Heterotypic protection of mice against chlamydial salpingitis and colonization of the lower genital tract with a human serovar F isolate of *Chlamydia trachomatis* by prior immunization with recombinant serovar L1 major outer-membrane protein

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Intrauterine infection of mice with a human genital tract isolate of *Chlamydia trachomatis* (serovar F) resulted in salpingitis. In some cases, oviduct damage was sufficient to cause infertility due to luminal blockage. Parenteral immunization with a purified, heterologous, recombinant major outer-membrane (rMOMP) preparation reduced the proportion of animals developing severe salpingitis by 77% compared with mock-immunized controls, but failed to reduce chlamydial colonization of the lower genital tract. In contrast, mice immunized with rMOMP directly into the Peyer's patches to stimulate mucosal immunity shed fewer chlamydiae from the vagina than controls, but showed little reduction in oviduct damage. No consistent correlation was observed between antibody levels to rMOMP in immunized mice and reduced lower genital tract colonization. Immunization with rMOMP via the presacral space, a route previously shown to stimulate mucosal immunity in the genital tract, produced high levels of circulating anti-rMOMP IgG but only traces of anti-rMOMP IgA in vaginal secretions. There was no difference in the severity of salpingitis in these animals compared with mock-immunized controls. Immunization with rMOMP conferred no protection against infertility resulting from direct inoculation of chlamydiae into the oviducts.

Introduction

The obligate intracellular pathogen *Chlamydia trachomatis* is a major cause of sexually transmitted disease worldwide. In women, infection of the upper genital tract with chlamydiae is associated with pelvic pain and salpingitis which may lead to infertility or ectopic pregnancy (Chow *et al.*, 1987; Robertson & Ward, 1988). In large areas of the world *C. trachomatis* is the major infectious cause of female infertility. The cost of treating chlamydial infections and their sequelae runs to billions of dollars annually for the USA alone (Washington *et al.*, 1987). In addition, *C. trachomatis* is also the world's

major cause of preventable blindness (trachoma). An effective chlamydial vaccine is a major public health goal.

Empirical attempts to vaccinate against trachoma by immunization with whole *C. trachomatis* resulted in evidence of short-term, serovar-specific (homotypic) immunity to ocular chlamydial infection (reviewed in Taylor-Robinson & Ward, 1989). Homotypic immunity would explain why repeat or multiple infections with *C. trachomatis* often involve immunologically distinct serovars (Barnes *et al.*, 1986). Protective immunity following repeated infection with *C. trachomatis* would explain why active trachoma in endemic areas is essentially a childhood disease (Ward *et al.*, 1990). However, delayed-type hypersensitivity responses to some chlamydial antigens, notably heat-shock proteins, may enhance disease severity (Morrison *et al.*, 1989). Accordingly, attention has focused on the use of purified or

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Abbreviation: rMOMP, recombinant major outer-membrane protein.

recombinant chlamydial antigens for vaccine development. The major outer-membrane protein (MOMP) of *C. trachomatis* is the main candidate antigen.

MOMP, a porin, is the principal protein at the surface of the infectious chlamydial elementary body. MOMP may play a significant role in the entry of chlamydiae into the host cell (Su *et al.*, 1988) and the initiation of intracellular development (Bavoil *et al.*, 1984). Polyclonal and monoclonal antibodies directed against the serovar- or sub-species specific, surface-exposed antigenic epitopes on MOMP neutralize the infectivity of *C. trachomatis* *in vitro* and *in vivo* (Lucero & Kuo, 1985; Zhang *et al.*, 1987). The sub-species specific epitope should, thus, stimulate heterotypic immunity, while additional homotypic immunity should arise if the vaccine and challenge strain share the same serovar-specific epitope. The primary structures of these epitopes have been identified with high precision (Baehr *et al.*, 1988; Conlan *et al.*, 1988; Stephens *et al.*, 1988). Moreover, recombinant MOMP (rMOMP) has been expressed at high levels in *Escherichia coli* (Pickett *et al.*, 1988), facilitating the preparation of antigen in sufficient quantity for animal studies.

