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Protective Efficacy of Major Outer Membrane Protein-Specific Immunoglobulin A (IgA) and IgG Monoclonal Antibodies in a Murine Model of *Chlamydia trachomatis* Genital Tract Infection

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The protective efficacy of immunoglobulin A (IgA) and IgG monoclonal antibodies (MAbs) specific for the major outer membrane protein of *Chlamydia trachomatis* MoPn was evaluated in a murine genital tract infection model. MAbs were delivered into serum and vaginal secretions of naive mice by using the backpack hybridoma tumor system, and protective efficacy was assessed over the first 8 days following challenge by quantitative determination of chlamydial recovery from cervicovaginal swabs, histopathological evaluation of genital tract tissue, and immunohistochemical detection of chlamydial inclusions. IgA and IgG significantly reduced the incidence of infection following vaginal challenge with 5 50% infectious doses, but such protection was overwhelmed by 10- and 100-fold higher challenge doses. Both MAbs also consistently reduced vaginal shedding from infected animals with all three challenge doses compared with the negative control MAb, although the magnitude of this effect was marginal. Blinded pathological evaluation of genital tract tissues at 8 days postinfection showed a significant reduction in the severity of the inflammatory infiltrate in oviduct tissue of infected IgA- and IgG-treated animals. Immunohistochemical detection of chlamydial inclusions revealed a marked reduction in the chlamydial burden of the oviduct epithelium; this finding is consistent with the reduced pathological changes observed in this tissue. These studies indicate that the presence of IgA or IgG MAbs specific to major outer membrane proteins has a marginal effect in preventing chlamydial colonization and shedding from the genital tract but has a more pronounced effect on ascending chlamydial infection and accompanying upper genital tract pathology.

Chlamydial infections of mucosal tissues elicit a broad spectrum of human disease, including blinding trachoma, pelvic inflammatory disease, and pneumonia. Natural immunity to reinfection does occur; however, it is limited by being serovar specific and relatively short lived (6, 7). The basis for this immunity is not understood, but identification of the protective component(s) of the immune response is central to the rational development of vaccines.

Of all of the immune effectors, the role of antibody in immunity to chlamydial infections has been the most intensively studied. Earlier reports have demonstrated a correlation between increased levels of chlamydia-specific antibody in secretions and either resolution of primary infection (3, 15) or immunity to reinfection (10, 13). In particular, elevated levels of secretory immunoglobulin A (sIgA) have shown a strong correlation with resolution of human endocervical infection (3) and resistance to reinfection of guinea pig eyes (10). The involvement of antibody in protection is supported further by several lines of direct evidence. Antichlamydial immunoglobulin fractions from either plasma or secretions of trachoma patients or immune mice can passively neutralize chlamydial infectivity for monkey eyes (2) and mouse lungs (24), respectively. Monoclonal antibodies (MAbs) specific for the chlamydial major outer membrane protein (MOMP) neutralize infectivity for monkey eyes, as well as for cultured cell lines, defining the MOMP as an important protective antigen (26). Finally, passive transfer of high-titer antichlamydial sera significantly reduces chlamydial shedding in the guinea pig vaginal infection model (14).

In contrast, data from two studies involving B-cell-deficient mice indicate that antibody is not required for resolution of primary respiratory or genital tract infections (12, 23) or for resistance to reinfection of the genital tract (12). Although antibody is not required for these events, these studies do not indicate whether antibody is a contributing component of protection or clearance in an immunocompetent animal, nor do they indicate whether antibody alone is capable of preventing infection, which is an important consideration for immunoprophylaxis. Furthermore, the importance of sIgA versus that of IgG in the control of this mucosal pathogen has not been established.

Because of the controversy surrounding the role of antibody in immunity to chlamydial infections, the experiments described here were designed to test directly whether antibody alone can protect naive mice from infection of the genital tract by the murine strain of *Chlamydia trachomatis* (MoPn) and also to compare the relative efficacies of IgA and IgG antibodies. The "backpack" tumor system (25) was used to deliver MoPn MOMP-specific MAbs into the serum and vaginal secretions of naive mice. Mice were implanted with syngeneic hybridoma tumors that produce either IgG or IgA MAbs spe-

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cific for the MOMP. Following MAb detection in vaginal secretions, the mice were challenged with various dosages of MoPn and protective efficacy was assessed by quantitatively measuring vaginal shedding. At the termination of the experiment, entire genital tracts were fixed and thin sections were used for histopathological evaluation and immunohistochemical detection of chlamydial inclusions.

MATERIALS AND METHODS

Chlamydiae. *C. trachomatis* MoPn (Wiess) was grown in HeLa 229 cells, and elementary bodies (EBs) were purified from infected cells by density gradient centrifugation as previously described (5). A single preparation of MoPn was aliquoted and stored at −70°C, and diluted material from that stock was used as challenge inocula for all of the experiments described here. The 50% infectious dose (ID_{50}) of MoPn was determined experimentally and calculated to be 15 chlamydial infection-forming units (IFUs) by the method of Reed and Muench (16).

Hybridomas. The murine myeloma line MOPC-315, obtained from the American Type Culture Collection, secretes an IgA MAb specific for the hapten dinitrophenol. The murine hybridomas MP-A5d and MP-33b, both of which secrete MAbs specific for the MOMP of *C. trachomatis* MoPn, were generated by the following procedure. Six-week-old female BALB/cAnHsd mice were immunized perorally on day 0 with 10^8 IFUs of MoPn EBs in 0.2 ml of 0.2 M sodium bicarbonate. Immunization was repeated at days 28 and 56. On day 59, immunized mice were euthanized, Peyer's patches were removed, and Peyer's patch lymphocytes were harvested (22). Peyer's patch lymphocytes were then mixed with SP2/0-Ag14 murine myeloma cells at a ratio of 5:1 and fused in 35% polyethylene 4000 (Sigma) as previously described (1). Fused cells were diluted to 6×10^5/ml in RPMI 1640 medium supplemented with 15% fetal bovine serum, 2 mM glutamine, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 24 mM sodium bicarbonate, 1 mM sodium pyruvate, 100 U of penicillin per ml, and 100 μg of streptomycin per ml and distributed into 96-well tissue culture trays (0.1 ml per well) that had been seeded the previous day with peritoneal exudate cells (10^4 per well). Hybridoma growth was selected in hypoxanthine-aminopterin-thymidine-containing RPMI 1640 medium as previously described (1). Screening of hybridoma supernatants was performed by both Western and dot immunoblot analyses as described elsewhere (4, 26), except that immunoreactivity was detected with alkaline phosphatase-conjugated secondary antibodies (Sigma). Hybridomas were routinely cultured in Dulbecco's modified Eagle medium (GibcoBRL, Life Technologies, Gaithersburg, Md.) containing 10% fetal bovine serum, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 50 μg of gentamicin per ml, 10 mM HEPES, and 1 mM sodium pyruvate at 37°C in a 5% CO_2 atmosphere.

Characterization of MAbs. The specificity and functional properties of each of the MAbs were determined by using purified or partially purified material. MP-33b (IgG3) was purified by protein A affinity chromatography (4), and IgA MAbs MP-A5d and MOPC-315 were partially purified from serum-free culture supernatants. For partial purification of IgA MAbs, exponentially growing cells were harvested by centrifugation for 5 min at 500 × *g*, washed twice with Hanks' balanced salt solution (GibcoBRL, Life Technologies), and finally resuspended at 10^6 cells per ml in Dulbecco's modified Eagle medium supplemented as described above, but without serum or HEPES. After 3 days of incubation at 37°C in 5% CO_2, cells were removed by centrifugation and the supernatants were filtered through 0.45-μm-pore-size filters, immediately precipitated via dropwise addition of saturated ammonium sulfate to 45% (vol/vol), and incubated overnight at 4°C. Precipitated protein was pelleted by centrifugation for 1 h at 12,000 × *g*, resuspended in phosphate-buffered saline (PBS), and dialyzed exhaustively against PBS. This procedure resulted in protein preparations that were at least 80% MAb as judged by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Coomassie staining (data not shown).

