli strains (400 µg protein ml⁻¹) were disrupted by boiling for 10 min in 0.0625 M-Tris/HCl (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol and 0.001% (w/v) bromophenol blue. Whole cell lysates were separated by SDS-PAGE on 12.5% (w/v) polyacrylamide using the Laemmli (1970) buffer system. Denatured proteins were electrophoresed onto nitrocellulose (Schleicher & Schuell) at 100 mA for 90 min using a semi-dry blotter (Ancos, Denmark) (Khyse-Andersen, 1984). The nitrocellulose was then blocked overnight at 4 °C in Tris-buffered saline (TBS; 2.24 g Tris.HCl, 10 g NaCl, pH 7.5) containing 0.05% (v/v) Tween-20 (TTBS) and 5% (w/v) dried skimmed milk (Marvel). Blots were incubated for 3 h at room temperature with primary antibodies diluted 1:200 in TTBS containing 5% (w/v) Marvel, then washed 3 × 5 min in TTBS. The blots were incubated for 1 h at room temperature with goat anti-rabbit IgG-alkaline-phosphatase conjugate (Bio-Rad) diluted 1:3000 in TTBS containing 5% (w/v) Marvel, followed by washing for 4 × 5 min in TTBS, then 3 × 5 min in TBS. Antigen-antibody complexes were visualized using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma) and the reactions were stopped by washing the blots in distilled water.

DNA sequencing. Cloning junctions and inserted DNA of recombinant plasmids were sequenced by the dideoxy chain termination method modified for double stranded DNA (Zhang et al., 1988). Briefly, 3 µg of plasmid DNA was incubated for 5 min at room temperature in 0.2 m-NaOH, 0.2 m-EDTA (total volume, 20 µl). The solution was neutralized by addition of 2 µl 2 m-NH₄-acetate (pH 4.6) and DNA was precipitated with 60 µg 100% ethanol. The DNA was pelleted, washed with 80% ethanol, repelleted, then sequenced using modified T7 polymerase (Sequenase; Cambridge Bioscience) and [α-32P]dATP (410 Ci mmol⁻¹; 1.5 × 10⁵ dpm mmol⁻¹; Amersham). The 17mer oligonucleotides (5'AACTCTCTCTGCGGCGCA-G and 5'TTCCGGTTTCTGGTG) used to prime sequences in both directions across the inserts were based on the published sequence of the Lamb gene (Clement & Hofnung, 1981).

Separation of cytoplasmic and outer membranes of E. coli JM109 and S. typhimurium SL3261. Cytoplasmic and outer membranes of recombinant E. coli and S. typhimurium SL3261 expressing LamB containing B₁ + B₂ were separated by the method of Osborn & Munson (1974). Briefly, 250 ml cultures in LB broth, induced with 0.1 mM-IPTG were pelleted, resuspended in 15 ml 0.75 m-sucrose, 10 ml-Tris-acetate pH 7.5, 100 µg lysozyme ml⁻¹ and incubated on ice for 2 min. Cells were spheroplasted by addition of 30 ml ice-cold 1.5 mM-EDTA over protein peaks were subjected to PAGE followed by either silver staining to detect LPS (Tsai & Frasch, 1982) or immunoblotting to detect recombinant LamB.

Immunization of mice with live aroA mutants of S. typhimurium expressing B₁ + B₂ recombinant LamB protein. Mice were immunized with a B₁ + B₂ recombinant which possessed smooth LPS. A stock of the immunizing strain was grown in LB broth containing ampicillin (125 µg ml⁻¹) and 0.1 m- IPTG (16 h at 37 °C without shaking). This was harvested by centrifugation (600 g) and resuspended in PBS (Dulbecco A) containing 10% (v/v) glycerol as a cryopreservative to a total count of 2 × 10¹¹ ml⁻¹ (at 60% viability). Cells were stored frozen at −20 °C until required. A control culture of non-recombinant S. typhimurium was similarly prepared. Female C3H/He/Ola mice (Olac Ltd), 6–10 weeks old, were used in all experiments. This mouse strain was chosen because it is known to be susceptible to genital tract infections with human strains of C. trachomatis (Tuffrey et al., 1986). On day one, 24 mice were immunized intravenously (i.v.) with 0.1 ml of the B₁ + B₂ LamB recombinant diluted in PBS to a total count of 2 × 10¹⁰ ml⁻¹. A further five mice received an equivalent dose of control S. typhimurium aroA. A second group of 24 mice were challenged per os (p.o.) with the recombinant (0.1 ml of culture at 2 × 10¹¹ ml⁻¹ total colony counts delivered using a gavage needle. A control group of five mice were similarly immunized with control S. typhimurium aroA. Six mice received no vaccine. All mice were housed in separate cages within the same room. At weekly intervals for six weeks, serum was obtained from mice (4 per group) which had received recombinant B₁ + B₂ (i.v. or p.o.) by tail-vein venepuncture whilst under metafane anaesthesia. After blood samples had been taken the mice were killed by CO₂. The liver and spleen were removed whole from each animal aseptically into 10 ml sterile distilled water. The small intestine between the caecum and stomach was removed intact and the contents collected by washing. Sera and clarified gut washes were stored at −80 °C. Livers and spleens were homogenized for 1 min in a Colworth stomacher and the resulting macerates plated on LB-agar containing ampicillin for recovery of organisms. On the sixth week following immunization, all control animals were processed as above.