The present study was initiated in the mouse model of chlamydial salpingitis (Tuffrey *et al.*, 1986a, 1990a) to explore whether rMOMP administered by routes associated with systemic or mucosal immune responses would elicit protective immunity against colonization or severe disease following challenge with heterotypic *C. trachomatis*.

Methods

Mice. Syngeneic C3H mice, 6–8 weeks old, were bred and maintained in the specific-pathogen-free unit at the Clinical Research Centre (Harrow, Middx, UK) or were obtained from OLAC (Harlan–Olac, Bicester, UK).

Chlamydiae. *C. trachomatis* strain NI 1, serovar F, was isolated from the endocervix of a contact of a man with non-gonococcal urethritis. For inoculation, chlamydiae from the ninth tissue-culture passage were suspended in 2SP cryopreservative (68.5 g sucrose, 2.268 g sodium dihydrogen phosphate, 100 ml foetal calf serum, water to 1000 ml). The inoculum was verified to be free of contaminating *Mycoplasma* or bacteria. Elementary bodies of *C. trachomatis* strain L1/440/LN, serovar L1, were purified by density gradient separation as previously described (Salari & Ward, 1981).

Preparation of recombinant MOMP. The cloning and expression of rMOMP was as previously described (Pickett *et al.*, 1988). The $\frac{3}{4}$ - or $\frac{1}{2}$ -length rMOMP (from the C-terminus) of *C. trachomatis* serovar L1 was purified from *E. coli* strain JM109 and adsorbed to alhydrogel as previously described (Conlan *et al.*, 1990), except that in experiments 2 and 3 (see below) rMOMP was initially solubilized in 8 M-urea in buffer (25 mM-Tris/acetic acid buffer, pH 5, containing 1 mM-EDTA and 0.1 M-2-mercaptoethanol) before direct adsorption to alhydrogel for 1 h at room temperature at a ratio of 1 mg rMOMP to 2 mg alhydrogel. Yield of rMOMP from *E. coli* broth culture was approximately 35 mg l⁻¹.

Vaccination of mice. Subcutaneous, intramuscular or intraperitoneal immunization with rMOMP was used to stimulate systemic immunity. Oral dosing with rMOMP, or direct inoculation into the presacral space or into surgically exposed Peyer's patches was used to induce mucosal immunity. Three experiments were performed. The numbers of mice used in each group are shown in the Tables. In experiment 1, mice were immunized on days 0, 14 and 21 with 20 μ g $\frac{3}{4}$ -length rMOMP which had been solubilized in 5% (w/v) SDS prior to adsorption onto alhydrogel. Controls were inoculated with alhydrogel alone. Immunization was by one of three different routes. For parenteral immunization, each dose was divided between subcutaneous (0.1 ml), intramuscular (0.1 ml) and intraperitoneal (0.2 ml) sites. To stimulate mucosal immunity, mice were inoculated directly into a surgically exposed Peyer's patch (0.1 ml) or were dosed orally with a gavage needle with 0.05 ml (25 μ g) rMOMP on alhydrogel in 5% (w/v) sodium bicarbonate. The mice were challenged by the intravaginal or intrauterine route with *C. trachomatis* strain NI 1 on day 28.

The second experiment focussed on mucosal immunity to urea-solubilized $\frac{3}{4}$ -length rMOMP. Mice were inoculated directly into a surgically exposed Peyer's patch with 100 μ g rMOMP fragment on alhydrogel (0.06 ml) on days 0, 7 and 14. A small sponge tampon containing a further 200 μ g rMOMP was inserted into the vagina on day 20 and removed on day 22 with the objective of inducing a secondary vaginal response to gut-primed mucosal immunity. One control group received topical intravaginal rMOMP without prior inoculation of Peyer's patches. A second control group was unimmunized. Vaginal washes were collected on days 23 and 28 prior to challenge with chlamydiae on day 30.

