Fc Receptor–Mediated Antibody Regulation of T Cell Immunity against Intracellular Pathogens

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Immunity to intracellular microbial pathogens, including Chlamydia species, is controlled primarily by cell-mediated effector mechanisms, yet, the absence of antibodies results in inefficient microbial clearance. We investigated the hypothesis that certain Fc receptor functions promote the rapid induction of elevated T helper type 1 (Th1) response, which effectively clears chlamydiae. FcR−/− mice exhibited a delayed and reduced frequency of Chlamydia-specific Th1 cells, compared to FcR+/+ mice. In vitro, antichlamydial antibodies increased the rate of Th1 activation by FcR+/+ but not FcR−/− antigen-presenting cells. FcR−/− dendritic cells and the T cell–associated IgG2A and IgA mediate enhanced Th1 activation by antibodies. Immunization with chlamydia-antibody complexes induced elevated and protective Th1 response. These results provide a mechanistic basis for requiring both T cell and humoral immune responses in protective immunity and vaccine evaluation. Findings offer a paradigm in host defense wherein different effector components function indirectly to maximize the principal effector mechanism.

Designing vaccines against Chlamydia trachomatis require a better understanding of the elements of protective immunity. The current paradigm is that immunity is mediated primarily by a Th1 response [1–5]; however, a complementary role for certain less defined B cell functions ensures an efficient microbial clearance, especially during reinfections [2, 6]. Thus, a potentially protective vaccine should elicit both T cell and humoral immune responses, but the precise role of antibodies has remained undefined. Among the antimicrobial mechanisms of antibodies [7], binding and neutralization of infectious chlamydia particles is apparently ineffective in vivo [8]. However, FcR-mediated effector functions of antibodies, including antibody-dependent cellular cytotoxicity (ADCC) and receptor-mediated endocytosis leading to enhanced antigen presentation, have a potential role in chlamydial immunity. Because there is limited ADCC against infected cells [9] and immunity is specific to chlamydial serovars [10] and effectors [3, 4], the role of antibodies in chlamydial immunity may be indirect—perhaps to enhance Th1 development via rapid antigen uptake, processing and activating specific Th1 cells. However, there is no physiologic evidence that antibody enhancement of T cell activation [11, 12] can be measured as a determinant of the efficacy of adaptive immunity against pathogens.

It is known that FcR-mediated uptake concentrates antigens in antigen-presenting cells (APCs), dramatically lowering T cell activating dose [13, 14], fostering rapid delivery of endosomes to lysosomes for processing and loading of peptides on major histocompatibility complex (MHC) molecules [15, 16], and facilitating an adjuvant advantage of 100–1000 fold [11, 13, 15, 17–19], including cross-priming [12]. Also, dendritic cells (DCs) are motile, highly potent primary APCs, widely distributed in mucosal and other tissues [18, 20], expressing the endocytic receptors, FcRs, and mannose receptors, for uptake of immune complexes (ICs) and glycosylated proteins, respectively [20, 21]. Most DCs possess high costimulatory ability as a result of elevated density expression of costimulators, such as interleukin (IL)–1, IL-12, intercellular adhesion molecule
1 (ICAM-1), leukocyte function antigen (LFA)–3, CD40, and B7 molecules [22–25]. The importance of DCs in antichlamydial immunity [22, 26, 27] is the result of their ability to transport antigen from the mucosal epithelium to the draining mucosal inductive sites [20, 28, 29], their efficient processing and presentation of chlamydial antigens [24, 26, 27, 30], and their proclivity for activating Th1 response [23, 25]. We tested the hypothesis that a major role for antibodies in chlamydial immunity is the enhancement of Th1 activation via FcR-mediated processes involving DCs. By use of Fc receptor knockout (FcRKO) mice, we demonstrated that specific antibodies and FcRs are crucial for the development of an enhanced and effective Th1 response during reinfections and that the process has significant clinical implications.

