

Comparison of multiple genital tract infections with *Chlamydia trachomatis* in different strains of female mice

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Received: July 26, 2005 Revised: September 1, 2005 Accepted: September 9, 2005

We have previously shown that female outbred CF-1 mice are susceptible to prolonged genital tract infection with the oculogenital serovars (D-K) of *Chlamydia trachomatis*, and that partial homotypic and heterotypic protection against reinfection is induced. To understand the possible role of inherent T-helper 1 (Th1)/Th2 polarity bias on both the course of infection and the level of acquired immunity induced by infection, 2 immunologically different and well-characterized inbred strains of mice, BALB/c and C57BL/6, were studied in this model. Groups of mice were inoculated intravaginally with *C. trachomatis* serovar D (Ct D) and monitored by culture to determine the duration of initial infection. Two months later, mice were reinfected, and monitored along with age- and condition-matched control groups. Plasma and vaginal secretions were collected for serologic analysis and specific delayed-type hypersensitivity was assessed by footpad swelling. Initial infection in C57BL/6 mice was comparable in duration to outbred CF-1 mice (median duration 42 versus 43.5 days), while BALB/c mice had a shorter median duration of initial infection (12 days). All strains had significantly shorter durations of infection following reinfection. BALB/c mice shed 4-10 times more inclusion-forming units (IFU) than both C57BL/6 and CF-1 mice on sample days during the first week of infection and all strains shed less IFU during reinfection. C57BL/6 and BALB/c mice had significantly lower anti-Ct D immunoglobulin G titers in both plasma and vaginal secretions than CF-1 mice following resolution of infection; the frequency of immunoglobulin A seropositive vaginal secretions was less in both inbred strains, being significantly less in the case of C57BL/6 mice. Qualitative analysis of the antigen specificity and isotype composition revealed differences among the mouse strains. All 3 strains had detectable levels of specific footpad swelling on day 14 of infection, whereas only BALB/c mice showed a significant response at 70 days post-infection. Significant differences between 2 strains of mice that differ in Th1/Th2 polarity bias were observed in: 1) the duration of infection; 2) the level of bacterial shedding during infection; and 3) the quantitative and qualitative cellular and humoral responses made in response to female genital tract infection with a human oculogenital isolate of *C. trachomatis*. In addition, a similar and significant level of partial acquired immunity to reinfection was observed in both strains, suggesting that inherent Th1/Th2 polarity bias present upon initial infection does not prevent the development of a protective immune response within the genital tract during infection with an oculogenital isolate of *C. trachomatis*.

Key words: Active immunity, *Chlamydia trachomatis*, female genital diseases, inbred C57BL mice, inbred BALB C mice

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen of humans, with an estimated 3 million new cases in the United States [1] and 89 million new cases occurring worldwide each year [2]. The majority of infections occur in women, in whom the incidence has been increasing since the mid-1990s

[2,3] despite the availability of antibiotics that are clearly effective at eradicating these infections [4,5]. It is generally accepted that most female *C. trachomatis* genital tract infections are asymptomatic, and that although prior infection with *C. trachomatis* confers some protection against reinfection, the protection is at best short-lived, and reinfection with the same serovar does occur [6,7]. Persistent infection and/or reinfection is linked to an increased incidence of upper genital tract disease [8,9] which, like most chronic chlamydial

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diseases, has an associated immunopathic component that may be related to the chlamydial antigens responded to during these chronic processes, especially heat shock protein 60 [10,11]. A number of animal models have been developed in order to understand elements associated with the course and sequelae of infection and to provide model systems in which to test potential intervention strategies, particularly vaccines.