The third experiment used urea-solubilized $\frac{1}{2}$ -length rMOMP which is more readily expressed and purified than the $\frac{3}{4}$ -length product. The experiment explored whether immunization of mice intraperitoneally or into the presacral space was effective at generating mucosal immunity. Groups of mice were immunized with 20 μ g urea-solubilized $\frac{1}{2}$ -length rMOMP on alhydrogel on days 0, 14 and 28. Immunization was either by the oral, subcutaneous, or intraperitoneal routes or into the presacral space (Parr & Parr, 1988), i.e. into the muscle that is attached to the ventral surface of the sacrum. For this latter injection the needle started between the base of the tail and the anus and passed about 8 mm towards the sacrum. Some mice were additionally given two intravaginal boosts with 50 μ g rMOMP (soaked into tampons) on days 32 and 44. Immunized mice and untreated controls were challenged with chlamydiae on day 53.

Infection of mice. All mice were given a single dose of 2.5 mg progesterone subcutaneously (Depo-Provera; Upjohn, USA) 7 d prior to challenge with approximately 5×10^4 inclusion-forming units (i.f.u.) *C. trachomatis* strain NI 1 in 2SP diluent. Progesterone treatment is not essential, but enhances genital tract infection with chlamydiae by altering the oestrous cycle to maintain the target epithelium (Tuffrey & Taylor-Robinson, 1981). Mice were challenged by one of three routes. In experiment 1, the challenge dose (0.05 ml) was introduced into the vagina soaked on a small sponge tampon to aid retention of the inoculum. Other groups of mice were inoculated directly into a uterine horn (0.05 ml). For fertility studies, mice were inoculated into one or both of the ovarian bursae. Details of animal handling and surgical procedures have been published previously (Tuffrey *et al.*, 1986a, 1990b). Relevant animal welfare legislation was strictly complied with.

Recovery of chlamydiae. Vaginal colonization was assessed using nasopharyngeal swabs (Medical Wire and Equipment Co.) at regular intervals from days 4 to 33 (see Tables). Each swab was expressed in 1 ml cold 2SP medium then stored in liquid nitrogen. Chlamydiae were cultured by centrifuge-assisted inoculation onto cycloheximide-treated McCoy cell monolayers by conventional procedures (Thomas *et al.*, 1977).

Antibody studies. Sera and cervical washes were obtained at various intervals after immunization and were stored at -20°C until required. Samples from mice in the same group were pooled. Samples were collected one week after each of the 2nd and 3rd immunizations in experiment 1, and 3 and 5 d after the intravaginal boost in experiment 2. In experiment 3, samples were obtained 3 d after the 2nd and 3rd immunizations and 5 d after each vaginal boost.

Antibodies to rMOMP, or to *C. trachomatis* strain L1/440/LN or NI 1 elementary bodies were detected by a redox-amplified ELISA (Ward *et al.*, 1990) or by indirect immunofluorescence (Thomas *et al.*, 1977).

Fertility studies. Intrabursal inoculation with *C. trachomatis* strain NI 1 was used for breeding studies because it results in a higher proportion of infertile mice than intrauterine inoculation, minimizing the number of laboratory animals required. Groups of challenged vaccinated or control mice were set aside for breeding. No vaginal swabs were taken from these animals so as to avoid any possible interference with their reproductive capacity. On day 14 after challenge (20 d after progesterone treatment) both vaccinated and control mice were placed with fertile males. Control groups were progesterone-treated mice inoculated with suspending buffer alone, plus a group of age-matched, progesterone-treated mice which had had no surgical intervention. The first vaginal plug was detected in the 2SP diluent control group, 10 d after placing with males, confirming that the oestrous cycle had recommenced following progesterone treatment.