MATERIALS AND METHODS

Chlamydia, animals, infection, and analysis of the course of the infection. Stocks and antigens of C. trachomatis agent of mouse pneumonitis (Chlamydia muridarum or MoPn) were prepared by propagating elementary bodies in McCoy or HeLa cells, as described elsewhere [31]. Female FcR$^{-/-}$ on (C57BL/6:129) background, lacking the activatory FcγRI (CD64) and FcγRIII (CD16) and inhibitory FcγRIIB1 (CD32), were developed by gene targeting inactivation by Dr. Jeffrey Ravetch (Memorial Sloan Kettering Cancer Institute, University of California, San Francisco). These animals and the wild-type (wt) control FcR$^{+/+}$ mice (aged 5–8 weeks) were obtained from Taconic Farms. Animals received food and water ad libitum and were maintained in Laminar flow racks under specific pathogen–free conditions of 12 h light and 12 h darkness.

Mice were infected intravaginally with 10$^5$ inclusion-forming units (IFUs) of MoPn per mouse in a volume of 30 μL of PBS while under phenobarbitol anesthesia [32]. The course of the infection was monitored by periodic cervicovaginal swabbing of individual animals and isolation of Chlamydia in tissue culture, according to standard methods [31]. Groups of animals were reinfected 85 days after the primary infection with 10$^5$ IFUs of MoPn per mouse and swabbed for isolation of chlamydiae and/or killed to determine the development of hydrosalpinx. For assessment of complication of hydrosalpinx, mice were surgically dissected to expose the entire reproductive system. The uterine and oviductal regions were inspected for visible evidence of classic hydrosalpinx in infected mice. Experiments were repeated 2 times.

Measurement of frequency of mucosal and systemic chlamydial-specific Th1 cells (Th1 frequency) after a secondary genital chlamydial infection of FcR$^{-/-}$ and wt FcR$^{+/+}$ mice. A modified procedure for the limiting dilution technique [33, 34] was used. In brief, T cells were isolated from the iliac lymph nodes (ILNs) and spleens of infected mice at the indicated times and were seeded in a serial doubling dilution into 96-well round-bottom tissue culture plates at 24 wells/dilution. The T cells were stimulated with APCs from wt mice (2 × 10$^5$ cells/well) and chlamydial antigen (10 μg/mL). Background cultures contained 24 wells with APCs and antigen. After 5 days of incubation, the supernatants were assayed for interferon-γ by a sensitive ELISA, and frequency of chlamydial-specific Th1 cells was calculated. T cells from naive mice have a Th1 frequency of 15 (range, 9–21) per 10$^6$ cells. ILNs, iliac lymph nodes.

Figure 1. Frequency of specific Th1 cells after a secondary genital chlamydial infection of wild-type (wt) and Fc receptor knockout (FcRKO) mice. A modified procedure of the limiting dilution technique [33] was used to assess Th1 frequency in infected mice [33, 34]. T cells isolated from the lymphoid tissues of infected mice at the indicated times and cultures were established by seeding T cells in a serial doubling dilution into 96-well round-bottom tissue culture plates, as described in Materials and Methods. After incubation, the supernatants were assayed for interferon-γ by a sensitive ELISA, and frequency of chlamydial-specific Th1 cells was calculated. T cells from naive mice have a Th1 frequency of 15 (range, 9–21) per 10$^6$ cells. ILNs, iliac lymph nodes.