In our laboratory, we employ a murine model of *C. trachomatis* female genital tract infection in which human oculogenital serovars are used to establish infection by direct, noninvasive intravaginal inoculation [12,13]. The resulting infection closely mimics the features of the uncomplicated and self-limiting infection that occurs in most women. Using this model, we reported significant differences in a number of infection characteristics among strains of *C. trachomatis* that loosely correlated with the prevalence of the serovars among human clinical isolates, especially for the most and least prevalent serovars, i.e., D and E versus H and I. In addition to duration of infection and upper genital tract progression, the humoral immune response to infection was shown to differ among mice infected with different serovars that correlated with the observed duration of infection [14]. Using the most (serovar D) and least (serovar H) virulent strains identified in our previous study, we have shown homotypic protection against reinfection following infection with either serovar, with heterotypic protection observed only with the more virulent serovar D strain [15]. The protection was incomplete, i.e., mice remained susceptible to reinfection but the duration was shorter and shedding was reduced, which may be analogous to the transient protection seen in humans. However, no specific cellular and/or humoral immune responses were associated with the protection observed.

Although disappointing from the perspective of not identifying immune responses that were linked to protection, these observations are both informative and encouraging. Being the first such comparative study using human oculogenital isolates, the differences observed in the mouse may help explain the difference in prevalence between these serovars among human isolates, as well as provide hope that the development of a broadly cross-protective vaccine is possible. These studies were conducted in an outbred mouse strain in order to introduce at least a minimal level of genetic diversity, which we thought might help identify immune response differences that correlated with protection. That not being the case, the present study was conducted in C57BL/6 and BALB/c inbred mice in order to assess

the role of the well-documented inherent T-helper 1 (Th1)/Th2 polarity bias of these mouse strains [16-19] on both the course of infection and the nature of the immune response to genital tract infection with the serovar D strain of *C. trachomatis* used in our previous studies. Once identified in this defined setting, the possible influence of these elements, whether genetically fixed or physiologically controlled, on the course and outcome of infection in women can be assessed and appropriate intervention strategies developed.

Although comparative studies employing this model in more than 1 strain of mouse have been conducted using *Chlamydia muridarum* (MoPn) [20,21], only 2 studies have used human oculogenital serovars (serovar F [22] and serovar E [20]) and neither of these studies used both of the inbred strains used here. In addition, numerous studies have demonstrated and characterized the acquired immunity to reinfection induced during MoPn infection, however only 1 study did so using a *C. trachomatis* strain belonging to the oculogenital biovar, serovar E, and that study was conducted in C3H/HeN mice [23]. Studies like the latter and the one presented here that utilize human isolates in this valuable model of human disease are essential to determining the translational value of the many MoPn studies conducted to date. This issue takes on particular significance given the differences that have been reported between MoPn and human oculogenital serovars in such infection and immunity-affecting characteristics such as sensitivity to interferon-gamma [24-26], elementary body-associated cytotoxicity [27,28], and replication and release kinetics [28]. In this context, it becomes increasingly important to establish independently the value of the murine model using human isolates of *C. trachomatis* as we move closer to developing vaccines and other intervention strategies that must take into account both the diversity that exists among the serovars that comprise the oculogenital biovar and the variety of disease outcomes that are associated with *C. trachomatis* genital tract infection. In the end, a high concordance must exist between an animal model and the human disease the model is designed to mimic if translationally significant insights are to be gained from the model's use as a research tool.

Materials and Methods

Animals and bacterium

Inbred BALB/c and C57BL/6, and outbred CF-1 female mice were purchased from Charles River Laboratories

(Wilmington, MA, USA) and were used at >8 weeks of age. Mice used as previously non-infected age-matched controls during the reinfection (2°) arm of the study were housed, manipulated, and otherwise treated identically to the test group during the initial infection (1°) arm. A serotyped pure and mycoplasma-free strain of *C. trachomatis* serovar D (Ct D) was used to infect mice in both arms of the study. All experiments were conducted in a BL-2 containment facility in compliance with animal care regulations and under protocols approved by the institutional research animal care committee.

Culture technique

The organism was propagated, titrated, and isolated in cycloheximide-treated McCoy cell monolayers employing standard techniques. Vaginal specimens collected through day 14 of the study were cultured in duplicate. One culture was used for enumeration of inclusion-forming units (IFU) and the other was used for secondary amplification if no IFU were seen in the companion culture. Specimens collected after 14 days were tested by secondary amplification only.

