

NOTES

Chronic Chlamydial Genital Infection in Congenitally Athymic Nude Mice

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Congenitally athymic nude mice and their heterozygous counterparts were inoculated intravaginally with the chlamydial agent of mouse pneumonitis, a *Chlamydia trachomatis* biovar. Heterozygous mice resolved their infections in 20 days, whereas nude mice developed chronic infections which lasted at least 265 days and did not resolve within the time course of the experiments. Heterozygous mice produced high levels of antibody in both serum and secretions in contrast to nude mice, which produced very low levels of antibody in serum alone.

The agent of mouse pneumonitis (MoPn) is a member of the *Chlamydia trachomatis* species and is a natural parasite of the mouse. The apparent natural site of infection with MoPn in the mouse is the respiratory tract; however, we have previously reported that MoPn can also produce an infection of the genital tract (2). The infection lasts about 20 days and results in immunity to reinfection (1). The resolution of infection occurs concomitantly with an increase in specific antibodies in both the serum and genital secretions, whereas delayed-type hypersensitivity responses develop somewhat later (1). The exact roles of both the humoral and cell-mediated immune responses in the resolution of MoPn genital infection are not known. Thus, the purpose of this study was to develop a model of long-term infection with *C. trachomatis* in the mouse which could be used to study immune function.

Female 8-week-old nude mice (*nu/nu*) and mice heterozygous for the nude trait (*+/nu*) on a BALB/c background were obtained from Harlan Sprague Dawley Inc., Indianapolis, Ind., and were maintained in Plexiglas isolators under specific pathogen-free conditions. MoPn was maintained in embryonated eggs, and mice were infected intravaginally with a semipurified elementary-body suspension containing approximately 10^6 inclusion-forming units by methods described previously (1). The infection was assessed by swabbing the genital tract with calcium alginate swabs (Calgiswab, type I; Spectrum Diagnostics, Glenwood Ill.) and then processing the swab for isolation of chlamydiae in McCoy cell cultures (1). Cultures were routinely passaged a second time before being designated negative.

Antibodies to MoPn were assessed in serum by indirect immunofluorescence with fluorescein-labeled rabbit anti-mouse immunoglobulin G (IgG) (H- and L-chain specific; Miles Laboratories, Inc., Elkhart, Ind.) or fluorescein-labeled rabbit anti-mouse gamma chain (Cappel Laboratories, Cochranville, Pa.) (2). IgA antibodies were measured in pooled genital secretions (five mice in each pool) by using fluorescein-labeled rabbit anti-mouse alpha-chain antibody (Cappel Laboratories).

Mitogenic responses to concanavalin A (ConA) and lipopolysaccharide (LPS) were determined on spleen cells ob-

tained from either *+/nu* or *nu/nu* mice by methods described previously (8). Levels of interleukin-2 (IL-2) were determined in the supernatants of 24-h cultures of nude mouse spleen cells stimulated with ConA (2 μ g/ml) by the method of Corley (3). Briefly, IL-2-dependent cells prepared from ConA-stimulated BALB/c spleen cells were cultured at 2×10^4 cells per well with geometric dilutions of the test supernatants. The incorporation of [3 H]thymidine (1 μ Ci/ml) over the final 4 h of 3-day-old cultures was measured, and the units of IL-2 were determined by probit analysis (4).

In the first experiment, 15 *nu/nu* and 14 *+/nu* mice were inoculated in the genital tract with MoPn. Ten *nu/nu* mice and nine *+/nu* mice were monitored at various intervals for the isolation of MoPn, while blood was obtained from the retroorbital plexus from the remaining animals for the determination of antibodies to MoPn. Genital secretions were also collected from these animals for the assessment of specific IgA antibodies (1). Eight of ten *nu/nu* mice and eight of nine *+/nu* mice became infected as a result of intravaginal inoculation (Fig. 1). The infection in the *+/nu* mice resolved in 17 to 20 days, whereas the infection became chronic in the *nu/nu* mice and persisted for as long as 147 days in eight mice. By 265 days after infection, four mice were still positive for MoPn, at which time the experiment was terminated. One *nu/nu* mouse never became infected, and another acquired the infection by day 182, possibly as a result of horizontal transmission since the mice were caged together. Most of the mice remained healthy for the duration of the experiment. Three *nu/nu* mice lost weight and eventually became moribund. When these mice were killed and necropsied, the lungs were found to be consolidated. The lungs were homogenized and inoculated into embryonated eggs for the isolation of chlamydiae. In all three cases chlamydiae were detected; thus, it appears that the mice had acquired a respiratory tract infection, possibly as a result of MoPn shed from the genital tract.