Cytokines, antibodies, and other reagents. ELISA kits for quantitating the amounts of murine cytokines in biological and culture fluids were purchased from BioSource International. Mouse anti–MoPn antibodies were prepared by 50% ammonium sulfate precipitation of gamma-globulin fraction of hyperimmune sera from C57BL/6 mice infected multiple times with MoPn by the genital route, as described elsewhere [31]. The presence of different antibody isotypes, including IgG2a, IgG2b, and IgA, in the anti-MoPn antibody preparation, was ascertained by ELISA [35]. High-titered mouse anti–Myco-

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Figure 2. Kinetics of Th1 activation by wild-type (wt) and Fc receptor knockout (FcRKO) antigen-presenting cells (APCs) in the presence of antichlamydial antibodies (left panel “A”). Purified cell preparations (T cells) were stimulated with splenic APCs from either FcR−/− or FcR+/+ mice plus chlamydial antigen, in the presence or absence of specific antichlamydial antibodies (anti-MoPn) or an irrelevant antibody preparation (anti-M. tuberculosis [anti-TB]), in tissue-culture plates for 1, 2, and 5 days. At the end of the incubation period, the supernatants were collected and assayed for interferon (IFN)-γ content by a quantitative sandwich ELISA [35]. The concentration of the cytokine in each sample (pg/mL) was obtained by extrapolation from a standard calibration curve generated simultaneously. Data are mean ± SD values of triplicate cultures for each experiment and are derived from at least 3 independent experiments. Control cultures containing T cells and APCs without chlamydial antigen showed no measurable amounts of IFN-γ; thus, the data are not shown. Also, cultures containing anti-TB antibodies did not show any measurable difference, compared with cultures without anti-MoPn antibodies, as described elsewhere [9]; therefore, the results are excluded here. Kinetics of Th1 activation by wt and FcRKO APCs in the presence of antichlamydial antibodies (left panel “B”). Shown is a reproduction of a segment of the results shown in left panel (A), in which the data from cultures containing APCs, chlamydial antigen, and anti–MoPn antibodies (left panel “C”) are presented for clarity of the comparison of the impact of FcRs on Th1 augmentation in the presence of antichlamydial antibodies.

*bacterium tuberculosis* (anti-TB) serum antibodies (prepared in a similar manner) were provided by Dr. Ram Navalkar (Morehouse School of Medicine, Atlanta).

**Immunohistochemistry.** At the indicated time periods after a reinfection (i.e., days 7 and 28), mice were killed, and a portion of the reproductive system between the cervix and the ovaries of each mouse (i.e., the cervix, uterus, and fallopian tubes) was excised and fixed in 10% formalin. The tissues were transferred to 70% ethanol and were treated with sodium borohydride, to remove excess aldehydes that could obscure epitopes and prevent antibody binding. Tissues were cut into 5-μm sections on a cryostat microtome, transferred onto slides, air dried, and fixed in acetone. Sections were washed and stained with primary monoclonal antibodies (MAbs) recognizing the indicated murine leukocytes antigens. Control sections were stained with isotype-matched irrelevant antibodies. Mouse reactive MAbs used were as follows: CD3ε (clone 145–2C11), CD4 (clone H129.19), CD8 (clone 5H10–1), CCR3 (polyclonal), and CCR5 (clone C34–3448). Binding of biotinylated species-specific antibodies was detected by streptavidin-peroxidase staining. Slides were viewed with Nikon Microphot Provia 400 daylight film. In some of the results, the percentage of positively stained cells in the sections or the intensity of staining for each leukocyte marker was determined by enumerating cells in 10 randomly chosen fields. In other results, the images were digitized with a Polaroid SprintScan 35-mm slide scanner and processed in Adobe Photoshop 5.0.