Murine model

Progesterone, in the form of medroxyprogesterone acetate (Depo-Provera; Upjohn), was administered subcutaneously in 2.5 mg doses, 10 and 3 days before infection. Groups of mice were inoculated intravaginally by direct instillation of 25 µL of transport medium containing $\sim 2 \times 10^7$ IFU of Ct D. Previously non-infected age-matched control mice for the reinfection arm of the study were given sterile transport media. Approximately 2 months later, all groups were again progesterone treated and infected as described above.

Specimen collection

On the day before infection, blood and vaginal secretions were collected for serologic analysis, and immediately prior to infection vaginal samples were obtained for culture and cytology. The presence of Ct D in the lower genital tract was determined by culturing the material obtained by swabbing the vaginal vault and ectocervix with a calcium alginate swab. All swabs were placed in transport media and frozen at -70°C until tested. An animal was considered culture positive if IFU were detected in either primary or secondary cultures. Blood from a small tail vein incision was collected in heparin-coated capillary tubes, diluted with phosphate-buffered saline (PBS), and the plasma was separated by

centrifugation. Vaginal secretions were collected over a 2-h period by absorption into a piece of surgical sponge, and eluted into 400 µL of PBS. Plasma and vaginal secretions were frozen at -20°C until tested.

Serologic analysis

Anti-chlamydial immunoglobulin G (IgG) and immunoglobulin A (IgA) titers in plasma and vaginal secretions and the isotype profile of plasma immunoglobulins were determined by indirect solid phase enzyme immunoassay using sodium dodecyl sulfate solubilized Ct D elementary bodies (EB) as antigen, and concentration-equilibrated, affinity-purified rabbit IgG anti-mouse isotype primary antibodies, affinity-purified horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibodies, and ortho-phenylenediamine substrate. Western immunoblot analysis was performed using a similar antigen preparation which had been sodium dodecyl sulfate polyacrylamide gel electrophoresis-separated and transferred to nitrocellulose paper. After incubation with sample (plasma at 1:200 and vaginal secretions at 1:20) and the same primary and secondary antibodies used in the enzyme immunoassay, reactive bands were visualized with 4-chloro-1-naphthol.

Assessment of delayed-type hypersensitivity

Mice were injected in one hind footpad with 25 µL of a formalin-killed (FK) suspension of Ct D EB, while the other footpad received a suspension of FK McCoy cells. Footpad thickness was measured at 24-h intervals with a dial-gauge caliper.

Statistical evaluation

Duration of infection data were analyzed using the Wilcoxon rank sum test; mean data were evaluated with the *t* test.

Results

Duration of infection

One hundred percent of naïve C57BL/6, BALB/c and CF-1 mice were shown to be susceptible to genital tract infection with Ct D (control groups in Table 1). C57BL/6 mice had a median duration of initial infection comparable to outbred CF-1 (42 versus 43.5 days), while BALB/c mice had a significantly shorter duration of infection (12 days; $p < 0.01$). When compared to their age-matched control groups, both inbred strains were significantly protected ($p \leq 0.01$) against reinfection following resolution of initial infection to a level similar

Table 1. Duration of genital tract infection in CF-1, C57BL/6 and BALB/c mice infected with *Chlamydia trachomatis* serovar D

Strain	Group	No. of mice	No. of culture-positive animals on reinfection day																	Median duration of infection (days)	Wilcoxon rank sum <i>p</i>					
			2	4	7	10	14	17	21	24	28	31	35	38	42	45	50	56	59		63	66	70	R vs C	C vs C	
CF-1	R	12	12	8	7	4	2	2	5	3	3	3	3	1	2	2	2	0	0	0	0	0	0	7	0.01	
	C	12	12	12	12	10	10	12	10	9	9	6	9	8	3	4	3	1	1	0	1	1	1	43.5		–
C57BL/6	R	11	9	6	2	1	0	0	2	2	1	0	0	1	0	0	0	0	0	1	1	0	4	0.01		
	C	12	12	12	11	6	8	3	3	3	4	3	3	2	4	2	1	0	2	0	0	0	42		NS	
BALB/c	R	12	11	7	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.5	<0.01		
	C	12	12	12	12	7	4	1	2	3	1	0	0	0	0	0	0	0	0	0	0	0	12		<0.01	

Abbreviations: R = reinfected; C = control; NS = not significant

to that seen in outbred CF-1 mice (Table 1). The susceptibility to reinfection was essentially 100% for all 3 strains (90-100% of mice were culture-positive on at least 1 occasion following reinfection).