+/nu mice developed high titers of IgG antibody to MoPn by day 17 of infection when antibody levels were determined with fluorescein-labeled rabbit anti-mouse IgG (H- and L-chain specific) (Table 1). In contrast, *nu/nu* mice had only minimal and delayed IgG responses. It is interesting that three of five infected nude mice eventually developed IgG antibodies to MoPn, albeit at low levels in comparison with

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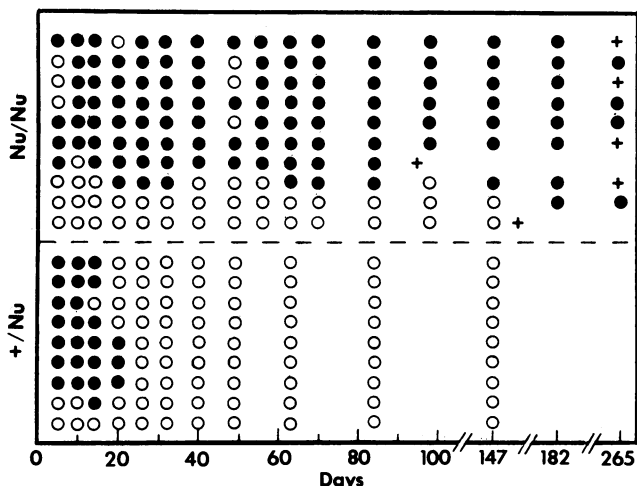


FIG. 1. Isolation of MoPn from genital swabs of *nu/nu* and *+/nu* mice. Each row represents the results of repeated isolations from one mouse. Symbols: ●, Isolation positive; ○, isolation negative; +, mouse either died or was sacrificed.

the *+/nu* mice. IgA antibodies to MoPn appeared in the pooled secretions of *+/nu* mice by day 18 (titer = 40) and attained a titer of 1,280 by day 35 which persisted until day 74. *nu/nu* mice, however, had no IgA antibodies in genital secretions at any time tested, including specimens taken as late as day 95 (data not shown).

The experiment was repeated with 10 *nu/nu* and 10 *+/nu* mice. Similar results to the first experiment were obtained. Nine of ten *+/nu* mice became infected but resolved their infections by day 20. In contrast, 7 of 10 *nu/nu* mice developed chronic genital infections and were still shedding MoPn 77 days after infection. Chlamydiae were also isolated from five of these mice on day 110. One *nu/nu* mouse was positive on days 5 and 9 but negative thereafter. Similar results were also obtained when antibody titers were determined, this time with fluorescein-labeled rabbit anti-mouse gamma-chain antibody. *+/nu* mice developed high titers (>1,280) by days 23 to 35. Titers in *nu/nu* mice remained negative until day 35, when antibody was detected in two mice at a titer of 10. By day 167, four of five mice were positive for IgG antibody to MoPn, with titers ranging from 10 to 40.

Normally, nude mice do not develop IgG antibody in response to antigenic challenge, but it has been shown that 9-month-old nude mice are capable of producing their own IL-2, whereas 2-month-old nude mice are unable to do so (5). As a result, the older nude mice are able to develop low levels of antibody to an antigenic stimulus as well as cytotoxic T cells. To explain the presence of IgG to MoPn in the nude mice, we attempted to determine whether any of the chronically infected nude mice in this study had T-cell function as measured by mitogenic responsiveness to ConA and the ability of the ConA-stimulated spleen cells to elaborate IL-2. Spleen cells were also tested for their response to LPS. The five *nu/nu* mice from the second experiment, two of which were positive for MoPn on day 110, were sacrificed on day 201, at which time they were about 9 months old, and their spleens were removed. Five 6-week-old *nu/nu* mice were used as controls. Spleen cells from the 6-week-old nude mice did not mount significant responses to ConA as determined by a one-tailed *t* test (Table 2). In contrast, 9-month-old infected nude mice did have significant re-

sponses to ConA ($P < 0.05$) which were two- to fourfold greater than those of the unstimulated control cultures. All of the mice had significant stimulation by LPS ($P < 0.005$) regardless of age, as would be expected since B cells are normal in nude mice. When ConA induction of IL-2 was measured, only the spleen cells from the older nude mice were able to produce significant levels. In fact, the level of IL-2 elaborated by the spleen cells from one nude mouse was about sevenfold greater than the pooled standards which were derived from ConA-stimulated BALB/c spleen cells.

