The Protective Effect of Antibody in Immunity to Murine Chlamydiae Genital Tract Reinfection Is Independent of Immunoglobulin A

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The resolution of primary and secondary chlamydial genital infection in immunoglobulin A (IgA)-deficient (IgA−/−) mice was not different from that in IgA+/+ mice. Furthermore, depletion of either CD4+ or CD8+ T cells prior to reinfection of IgA−/− or −/+ mice had limited impact on immunity to reinfection. Thus, although antibody contributes importantly to immunity to chlamydial genital tract reinfection, IgA antibodies are not an absolute requirement of that protective response.

Sexually transmitted infections due to Chlamydia trachomatis cause considerable morbidity and socioeconomic burden worldwide. Four million new cases of chlamydial sexually transmitted diseases are reported annually in the United States, and costs associated with the management of these infections exceed $2 billion (7). Vaginal inoculation of the female mouse with Chlamydia muridarum (C. trachomatis strain mouse pneumonitis) closely mimics acute genital infection of women and provides a reasonable model in which to study adaptive immunity (8). Female mice develop a self-limiting infection that originates in the lower genital tract, ascends approximating infection that originates in the lower genital tract, ascends approximately 4 weeks (1, 9). Mice that resolve infection are markedly resistant to reinfection (9), and CD4+ Th1 T-cell responses are arguably the most vital elements of protective immunity (3, 4, 6, 10, 11, 15, 20). Recently, however, we have demonstrated that antibodies (B cells) play a key role in adaptive immunity to genital tract reinfection (10, 11).

Chlamydiae predominantly infect mucosal epithelial cells and cause disease at mucosal surfaces, and thus the mucosal immune response has long been predicted to be important in antichlamydial adaptive immunity. Antichlamydial immunoglobulin A (IgA) antibodies are found in both the serum and genital tract secretions following murine chlamydial genital infection (9), and antichlamydial IgA antibodies have been associated with resolution of infection in women (2). Our previous studies reveal an important role for antibody in adaptive immunity to chlamydial genital tract reinfection (10, 11). In those studies we demonstrate that mice deficient in both CD4+ T cells and antibody are unable to resolve secondary chlamydial infection, whereas mice deficient in only CD4+ T cells or B cells resolve chlamydial reinfection. Those results clearly define a previously unrecognized role for antibody in immunity to chlamydial genital tract reinfection. However, the results could not distinguish the relative contribution of IgA in immune protection because the antibody deficiency was panspecific (i.e., absence of all classes of immunoglobulins). Knowing whether the protective efficacy of the antichlamydial antibody response is solely dependent on IgA antibodies is of importance not only because chlamydia cause mucosal infection, but also because the composition of experimental chlamydial vaccines and vaccination protocols will be impacted by the need to elicit antichlamydia IgA responses. In the present study we evaluated the role of IgA antibodies in adaptive immunity to chlamydial reinfection using mice with a targeted disruption in the switch region and a-heavy chain locus (IgA−/−).

Breeding pairs of C57BL/6 × 129 IgA-deficient (IgA−/−) mice and C57BL/6 × 129 F2 (IgA+/−) mice (wild-type control) were generated as previously described (5) and provided as a kind gift by I. N. Mbawuike, Baylor College of Medicine, Houston, Tex. All animal procedures were in accordance with institutional policies for animal health and well-being and were approved by the institutional animal care and use committee. The targeted mutation was confirmed as described previously (22). Methodologies used for infection, enumeration of inclusion forming units (IFUs), T-cell subpopulation depletion, and antichlamydial antibody titration have been reported in detail previously (9, 11) and are only briefly described here. Eight- to 12-week-old female mice were treated with Depo-Provera 5 days prior to infection. Mice were inoculated vaginally with 100 50% infective doses of C. muridarum (5 × 104 IFUs), and infection was followed by enumeration of IFUs from vaginal-cervical swabs collected at various times throughout the course of infection. To assess the role of IgA in adaptive immunity to chlamydial reinfection, mice that had resolved primary infection were depleted of either CD4+ or CD8+ T cells prior to secondary infectious challenge. Groups of mice were injected with anti-CD4, anti-CD8, or phosphate-buffered saline (PBS) on days 56, 57, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, and 88 after primary infection. Depo-Provera-treated mice (day 57 after primary infection) were rechallenged (secondary infection) on day 62 after primary infection. The T-cell depletion scheme described above has been shown to effectively deplete CD4+ and CD8+ T-cell subpopulations prior to infectious challenge and throughout the course of the study period (9) and was confirmed for these studies (data not shown). Antichlamydial antibody titers were determined using a C. muridarum elementary body enzyme-linked immunosorbent assay and isotype-specific detection antibodies (9).
The course of primary chlamydial genital tract infection of IgA<sup>+</sup> and IgA<sup>-</sup> mice was indistinguishable from that of IgA<sup>+</sup> mice (Fig. 1). Neither the shedding of infectious chlamydiae nor the duration of infection was different between the strains at any of the time points analyzed. The finding that IgA<sup>+</sup> mice resolved primary infection comparably to IgA<sup>-</sup> mice was not unexpected. We had previously shown the resolution of primary infection in antibody-deficient mice was nearly identical to that in antibody-positive wild-type mice (21) and thus anticipated that animals deficient in only IgA would respond similarly.