Shedding of infectious units

During the course of initial infection, the number of infectious units of chlamydia shed by culture-positive mice during the acute phase of infection varied significantly between the mouse strains (Table 2). C57BL/6 and CF-1 mice, while having longer median durations of infection, shed less IFU than BALB/c mice, which shed 4-10 times more IFU on sample days during the first 2 weeks of infection. Following reinfection, a similar pattern was observed in the level of shedding among strains, although in all 3 mouse strains the number of IFU shed by culture-positive reinfected mice was significantly less than in control mice during the first week of infection (Table 2).

Humoral immune response

Table 3 summarizes the quantitative data relating to the humoral responses of naïve mice to infection with Ct D. Both C57BL/6 and BALB/c mice had significantly less (*p*<0.05) anti-Ct D IgG in both plasma (mean titers [\log_2] 12.2 and 11.8) and vaginal secretions (mean titers [\log_2] 6.9 and 6.3) than CF-1 mice following resolution of infection (mean titers [\log_2] 14.4 and 8.5). In addition, the frequency of IgA seropositive vaginal secretions was less in both inbred strains (2 C57BL/6 and 7 BALB/c), being significantly less in the case of C57BL/6 mice (*p*<0.05).

Analysis of antigen specificity

Qualitative and semi-quantitative analysis of the antigen specificity of plasma IgG was similar for BALB/c and CF-1 mice, with all mice of both strains reacting with 60 and 46.5 kD proteins and quantitative differences (assessed by reaction intensity) attributed to the differences in quantity of IgG in the specimens as noted

Table 2. Primary culture results on days 2 through 14 of genital tract infection with *Chlamydia trachomatis* serovar D in CF-1, C57BL/6 and BALB/c mice

Strain	Group	No. of mice	Mean IFU of primary culture-positive mice (primary positive/total positive)					Median duration of infection (weeks)
			Range of IFU in primary culture-positive mice					
			Day 2	Day 4	Day 7	Day 10	Day 14	
CF-1	R	12	600 (12/12) 40-2140	120 (6/8) 20-550	120 (6/7) 10-290	170 (1/4) 170	60 (2/2) 30-80	7
	C	12	5120 (12/12) 1180-12,380	3800 (12/12) 890-15,730	2560 (12/12) 70-10,670	150 (7/10) 80-270	40 (4/10) 10-70	43.5
C57BL/6	R	11	730 (9/9) 50-2480	130 (4/6) 20-470	270 (1/2) 270	0 (0/1) 0	0 (0/0) 0	4
	C	12	9380 (12/12) 2840-25,120	1760 (12/12) 100-5390	220 (11/12) 10-920	20 (5/6) 10-30	40 (5/8) 10-120	42
BALB/c	R	12	5620 (11/11) 610-19,940	470 (5/7) 20-1650	2910 (5/5) 10-14,080	120 (3/3) 80-190	0 (0/0) 0	5.5
	C	12	36,480 (12/12) 11,550-141,900	18,600 (12/12) 7260-50,780	1670 (11/12) 20-8250	910 (7/7) 10-3630	390 (4/4) 100-670	12

Abbreviations: IFU = inclusion-forming units; R = reinfected; C = control

Table 3. Humoral immune response to genital tract infection with *Chlamydia trachomatis* serovar D in CF-1, C57BL/6 and BALB/c mice immediately before reinfection

Strain	No. of mice	Mean titers (log ₂) of seropositive animals (no. seropositive)			Median duration of infection (weeks)	
		Plasma IgG	Vaginal IgG	Vaginal IgA	Initial	Reinfection
CF-1	12	14.4 (12)	8.5 (12)	6.1 (11)	43.5	7
C57BL/6	11	12.2 (11) ^a	6.9 (10) ^a	4.0 (2) ^b	42	4
BALB/c	12	11.8 (12) ^a	6.3 (10) ^a	6.6 (7)	12	5.5

Abbreviations: IgG = immunoglobulin G; IgA = immunoglobulin A

^aValue varies significantly ($p < 0.05$) from value obtained for CF-1 mice.