In this study, we demonstrated that infection of nude mice with MoPn results in a chronic infection which does not resolve. It is significant that this model uses a *C. trachomatis* biovar, which is a natural parasite of the mouse and can persist in the nude mouse much as *C. trachomatis* persists in human genital infections. These data are in direct contrast to those of Tuffrey et al. (9), who reported that there was no difference in the duration of infection between *nu/nu* and *+/nu* mice infected with lymphogranuloma venereum strain of *C. trachomatis* (SA-2f). Both groups of mice resolved their infections in about 60 days. These investigators could not explain these results, but this may have been due to the artificial nature of their system since the SA-2f strain of *C. trachomatis* is not a natural parasite of the mouse. Moreover, treatment of the mice with progesterone was required for infection to be established (9). No such manipulation is required to produce infection with MoPn.

Although the results of this study indicate that T cells are required for the resolution of MoPn genital infection, the exact role of the T cell cannot yet be determined. However, other studies involving MoPn respiratory infections of nude mice have indicated that both humoral and cell-mediated immunity may be important (10, 11, 12), thereby suggesting possible roles for helper T cells, delayed-type hypersensitivity T cells, or cytotoxic T cells. Our previous studies with the guinea pig-GPIC model (i.e., with the agent of guinea pig inclusion conjunctivitis) have also indicated that both humoral and cell-mediated immunity are required for the resolution of chlamydial genital infection (6, 7). In fact, when GPIC-infected guinea pigs were treated with antithymocyte serum, a low-grade chronic infection developed which did not resolve until the treatment was withdrawn, which was very much analogous to MoPn infection of the nude mouse. The MoPn-nude mouse model provides a unique opportunity to investigate the contribution of various T-cell subpopulations to the development of immunity to chlamydial genital infection.

TABLE 1. Antibody titer (IgG) to MoPn in serum

Group	Animal	Postinfection antibody titer at (days) ^a :					
		17	24	35	53	74	94
<i>nu/nu</i>	1	<10	<10	<10	<10	<10	<10
	2	10	40	<10	40	10	ND
	3	<10	<10	20	10	20	40
	4	<10	<10	<10	ND	ND	ND
	5	10	20	10	40	ND	ND
<i>+/nu</i>	1	320	>1,280	>1,280	>1,280	>1,280	>1,280
	2	>1,280	>1,280	>1,280	>1,280	>1,280	>1,280
	3	1,280	640	>1,280	ND	>1,280	ND
	4	640	>1,280	>1,280	>1,280	>1,280	>1,280
	5	>1,280	>1,280	>1,280	>1,280	>1,280	ND

^a At 7 days postinfection, the antibody titers for all animals were <10. ND, Not done.

TABLE 2. Response of spleen cells to mitogenic stimulation with ConA and LPS and production of IL-2

Group	Animal	Spleen cell response (cpm, mean \pm SEM)			Production of IL-2 (U)
		Control	ConA	LPS	
Pooled standard	1				3.2
	2				2.6
<i>nu/nu</i> (6 wk old)	1	606 \pm 26	564 \pm 42	14,262 \pm 756	0.0
	2	622 \pm 39	861 \pm 68	17,648 \pm 166	ND ^a
	3	347 \pm 22	524 \pm 42	19,504 \pm 468	0.0
	4	647 \pm 40	783 \pm 16	23,601 \pm 325	0.0
	5	388 \pm 35	451 \pm 26	26,137 \pm 802	ND
	Mean	522 \pm 63	636 \pm 78	20,230 \pm 2,110 ^b	ND
<i>nu/nu</i> (infected, 9 mo old)	1	2,235 \pm 99	4,322 \pm 195	32,075 \pm 1,357	3.0
	2	1,808 \pm 138	8,877 \pm 459	24,878 \pm 633	21.5
	3	1,964 \pm 85	4,957 \pm 611	25,354 \pm 802	3.5
	4	1,645 \pm 116	2,621 \pm 30	27,645 \pm 318	1.6
	5	2,200 \pm 142	1,960 \pm 91	30,837 \pm 1,331	0.8
	Mean	1,970 \pm 112	4,547 \pm 1,211 ^c	28,157 \pm 1,438 ^b	

^a ND, Not done (insufficient cells).

^b Statistically significant at $P < 0.005$.

^c Statistically significant according to a one-tailed t test at $P < 0.05$.

When antibody levels were determined in this study, no IgA response was observed in the genital secretions of *nu/nu* mice, and only minimal IgG levels were observed in the serum. When T-cell responsiveness was assessed by blast transformation by ConA and IL-2 activity in ConA-stimulated spleen cells, the chronically infected nude mice were found to have significant activity compared to young uninfected nude mice, a phenomenon which has been reported previously (5). These results may explain the appearance of IgG antibody in the infected *nu/nu* mice, although the status of the T-cell responsiveness when antibody first appeared in these animals could not be determined. It should be noted that even though some T-cell activity and low levels of specific antibody were present, the mice were still unable to resolve the infection.

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