MAb isotypes were determined by enzyme-linked immunosorbent assays (ELISA) using class- and subclass-specific secondary antibodies (Southern Biotechnology Associates, Birmingham, Ala.). Western and dot blot analyses were conducted as described elsewhere (4, 26), except that for both procedures, detection of IgA MAb immunoreactivity required the use of a rabbit anti-mouse IgA (alpha chain)-specific secondary antibody (Zymed) at a 1:1,000 dilution prior to incubation with ^{125}I-labeled protein A.

To determine whether hybridoma MP-A5d and myeloma MOPC-315 secrete dimeric and higher polymers of IgA, 0.2 μg of MAb was denatured in SDS sample buffer, heated to 100°C for 30 s, and then electrophoresed on 7.5% polyacrylamide gels. Following transfer to nitrocellulose, immunoglobulin heavy chains alpha and gamma were detected with specific rabbit antibodies (Zymed) and ^{125}I-labeled protein A as described above.

In vitro neutralization of MoPn infectivity by MAbs was assayed on Syrian hamster kidney (HaK) and HeLa 229 cells cultivated in 96-well tissue culture trays as previously described (18).

Design of MAb-mediated protection experiments. The rapid growth rates of and subtle differences between the hybridoma tumors used in these studies required that parameters for backpack tumor development and timing of antibody measurements and vaginal infection for each tumor line be established first in small pilot experiments. From those experiments, it became evident that once a MAb could be detected in vaginal secretions (9 to 12 days postimplantation of tumor cells), a maximum of 8 days remained for efficacy evaluation because of the negative health effects caused by tumor growth. This information resulted in the experimental design described below. All experiments involving animals were conducted under veterinarian supervision, in strict accordance with National Institutes of Health animal care and use guidelines.

Experiments were conducted with 7- to 9-week-old female BALB/cAn mice (National Institutes of Health). Pre-experimental blood and vaginal secretion samples were collected at 5 days prior to hybridoma tumor implantation and tested for the presence of MoPn-specific antibodies by ELISA. No reactivity was detected in any animal at that time. All mice were given 2.5 mg of medroxyprogesterone acetate (Depo-Provera; Upjohn, Kalamazoo, Mich.) in 0.1 ml of saline subcutaneously at 10 and 3 days prior to vaginal challenge. At 12 (MOPC-315) or 9 (MP-A5d and MP-33b) days prior to challenge, mice received subcutaneous implants of 2×10^6 (MOPC-315) or 1×10^6 (MP-A5d and MP-33b) actively growing hybridoma cells in 0.5 ml of Hanks' balanced salt solution in the upper back (9). At the indicated times postimplantation of tumors, when the average tumor diameter was approximately 1 cm, vaginal secretions were collected and MAb levels were quantitatively determined by ELISA (see below). Later on the same day, serum samples were collected and the animals were challenged vaginally with 5 ID_{50} (75 IFUs), 50 ID_{50} (750 IFUs), or 500 ID_{50} (7,500 IFUs) in 5 μl of 10 mM phosphate, pH 7.2, containing 0.25 M sucrose and 5 mM L-glutamic acid (SPG). Following the challenge, chlamydial shedding was monitored by vaginal swabbing at days 2, 4, 6, and 8 as described below. At 8 days postinfection, all surviving animals were swabbed and then sacrificed for excision of whole genital tracts, which were fixed in 10% buffered formalin.

Measurement of MAb levels in serum and vaginal secretions. MAb levels in serum and vaginal secretions were quantified by ELISA by using known amounts of purified MAbs as standards. Vaginal secretion and blood samples were obtained at the times described above. Vaginal secretions were collected by alternately delivering and withdrawing 70 μl of PBS six times with a micropipettor and large-orifice micropipette tips. This was done twice for each animal, and the two washes were pooled and microcentrifuged at high speed for 10 min to remove mucus. After centrifugation, 100 μl of the clarified wash was split into two 50-μl aliquots; one was used immediately for analysis by ELISA, and the other was stored at −70°C as a backup. Blood was collected from the tail vein, and the resultant serum was stored at −70°C for later analysis by ELISA.

Individual wells of 96-well microtiter plates (Immulon 2; Dynatech Laboratories Inc., Alexandria, Va.) were coated with 0.1 ml of formalin-fixed MoPn EBs (10 μg/ml) in 0.05 M Tris buffer (pH 7.5) containing 0.15 M NaCl by overnight incubation at 4°C. After antigen removal, the plates were washed three times with 12 mM Tris buffer (pH 7.4) containing 137 mM NaCl, 3 mM KCl, and 0.05% Tween 20 (TBS-Tween) and blocked for 90 min at 37°C with TBS-Tween containing 2% bovine serum albumin (BSA). After washing once with TBS-Tween, 100 μl of sera or vaginal secretions diluted with TBS-Tween-BSA, and purified MAb standards diluted with TBS-Tween-BSA, an appropriate amount of normal mouse sera or secretions was added to individual wells in triplicate and the plates were incubated for 90 min at 37°C. The plates were then washed five times with TBS-Tween, 100 μl of a 1:250 dilution of alkaline phosphatase-conjugated rabbit anti-mouse alpha or gamma chain-specific secondary antibody (Zymed Laboratories Inc., South San Francisco, Calif.) was added per well, and the plates were incubated for 90 min at 37°C. The plates were then washed five times with TBS-Tween, and 100 μl of substrate (5 mg of *p*-nitrophenylphosphate in 10 ml of 0.1 M 2-amino-2-methyl-1,3-propanediol, pH 10.3) was added per well. Negative controls consisted of pooled pre-experiment sera or vaginal secretions. MAb concentrations were determined by comparison of A_{405} readings of the unknown samples with those from dilutions of purified MAb standards.

Measurement of chlamydial shedding. Chlamydial shedding was measured by quantifying the number of IFUs recovered from cervicovaginal swabs taken at the times described above. Samples were obtained by inserting a calcium alginate swab (Calgiswab, type 1; Spectrum Laboratories Inc., Houston, Tex.) into the vagina and rotating it 10 times. The swab tip was then cut off into 1 ml of SPG with two 5-mm-diameter glass beads contained in a sterile 2.0-ml screw-cap microcentrifuge tube kept on ice. The sample was vortexed vigorously for 30 s at room temperature, split into three 300-μl samples, and stored at −70°C. One freeze-thaw cycle did not effect recovery of MoPn (data not shown).

Once all of the samples were collected, recovery was determined by plating onto 24-h confluent HeLa 229 monolayers in 96-well microtiter trays. Monolayers were washed once with 200 μl of Hanks' balanced salt solution, and 50 μl of undiluted material or material diluted 10^{-1} or 10^{-2} was inoculated in triplicate. The plates were centrifuged at 700 × *g* for 1 h at 24°C and then incubated at 37°C for 30 min on a rocking platform. The inoculum was removed, and the monolayers were washed once with 200 μl of Hanks' balanced salt solution. The cells were then fed with 200 μl of minimal essential medium supplemented with 10% fetal bovine serum and 0.7 μg of cycloheximide per ml and incubated at 37°C with 5% CO_2 for 28 h. The monolayers were then washed once with 200 μl of PBS, fixed with 100 μl of absolute methanol, and stained by indirect immunofluorescence by using EVI-H1, a genus-specific MAb specific for chlamydial lipopolysaccharide. The inclusions contained within three microscopic fields (to-