^bFrequency of seropositive animals varies significantly ($p < 0.05$) from CF-1 mice.

above. Semi-quantitatively, reactivity of plasma IgG from C57BL/6 mice was less, with 1 low titer animal having no detectable reactivity and 2 additional animals having no reactivity against the 46.5 kD protein. Table 4 depicts the Western immunoblot analysis of plasma samples from representative mice collected following infection and immediately prior to reinfection. The mice are grouped by strain and ordered based on the infection duration following reinfection. Table 5 presents the results of the same analysis performed on vaginal secretions pooled based on the duration of secondary infection relative to the median duration of secondary infection for the strain. Qualitative assessment revealed reactivity in all pooled specimens against the 60 and 46.5 kD proteins, with semi-quantitatively less activity observed in the pools from mice with infection durations greater than the median duration for the group.

Isotype analysis

Plasma anti-Ct D immunoglobulin isotype analysis (Table 6) demonstrated differences among the strains in the pattern of anti-Ct D IgG isotype response as measured by the ratio of one isotype to another. In all strains, the predominant Ct D reactive isotype was the IgG subclass IgG2b, the only isotype detected at any significant level in the plasma of C57BL/6 mice. IgM was detected at low levels in both CF-1 and C57BL/6 and was not detected in BALB/c mice. The IgG subclass IgG2a was present in a similar proportion to IgG2b in both BALB/c (1:2.0) and CF-1 (1:2.1) plasma, with the 2 remaining isotypes, IgG1 and IgG3, being present in a noticeably higher percentage of total IgG from *C. trachomatis*-infected BALB/c mice (67%) as compared to CF-1 (14%) and C59BL/6 mice (4%).

Specific cell-mediated immunity

During the course of primary infection, specific cell-mediated immunity was assessed by specific footpad

swelling to intact FK Ct D EB (Table 7). All 3 strains had detectable levels of specific swelling on day 14 of infection, whereas only BALB/c mice showed a significant response at 70 days post-infection.

Discussion

In previous studies it was not possible to identify cellular or humoral elements among the immune responses analyzed that correlated with either homotypic or heterotypic protection following infection [15] or systemic immunization (unpublished observation) with oculogenital serovars of *C. trachomatis*. The present study was conducted in C57BL/6 and BALB/c inbred mice in order to assess the role of inherent Th1/Th2 polarity bias on the course of infection and nature of the immune response to female genital tract infection with the serovar D strain of *C. trachomatis* characterized in previous studies [14,15,28]. This strain was selected because it represents one of the more prevalent serovars that infect humans and it provided significant levels of both homotypic and heterotypic protection against female genital tract infection in the mouse following infection and systemic immunization.

In this study, we observed that inbred strains C57BL/6 and BALB/c were equally susceptible to infection at a level equivalent to the outbred CF-1 strain used in our previous studies. This is in keeping with published studies which support the notion that all genetically intact mouse strains are susceptible to genital tract infection in this model of non-invasive intravaginal inoculation, not only with human isolates of *C. trachomatis* but also with the *C. muridarum* biovar (MoPn). Once established, the median duration of genital tract infection with the serovar D strain in C57BL/6 was significantly longer compared to BALB/c mice, 42 and 12 days respectively, while the level of shedding during the acute phase of initial

Table 4. Serologic analysis of plasma immunoglobulin G (IgG) from representative mice following infection and immediately before reinfection