Determination of antibody in sera and gut washes of immunized mice by amplified ELISA. Antibodies to S. typhimurium aroA and C. trachomatis serovar B were detected by-redox-amplified ELISA (Johansson et al., 1985). S. typhimurium aroA or C. trachomatis serovar B whole cells at a protein concentration of 0.05 mg ml⁻¹ were coupled to microtitre PVC microtitre trays (Flow laboratories) by overnight incubation in carbonate buffer at 18 °C (1.6 g l⁻¹ Na₂CO₃, 2.9 g l⁻¹ NaHCO₃, 0.2 g l⁻¹ Na₂SO₄, pH 9.0). Excess antigen was removed by washing the trays three times with 50 mM-ethanolamine/100 mM-NaCl (pH adjusted to 10.5 with 100 mM-citric acid). The antigen-coated, washed trays were blocked for 1 h at 37 °C with PBS (Dulbecco A) containing 5% (v/v) normal goat serum (100 µl per well). The blocking solution was drained and test sera or gut washes added at 1:100 or 1:5 dilution respectively (50 µl per well). The diluent was blocking solution containing 0.05% (v/v) W1 detergent (Sigma). The trays were sealed and incubated for 3 h at 37 °C, then washed four times. Goat anti-mouse alkaline phosphatase conjugates specific for IgA or IgG (Zymed laboratories) were added at 1:1000 dilution (50 µl per well) and incubation continued for 45 min. After a further six washes, NADP substrate (0.2 mM-NADP, 10 mM-MgCl₂, 1 mM-ZnCl₂, 150 mM-Na₂S₂O₃ in 50 mM diethanolamine buffer, pH 9.5) was added (50 µl per well). This solution was filtered through a 0.22 µm filter immediately before use. Incubation continued for 40 min at 18 °C. After this time 0.1 µl amplifier was added to each well. Amplifier solution consisted of 0.2 g l⁻¹ alcohol dehydrogenase, 0.15 g l⁻¹ diaphorase, 0.28 g l⁻¹ p-iodonitrotetrazolium violet (all reagents from Sigma) in 20 mM-sodium phosphate buffer (pH 7.2) containing 4% (v/v) ethanol. The reaction was stopped by addition of 3% (v/v) H₂SO₄ (25 µl per well) and the reaction product measured as A₅₉₅.
Results and Discussion

Expression of epitopes in E. coli

*C. trachomatis* epitopes B₁ and B₂ are shown in Fig. 1; they are identical to the sequences occurring in *C. trachomatis* serovar B/Jali-20/OT VS4 (Hayes et al., 1990). Sequence B₁ contains the known B-complex-specific, surface-exposed epitope IFDV (Conlan et al., 1988). Sequence B₂ contains a species-specific epitope, LNPTIA, which is not surface-exposed (Stephens et al., 1988; Conlan et al., 1988; Baehr et al., 1988) as well as an additional surface exposed, B-complex specific region AGDV (Conlan et al., 1989). Epitopes B₁ and B₂ were expressed as fusions with LamB both individually and in tandem, as shown in Table 1. An additional sequence from VS1 of serovar A, containing serovar A- and C-subspecies-specific epitopes (Hayes et al., 1990) was expressed in *S. typhimurium* but was not used for animal inoculations since it was poorly detected in Western blots and the recombinant salmonellae expressing this epitope consistently possessed rough LPS.

Immunoblots of *E. coli* strains expressing epitopes B₁ and B₂ as recombinant LamB proteins are shown in Fig. 2 (a and b). In VS4 of serovar B MOMP, the sequence 288SAETIFDVTTLNPTIAGAGDV308 contains epitopes B₁ (residues 288-298) and B₂ (residues 298-308) which overlap each other by one amino acid. Interestingly, the double construct B₁ + B₂ in the reversed order, ie (P)LNPTIAGAGDV(D)SAETIFDVTTL(D) appeared to express both epitopes, since a non-truncated recombinant protein was obtained. However, only the proximal B₂ was detected by immunoblotting (data not shown). DNA sequencing confirmed the correct orientation of both epitopes in the double recombinant. Presumably the lack of reactivity of B₁ in this construct reflects stoichiometric constraints on the ordering of epitopes within the LamB vector.