**Effect of specific antichlamydial antibodies on chlamydial antigen presentation for Th1 activation by APCs from FcR−/− and FcR+/+ mice.** To assess the effect of specific antichlamydial antibodies on APCs from either FcRKOs or control mice, 2 × 10^6 splenic cells (or 1 × 10^4 bone marrow–derived DCs [BMDCs]) were cocultured with 2 × 10^7 nylon-wool–purified T cells in the presence or absence of chlamydial antigen (10 μg/mL) and specific anti–MoPn antibodies (50 μg/mL) in 96-well tissue culture plates for 24, 48, and/or 120 h. Anti-isotype antibodies (i.e., rabbit anti–mouse IgG1, IgG2a, IgG2b, IgG3, and IgA) were used at 50 μg/mL in culture to determine the antibody isotype or isotopes responsible for augmenting T cell activation. Preliminary studies indicated that there was no significant difference between the effect of equivalent titers of whole and F(ab)2 anti-isotype antibodies on the Th1 enhancement caused by anti–MoPn IgG2a or IgA in this culture system. Therefore, the more readily available whole anti-isotype antibodies were used in the reported results. At the end of each incubation period, the supernatants were collected and assayed for IFN-γ content by a quantitative ELISA [9]. The results were derived from at least 3 independent experiments.

**Induction of augmented protective Th1 response by chlamydia-antibody complexes.** Four groups of mice were vac-
Figure 3. Immunohistochemical localization of Th1 cells in the genital mucosa of infected wild-type (wt) and Fc receptor knockout (FcRKO) mice. Genital tissue sections from reinfected wt and FcRKO mice were analyzed by immunocytochemistry for the murine chemokine receptor CCR5 expressed on Th1 (CD4 and CD8) and dendritic cells by use of the biotin-streptavidin-peroxidase detection system. Slides were viewed with Nikon Microphot Provia 400 daylight film. Images were digitalized with a Polaroid SprintScan 35-mm slide scanner and processed in Adobe Photoshop 5.0.

cinated 2 times by the intranasal route at 3-week intervals, as follows. Group 1 received 10^7 IFUs ultraviolet (UV)–inactivated MoPn in 30 μL of PBS; group 2 received 10^7 IFUs UV-inactivated MoPn mixed with anti–MoPn antibodies (1:100 titer); group 3 received equivalent amounts of anti–MoPn antibodies as group 2; and group 4 received 10^7 IFUs live MoPn. Two weeks after the last immunization, the frequency of MoPn-specific Th1 cells in the ILNs were determined, as described elsewhere [35]. Also, purified immune T cells were adoptively transferred from vaccinated mice into naive mice, and the latter were challenged with 10^4 live MoPn after 24 h [34]. Protective adoptive immunity was measured by isolation of chlamydiae from cervicovaginal swabs at 7 days after challenge, as described elsewhere [31]. The results were collated as mean ± SEM of 2 independent experiments.

Statistical analysis. The levels of IFN-γ in samples and IFUs from different experiments were analyzed and compared by performing a 1- or 2-tailed Student’s t test, and the relationship between different experimental groupings was assessed by analysis of variance. Minimal statistical significance was judged at P<.05.

RESULTS

Frequency of specific Th1 cells after a secondary genital chlamydial infection of wt and FcRKO mice. We tested the hypothesis that the high intensity and delayed resolution of secondary genital chlamydial infection exhibited by FcRKO mice [9] is the result of a slow rate and low Th1 activation during reinfections. Results presented in figure 1 indicate that the frequency of mucosal Th1 induced by wt FcR^{−/−} mice was ~6-fold higher than that of FcR^{+/−} mice by the first week of infection. In addition, T cell response was elicited in the mucosal and systemic lymphoid tissues in wt mice by the first week, but systemic response increased further by ~40% at week 4 after reinfecion. On the other hand, a low Th1 response was first detectable in the genital mucosal draining ILNs of FcRKO mice by the first week, whereas systemic response was undetectable, suggesting that the local immune response preceded the systemic response.

The systemic Th1 response in the FcRKO mice was detected at the later time interval (4 weeks) at a level ~5-fold less than the systemic response of the wt mice. Thus, the induction of Th1 response in FcRKO mice is delayed and suppressed, suggesting that an inadequate T cell response may be responsible for the greater intensity of secondary infection in the mice [9]. Furthermore, when groups of reinfected wt and FcRKO animals were evaluated for tubal complications, the incidence of hydrosalpinx formation was 1 in 10 infected wt mice, whereas all 10 FcRKO mice developed hydrosalpinx. The results indicated that the delayed and low capacity to induce adequate Th1 response allowed for increased ascending infection and consequently high incidence of development of tubal complications.

