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Reinfection of the mouse genital tract with *Chlamydia trachomatis*: the relationship of antibody to immunity

M. Tuffrey, P. Falder and D. Taylor-Robinson
Division of Sexually Transmitted Diseases, MRC Clinical Research Centre, Harrow, Middlesex HA1 3UJ

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Summary. Progesterone-treated CBA mice which had had genital infections with a fast, human strain of *Chlamydia trachomatis* either 8, 16, 58, or 69 weeks previously were rechallenged through the uterine wall, along with groups of untreated controls. Serum IgG antibody and/or local IgA antibody was measured using a micro-immunofluorescence technique. Although the infection was self-limiting, chlamydiae were cleared significantly more quickly from the previously infected groups than from their controls in all experiments. However, mice which had had a previous infection recently and which had high titres (geometric mean 1: $\geqslant$ 2048) of serum IgG antibody and local IgA antibody immediately before rechallenge, were as susceptible as mice which had had a distant past infection and which had much lower titres (geometric mean 1:48) of serum IgG antibody. Thus, some immunity was induced in the mouse model, but pre-existing antibody seemed to be of little importance in this, and did not influence the initial susceptibility to reinfection.

Key words: *Chlamydia trachomatis*, reinfection, antibody/immunity relationship

Chlamydia trachomatis organisms cause a wide variety of diseases including non-gonococcal urethritis in men and 'non-specific' cervicitis and acute salpingitis in women, as well as being associated with various reproductive problems (Taylor-Robinson & Thomas 1980). Little is known about the mechanisms of pathogenicity although genital-tract disease is thought to have an immunological basis and not be due to the direct effect of the chlamydiae on the tissues. Furthermore, the extent to which immunity to chlamydiae develops in the genital tract is not clear, nor are the mechanisms understood. In an attempt to contribute to the understanding of these problems, we have developed a mouse model of chlamydial genital-tract infection. This was achieved in female CBA mice by pre-treatment with progesterone, and by flooding the entire genital tract with organisms introduced through the uterine wall (Tuffrey & Taylor-Robinson 1981). Infection persisted for 6 weeks or more but was self-limiting. To study whether thymus-dependent (T) lymphocytes were involved in recovery from infection, we infected nu/nu mice, which have an impairment of T-cell function, along with normal immunocompetent mice (Tuffrey *et al.* 1982). Although there were larger numbers of organisms in the nu/nu mice, the duration of infection in the nude mice, despite their failure to mount an antibody response, was about the same as in the immunocompetent mice. We concluded that T-lymphocytes and T-lymphocyte-dependent antibody had little effect on the course of the self-limited chlamydial genital-tract infection. However, this is only one aspect of immunity, and the experiments to be described here were

designed to investigate whether previously infected animals which had recovered were susceptible to reinfection or whether immunity had developed. Such information should be valuable in any logical immunization approach to the control of chlamydial genital-tract infections.

Materials and methods

Mice. Adult inbred strain CBA mice which had been bred and maintained before experimentation in the specific-pathogen-free unit at the Clinical Research Centre (CRC) were used.

C. trachomatis strain. The 'fast' human strain of *C. trachomatis*, designated SA–2f, was used. This is a genital strain related to the LGV serotypes (Wang & Grayston 1971).

Infection of mice. The mice were given the progesterone preparation Depo-Provera (Upjohn) in two subcutaneous doses of 2.5 mg 1 week apart before an intrauterine injection of strain SA–2f. This technique has been described previously (Tuffrey *et al.* 1982). One group of mice was 'primed' with an intravenous inoculation of live SA–2f organisms.

Detection of chlamydiae. The vagina of each mouse was swabbed with a cotton-wool-tipped nasopharyngeal swab (Medical Wire and Equipment Company Ltd) at approximately weekly intervals from day 5 to day 69 after inoculation. Each swab was expressed in 1 ml of cold sucrose-phosphate transport medium which was stored in liquid nitrogen until inoculation into cycloheximide-treated McCoy cells for the isolation of chlamydiae, as described previously (Thomas *et al.* 1977).

Detection of antibody. Most of the mice were tested at some stage during the experiments for chlamydial serum IgG antibody and/or local IgA antibody. Serum samples were obtained from tail bleeds and samples for local antibody were obtained by rinsing the vagina with 25 μl of PBS using a plastic pipette tip. Samples were stored at $-20°C$ and a micro-immunofluorescence technique was employed using either fluorescein-labelled anti-mouse IgG or anti-mouse IgA conjugate (Thomas *et al.* 1976).