Animal	Mouse strain																			
	CF-1						C57BL/6						BALB/c							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
	Infection duration (days)																			
Initial	50	31	53	35	50	56	7	28	59	14	31	17	14	10	10	7	10	10		
Reinfection	2	4	4	42	50	50	2	2	2	4	24	66	<2	2	2	7	7	10		
	Anti-serovar D enzyme immunoassay titer (log ₂)																			
Plasma IgG	15	12	16	16	15	14	10	9	15	15	12	9	11	14	11	12	12	11		
Vaginal IgG	9	6	11	11	9	9	5	4	9	9	6	<4	6	8	5	7	6	5		
Vaginal IgA	5	7	6	7	6	7	<2	<2	6	<2	2	<2	<2	6	<2	8	6	<2		
	Plasma IgG Western immunoblot reactions ^a																			
MW (kD)	NR																		MW (kD)	
140																			140	
120																			120	
115																			115	
110																			110	
106																			106	
100																			100	
96																			96	
90																			90	
78																			78	
72																			72	
66																			66	
64																			64	
62																			62	
61.5																			61.5	
60	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	60	
58																			58	
56																			56	
49	●																		49	
46.5	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	46.5	
44.5																			44.5	
43																			43	
40																			40	
36																			36	
34.5	●																		34.5	
33																			33	
29																			29	
26																			26	
20																			20	
15.5	●	●	●	●	●	●	●											●	●	15.5

Abbreviations: IgA = immunoglobulin A; MW = molecular weight; NR = no reaction

^aReaction intensity: ● = weak; ● = moderate; ● = strong.

infection was significantly greater in BALB/c compared to C57BL/6 mice. In both assessments, initial genital tract infection of C57BL/6 was similar to outbred CF-1 female mice. In the only comparable study in which MoPn was used to infect both C57BL/6 and

BALB/c mice, a statement without supporting data was made that no differences were observed in either the duration or intensity of infection between these 2 inbred mouse strains, although a higher incidence and greater severity of upper genital tract disease was observed in

Table 5. Western immunoblot analysis of anti-serovar D immunoglobulin G in vaginal secretions collected immediately before reinfection and pooled based on the duration of infection after rechallenge^a

	Mouse strain						MW (kD)
	CF-1		C57BL/6		BALB/c		
	<Median duration	>Median duration	<Median duration	>Median duration	<Median duration	>Median duration	
MW (kD)							MW (kD)
115							115
110							110
100							100
90							90
78							78
72							72
66					●		66
62							62
60	●	●	●	●	●	●	60
58							58
56							56
49							49
46.5	●	●	●	●	●	●	46.5
40							40
36							36
33							33
29							29
26							26
20							20
15.5	●	●					15.5

Abbreviation : MW = molecular weight

^aReaction intensity: ● = weak; ● = moderate; ● = strong.

BALB/c mice [29]. In an independent study it was reported that the incidence of infertility was higher in BALB/c compared to C57BL/6 mice following infection with MoPn [30]. However in another study that focused on age as a susceptibility and outcome-influencing variable, a semi-quantitative assessment of shedding during infection with MoPn suggested that BALB/c mice shed more infectious units during the acute phase and shed longer than C57BL/6 mice [31]. Although fertility

was not assessed during the study presented in this report, we did not observe any gross pathologic abnormalities in the upper genital tract of mice infected with the same serovar D strain of *C. trachomatis* during either the initial or reinfection arms of this study. Thus, and consistent with the less invasive character of human oculogenital biovar strains in this model [15,22,32], we assume in the absence of visible hydrosalpinx, which has been used a surrogate marker of infertility [23],

Table 6. Isotype analysis of plasma anti-chlamydial immunoglobulins after genital tract infection with *Chlamydia trachomatis* serovar D (Ct D) in CF-1, C57BL/6 and BALB/c mice

Strain	No. of mice analyzed	Mean specific anti-Ct D activity at OD ₄₉₀ for isotype ^a					
		IgG1	IgG2a	IgG2b	IgG3	IgM	IgA
CF-1	6	0.020	0.158	0.298	0.056	0.018	0.001
C57BL/6	6	0.003	0.008	0.279	0.008	0.014	0.001
BALB/c	6	0.026	0.065	0.130	0.060	0.000	0.001
Controls	6	0.00	0.00	0.00	0.00	0.00	0.00