Kinetics of Th1 activation by wt and FcRKO APCs in the presence of antichlamydial antibodies. The presence of antichlamydial antibodies (anti–MoPn antibodies), antigen, and
FcR-positive APCs resulted in a faster activation of Th1 cells and ultimately a greater overall Th1 response as compared with cultures containing FcRKO APCs, antigen and anti-MoPn, or FcR-positive APCs plus antigen without anti–MoPn antibodies (figure 2A). In fact, as more precisely depicted in figure 2B, within 24 h of incubation, the presence of anti–MoPn antibodies caused an ∼5-fold increase in Th1 activation over cultures containing FcRKO APC plus anti–MoPn antibodies or cultures without antibodies. The rapid Th1 response with 24 h in the presence of anti–MoPn antibodies approached the level of a 5-day antigen plus wt APC culture. As reported elsewhere [9], control cultures containing an irrelevant antibody preparation did not enhance T cell activation (data not shown). Thus, specific antichlamydial antibodies and FcR-positive APCs lead to a faster rate of Th1 activation against *Chlamydia*.

**Immunohistochemical localization of Th1 cells in the genital mucosa of infected wt and FcRKO mice.** When genital mucosal tissue sections from wt and FcRKO mice were analyzed by immunocytochemistry for the murine leukocytes antigens CD3e, CD4, CD8, CCR3, and CCR5 1 week after reinfection, FcRKO mice contained ∼25% of the cells bearing CD3, CD4, and CCR5 present in wt mice. However, equivalent proportions of cells bearing CD8 and CCR5 were detected in the genital tissues from wt and FcRKO mice, which suggests that FcR-deficiency affected the induction of CD4+, but not CD8+, Th1 cells. Figure 3 shows the digitalized images of the CCR5-stained tissues from wt and FcRKO mice. The preponderance of CCR5-positive cells, indicative of Th1 cells, such as CD4+ Th1, CD8+, and DCs, in the wt mice, but the paucity or absence of these cells in the FcRKO mice can be clearly observed. Therefore, the greater disease and delayed clearance of secondary chlamydial infection in FcRKO mice is associated with the low induction and recruitment of Th1 cells into the genital mucosa.

**Role of DCs and specific antibody isotypes in FcR-mediated enhanced Th1 activation.** Results presented in figure 4 (splenic APCs) and figure 5 (BMDCs) reveal that the presence
Table 1. FcR-mediated induction of high frequency of protective Th1 cells.

<table>
<thead>
<tr>
<th>Group</th>
<th>Frequency of Th1 cells per 10⁶ cells (range)</th>
<th>Protectiona</th>
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<tbody>
<tr>
<td>UV-MoPn only</td>
<td>50 (38–72)</td>
<td>0/6 (6.1 × 10⁶ ± 1250)</td>
</tr>
<tr>
<td>UV-MoPn + Anti-MoPn</td>
<td>1600 (1067–2133)</td>
<td>9/10 (86 ± 23)</td>
</tr>
<tr>
<td>Anti-MoPn only</td>
<td>10 (1–13)</td>
<td>0/6 (1.2 × 10⁶ ± 2280)</td>
</tr>
<tr>
<td>Live MoPnb</td>
<td>2600 (1733–3467)</td>
<td>10/10 (10)</td>
</tr>
</tbody>
</table>

NOTE. IFUs, inclusion-forming units; UV, ultraviolet.

Data are no. of negative/total no. of mice challenged (mean ± SEM in IFUs/mL of total mice in each group). Protection was measured by IFUs of chlamydia recovered from cervicovaginal swabs and expressed as both the number of mice that were resistant to infection out of the total mice challenged.

Live MoPn delivered intranasally induces a potent genital mucosal Th1 response that is protective against genital chlamydial infection [35] and thus was used as the positive control.

