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Primary Murine *Chlamydia trachomatis* Pneumonia in B-Cell-Deficient Mice

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Mice were rendered deficient in B-cell activity by treatment with anti-μ antibody from birth. These animals were then infected intranasally with murine *Chlamydia trachomatis* (murine pneumonitis agent [MoPn]). They produced neither local nor systemic antibody to MoPn but had intact delayed-type hypersensitivity to MoPn. Anti-μ-treated mice were not significantly more susceptible to primary invasive infection with MoPn than were control mice, and unrestricted multiplication with MoPn did not occur. The dominant immune response controlling this type of infection is not likely to be antibody.

The role of antibody in host defense against *Chlamydia trachomatis* is unclear. In human cervical infection, an inverse correlation has been observed between the amount of local antibody present and the number of chlamydial organisms isolated. The relationship was most striking for secretory immunoglobulin A (1).

In the guinea pig model of primary genital mucosal infection, local antibody appeared to play an important role in host defense against the guinea pig inclusion conjunctivitis agent (*Chlamydia psittaci*) (8, 10), although a T-cell-dependent component of cell-mediated immunity may have played a role as well (9).

We have developed a murine model of chlamydial pneumonia by using the nude athymic mouse (*nu/nu*) and its furred heterozygous littermate (*nu/+*) (13–15). Host defense is strongly T cell dependent in this model in that the nude mouse is significantly more susceptible to the murine pneumonitis agent (MoPn) than the *nu/+* mouse is. The *nu/nu* mouse is deficient in its cell-mediated immune response to MoPn (12) but, in addition, does not produce antibody in response to MoPn infection (13–15).

For this reason, experiments were done giving antibody locally (15) or systemically (14) at the time of or just before infection with MoPn. These experiments thus mimicked rechallenge rather than primary infection in evaluating the role of antibody, since antibody was present very early in infection. With the model of MoPn pneumonitis currently used (using mice that are "superclean" with a very limited defined flora), local (but not systemic) antibody present at the time of infection has had a partially protective role in this model of nonprimary infection (15). To obtain protection, either antibody was given intranasally just before infection with MoPn, or the organisms were pretreated with antibody before infection.

We thus decided to investigate the role of antibody in host defense against a primary infection with MoPn in our model of invasive (rather than primarily mucosal) infection. To do this, we used the B-cell-deficient mouse.

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MATERIALS AND METHODS

Production of B-cell-deficient mice. Production of B-cell-deficient mice was attained by treatment of BALB/c (+/+) mice from birth with goat antibody to mouse μ chain. This is a well-described standard method (4, 6, 7). The exact method used was that of Grun and Weidanz (4), using antiserum prepared in goats (anti-μ) against a purified myeloma protein MOPC 104E (μ, λ). This material was heat inactivated, sterilized by filtration, and diluted 1:4 in phosphate buffer. Daily injections were given to newborn mice for several days, followed by thrice-weekly injections for the duration of the experiments. No azide preservative was used. Control BALB/c mice received the same amount of normal goat serum (NGS). Both male and female mice were used.

To monitor for B-cell deficiency, antibody to MoPn was measured (see below) after infection. Lymphocyte transformation to T- and B-cell mitogens was monitored before infection after 6 weeks of anti-μ treatment. By these criteria, the mice were rendered B cell deficient by anti-μ treatment.

The mice used were pathogen-free superclean mice shown to be free of *Mycoplasma* sp., *Chlamydia* sp., and viral and pathogenic bacteria by culture and serologic methods (12, 15).

MoPn. The mouse pneumonitis biovar of *C. trachomatis* was obtained as described previously and maintained in the yolk sacs of embryonated hen's eggs (12–15). Dilutions of the organism for intranasal inoculation were made in McCoy modified 5A medium (Difco Laboratories, Detroit, Mich.). Mice were inoculated with 1×10^3 to 5×10^4 inclusion-forming units (IFU) of MoPn in a volume of 0.05 ml. Mortality was monitored daily.

MoPn antigen. The antigen used for antigen-specific ear swelling (SES) was an elementary body preparation grown in cell culture (HeLa-229 cells) to avoid the problem of contamination of test material with egg yolk material (12). It was purified by Renografin (E. R. Squibb & Sons, Princeton, N.J.) gradient separation. The elementary body titer was 10^9 IFU/ml, and the protein content was 1.43 mg/ml (12). The antigen was inactivated by UV light. Control material con-

TABLE 1. Response of NGS- and anti-μ-treated +/+ mice to ConA and LPS

Addition	Lymphocyte transformation[a]	
	NGS group	Anti-μ group
ConA	170,514 ± 50,952	147,332 ± 62,974
LPS	31,882 ± 1,433	4,537 ± 863[b]

[a] Results expressed as mean counts per minute ± standard deviation in five mice per group.
[b] $P < 0.005$ compared with +/+ mice (Student's t test).

tained HeLa-229 protein (2.16 mg/ml), but not elementary body antigen.