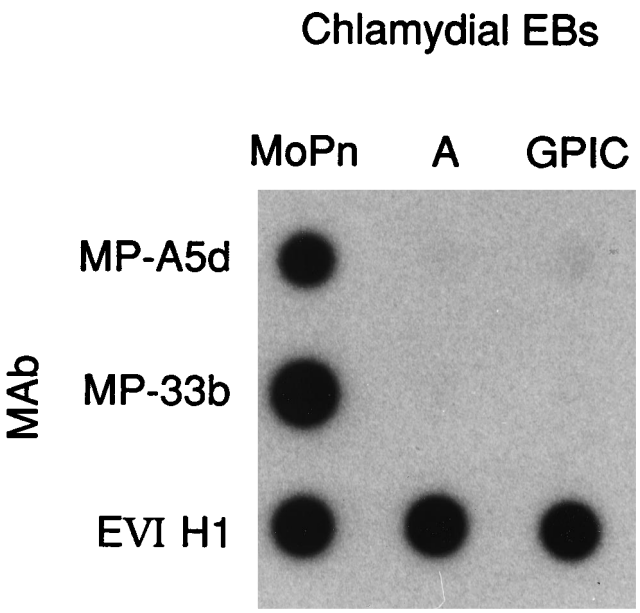


FIG. 1. Dot blot analysis of the specificity and surface reactivity of MAbs MP-A5d and MP-33b with viable chlamydiae. MP-A5d (IgA) and MP-33b (IgG3) were specific for *C. trachomatis* MoPn EBs when compared with genus-specific EVI-H1 (antilipopolysaccharide IgG2a).

tal magnification, ×100) were counted for triplicate wells, and the mean value was used to calculate the total number of IFUs in a particular sample.

Pathological evaluation and immunodetection of chlamydial inclusions. At the completion of both protection experiments (day 8 postinfection), all of the remaining animals were sacrificed and their entire genital tracts were removed and fixed in 10% buffered formalin. Fixed tissues were embedded in paraffin and sectioned for hematoxylin-eosin staining followed by blinded histopathological evaluation by a veterinary pathologist (Histo-Path of America, Temple Hills, Md.). Each region of the female mouse genital tract (vagina-cervix, uterus, and oviduct) was examined for pathological changes and then scored for both type and severity of inflammation. Acute, subacute, or chronic inflammation was characterized by primarily polymorphonuclear, mixed polymorphonuclear and mononuclear, or mononuclear and macrophage cellular infiltrates in the submucosae, respectively. Severity was scored on a relative basis as mild, moderate, or marked on the basis of changes in the epithelium, the intensity of submucosal and lumenal cellular infiltrates, and overall levels of edema and tissue destruction.

Chlamydial inclusions were detected in longitudinal thin sections of the entire genital tract by indirect immunoperoxidase staining (9a). Briefly, microscope-mounted tissue sections were deparaffinized with xylene and then transferred to 30% methanol containing 0.5% H_2O_2 to block endogenous peroxidase activity. Tissues were rehydrated with graded ethanol solutions and PBS; this was followed sequentially by blocking of nonspecific antibody binding by incubation in 3% horse serum and blocking of nonspecific avidin and biotin binding by using an avidin-biotin blocking kit (Vector Laboratories, Burlingame, Calif.). MoPn inclusions were detected with sequential incubations in MAb MP-33b, biotinylated horse anti-mouse immunoglobulin (Vector Laboratories), and streptavidin-peroxidase (Biogenex, San Ramon, Calif.). Use of 3,3′-diaminobenzidine substrate (Vector Laboratories) for color development was followed by counterstaining with 3% methyl green.

RESULTS

Characterization of MAbs. To compare the protective efficacies of IgA and IgG against chlamydial genital tract infection, a MAb of each isotype having similar specificity was selected. Dot blot analysis (Fig. 1) demonstrated that both MP-A5d (IgA) and MP-33b (IgG3) reacted with the native surface of MoPn EBs and not with *C. trachomatis* serovar A or *C. psittaci* GPIC, when compared with EVI-H1, a genus-specific IgG2a MAb. Immunoblot analyses (Fig. 2) using EB lysates confirmed the specificity of MP-A5d and MP-33b for MoPn and also indicated that both MAbs were specific for the MOMP of MoPn.

Figure 3 shows immunoblots of purified MAbs in which the heavy chains were detected by specific antibodies to determine whether polymeric IgA was produced by MP-A5d and MOPC-315, the negative control IgA MAb used in the protection experiments described below. Fully reduced and denatured MOPC-315 (lane 1) and MP-A5d (lane 2) contained alpha chains that migrated at 55 to 60 kDa. When those same two MAbs were denatured under nonreducing conditions (lanes 3 and 4), both monomeric IgA and dimeric IgA (dIgA) were present. The apparent molecular masses of monomeric IgA and dIgA were lower than expected because of the absence of covalent linkages between heavy and light chains in IgA from BALB/c mice (21) and hence the dissociation of light chains in SDS sample buffer. For comparison, IgG3 MAb MP-33b sol-

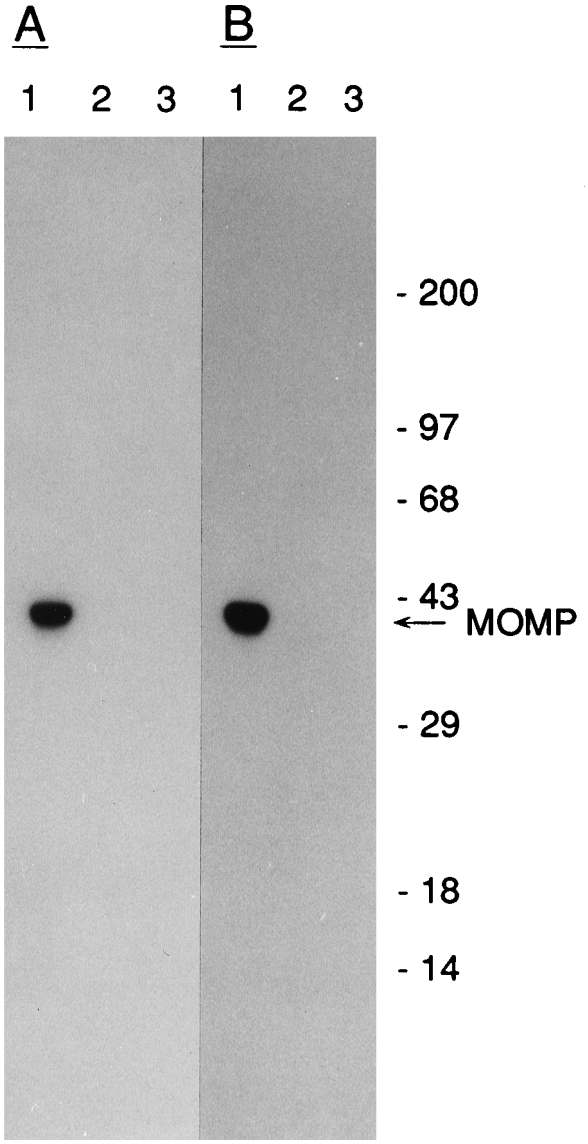


FIG. 2. Immunoblot analysis of reactivity of MP-A5d (IgA) and MP-33b (IgG3) with lysates of chlamydial EBs. Lanes: 1, *C. trachomatis* MoPn EBs; 2, *C. trachomatis* serovar A EBs; 3, *C. psittaci* GPIC EBs. Duplicate immunoblots were reacted with MP-33b (A) and MP-A5d (B). Molecular size markers are indicated on the right in kilodaltons. Both MAbs were specific for the MOMP of MoPn.