Histology. Mice were killed between days 29 to 74 days after infection (experiment 1) or between 12 to 35 days post-infection (experiments 2 and 3). The oviducts and uterus on both sides were fixed in 10% (v/v) buffered formalin before paraffin embedding. Sections 4 μm thick were cut at various levels through the tissue and stained with conventional haematoxylin and eosin. Inflammatory changes were graded from – to +++ as described previously (Tuffrey *et al.*, 1986a).

Electron microscopy. Mouse oviduct was processed for scanning electron microscopy as previously described (Tuffrey *et al.*, 1990a). Specimens were examined at 20 kV in a 2 nm resolution Hitachi S500 scanning electron microscope equipped with a field-emission electron gun.

Results

Effects of chlamydial infection on the oviduct of unimmunized mice

The morphological effects of intrauterine challenge with *C. trachomatis* strain NI 1 are summarized in the scanning electron micrographs (Fig. 1). The mucosal surface of the normal mouse oviduct consists of both ciliated and non-ciliated epithelia (Fig. 1a). Fifteen days after challenge with strain NI 1 there was a severe loss of ciliated epithelial cells (Fig. 1b). This has been associated with impairment in transportation of eggs (Tuffrey *et al.*, 1990a). The lumen of infected oviducts almost invariably contained an exudate (Fig. 1c), characterized by light microscopy (data not shown) as consisting of mucus, epithelial debris and the acute inflammatory cells characteristic of salpingitis. This exudate is frequently sufficient to block the oviduct lumen (Tuffrey *et al.*,

1986b). Hydrosalpinx, characterized by severe ballooning of the oviduct (Fig. 1d) and further mucosal destruction, was observed in approximately half of the tubes.

Re-isolation of chlamydiae from the lower genital tract in test and control mice challenged by the intrauterine route

Chlamydial isolation rates for mice in experiment 1, which had been vaccinated either parenterally or via a Peyer's patch with solubilized rMOMP adsorbed onto alhydrogel, were compared with control mice immunized with alhydrogel alone (Table 1). Chlamydiae were isolated from the genital tract on at least one occasion from 7 out of 8 (88%) control mice and from 4 out of 6 (67%) of the parenterally vaccinated mice. Chlamydial colonization was reduced from 25 to 15 d in the parenterally immunized mice compared to controls. Immunization with rMOMP into surgically exposed Peyer's patches delayed vaginal colonization with chlamydiae by 5 d in the test animals compared with controls. Overall, the number of mice from which chlamydiae were recovered on at least one occasion was also reduced in the immunized animals from 7 out of 8 (88%) compared to 5 out of 8 (63%) in the control group. Moreover, in the latter group chlamydiae were isolated on only one occasion when, generally, only a single inclusion body was seen. A total of 383 i.f.u. were recovered from the six control mice found to be infected with chlamydiae over the first 11 d, but in the same period only 7 i.f.u. were recovered from the three mice found to be infected with chlamydiae following immunization with rMOMP via a Peyer's patch. Thus immunization of the gut with heterotypic serovar L1 rMOMP via the Peyer's patches markedly reduced vaginal shedding of *C. trachomatis*, strain NI 1, serovar F.

Mice challenged by the intravaginal route

In the natural infection, vaginal colonization precedes ascending chlamydial infection of the upper genital tract. The oral and Peyer's patch routes of mucosal immunization were less effective against intravaginal challenge than against intrauterine challenge (cf. Tables 1 and 2). There was no evidence of protection against intravaginal challenge in mice immunized via surgically exposed Peyer's patches, and only weak evidence of protection in mice orally dosed with rMOMP. In the orally immunized group, 13 out of 14 (93%) animals and 39% of swabs were positive for chlamydial isolation in the control mice compared with 8 out of 13 (62%) and 22%, respectively.