Statistical analyses. The data were analysed using the statistical package GLIM (Baker & Nelder 1978). Statistical analysis took the form of logistic regression yielding log-likelihood ratio χ^2 tests.

Results

Challenge after intravenous inoculation

A group of eight mice were inoculated intravenously with 2×10^8 inclusion-forming units (i.f.u.) of SA–2f and were challenged genitally with 2×10^8 i.f.u. 12 days later, at the same time as a control group of eight mice (Table 1). Serum antibody was not detected in either group at the time of challenge, although titres of 1:2048 were recorded 63 days after challenge. All the mice in both groups became infected genitally; on Day 41, only half of the mice in the control group were infected, whereas chlamydiae were detected in all of those in the intravenously inoculated group. Despite this, infection in practically all mice in both groups had been eliminated by Day 69.

Rechallenge after distant previous genital infections

As part of the preceding experiment, a group of eight mice which had had a genital infection (dose: 1×10^8 i.f.u.) 58 weeks previously, were rechallenged genitally with 2×10^8 i.f.u. (Table 1). The serum IgG antibody titres at the rechallenge ranged from 1:64 to 1:1024 (geometric mean 1:287). Despite this, all the mice became infected. Elimination of infection, however, seemed to be marginally more rapid than in the previously uninfected control group. Thus, all mice in the control group were still infected on Day 26, whereas chlamydiae were iso-

Table 1. Result of intrauterine inoculation of SA–2f (2×10^8 i.f.u./0.1 ml) into progesterone-treated CBA mice which have or have not had a previous infection

Previous treatment	No. of mice in group	Number of mice from which chlamydiae were isolated on indicated day after rechallenge														
		5	8	12	15	19	22	26	29	33	36	41	47	54	57	69
None	8	8 (7–118)	7 (5–110)	nt	6 (2–16)	8 (1–115)	8 (1–30)	8 (1–42)	6 (1–26)	4 (1–53)	5 (1–44)	4 (4–67)	4 (2–39)	3 (2–9)	3 (1–4)	1 (9)
Intravenous inoculation 12 days previously	8	8 (1–324)	7 (1–244)	8 (9–472)	8 (3–43)	7 (1–207)	8 (5–182)	7 (14–56)	8 (1–15)	7 (1–104)	6 (1–97)	8 (4–342)	8 (1–116)	7 (3–37)	5 (1–31)	1 (5)
Genital inoculation 58 weeks previously*	8	5 (2–159)	7 (1–119)	nt	5 (3–14)	7 (1–30)	7 (3–102)	5 (6–24)	4 (1–5)	3 (1–7)	4 (5–39)	3 (2–61)	1 (7)	1 (1)	1 (4)	0

Ranges of number of inclusions per McCoy cell monolayer are shown in parentheses.

* Geometric mean serum IgG antibody titre 1:287.

nt, Not tested.

lated from only five of the eight mice which had been infected previously. On Day 47, half of the mice in the control group were infected compared with one in the previously infected group. However, the time of total elimination from all mice was about the same in both groups.

Statistical analysis, after adjustment for the effect of interval after infection, confirmed that the chance of mice having a chlamydial infection during the course of the experiment was significantly greater in the group of mice pre-immunized intravenously than in the 'non-immune' control group, and that the latter mice were more likely to be infected during the experiment than those which had had a previous genital infection. ($\chi^2 = 48.1$, 2 d.f., $P < 10^{-10}$). The effect of interval was to decrease the probability of infection as time went on.