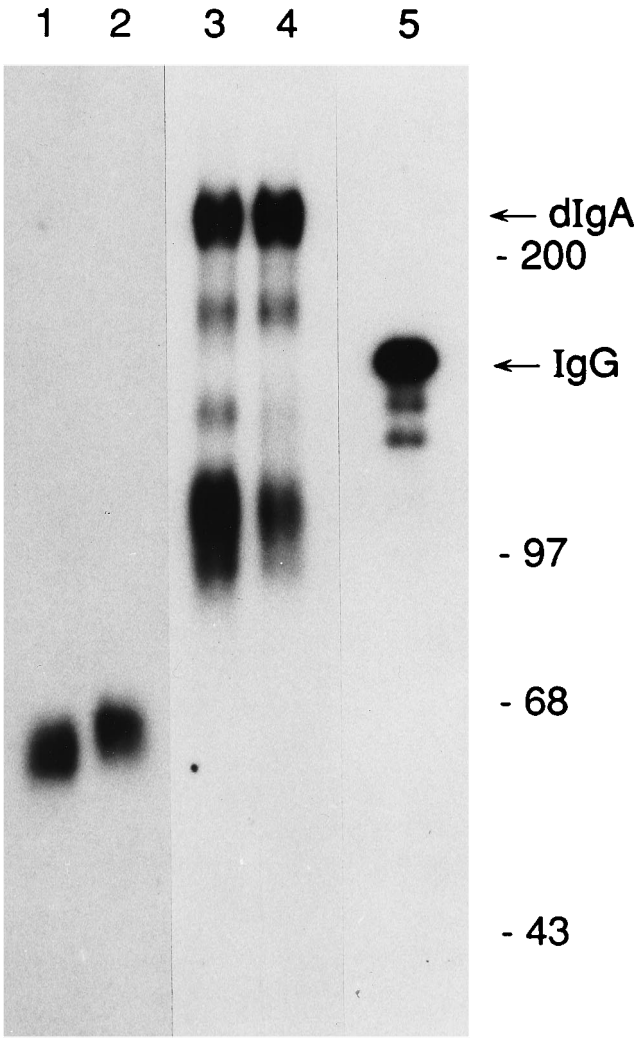


FIG. 3. Detection of dIgA by immunoblot analysis of MAbs MP-A5d and MOPC-315 solubilized under reducing and nonreducing conditions. Lanes: 1 and 3, MOPC-315 solubilized under reducing (lane 1) and nonreducing (lane 3) conditions; 2 and 4, MP-A5d solubilized under reducing (lane 2) and nonreducing (lane 4) conditions; 5, MP-33b (IgG3) solubilized under nonreducing conditions. Molecular size markers are indicated on the right in kilodaltons. Both preparations of MOPC-315 and MP-A5d contained monomeric IgA and dIgA.

ubilized under nonreducing conditions is also shown (lane 5). These results indicated that dIgA was produced by MP-A5d and MOPC-315, theoretically allowing the specific recognition and transcytosis of these MAbs onto mucosal surfaces by polymeric immunoglobulin receptors located on epithelial cells.

Both MP-A5d and MP-33b were unable to neutralize the in vitro infectivity of MoPn for either HaK or HeLa 229 cells (data not shown). In fact, this group has never observed neutralization of MoPn by any antibody preparation, monoclonal or polyclonal, and so it was decided to proceed with these antibodies in the in vivo protection experiments because they exhibited strong reactivity with surface-exposed MOMP epitopes, characteristics similar to those of other MAbs that have been previously shown to neutralize infectivity for human *C. trachomatis* biovars for cultured cell lines, as well as relevant animal tissue (26).

Antibody-mediated protection experiments. The backpack tumor system (25) was used to deliver the MAbs described above into serum and secretions of naive female BALB/c mice, and the protective efficacy of MP-A5d and MP-33b was assessed by comparing their effects on vaginal shedding with that of the negative control (MOPC-315) at various times postchallenge. The data presented here result from two experiments. Experiment 1 tested the ability of each MAb to protect naive mice from vaginal challenge with three different doses, and experiment 2 was designed to confirm the protection against the low challenge dose seen in experiment 1 by using larger numbers of animals.

Experiment 1. Three different challenge doses, termed low, medium, and high, corresponding to 5, 50, and 500 ID_{50}, respectively, were used in experiment 1. Group sizes were as follows: 30 animals bearing MP-33b tumors (10 for each challenge dose), 33 animals bearing MP-A5d tumors (11 for each challenge dose), and 33 animals bearing MOPC-315 tumors (11 for each challenge dose). All animals carrying either MP-A5d or MOPC-315 tumors had detectable MAb in serum and vaginal secretions. All animals carrying MP-33b tumors had MAb in serum, and 25 (83%) of 30 had detectable MAb in vaginal secretions. Group mean levels of MAb in serum and vaginal secretions measured at the time of challenge are presented in Table 1. The mean MAb concentration in MP-33b animal serum was 4- and 11-fold greater than that of MOPC-315 and MP-A5d group animals, respectively. MAb levels in vaginal secretions were also highest in the MP-33b group, although the difference between the groups was less than twofold. These differences translated into more MAb in vaginal

TABLE 1. MAb levels in sera and vaginal secretions of mice in experiments 1 and 2

MAb-tumor group	No. of mice[a]	Mean MAb concn (ng/ml) in secretions (SD, SEM)[b]	Mean MAb concn (μg/ml) in sera (SD, SEM)[c]	Ratio of MAb concns in secretions/sera	Correlation[d]	
					r	*P*
Expt 1						
MOPC-315 (IgA)	33	166 (173, 30)	92 (66, 12)	1.8	0.53	0.002
MP-A5d (IgA)	33	238 (298, 52)	35 (29, 5)	6.7	0.73	<0.001
MP-33b (IgG)	30	305 (374, 68)	395 (67, 12)	0.8	0.50	0.005
Expt 2						
MOPC-315 (IgA)	25	434 (289, 57)	86 (29, 6)	5.0	0.58	0.003
MP-A5d (IgA)	25	326 (335, 67)	46 (21, 4)	7.1	0.82	<0.001
MP-33b (IgG)	25	449 (541, 108)	341 (69, 14)	1.3	0.07	0.739

[a] Total number of animals involved in statistical analysis of each group.
[b] Mean MAb concentration in vaginal secretions of each group.
[c] Mean MAb concentration in serum of each group.
[d] Correlation between MAb concentrations in serum and secretions of individual animals within each group determined by using the nonparametric Spearman rank correlation test. *P* values are from two-tailed analyses.

secretions per unit of MAb in serum for MOPC-315 and MP-A5d than for MP-33b (Table 1). These results indicated that IgA was more efficiently translocated from serum onto mucosal surfaces, possibly because of the recognition and translocation of dIgA by polyimmunoglobulin receptors on genital tract epithelial cells. Finally, correlation analysis revealed a significant correlation between MAb levels in serum and secretions within individual animals in all three groups from experiment 1 (Table 1).

Postchallenge recovery of MoPn from cervicovaginal swabs in experiment 1 is presented in Fig. 4. Figure 4A shows the number of animals in each tumor group from which any MoPn was recovered at days 2, 4, 6, and 8 following vaginal challenge with the various doses described above. With the low challenge dose (5 ID_{50}), there were reduced percentages of infected animals in both the MP-A5d and MP-33b groups compared with the MOPC-315 control group at all culture time points. The consistent reduction in the number of infected animals over time suggested that the presence of MOMP-specific MAb in serum and vaginal secretions had affected the ability of MoPn to colonize the murine genital tract. The effects obtained with the low challenge dose were not obtained with the 10- and 100-fold higher doses, indicating that the protection afforded by the MAbs was limited and could be overwhelmed. It should be noted that the reason for the decrease in the number of animals over time in each group was due to the fact that some animals had to be euthanized prior to day 8 because of rapid tumor growth and declining overall health. Quantitative determinations of chlamydial recovery also allowed us to determine whether the MOMP-specific MAbs reduced shedding relative to the negative control. Comparison of recovery means for each group with each challenge dose was done by using only data from those animals that were shedding MoPn at each time point, for the most conservative estimate of the effect of the MOMP-specific MAbs (Fig. 4B). At all time points and with all challenge doses, the mean recovery of MoPn was lower from animals bearing either MP-A5d or MP-33b tumors than from animals bearing the negative control MOPC-315 tumors. MP-33b was more effective in reducing shedding than was MP-A5d with all challenge doses on the basis of the number of time points at which either group was significantly different from MOPC-315 ($P < 0.05$, two-tailed Wilcoxon test; Fig. 4B). These data indicate that at the levels measured during this experiment, the presence of IgG or IgA MAb can reduce vaginal shedding but has minimal to no effect on the resolution of an established primary infection.