Fig. 1. Scanning electron micrographs showing the effects of infection with *C. trachomatis* strain NI 1 on the epithelial surface of the mouse oviduct. Twenty control or infected oviducts were examined. (a) Normal, uninfected oviduct showing ciliated and non-ciliated epithelium. The bar represents 20 µm. (b) The luminal epithelium of the oviduct 15 d after intrauterine infection with *C. trachomatis* strain NI 1. Note the denudation of the ciliated epithelium and the presence of surface debris. The bar represents 20 µm. (c) Transverse section of murine oviduct 15 d after intrauterine infection with chlamydiae showing blockage of the lumen with cellular debris. The bar represents 50 µm. (d) Transverse section of an oviduct with hydrosalpinx, 27 d after intrauterine infection with *C. trachomatis* strain NI 1. The bar represents 0.5 mm.

Table 1. Vaginal colonization of vaccinated and control C3H mice after intrauterine challenge with *C. trachomatis* strain NI 1

Immunization route and group of mice	No. of mice from which chlamydiae were isolated on indicated day after intrauterine challenge:							No. of mice from which chlamydiae were isolated on at least one occasion/no. of mice tested (%)	Range of i.f.u. per monolayer	No. of swabs from which chlamydiae were isolated/no. of swabs tested (%)
	5	8	11	15	22	25	29			
Parenteral										
Control	3/8	2/8	4/8	0/8	2/8	3/8	0/8	7/8 (88%)	1-47	14/56 (25%)
Test	3/6	1/6	3/6	2/6	0/6	0/6	0/6	4/6 (67%)	1-104	9/42 (21%)
Via Peyer's patch										
Control	6/8	4/8	2/8	0/8	1/8	1/8	0/8	7/8 (88%)	1-113	14/56 (25%)
Test	0/8	3/8	3/8	0/8	1/8	0/8	0/8	5/8 (63%)	1-6	7/56 (13%)

Table 2. Vaginal colonization of vaccinated and control C3H mice after intravaginal challenge with *C. trachomatis* strain NI 1

Immunization route and group of mice	No. of mice from which chlamydiae were isolated on indicated day after intravaginal challenge:							No. of mice from which chlamydiae were isolated on at least one occasion/no. of mice tested (%)	Range of i.f.u. per monolayer	No. of swabs from which chlamydiae were isolated/no. of swabs tested (%)
	5	8	11	14	20	23	32			
Oral										
Control	NT	9/14	NT	3/13	5/14	8/14	2/14	13/14 (93%)	1-82	27/69 (39%)
Test	NT	8/13	NT	2/13	1/13	2/13	1/13	8/13 (62%)	1-122	14/65 (22%)
Via Peyer's patch										
Control	1/8	4/8	2/7	0/8	1/8	NT	NT	6/8 (75%)	2-25	7/30 (18%)
Test	5/8	2/8	4/7	0/8	0/8	NT	NT	7/8 (88%)	1-12	11/39 (28%)

NT, Not tested.

Effect of immunization on salpingitis

None of the mice inoculated intravaginally in experiment 1 developed salpingitis. In 10 years of experience of this mouse model salpingitis has not been observed following intravaginal challenge with *C. trachomatis*. Our data are, therefore, restricted to animals challenged via the intrauterine route. In experiment 1, histological studies on oviducts 29 to 74 d after challenge (after the colonization study) showed that parenteral immunization with rMOMP reduced the incidence of late salpingitis from 4 out of 7 (57%) in the control mice (3 out of 4 of which had hydrosalpinx) to 2 out of 6 (33%) in the immunized mice (none of which had hydrosalpinx). When pairs of test and control mice killed on the same day were compared (Table 3), the lesions seen in control mice were more severe than those in the test mice in 50% of comparisons and of equal severity in 33% of cases. Immunization via Peyer's patches reduced chlamydial shedding, but as 3 out of 8 controls had inflammatory changes compared with 4 out of 8 test animals, this was not associated with a reduction in the prevalence or severity of salpingitis.