Expression of epitopes in *S. typhimurium* SL3261

*S. typhimurium* SL3261 (aroA) was transduced with φP22 carrying recombinant plasmids, since direct transformation of smooth *Salmonella* strains is very inefficient (MacLachlan & Sanderson, 1985) and selects for rough mutants (Brown et al., 1987). The receptor for φP22 is not present on rough salmonellae, ensuring that transductants are smooth. Only smooth salmonellae will

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Table 1. Summary of chlamydial epitopes fused with LamB

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<thead>
<tr>
<th>Epitopes</th>
<th>Amino acid sequence</th>
<th>No. of residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>(P)SAETIFDVTTL(D)</td>
<td>13</td>
</tr>
<tr>
<td>B₂</td>
<td>(P)LNPTIAGAGDV(D)</td>
<td>13</td>
</tr>
<tr>
<td>B₁ + B₂</td>
<td>(P)SAETIFDVTTL(DP)LNPTIAGAGDV(D)</td>
<td>26</td>
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Brackets indicate non-native sequences.
colonize mice. Fig. 2c shows immunoblots of S. typhimurium SL3261 expressing recombinant LamB fused with epitopes B1 + B2. These epitopes were expressed in S. typhimurium SL3261 at equivalent levels to those observed in E. coli JM109.

Location of recombinant LamB proteins within the bacterial outer membrane

To verify the location of the expressed proteins, cytoplasmic and outer membrane fractions of recombinant E. coli and S. typhimurium were separated by sucrose density gradient centrifugation, then subjected to PAGE followed by silver staining (Fig. 3a and b). The outer membrane component LPS was located in fractions towards the bottom of the gradient. In identical gels immunoblotted and probed with anti-B1 (Fig. 3c and d), the recombinant LamB protein was located exclusively in those fractions which contained LPS, indicating that it is present in the outer membrane in both E. coli and S. typhimurium. From the proposed model of LamB folding (Boulain et al., 1986; Charbit et al., 1991) it would be expected that the inserted C. trachomatis epitopes should reside within a surface-exposed loop. However, attempts to label these epitopes at the surfaces of recombinant E. coli and S. typhimurium with immunogold were unsuccessful. The long side chain of smooth LPS may prevent antibody binding to the surface-exposed LamB, blocking surface labelling of the recombinant bacteria with immunogold. However, Brown et al. (1987) demonstrated that expression at the surface of salmonellae is not necessary to provoke B- and T-cell responses to cloned proteins.
Recovery of B1 + B2 LamB recombinant S. typhimurium aroA mutants from immunized mice

These were the first experiments to use recombinant chlamydial antigens expressed in salmonellae to elicit immune responses in the mouse model of chlamydial infection. Ampicillin-resistant bacteria were recovered from the livers of mice immunized i.v. for up to two weeks post-challenge and from the spleens of these animals for up to four weeks (Table 2). Bacteria were also recovered from the livers of p.o. immunized mice for up to two weeks. However, no bacteria were isolated from the spleens of p.o. immunized animals after the first week. In all cases the numbers of bacteria recovered were low (10–30 organisms per organ) and they were confirmed to be carrying the recombinant lamB plasmid by PCR, using as primers the lamB-specific oligonucleotides described above (data not shown).

Detection of antibodies in immunized mice to S. typhimurium and C. trachomatis

A live attenuated S. typhimurium aroA mutant containing recombinant LamB protein incorporating C. trachomatis epitopes B1 + B2 was assessed for its ability to generate anti-chlamydial antibodies when inoculated into mice. Two routes of administration were tried; i.v. immunization with S. typhimurium aroA expressing LamB (B1 + B2), which produced circulating IgG antibody to S. typhimurium and to C. trachomatis in only 5/24 and 3/24 animals respectively (Fig. 4a), and oral immunization, which produced circulating IgG to S. typhimurium in 15/19 animals although only 5/19 had circulating IgG to C. trachomatis (Fig. 4b). Antibody titres to C. trachomatis were generally much lower than for S. typhimurium, as would be expected given the much lower mass of chlamydial epitopes. There was little correlation between the levels of antibody to S. typhimurium and C. trachomatis in the p.o. immunized animals. Neither IgA nor IgG antibodies to either S. typhimurium or C. trachomatis were detected in the gut washes, nor was IgA antibody to either antigen detected in the sera.

Brown et al. (1987) elicited high-titre antibodies to β-galactosidase expressed as cytoplasmic inclusions in S. typhimurium aroA when intravenously administered. However, this group used a different strain of mouse (BIOXAJ) to that used in the present study. Moreover, the β-galactosidase in their immunogen represented up to 2% of the total protein whereas the C. trachomatis-specific epitope present in our vaccine strain was much less than this as judged subjectively by the quantity of LamB present on SDS-PAGE profiles.