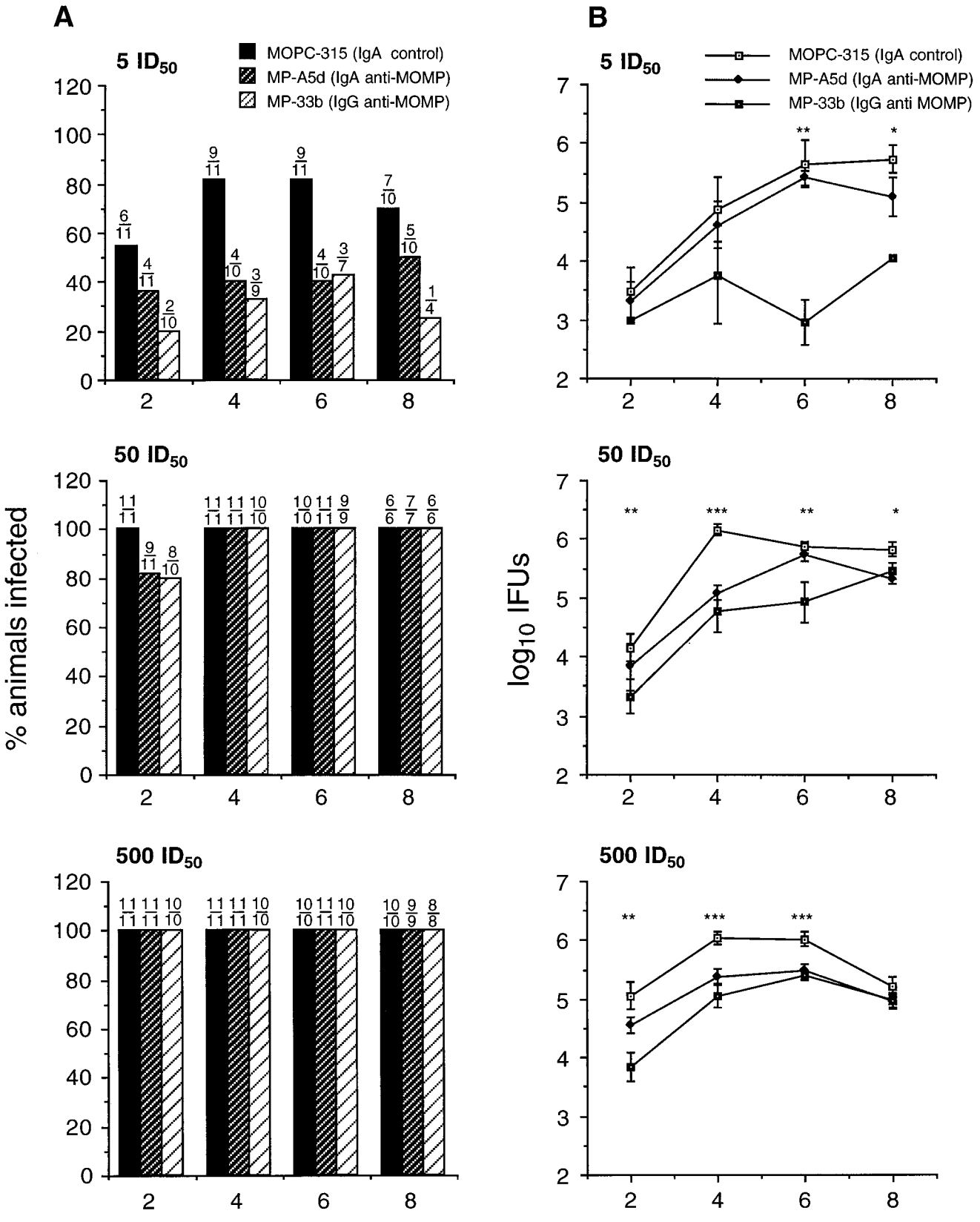
Experiment 2. Experiment 2 was designed to confirm the protection obtained with the low challenge dose used in experiment 1 with larger group sizes. Each MAb-tumor group contained 25 mice, and all animals were challenged with 5 ID_{50} immediately following measurement of MAb levels in vaginal secretions. All animals in experiment 2 had MAb in serum, and all but one animal in the MOPC-315 group and two animals in the MP-33b group had detectable MAb in vaginal secretions. Group mean MAb levels in serum and vaginal secretions measured at the time of challenge are presented in Table 1. In general, the relative MAb concentrations among the three groups were similar to those measured in experiment 1. Table 1 also shows that there was a significant correlation between MAb concentrations in serum and secretions within individual animals in the MOPC-315 and MP-A5d groups, but unlike experiment 1, that correlation was absent in animals from the MP-33b group.

The percentage of animals within each MAb-tumor group from which any MoPn organisms were recovered from cervicovaginal swabs at 2, 4, 6, and 8 days postchallenge and the corresponding quantitative infected-animal recovery means are depicted in Fig. 5. As in experiment 1, the MP-A5d and MP-33b tumor groups had a lower incidence of infection than did the negative control group with the low challenge dose of 5 ID_{50}; however, in experiment 2 the differences were significantly different ($P < 0.05$, two-tailed Fisher's exact test), with MP-33b different from MOPC-315 at all four time points and MP-A5d different from MOPC-315 at days 4 and 6 (Fig. 5A). Recovery of MoPn from infected MP-A5d animals was not significantly different from that from the MOPC-315 group at any time point ($P > 0.05$, two-tailed Wilcoxon test; Fig. 5B). The small number of infected MP-33b animals at all time points prevented a similar comparison.

The combined chlamydial recovery results from experiments 1 and 2 indicated that both IgA and IgG antibodies with singular specificity had the ability to prevent infection of the murine genital tract following a challenge with 5 ID_{50} (75 IFUs) and that this protection was overwhelmed by 10- and 100-fold higher challenge doses. Furthermore, both anti-MOMP IgA and IgG reduced vaginal shedding of MoPn, especially with the medium and high challenge doses; however, this effect was not seen at all time points postinfection.

Histopathology. Histopathological evaluation was performed by using the entire genital tracts of 102 of the 104 animals that survived to day 8 postchallenge and was scored for both the type and severity of the inflammatory response. Figure 6A to D shows representative examples of hematoxylin-and-eosin-stained control oviduct tissue and MoPn-infected oviduct tissue exhibiting a marked inflammatory response. Those animals that were not shedding MoPn had no detectable inflammation at the time of sacrifice. Most of the inflammation seen in all groups was subacute (94%), and the rest was acute. Comparative histopathological analysis of vaginal, cervical, and uterine tissues of all of the challenge groups revealed a lower cumulative incidence of inflammation in anti-MOMP-treated animals than in control mice, but because only a mild inflammatory response was observed in these tissues it was not possible to analyze the findings objectively and they are therefore not presented. Conversely, histopathological analysis of oviduct tissue revealed substantial differences in the incidence of marked inflammation between control and anti-MOMP-treated animals. Most of the control mice given high and moderate challenge inocula showed marked subacute inflammation in the oviducts, whereas both IgA and IgG anti-MOMP-treated groups showed a reduction in the percentages of animals with marked inflammation (Fig. 7). This was particularly true for the IgG group, in which none of the animals given any challenge dose had marked inflammation of the oviduct tis-

FIG. 4. Recovery of MoPn from cervicovaginal swabs following vaginal challenge with three different doses in experiment 1. (A) Percentage of animals shedding MoPn at each culture time point. The fraction above each bar indicates the number of animals from which any MoPn was recovered over the total number challenged in that group. There were no significant differences ($P > 0.05$, two-tailed Fisher's exact test) between either MP-A5d or MP-33b and the negative control MOPC-315 group. (B) Mean recovery of MoPn from animals shedding MoPn ($\pm$ the standard error of the mean). The number of animals included in each mean is the numerator of each fraction above the corresponding bar in A. The asterisks at each time point indicate statistically significant differences between group means ($P < 0.05$, two-tailed Wilcoxon nonparametric test) as follows: *, MP-A5d differs from MOPC-315; **, MP-33b differs from MOPC-315; ***, both MP-A5d and MP-33b differ from MOPC-315.