In experiment 2, the mice were killed from 12 to 24 d after intrauterine challenge, when salpingitis should be

at its most severe. All mice immunized via the Peyer's patches developed salpingitis, although there was some evidence of a reduction in severity. No immunized animals had lesions that were more severe than in the unimmunized controls, while 44% of the immunized animals had less severe lesions. Taken together, these data indicate that the vaccine had a modest protective effect. Topical intravaginal rMOMP alone, failed to reduce the severity of the salpingitis.

In experiment 3, parenteral immunization with rMOMP had little effect on the prevalence of salpingitis 14 to 35 d after intrauterine challenge. Thus 7 out of 8 control mice had salpingitis, compared with 5 out of 8 mice immunized subcutaneously, 7 out of 8 immunized intraperitoneally, 6 out of 8 immunized orally and 8 out of 8 immunized by the presacral route. However, the salpingitis in immunized animals was again generally less severe than in control animals (Table 3), indicating some protection from rMOMP immunization. Salpingitis was less severe than in the controls in all mice immunized subcutaneously and in 75% of mice immunized intraperitoneally or orally with an intravaginal booster.

Table 4 summarizes salpingitis in the 55 pairs of control and test mice immunized with rMOMP in all

Table 3. *Oviductal histological changes in mice challenged after immunization (test) compared with those in mice after primary genital inoculation with chlamydial strain NI 1 (control)*

Experiment 1: immunized with $\frac{1}{2}$ -length rMOMP; mice killed 29–74 d post-infection. Experiment 2: mice killed 12–24 d post-infection. Experiment 3: immunized with $\frac{1}{2}$ -length rMOMP; mice killed 14–35 d post-infection.

Experiment no.	No. of pairs of mice (test versus control)	Immunization route and schedule for test mice*	Histological evaluation of salpingitis in pairs of mice (test versus control) killed on the same day†		
			Test more severe	Test less severe	Same
1	6	s.c., i.p. and i.m.	1 (17%)	3 (50%)	2 (33%)
	8	Peyer's patch	2 (25%)	2 (25%)	4 (50%)
2	9	Peyer's patch	0	4 (44%)	5 (50%)
3	4	s.c.		4 (100%)	
	4	s.c. and i.vag. booster		4 (100%)	
	4	i.p.		3 (75%)	1 (25%)
	4	i.p. and i.vag. booster	1 (25%)	3 (75%)	
	4	Oral	1 (25%)	2 (50%)	1 (25%)
	4	Oral and i.vag. booster	1 (25%)	3 (75%)	
	4	Presacral		2 (50%)	2 (50%)
	4	Presacral and i.vag. booster	1 (25%)	2 (50%)	1 (25%)
Total	55		7 (12.7%)	32 (58.2%)	16 (29.1%)

* Immunization route subcutaneous (s.c.), intraperitoneal (i.p.), intramuscular (i.m.) or intravaginal (i.vag.).

† Inflammatory changes were graded from – to +++ as described by Tuffrey *et al.* (1986a).

Table 4. *Comparison of immunization routes*

Routes of immunization of test mice*	No. of pairs of mice (test vs. control)	Histological evaluation of salpingitis in pairs of mice (test vs. control) killed on the same day (%)†		
		Test more severe	Test less severe	Same
Parenteral routes s.c., i.p. and i.m.	22	2(9%)	17(77%)	3(14%)
Peyer's patch	17	2(12%)	6(35%)	9(53%)
Oral	8	2(25%)	5(63%)	1(13%)
Presacral	8	1(12.5%)	4(50%)	3(37.5%)

* See Table 3.