Abbreviations: OD₄₉₀ = optical density at 490 nm; IgG = immunoglobulin G; IgM = immunoglobulin M; IgA = immunoglobulin A

^aThe value presented is the difference between the activity measured in the antigen-containing well (sodium dodecyl sulfate solubilized Ct D elementary bodies) and the nonspecific activity detected in a non-antigen containing well, using concentration-equilibrated, affinity-purified monoclonal anti-isotype primary antibodies. The plasma specimens were diluted 1:128 and the control group consisted of 2 non-infected mice of each strain.

Table 7. Specific footpad swelling to elementary bodies (EB) after genital tract infection with *Chlamydia trachomatis* serovar D in CF-1, C57BL/6 and BALB/c mice

Strain	Assessment	Mean percent increase ^a (± SD) in footpad thickness at indicated time after footpad injection			
		24 h	48 h	72 h	96 h
CF-1	14 days post-infection	9.0 (± 4.6) ^b	21.0 (± 13.6) ^b	14.6 (± 4.7) ^b	14.4 (± 6.2) ^b
	70 days post-infection	7.0 (± 7.1)	10.3 (± 6.1)	14.1 (± 10.2)	13.7 (± 9.0)
	Control	2.7 (± 1.0)	5.5 (± 4.8)	3.5 (± 3.6)	4.5 (± 2.3)
C57BL/6	14 days post-infection	15.6 (± 12.7) ^b	22.8 (± 10.0) ^b	21.8 (± 11.6) ^b	24.4 (± 9.8) ^b
	70 days post-infection	7.2 (± 6.4)	10.8 (± 8.4)	12.5 (± 9.8)	12.4 (± 7.6)
	Control	3.7 (± 5.1)	7.3 (± 4.2)	3.7 (± 5.8)	6.1 (± 6.4)
BALB/c	14 days post-infection	14.8 (± 7.8) ^b	19.5 (± 6.3) ^b	19.0 (± 7.6) ^b	17.6 (± 11.9)
	70 days post-infection	21.6 (± 4.0) ^b	25.1 (± 8.3) ^b	22.0 (± 4.2) ^b	25.2 (± 5.4) ^b
	Control	1.7 (± 4.0)	4.9 (± 1.9)	5.6 (± 1.0)	4.7 (± 4.8)

Abbreviation: SD = standard deviation

^aPercent increase = (thickness EB foot – thickness McCoy foot/thickness McCoy foot) × 100.

^bSignificant difference from control at $p < 0.05$; each group consisted of 4 animals.

that fertility was not affected following infection with this serovar D strain of *C. trachomatis*. However, observations of non-hydrosalpinx-associated infertility in C3H mice following infection with serovar E [23,33] suggests the value of conducting fertility assessment even in the absence of gross upper genital tract pathology.

Following resolution of an initial infection with serovar D of *C. trachomatis*, we observed a similar level of protection against reinfection in both C57BL/6 and BALB/c mice that was comparable to that observed in CF-1 mice. This protection, although partial, was significant and similar in nature to the protection seen following MoPn infection, reducing both the median duration of infection and the level of shedding during the acute phase of infection compared to age-matched controls during an initial infection. The fact that in all 3 strains of mice the level of shedding detected on day 2 was less than in controls suggests a common mechanism, and implies that either the epithelial attachment/uptake of EB was interrupted and/or the initial cycle of replication/maturation was rendered less efficient. The underlying mechanism of the former is usually associated with neutralizing antibodies and Th2-type immune response, while the latter results from Th1-type responses that enhance innate immunity.