A
B
5 ID50
50 ID50
500 ID50
MOPC-315 (IgA control)
MP-A5d (IgA anti-MOMP)
MP-33b (IgG anti-MOMP)
% animals infected
log10 IFUs
Days

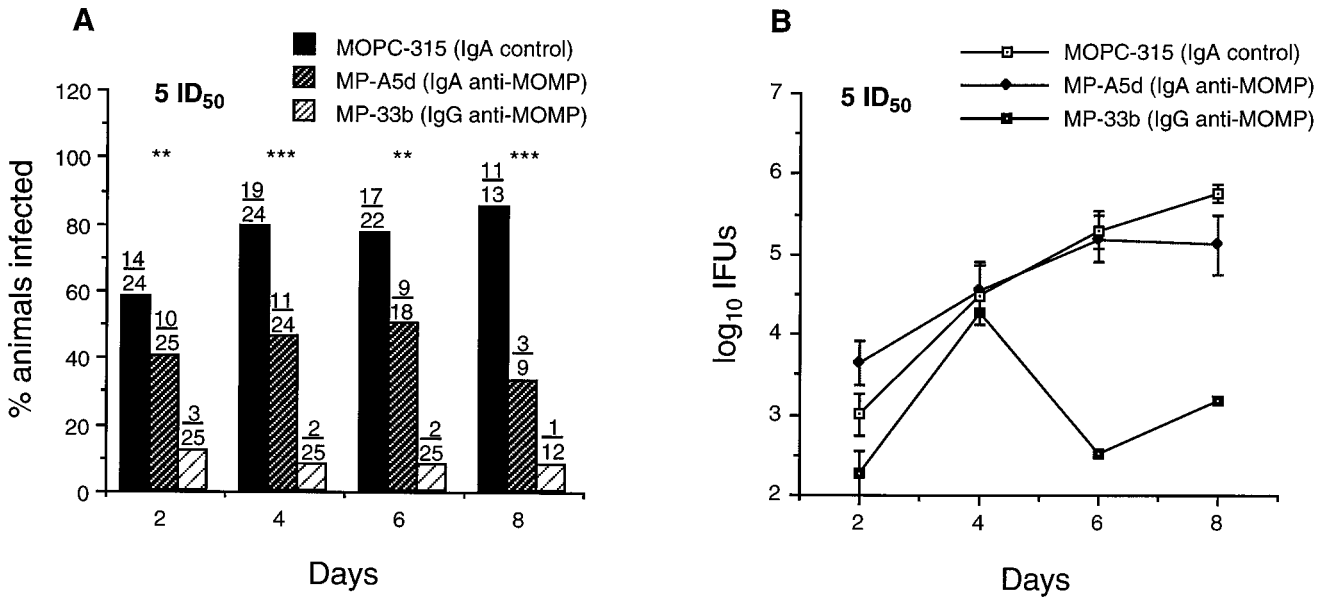


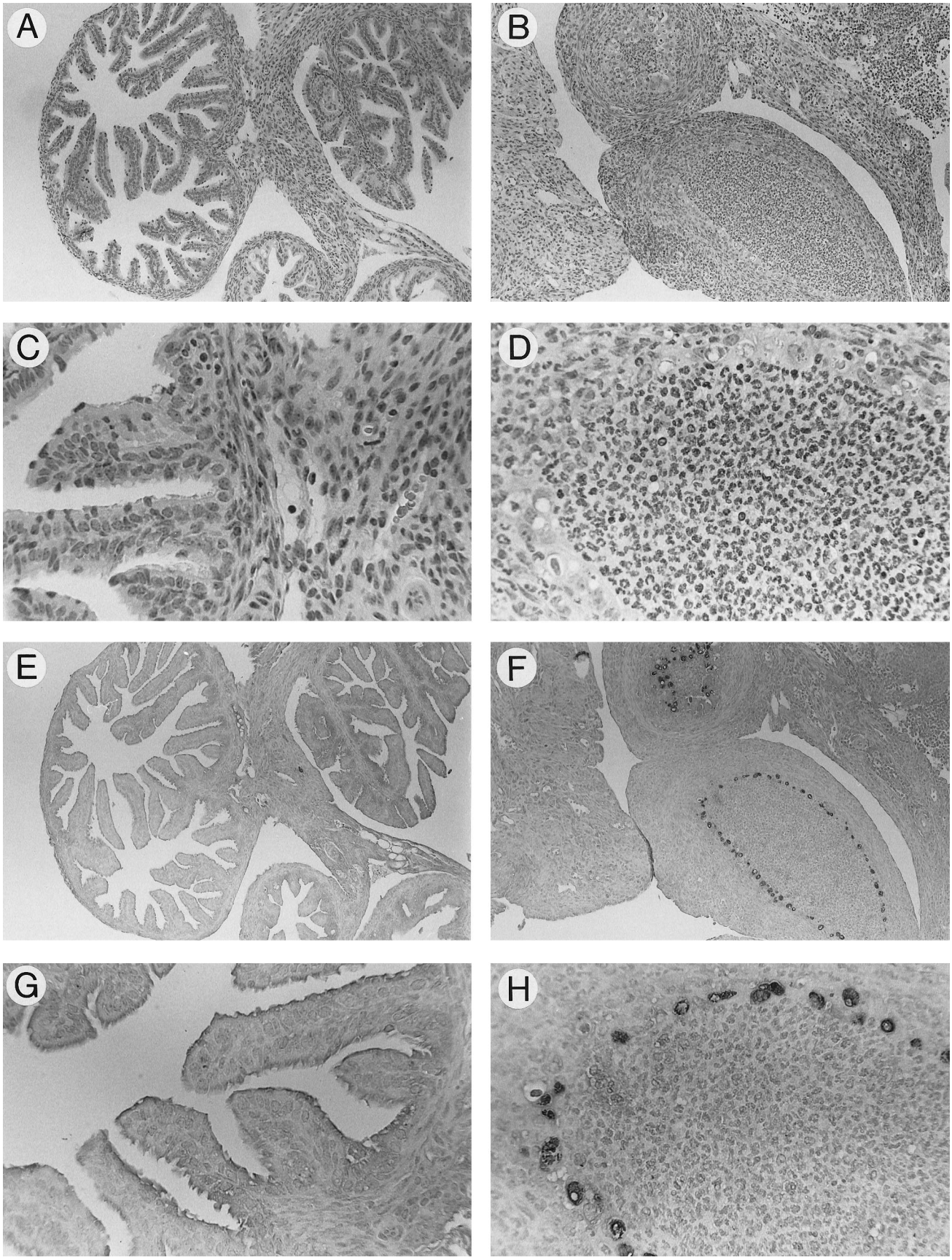
FIG. 5. Recovery of MoPn from cervicovaginal swabs following vaginal challenge with 5 ID_{50} in experiment 2. (A) Percentage of animals shedding MoPn at each culture time point. The fraction above each bar indicates the number of animals from which any MoPn was recovered over the total number challenged in that group. The asterisks at each time point indicate statistically significant differences between group means ($P < 0.05$, two-tailed Fisher's exact test) as follows: **, MP-33b differs from MOPC-315; ***, both MP-A5d and MP-33b differ from MOPC-315. (B) Mean recovery of MoPn from animals shedding MoPn (± the standard error of the mean). The number of animals included in each mean is the numerator of each fraction above the corresponding bar in A. There were no significant differences ($P < 0.05$, two-tailed Wilcoxon nonparametric test) between either MP-A5d or MP-33b and the negative control MOPC-315 group.

sues. These data demonstrate that the presence of IgG or IgA MAbs specific to MOMP had a significant effect on both the incidence and severity of the inflammatory response in the oviducts of mice 8 days post vaginal infection. Moreover, as was seen in the results from the protection and shedding experiments, the IgG MAb was more efficacious than the IgA MAb in reducing oviduct pathology.