By contrast to i.v. immunization, p.o. administration of the recombinant LamB aroA strain resulted in

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Table 2. Recovery of bacteria from livers and spleens of mice immunized with S. typhimurium LamB (B1 + B2)

<table>
<thead>
<tr>
<th>No. of mice positive</th>
<th>i.v. immunized</th>
<th>p.o. immunized</th>
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<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Spleen</td>
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<tr>
<td>Time (weeks)</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>2/4</td>
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<td>2</td>
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<tr>
<td>6</td>
<td>0/4</td>
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* Five mice in the p.o. immunized group died during the experiment and were not available for testing.
NT, not tested.

Fig. 4. Detection of IgG antibodies to S. typhimurium and C. trachomatis serovar E in mice. Control group 1, normal control sera; same mice in (a) and (b). Experimental groups; immunized i.v. (a) or p.o. (b) with recombinant S. typhimurium aroA, sampled 1–6 weeks after challenge as shown. Control group 2, inoculated i.v. (a) or p.o. (b) with non-recombinant S. typhimurium aroA, sampled 5 weeks post challenge. Each pair of columns represents a single mouse. Filled columns represent IgG to S. typhimurium; hatched columns represent IgG to C. trachomatis.
total number of mice responding and the antibody titres remained low. Charbit et al. (1987) also found only low levels of antibody to a hepatitis B virus epitope inserted into the LamB protein, although this group used E. coli rather than S. typhimurium as the bacterial host. Leclerc et al. (1989) found the route of inoculation to be important; i.v. and i.p. administration of live recombinant E. coli being more effective than subcutaneous administration, however, mucosal immunity was not sought by those authors.

Surprisingly, no IgA antibody to C. trachomatis or to S. typhimurium was found in any of the test samples, including the sera. Maskell et al. (1987) using S. typhimurium aroA mutants expressing the B subunit of E. coli heat labile toxin, demonstrated a consistent IgA response in both the sera and gut washes of recipient Balb/C mice. In that study, the livers and spleens of orally-immunized mice were colonized by bacteria for up to 5 weeks, longer than in the present study, which might partly explain the differences in IgA production. Sigwart et al. (1989) also demonstrated IgA antibodies to S. dublin aroA in orally immunized Balb/C mice. In their study, colonization of the liver and spleen was similar to the results reported here, but S. dublin was recovered from the small intestine for up to 3 weeks following oral administration. In our study, the recombinant S. typhimurium was cleared rapidly (8 d) from the small intestine of C3H/He/Ola mice. Long-term colonization of the small intestine and particularly the gut-associated lymphoid tissue (GALT) should favour a local IgA response, thus the transient passage of S. typhimurium through the gut observed here is probably an insufficient stimulus to elicit secretory IgA. This may reflect the mouse strain we used; C3H/He/Ola mice are susceptible to chlamydial salpingitis (Tuffrey et al., 1986) but are less susceptible to natural S. typhimurium infections than Balb/C mice (Hormaeche, 1979). Moreover, surface expression of the recombinant LamB protein might also alter the colonization properties of S. typhimurium. Thus the mouse and Salmonella strain, the carrier protein and the properties of the inserted epitopes are all likely to influence the immunogenicity of attenuated Salmonella constructs in mice.

At least one attenuated Salmonella, S. typhi Ty21a, has already proved acceptable for use in man. In some systems Salmonella vectors have achieved adequate mucosal immunity. Our results suggest this may not be true of all systems, indicating a need to modify our vaccination strategy if effective mucosal immunization is to be achieved. The choice of mouse strain is limited by murine genotypic factors affecting the establishment of colonization and chlamydial salpingitis. We believe that the strategy should be to identify a stable Salmonella host capable of colonizing the mouse intestine for 3–4 weeks following oral administration. There is a considerable range of S. typhimurium and S. enteritidis strains to choose from (reviewed by Curtiss et al., 1989). Secondly, the ability of the chlamydial-Salmonella recombinant to colonize the mouse gut should be determined prior to immunization and challenge studies. Finally, incorporation of T-cell epitopes of MOMP (Allen et al., 1990; Holland et al., 1990) as well as important neutralizing epitopes from other chlamydial surface antigens may enhance the immunogenicity of the constructs. Brown et al. (1987) have demonstrated that cloned antigens expressed in aroA mutants of S. typhimurium can induce cellular as well as humoral immune responses. Each model system of human infection may pose its own problems. We believe such studies to be of fundamental importance, given the present inability to control a large number of diseases affecting respiratory, mucosal and ocular surfaces by immunization.

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References