Because a large challenge dose of organisms has been used in the experiments described above, three groups of mice were challenged with a smaller dose of 1×10^4 i.f.u. Mice in one of the groups had been infected in the genital tract 69 weeks previously (dose: 2×10^8 i.f.u.), while those in another group had had an additional genital infection 58 weeks before that (dose: 1×10^8 i.f.u.). The results of this experiment are shown in Table 2. The serum antibody titres before rechallenge for the group of mice which had sustained a single infection 69 weeks previously ranged from 1:8 to 1:512 (geometric mean 1:48), while those for the group of mice which had had two previous infections ranged from 1:8 to 1:1024 (geometric mean 1:287). Two mice in the latter group never became infected, and on Day 12, when chlamydiae were isolated from eight of nine mice in the control group, they were isolated from only four of seven mice in each of the other groups. This was a trend which continued throughout the experiment. Statistical analysis of these results showed that the previously uninfected mice had the highest probability of being infected during the period of the experiment, those that had

Table 2. Result of intrauterine inoculation of SA–2f (1×10^4 i.f.u./0.1 ml) into progesterone-treated CBA mice which have or have not had a previous infection

Previous treatment	No. of mice in group	Number of mice from which chlamydiae were isolated on indicated day after rechallenge							
		5	12	18	29	36	43	50	57
None	9	6 (7–351)	8 (1–40)	6 (1–6)	8 (12–143)	8 (2–26)	7 (1–54)	4 (1–3)	0
One genital infection* 69 weeks previously	7	5 (35–201)	4 (14–94)	2 (3, 4)	5 (1–204)	5 (1–42)	2 (2, 3)	0	0
Two genital infections 69 weeks and 58 weeks† previously	7	5 (1–234)	4 (2–112)	1 (3)	4 (1–356)	3 (1–9)	2 (1, 2)	0	1 (4)

Ranges of number of inclusions per McCoy cell monolayer are shown in parentheses.
* Geometric mean serum IgG antibody titre 1:48.
† Geometric mean serum IgG antibody titre 1:287.

had two previous infections had the lowest probability and mice which had experienced a single previous infection were intermediate in category ($\chi^2 = 17.7$, 2 d.f., $P < 0.001$).

Rechallenge after recent previous genital infections

In the first of these experiments, mice which had had a single previous genital infection (dose: 1×10^6 i.f.u.) were rechallenged 8 weeks later with 1×10^4 i.f.u. All these mice had serum IgG antibody titres in excess of 1:2048 three weeks before rechallenge, that is 40 days after the first inoculation. Local IgA antibody was detected in vaginal washings from nine of 10 mice 10 days before rechallenge. In the second experiment, mice which had had a single previous genital infection (dose: 1×10^6 i.f.u.) were rechallenged 16 weeks later with 1×10^4 i.f.u. Antibody studies were not performed. The results of these experiments are shown in Table 3. There was no evidence that pre-existing antibody diminished susceptibility to reinfection, but an 'immune effect' was seen by Day 21 when most of the control mice were still infected whereas only about half of the previously infected mice were infected. Statistical analysis of these results also revealed that there was a significantly higher rate of infection existing in mice in the control groups than in those that had been infected previously ($\chi^2 = 16.2$, 1 d.f., $P < 0.0001$).

Discussion

Except for the studies of Barron *et al.* (1981), who were able to infect the mouse genital tract for a short period using the MoPn

Table 3. Result of intrauterine inoculation of SA–2f (1×10^4 i.f.u./0.1 ml) into progesterone-treated CBA mice which have or have not had a previous infection

Previous treatment	No. of mice in group	Number of mice from which chlamydiae were isolated on indicated day after rechallenge						
		8	15	21	28	41	48	55
Experiment 1								
None	10	10 (4–396)	8 (2–75)	8 (1–223)	nt	7 (2–19)	6 (1–28)	3 (1–5)
One genital infection* 8 weeks previously	10	9 (1–21)	9 (1–33)	5 (5–30)	nt	3 (3–135)	2 (1, 2)	0
Experiment 2								
None	9	8 (2–144)	8 (1–125)	9 (1–82)	5 (2–30)	8 (1–536)	7 (2–14)	1 (1)
One genital infection 16 weeks previously	9	7 (1–29)	8 (1–106)	4 (1–5)	3 (1–4)	6 (4–50)	2 (1, 4)	0

Ranges of number of inclusions per McCoy cell monolayer are shown in parentheses.
* Geometric mean serum IgG 1: $\geqslant$2048, local IgA +ve.
nt, Not tested.