Quantitative and qualitative serologic analysis performed on plasma and vaginal secretions collected following resolution of initial infection and shortly before reinfection revealed similarities and differences that possibly arose from the inherent Th1/Th2 polarity bias that exists between these 2 strains. Both strains responded to infection with the production of similar levels of circulating and vaginally secreted IgG. Western immunoblot analyses of individual plasma and vaginal

secretions pooled based on the duration of secondary infection, although in some aspects dissimilar, were not of any value in identifying a link between a given response(s) and either the duration of infection or level of shedding observed following reinfection. In these assessments of the humoral response, CF-1 mice responded to initial infection with higher detectable IgG levels to a more diverse pattern of serovar D antigens. As was the case with the results observed for the inbred mouse strains, the particulars of these responses were not informative.

However, in 2 other assessments of humoral response, differences were observed between C57BL/6 and BALB/c that might help explain the significant level of acquired immunity against reinfection that is induced in all strains of female mice following infection with either human oculogenital serovars of *C. trachomatis* or MoPn. Firstly, the isotype profile of C57BL/6 mice following resolution of initial infection was almost exclusively IgG2b with a small but detectable level of IgM, while BALB/c mice had a more balanced isotype profile that included equal proportions of IgG2a and IgG3 and an IgG2a to IgG1 ratio of 2.5:1 and no detectable IgM; and secondly, fewer C57BL/6 mice, 2 of 11, had detectable levels of IgA in vaginal secretions than BALB/c mice, 7 of 12. The isotype profile of CF-1 mice was more similar to the BALB/c profile, with an IgG2a to IgG1 ratio of 7.9:1 and a low level of IgM, and with 11 of 12 mice having detectable IgA in vaginal secretions. In all strains and although predictably weak in BALB/c mice perhaps because of this strain's inherent Th2 polarity bias, the isotype profiles were consistent with a Th1-type immune response. Th1 responses have been shown to be associated with both resolution of

infection [20,29] and the protective immunity induced by infection with MoPn in both C57BL/6 and BALB/c mice [34,35], as well as in C57BL/6 [20] and C3H/HeN mice [20,36] following infection with *C. trachomatis* strains identified as serovar E. Interestingly, IgG2b was the predominant IgG isotype in C57BL/6 mice, being essentially a monotypic response. This might reflect a hyper-responsiveness of C57BL/6 mice to the IgG2b promoting influence of both macrophage inflammatory protein-1alpha [37] and transforming growth factor-beta [38], both of which have been shown to be up-regulated during genital tract infection with MoPn and to play a role in the course and outcome of infection with this agent [29,39].

Finally, and as a more direct measure of a Th1-directed response, all 3 strains developed a detectable delayed-type hypersensitivity (DTH) reaction by at least day 14 of infection, which coincided with the time point when a significant decrease in the level of shedding was observed, after which culture positivity was intermittent until ultimate resolution of infection occurred. An interesting observation is that although both C57BL/6 and CF-1 mice had detectable but not statistically significant DTH reactions at 70 days post-infection compared to non-infected controls, BALB/c mice had a DTH reaction that persisted and was stronger in magnitude at 70 days than that detected in the day 14 post-infection group. The strength and durability of this Th1-type response compared to C57BL/6 mice was unexpected given the Th2 polarity bias of BALB/c mice. However, it does provide a possible explanation of the results observed in this study as well as possible insight into the nature of the diversity that is seen in women during *C. trachomatis* genital tract infection.

In conclusion, inbred C57BL/6 and BALB/c mice were shown to be as susceptible to genital tract infection with Ct D as the outbred CF-1 mouse strain used in previous studies. While significant differences in duration of infection, level of shedding, and immune responses to initial infection were observed between the mouse strains, the level of protection against reinfection was similar and significant. Although no specific response could be linked to the observed protection, the results suggest that, regardless of the possible impact on innate responses during initial infection, Th1/Th2 polarity bias does not prevent the induction of what appears to be Th1-dominant immune response during female genital tract infection with *C. trachomatis*. However, without more detailed analysis of the cytokines, immunoglobulin specificities, and cell types

that are actually involved in this response, this conclusion and its extension to human female genital tract infection with *C. trachomatis* will remain speculative.

Acknowledgment

This work was supported in part by Public Health Service grant AI-23792 from the National Institute of Allergy and Infectious Diseases.

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