Detection of chlamydial inclusions in genital tract tissue. Fixed, paraffin-embedded tissues were also sectioned for immunohistochemical detection of chlamydial inclusions. Figure 6E to H presents representative fields of normal and MoPn-infected oviduct tissues showing chlamydial inclusions localized to the mucosal epithelium. For each animal, a single longitudinal section of the entire genital tract was stained and each region (vagina-cervix, uterus, and oviduct) was evaluated for the presence of inclusions. In no instance were inclusions detected in tissues from animals that were not shedding MoPn at the time of sacrifice. The stained tissues were analyzed in a qualitative manner because a meaningful quantitative analysis would have required that chlamydial inclusions be enumerated in several serial sections from each animal; this was not possible because of the extremely high number of inclusions seen in many of the tissues.

In the medium and high challenge dose negative control groups, in which all animals became infected, inclusions were sparsely distributed in the vaginal-cervical epithelium of all animals and in the uterine epithelium of 45% of the animals. A much higher density of inclusions was seen in the epithelium of virtually all oviducts in these challenge groups. The uteruses of all medium and high challenge dose negative control animals were inflamed and denuded, suggesting that the chlamydiae had already infected and destroyed the uterine epithelium by 8 days postinfection. The distribution and numbers of inclusions in the low challenge dose control group were not as uniform. When stained tissues from IgA and IgG anti-MOMP antibody-treated animals were compared with the negative controls, there were noticeably fewer inclusions in all regions of the genital tract, particularly in the IgG-treated animals. Of potentially greatest significance was the reduction of detectable oviduct infection in animals bearing anti-MOMP hybridoma tumors. Figure 8 shows the substantial reduction in the percentage of oviducts with any detectable inclusions in the IgA and IgG anti-MOMP antibody-treated animals compared with control animals with all of the challenge doses. The absence of chlamydial inclusions in a single thin section is not proof of a complete lack of oviduct infection; however, these findings do indicate that the presence of MOMP-specific antibody reduced the infectious burden in the oviduct relative to the negative control antibody at day 8 postinfection. The reduced chlamydial burden in the oviducts is consistent with the absence or reduced intensity of inflammation in the oviducts of IgG and IgA anti-MOMP antibody-treated mice. Collectively, these findings strongly support a protective role for anti-MOMP antibodies in reducing the infectious burden and accompanying pathological changes of the upper genital tracts of vaginally challenged mice. It cannot, however, be determined whether infection would eventually have ascended into those oviducts that were not infected at day 8 postinfection.

FIG. 6. Examples of histopathology and immunohistocytochemistry of oviduct tissue. Representative examples of normal tissue (A, C, E, and G) and MoPn-infected tissue (B, D, F, and H) were compared by using hematoxylin-eosin (A, B, C, and D) and immunostaining of MoPn inclusions (E, F, G, and H). (C, D, G, and H) Higher magnifications of fields within A, B, E, and F, respectively. (B and D) Representative examples of marked inflammation exhibiting intense submucosal and lumenal infiltrates and edema. (F and H) Representative examples of ascending MoPn infection to the oviduct epithelium in control animals at day 8 postchallenge. Magnifications: A, B, E, and F, ×100; C, D, G, and H, ×400.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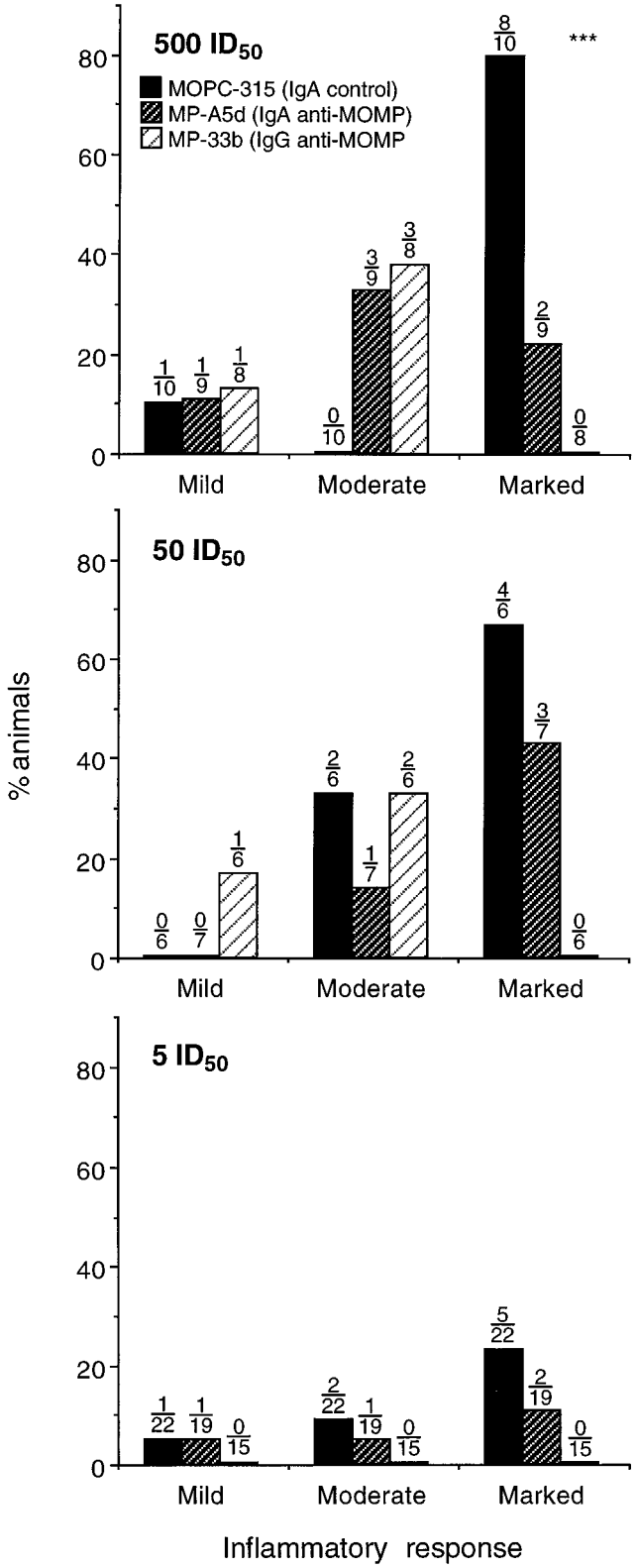

FIG. 7. Incidence and intensity of oviduct inflammation in all challenge groups. The fraction above each bar indicates the number of animals exhibiting the designated degree of inflammation over the total number of animals examined in that challenge group. The asterisks indicate that both MP-A5d and MP-33b are statistically significantly different from MOPC-315 ($P < 0.05$, two-tailed Fisher's exact test).

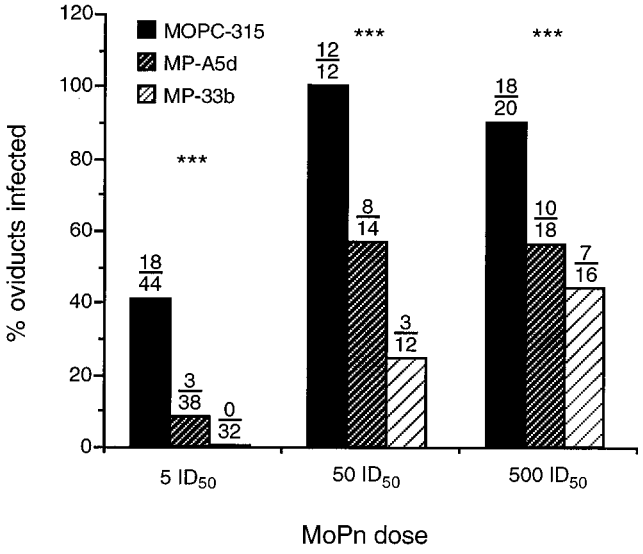

FIG. 8. Incidence of MoPn infection in oviduct tissue. The fraction above each bar indicates the number of oviducts with any detectable MoPn inclusions over the total number of oviducts examined in that challenge group. The asterisks indicate that the group means of both MP-A5d and MP-33b are statistically significantly different from that of MOPC-315 ($P < 0.05$, two-tailed Fisher's exact test).