The protective role of antibody in murine chlamydial genital infection has only been clearly established in immunity to reinfection (10, 11). The experimental approach used previously for demonstrating the role of antibody in immunity to reinfection was used in the present study to determine if the protective effect of antibody was limited to IgA or if other immunoglobulin classes contributed to the protective response. The ability of IgA<sup>+</sup> and IgA<sup>-</sup> mice to resolve a secondary infectious challenge was evaluated in nondepleted (PBS-treated), CD4<sup>+</sup> T-cell-depleted (anti-CD4-treated), or CD8<sup>+</sup> T-cell-depleted (anti-CD8-treated) mice that had resolved primary infection. Both IgA<sup>+</sup> and IgA<sup>-</sup> mice were markedly immune to reinfection (PBS treated; Fig. 2A). Similarly, depletion of CD8<sup>+</sup> T cells had no effect on the course of secondary infection (Fig. 2B). Although depletion of CD4<sup>+</sup> T cells extended the duration of secondary infection (Fig. 2C) (compared to nondepleted and CD8-depleted mice), in both IgA<sup>+</sup> and IgA<sup>-</sup> mice, CD4-depleted mice shed far fewer infectious bacteria than during primary infection (4 to 5 log<sub>10</sub> lower IFU counts) and resolved secondary infection in the absence of CD4<sup>+</sup> T cells. A greater number of CD4-depleted mice remained culture positive for a longer duration than CD8-depleted or nondepleted mice (Table 1), but the magni-

![FIG. 1. Primary C. muridarum genital tract infection of female IgA<sup>+</sup> and IgA<sup>-</sup> mice. Depo-Provera-treated mice were infected intravaginally with approximately 50,000 IFUs of C. muridarum elementary bodies. Infection was monitored by swabbing the vaginal vault and enumerating IFUs on HeLa cell monolayers (9). Data are presented as log<sub>10</sub> IFUs and represent the means ± standard errors of the means of triplicate determinations of 18 mice per group. IFUs recovered from IgA<sup>+</sup> and IgA<sup>-</sup> mice were not statistically different at any time point (Student’s t test).](http://iai.asm.org/)
IgA

Heightened IgG responses have been reported previously for antichlamydial IgG2a and IgG2b responses in those mice. The role of antibody in immunity to chlamydial reinfection (11). Antichlamydial antibody responses were evaluated in the groups of IgA+/+ and IgA−/− mice following chlamydial infection (data not shown). Briefly, all mice developed high titers of antichlamydial antibody. IgA−/− mice had somewhat higher total antichlamydial antibody titers than IgA+/+ mice, 65,536 versus 16,384, respectively. Analysis of the immunoglobulin isotype specificity of the antichlamydial responses confirmed the IgA deficiency in IgA−/− mice and indicated heightened antichlamydial IgG2a and IgG2b responses in those mice. Heightened IgG responses have been reported previously for IgA−/− mice (5, 12). IgG1 responses were less than a titer of 16 in both strains of mice, which is typical for murine chlamydial genital tract infection (9).

Previous studies have convincingly shown that B cells play an important role in immunity to chlamydial genital tract reinfection in the mouse (10, 11), and a protective role for antibody has been demonstrated in the guinea pig model of chlamydial genital tract infection (17–19). The purpose of the present study was to specifically address the relative contribution of IgA in this protective response by using IgA-deficient mice and the experimental design used previously to demonstrate the role of antibody in immunity to chlamydial reinfection (11). We reasoned that, because chlamydia are mucosal pathogens and antichlamydial IgA has been associated with lower infectious burdens in women (2), IgA may play a dominant role in protective immunity to reinfection. Our results demonstrate that antichlamydial IgA is not a required component of adaptive antibody-mediated immunity to reinfection. However, the possibility that antichlamydial IgA antibodies function in immunity in the immunocompetent host cannot be ruled out by the results of this study. Thus, the data should not be interpreted to imply that IgA antibodies are not involved in immunity to reinfection in the immunocompetent host. Rather IgA may function in immunity to reinfection, but, because other antibody subclasses also confer protective immunity to reinfection, the elimination of a single antibody class (e.g., IgA) minimally impacts the course of secondary infection. Similarly, if the principal function of IgA is to prevent the establishment of intracellular infection by blocking the attachment of chlamydiae to host cells, we cannot completely rule out the possibility that the challenge dose used for reinfection overwhelmed the neutralizing function of IgA. The contribution of both antichlamydial IgG and IgA to protective immunity is supported by numerous in vitro studies showing the neutralizing capabilities of those antibodies (13, 14, 16, 23, 24) and in vivo data showing antichlamydial IgA as well as IgG in genital tract secretions (2, 9). In summary, the protective efficacy of the antichlamydial antibody response to murine chlamydial genital tract reinfection is not solely dependent on IgA and other classes of antibodies effectively protect against reinfection.

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REFERENCES


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