† Inflammatory changes graded as in Table 3.

three experiments. The aggregated results show that salpingitis in mice parenterally immunized with rMOMP was less severe than in the unimmunized controls in 77% of cases. By contrast, immunization with rMOMP via the Peyer's patch showed little reduction in the severity of salpingitis (Table 4), despite the associated reduction in chlamydial shedding (Table 1). Immunization via the oral or presacral routes, which was also designed to induce mucosal immunity, was also less protective than the parenteral route. Taken together, this

suggests that the protective mechanisms reducing disease severity were not the same as those which reduced colonization.

Effect of immunization on fertility following C. trachomatis challenge

The fertility of unimmunized control mice, or of mice vaccinated with rMOMP either parenterally or via the Peyer's patches was compared over an 8 month period

Table 5. Fertility in immunized and control C3H mice 8 months after intrabursal challenge with *C. trachomatis* strain NI 1

Immunization schedule	Challenge inoculum	No. of mice litters/ no. of mice in group (%)
Untreated controls	None	9/10 (90%)
	2SP buffer	5/6 (83%)
Parenteral alum	Unilateral NI 1	4/10 (40%)
Parenteral rMOMP	Unilateral NI 1	2/10 (20%)
Parenteral alum	Bilateral NI 1	0/9 (0%)
Parenteral rMOMP	Bilateral NI 1	0/10 (0%)
Alum via Peyer's patch	Unilateral NI 1	1/10 (10%)
rMOMP via Peyer's patch	Unilateral NI 1	2/10 (20%)
Alum via Peyer's patch	Bilateral NI 1	0/10 (0%)
rMOMP via Peyer's patch	Bilateral NI 1	1/9 (11%)

following direct inoculation into the ovarian bursa with *C. trachomatis* strain NI 1. This route achieves the highest levels of infertility in unprotected animals (Tuffrey *et al.*, 1986b). Control mice were inoculated with 2SP buffer alone or were uninoculated. A summary of the results is presented in Table 5.

Of the untreated mice, 9 out of 10 gave birth to 1–3 litters (average litter size = 5–6) over the study period as did 5 out of 6 mice inoculated with 2SP buffer alone. No litters were born to any of the parenterally vaccinated or control-treated mice infected bilaterally with *C. trachomatis* strain NI 1. Of the parenterally vaccinated animals in this group which had been challenged unilaterally, 2 out of 10 gave birth to 1 or 2 litters, whilst 4 out of 10 of the alhydrogel-treated controls in this group also littered. Thus, parenteral vaccination with rMOMP did not prevent infertility caused by chlamydial infection of the genital tract.

In mice immunized via the Peyer's patches, bilateral intrabursal inoculation with strain NI 1 rendered all 10 alhydrogel-treated controls in this group infertile and only 1 out of 9 mice vaccinated with rMOMP gave birth to a single litter of three offspring. Unilateral challenge with strain NI 1 also resulted in infertility in mice immunized via Peyer's patches and control mice. Of the alhydrogel control mice, 1 out of 10 gave birth to a single litter, whilst 2 out of 10 rMOMP-vaccinated mice also littered. Thus, prior immunization with rMOMP via surgically exposed Peyer's patches also failed to protect the fertility of mice with upper genital tract chlamydial infection.

Antibody studies

Sera and cervical washes were collected at various times following immunization with rMOMP adsorbed to alhydrogel or with alhydrogel alone. The most detailed antibody observations using conventional techniques (immunofluorescence) were carried out in experiment 3.

In this experiment, mice were immunized with rMOMP via the subcutaneous, intraperitoneal, oral or presacral routes with or without an additional intravaginal booster. Pooled serum samples and cervical washes from pairs of mice were taken on days 17, 31, 37 and 50, and antibody titres were determined by indirect immunofluorescence. Circulating IgG to both rMOMP and homologous serovar L1 elementary bodies was detected at similar levels in mice immunized via the intraperitoneal or presacral routes, with a mean titre of 1:1024 on day 37. Similar titres of IgG antibodies to rMOMP were detected in the mice immunized subcutaneously. These antibody titres were reduced by about one doubling dilution when strain NI 1 elementary bodies were used as antigen. No circulating IgG antibodies were found in the group immunized orally. Low levels of vaginal IgA were detected in imprint material from the antigen-soaked tampons from all groups assayed at day 46, except the subcutaneously immunized mice. IgG was present in tampon imprints from all groups.