agent, no work, apart from our own, has been reported on *C. trachomatis* infections of the mouse genital tract. The results of the experiments reported here show that previous infections of the genital tract can produce some immunity in that chlamydiae are cleared more rapidly following rechallenge. However, the susceptibility of previously infected mice to initial infection on rechallenge is the same as that of previously uninfected mice. Thus, mice which had pre-existing antibody titres of $1:\geqslant 2048$, infected 8 weeks previously were as susceptible as mice which had antibody titres of 1:48, infected 69 weeks previously. Although previously infected mice cleared chlamydiae more rapidly than the controls, it seems that this was not dependent on antibody. Had pre-existing local IgA or serum IgG antibody been important, the pre-immunized mice would have been expected to have had an advantage, and to have begun to clear the chlamydiae during the first 10–12 days after rechallenge when antibody was present only in these mice and before actively induced antibody had time to appear. In fact, the more rapid elimination from the pre-immunized mice was not seen until much later in the course of infection when antibody in all the groups would probably have been boosted to about the same titre. These results are consistent with those we obtained in experiments with nude mice (Tuffrey *et al.* 1982) in which antibody was not detected at any time. Despite this, there was no statistically significant difference between the duration of infection seen in the nude mice and that seen in normal immunocompetent mice with high titres of antibody.

Although antibody appears to play very little part in the elimination of chlamydiae, the 'immune effect' must be associated in some way with specific factors 'memorized' as a result of the previous infection. Chlamydial genital infections in nude mice lasted for approximately the same length of time indicating that this system is not strongly T cell-dependent. However, the numbers of chlamydiae detected in specimens from these T cell lymphocyte-impaired animals were significantly larger than in specimens from immunocompetent mice. Thus, T lymphocytes could have some effect in inhibiting the number of organisms contributing to the infection. It is possible that non-specific immunological factors contribute considerably to the control of chlamydial genital-tract infections. Nude mice are known to have a keen non-specific immunological capacity which compensates in some part for their lack of T lymphocytes. The ability of the mice to eliminate chlamydiae in the experiments described here could be due to sensitized T lymphocytes modifying the functions of normal macrophages to confer special bactericidal activity on them. Transfer of either antibody or spleen cells from infected donors to recipient mice followed by challenge is being undertaken by us to further clarify this situation. Preliminary results support these findings and indicate the lack of protection afforded by passive transfer of antibody.

There are difficulties in relating closely our observations to those of others working with different model systems. It is likely that there are differences between the immune mechanisms which operate in the lung and in the genital tract, the uterus being regarded as an immunologically privileged site, and there may be differences between animal species in this regard. In addition, the development of immunity will be influenced according to whether the chlamydial infection is systemic or mainly local. Williams *et al.* (1981), who used the MoPn chlamydial agent to infect the lungs of nude and immunologically competent mice, found that susceptibility and resistance to infection in this model were T cell-dependent functions. They were able to pre-immunize immunocompetent mice and transfer immunity to nude mice by passive transfer of antisera which had high titres of both IgA and IgG antibodies, and also by transfer of immune spleen cells. We have so far been unable to detect chlamydial IgA antibody in the sera of

our infected mice, which could indicate an important difference between the two systems. Kuo *et al.* (1982), who inoculated trachoma and lymphogranuloma venereum biotypes of *C. trachomatis* intravenously to induce systemic infection in mice, were able to show that immunity could be conferred by passive transfer of spleen cells but not serum. Intravenous rechallenge resulted in rapid elimination of the organisms.

Some immunity to reinfection of the genital tract with *C. trachomatis* has been described in marmosets and baboons. Johnson *et al.* (1981) showed that female marmosets reinoculated intravaginally on one or more occasions with either of two serotypes of *C. trachomatis* were infected for a shorter duration and that immunity was not serotype-specific but cross-protective. Although there appeared to be some association between pre-existing high antibody titres and the curtailment of infection, this was not statistically significant. Digiacomo *et al.* (1975) induced chlamydial urethral infection in two male baboons and found that intraurethral reinoculation of homologous and heterologous chlamydial strains resulted in relatively shorter periods of infection but that antibody titres and type patterns were not affected. They concluded that immunity was most likely cell-mediated.

Although some immunity was induced in our mouse model in that recovery occurred a little more rapidly following previous genital infections, pre-existing antibody seemed to be of little importance in this and did not increase resistance to reinfection. In general, these findings would appear to support the opinions of Grayston & Wang (1978) and others that control of chlamydial genital infections through immunization is not a promising approach. It is interesting that a single intravenous dose of chlamydiae resulted in the prolongation of infection. We are at present investigating the immune capacity of mice which have undergone multiple vaccination procedures to see whether such treatments might be more successful in prevention.

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