Table 1 shows that FcR-mediated induction of high frequency of Th1 cells as a vaccine strategy against chlamydia. Table 1 shows that when groups of mice were vaccinated by the intranasal route with UV-inactivated MoPn mixed with anti–MoPn antibodies, the frequency of chlamydial-specific Th1 cells in the genital mucosa-draining ILNs was 1600 Th1 cells per 10⁶ ILNs (range, 1067–2133 per 10⁶ cells). This response was 32-fold greater than the Th1 frequency obtained with UV-inactivated MoPn alone and was comparable to the response in mice that received live MoPn (2600 Th1 cells per 10⁶ ILNs; range, 1733–3467 per 10⁶ ILNs), which was shown elsewhere to induc high levels of protective Th1 response [35]. The induction of protective Th1 cells was verified by the ability of immune T cells from either live-MoPn–exposed or UV-MoPn mixed with anti–MoPn antibodies to transfer protection against genital chlamydial infection (table 1). Thus, immunization with ICs comprising chlamydiae and specific antichlamydial antibodies was capable of rapidly boosting high frequency of Th1 cells that conferred protective immunity against chlamydia.

DISCUSSION

These studies reveal that antibodies participate in antichlamydial immunity by facilitating rapid and elevated Th1 response via FcR-mediated processes and that DCs may play a pivotal role in Th1 augmentation during reinfections in vivo. FcR-mediated enhancement of Th1 response is therefore a component of the memory response that drives the induction of faster and higher immune effectors that rapidly clear reinfections. In corroborating reports, FcRKO mice were more susceptible to influenza infection, even in the presence of anti-influenza antibodies, a mechanism that was NK cell independent, although Th1 enhancement was not investigated [36]. Delayed induction or inadequate Th1 response lead to ascending infection and complications of genital chlamydial infection in animals [37] and humans [38]. A potential intervention strategy is the use of Th1 enhancers or immunomodulators in combination with antimicrobial therapy [39–41], which requires establishing the optimum level of Th1 that mediates microbial clearance without pathology.

Only the cell-mediated immunity–associated IgG2a and IgA could perform antibody enhancement of Th1 activation. Thus, cross-linking the FcR (CD89) on mucosal DCs caused cell activation, which suggests a major role in antigen sampling and presentation in mucosal epithelia for an enhanced Th1 cell activation [42]. Mechanistically, FcR delivery of antigen increases the antigen-capturing and internalization ability of APCs and enhances both the antigen processing capacity and the T cell–activating mode, leading to a rapid and augmented T cell response. IL-12 delivered by APCs is important for signaling to signal transducers and activators of transcription type 4 (STAT4), which stimulates IFN-γ gene expression and activation-induced differentiation of naive T cells to the Th1 phenotypes [43].

Therefore, a plausible mechanism for FcR-mediated acceleration of Th1 activation is rapid uptake of opsonized antigen, followed by efficient processing in targeted intracellular endosomal and cytoplasmic compartments, involving stimulation of proteosomal, lysosomal, or endosomal enzymes, and induction of transcription factors, such as STAT2 and STAT4, that are associated with Th1 cell activation [44–46]. Thus, FcR-mediated internalization enhances timely endosomal transport, processing, and loading onto MHC molecules, which is dependent on induction of the cellular signaling and activation system via the protein tyrosine kinase PTK syk [15]. FcR-mediated antigen uptake also promotes DC maturation and facilitates immune response different antigens [15]. Certain antibody isotypes may direct an antigen to a specific intracellular environment that favors Th1 versus Th2 activation by prefer-
potential induction of certain transcription factors [21, 47], and this targeted signaling may involve specific FcRs for IgG2a and IgA. Finally, FcR-mediated delivery of antigen could potentially constitute an effective vaccination strategy and an adjuvant for boosting immune response against pathogens or tumors [48].

References