Discussion

Parenteral immunization with rMOMP reduced the duration of colonization and also reduced the severity of inflammatory changes in the oviduct (salpingitis). This protective effect of parenteral immunization with rMOMP against salpingitis was unexpected. Previous studies in this model showed that prior chlamydial infection with live organisms was associated with more severe salpingitis (Tuffrey *et al.*, 1990b), possibly as a result of delayed-type hypersensitivity responses to chlamydial heat-shock proteins similar to those observed in the guinea pig (Morrison *et al.*, 1989). Reduction in the severity of salpingitis in animals parenterally immunized with rMOMP coincided with high levels of circulating IgG to the protein, although it is not known whether this association was significant. Parenteral immunization

might promote antibody-dependent cell-mediated cytotoxic attack (ADCC) on cells infected with chlamydiae. However, it seems more likely that parenteral MOMP immunization sensitizes T cells to chlamydial antigen, leading to the production of chlamydia-stimulating cytokines such as gamma interferon or tumour necrosis factor (Shemer-Avni *et al.*, 1988). Salpingitis is one of the most significant complications of chlamydial genital tract infection because of its impact on fertility. Unfortunately, the reduced severity of salpingitis following parenteral immunization with rMOMP was insufficient to prevent infertility following direct inoculation of chlamydiae into the ovarian bursa. This severe and unnatural challenge probably overwhelmed the modest degree of protection achieved by parenteral immunization with rMOMP.

Direct immunization with rMOMP into the Peyer's patches was more effective at reducing chlamydial colonization than parenteral immunization, despite the absence of significant local antibody. Priming of the common mucosal immune system via the gut-associated lymphoid tissue (GALT) may not lead to the appearance of specific antibodies at distant mucosal surfaces in the absence of local secondary stimulation with specific antigen (Allardyce & Bienenstock, 1984). However, neither topical intravaginal boosts with rMOMP, nor immunization via the presacral space achieved significant levels of antibody in genital tract secretions prior to challenge.

Some important preliminary conclusions can be drawn. Firstly, it appears that rMOMP does have some protective effect on both chlamydial colonization and subsequent tubal damage. Secondly, heterotypic protection appears to be feasible given that serovar F MOMP is quite distinct in its critical, surface-exposed, variable regions from the strain L1 rMOMP antigen used for immunization (Yuan *et al.*, 1989). Serovar F is also unusual in that its species-specific epitope is surface exposed (Collett *et al.*, 1989). This may have contributed to heterotypic protection. Thirdly, there was no evidence that vaccination with rMOMP showed the same tendency as vaccination with viable organisms of increased disease severity on subsequent challenge with live chlamydiae. Finally, mucosal and parenteral immunization appeared to have distinct effects on chlamydial colonization and tubal damage, respectively. Either effect would be valuable for controlling human infertility and ectopic pregnancy due to chlamydial infection.

Further experiments are necessary to enhance the weak protective effect of rMOMP. One strategy currently being explored is the incorporation of type-specific neutralizing epitopes into the recombinant vaccine, either by recombinant DNA techniques or by cross-linking synthetic peptide epitopes onto a MOMP

backbone. An alternative strategy is the use of live vectors which stimulate mucosal immunity by their persistent presentation of recombinant antigen. The recent engineering of MOMP epitopes into recombinant *E. coli* enterotoxoid (Hayes & Clark, 1990) so that they bind to the antigen-presenting M cells of the gut, is one such strategy. The present experiments indicate that rMOMP, suitably presented, is capable of eliciting weak protective immunity. The challenge is to augment protection to useful levels.

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