DISCUSSION

The role of antibody in protective immunity to chlamydial infections of mucosal tissues has remained unclear. We have utilized the backpack tumor system (25) to deliver high levels of MAbs specific for surface-exposed MOMP epitopes of *C. trachomatis* MoPn into serum and vaginal secretions of naive mice, allowing the antibody effects to be assessed in the absence of other acquired immune responses. These studies have shown that the presence of such antibodies had positive effects on the incidence of infection, shedding, and ascending infection and the development of upper genital tract disease over the first 8 days of a 21- to 28-day infection. This work provides the first direct evidence that delivery of chlamydia-specific antibody from the submucosae into secretions can protect an animal from chlamydial infection. These results extend and confirm earlier work that demonstrated antibody-mediated protection from infection by either preincubation of chlamydiae with specific antibody (26) or topical application of such antibody to the mucosal surface immediately prior to challenge (24) and also the reduction of chlamydial shedding following passive transfer of chlamydia-specific antiserum (14).

Quantitative recovery of MoPn from cervicovaginal swabs demonstrated that both IgA and IgG significantly reduced the incidence of genital tract infection following vaginal challenge with 5 ID_{50} but had a limited ability to reduce shedding in animals that became infected. With 10- and 100-fold higher challenge doses, the protective effects were overwhelmed but the modest reduction of vaginal shedding was maintained. Su et al. (19) found a similar protective effect in mice vaccinated with a MOMP synthetic peptide immunogen that were challenged vaginally with human *C. trachomatis* serovar D. In those studies, mice that had high IgG anti-MOMP antibody levels in serum and detectable antibodies in vaginal washes showed a decrease in the infection rate that was accompanied by a modest reduction in vaginal shedding. It was not possible for Su et al. to address the effect of anti-MOMP antibodies on disease outcome however, since the human *C. trachomatis* strain used

in their work failed to generate a significant inflammatory response in the submucosa of the genital tract. Although strictly speculative, protection from a challenge of 5 ID_{50} may be significant when put in the context of available information on the transmission rate of human chlamydial genital serovars. Vaginal challenge with 5 ID_{50} caused infection in 80% of the negative control animals in this study, compared with a transmission rate in humans of about 60 to 70% (11). This suggests that the transmitted dose in humans might be lower than 5 ID_{50}, and therefore protection from such a challenge dose may be clinically important; however, the dose transmitted between humans is not known. The recovery data were supported by immunochemical staining, which showed an overall reduction of the chlamydial burden, as well as a reduction in the incidence of ascending infection. Animals treated with either antibody had a reduced incidence of inflammation in the vagina, cervix, and uterus and a reduction in both the incidence and severity of oviduct inflammation at 8 days postinfection. The reduction of ascending infection and severity of the inflammatory infiltrate in the oviduct might represent a very important finding in that the equivalent human organ, the fallopian tube, is the primary site of complicated chlamydial infections with sequelae that include pelvic inflammatory disease, ectopic pregnancy, and infertility. Our findings suggest that anti-MOMP antibodies might have a significant effect in either reducing or preventing chlamydia-caused fallopian tube tissue injury.

It is curious that the IgA and IgG MOMP-specific MAbs had protective effects in vivo but were unable to neutralize MoPn infectivity in vitro under conditions in which other *C. trachomatis* serovars are neutralized by MAbs specific for surface-exposed MOMP epitopes (18). We could not conclude that these two MAbs were specific for nonneutralizing epitopes because we have never been able to neutralize MoPn infectivity with any antibody preparation in the absence of complement, including high-titer convalescent-phase sera. This fact may be due to different surface characteristics of MoPn, different mechanisms for adherence and/or internalization, inappropriate neutralization assays or target cell lines, or any combination of the above. It is not unreasonable, however, that we observed differences in in vitro and in vivo protection results since antibodies may function by a multitude of effector mechanisms in vivo in addition to direct neutralization of chlamydial infectivity. These could include, but not be limited to, opsonization and clearance by macrophages-polymorphonuclear cells and complement-mediated killing of chlamydiae in the case of the IgG anti-MOMP antibody.

The reasons for the greater efficacy in these studies of the IgG MAb than that of the IgA MAb in virtually all aspects of controlling chlamydial infection and genital tract tissue disease are unknown. Of significance in this regard is the fact that we were unable to determine directly whether the MP-A5d in vaginal secretions was actually sIgA, even though the amount of IgA in secretions per unit of serum relative to that of IgG suggests that the IgA was sIgA. It is possible, for whatever reason, that the backpack tumor system is not effective in delivering dIgA as sIgA into genital tract secretions. In the absence of this information, it is premature to conclude that IgG antibodies are more effective than IgA antibodies since sIgA is known to be more resistant than dIgA to proteases present in secretions and is therefore thought to be more effective (8, 20). The slightly higher mean level of the IgG MAb in vaginal secretions was an unexpected finding and may have been due to the fact that most of the dIgA in the bloodstream is cleared by the liver into bile (17). Conversely, enhanced levels of IgG in secretions could be the result of serum transudation onto the genital tract mucosae. The upper genital tract (uterus and oviducts) is highly vascularized, and this could lead to substantial transudation of serum antibodies directly onto these surfaces, as well as their deposition onto the much less vascularized mucosal surfaces of the cervix and vagina. It is possible that the enhanced level of protection observed in the oviducts of IgG-treated mice was directly related to increased levels of serum antibodies at this tissue site as a result of its greater vascularization.

Previous work involving B-cell-deficient mice has indicated that antibody is not required for resolution of pulmonary (23) or genital tract (12) infection by MoPn or for immunity to reinfection (12). The results from the studies described here do not argue against these conclusions but rather indicate that antibody can partially protect naive animals from genital tract infection, particularly against upper genital tract disease, an issue not addressed by the earlier work. The lack of a requirement for antibody in resistance to reinfection may be in part due to compensatory immunologic activities elicited in animals that are rendered B cell deficient from birth and does not indicate whether antibody plays a contributing role in natural resistance to reinfection in a healthy, immunocompetent animal.

Such considerations of earlier work, combined with data from the experiments described here, make it premature to discount the potential value of immunization designed to elicit antibody responses against chlamydiae. The prospects are not good for the development of vaccines that provide sterilizing immunity against chlamydial infections. It may, therefore, be reasonable to pursue vaccine strategies that are capable of preventing ascending chlamydial infections and their accompanying long-term sequelae, such as pelvic inflammatory disease. Certainly, the data presented here do not conclusively demonstrate that antibody alone can achieve these goals, but they do strongly suggest that antibody has the potential to reduce the risk and severity of pelvic inflammatory disease. Further utilization of the murine model of chlamydial genital tract infection will allow us to determine whether similar protective effects can be achieved following parenteral and/or mucosal immunization with recombinant MOMP, or synthetic peptides corresponding to T-helper and B-cell epitopes, to determine whether such protection can be maintained throughout the course of a primary infection, and whether a protective response can be recalled after extended periods following immunization when peak antibody titers have diminished. Aside from the issues of duration and recall of antibody-mediated protection in the murine model, it remains unclear whether protection from MoPn genital tract infection has relevance to protection from infection by human oculogenital serovars. This issue can be most decisively addressed with a primate model of chlamydial genital tract infection employing human *C. trachomatis* biovars. Lastly, the levels of serum and secretory anti-MOMP antibodies described in this study almost certainly exceed the levels of antichlamydial antibodies normally generated as a result of natural chlamydial infection. Thus, it is not known if decreased levels of serum or secretory antibodies would have the same beneficial effect against upper genital tract pathology in humans.

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