SES. The mouse ear swelling test (2, 12) was used to measure delayed-type hypersensitivity to MoPn by methods we have previously used (12). In brief, the thickness of both pinnae was measured with an industrial micrometer. After base-line measurements, MoPn elementary body antigen in 0.03 μl of phosphate-buffered saline was injected in one ear and control material was injected in the other. The thickness was again measured at intervals of 3 to 24 h after injection. SES was equal to the difference in swelling of the antigen-injected ear (thickness after injection minus base line in that ear) minus the swelling in the ear injected with phosphate-buffered saline (thickness after injection minus base line in that ear). Furthermore, mouse ears with SES were examined histologically to determine that the infiltrate consisted of monocytes, macrophages, and heterophils at 24 h, a result consistent with our previous studies (12).

Quantitative culture of MoPn in mouse lungs. Quantitative culture of MoPn in mouse lungs was done by using culture in McCoy cell monolayers as described previously (13).

Mitogenic lymphocyte transformation. Lymphocyte transformation was done on NGS and anti-μ-treated BALB/c mice by the procedure of Lammert and Wyrick (5). Concanavalin A (ConA [Sigma Chemical Co., St. Louis, Mo.]) was used at 0.4 μg per well (the optimal concentration). Lipopolysaccharide (LPS [Sigma]) was used at 5 μg per well. All cultures were incubated in quintuplicate for 48 h for each mouse before the addition of tritiated thymidine (5). The mean counts per minute with mitogen minus base line was then calculated for each mouse. Reported counts per minute are the mean ± standard deviation for five mice in each group.

Antibody determinations. Antibody determinations were done on serum and lavage samples by the microimmunofluorescence method previously described (11, 13–15). Lavage samples were obtained by lavage of each lung with a standardized minimal volume (3 ml) of phosphate-buffered saline.

Statistics. Statistical methods used were Student's t test with correction for unequal variances, the chi-square test, and the Wilcoxon test.

TABLE 2. Geometric mean antibody titers to MoPn[a]

Sample, day, and treatment	Titer			
	Immunoglobulin G		Immunoglobulin A	
	Mean	Range	Mean	Range
Serum, day 10				
NGS	32	All 32	<8	All <8
Anti-μ	<8	All <8	<8	All <8
Serum, day 15				
NGS	355	256–512	<8	All <8
Anti-μ	<8	All <8	<8	All <8
Serum, day 25				
NGS	2,048	1,024–4,096	21	16–32
Anti-μ	<8	All <8	<8	All <8
Tracheal lavage, day 25				
NGS	24	16–64	73	32–128
Anti-μ	<8	All <8	<8	All <8

[a] Number of mice ranged from three to five per group per day.

TABLE 3. SES in NGS- and anti-μ-treated BALB/c mice at day 25 postinfection with MoPn

Group[a]	Infection	SES[b]
A	Control (uninfected)	1.5 ± 1.9
B	NGS	6.5 ± 4.9
C	Anti-μ	7.8 ± 4.4

[a] A versus B and A versus C, $P < 0.05$ (Student's t test); B versus C, P = not significant.
[b] SES was measured at 24 h after each ear injection.

RESULTS

Table 1 shows the results of mitogenic stimulation of uninfected NGS and anti-μ-treated mice with ConA and LPS. ConA responses were intact in the anti-μ-treated mice, but B-cell stimulation (by LPS) was markedly impaired. The mice had been treated with anti-μ antibody for 6 weeks at the time of testing.

Table 2 shows the results of antibody measured in serum and lavage fluid 10 to 25 days after infection with MoPn. Antibody formation is markedly impaired in anti-μ-treated mice. Immunoglobulin M values were <8 in both groups.

Table 3 shows that delayed-type hypersensitivity measured by antigen-specific SES is intact in anti-μ-treated mice.

Mice were then infected with 5×10^3 MoPn (experiment 1) or 1×10^3 IFU (experiment 2), and mortality was monitored daily (Table 4). Table 4 shows two similar experiments (experiments 1 and 2) and one with a significantly higher inoculum of MoPn (experiment 3). There was no significant difference in mortality between anti-μ-treated and control mice in any experiment. Although experiment 2 showed a trend for greater susceptibility of anti-μ-treated mice ($P > 0.07$ by the Wilcoxon two-tailed test), it was not statistically significant and was not confirmed by experiment 1 ($P > 0.20$). Further, the surviving anti-μ-treated mice all appeared clinically well. The mice in experiment 2 were kept for 31 days total, and no further mortality occurred. If a much

TABLE 4. Mortality in NGS- and anti-μ-treated mice

Expt and treatment	No. dead/no. infected at day after infection			
	10	15	20	25
1				
NGS	0/10	2/10	3/10	4/10
Anti-μ	1/10	1/10	2/10	2/10
2				
NGS	0/10	0/10	1/10	1/10
Anti-μ	0/10	2/10	3/10	3/10
3				
NGS	3/10	7/10		
Anti-μ	4/10	7/10		

higher dose of MoPn was used (5×10^4, experiment 3), there was 70% death in each group by day 15 ($P > 0.30$ by the Wilcoxon test). The remaining mice were sacrificed at day 15 to recheck for inhibition of serum antibody formation. Serum antibody was present in all NGS-treated survivors and no anti-μ-treated survivors at this time.

Finally, quantitative culture of lungs in five NGS-treated and five anti-μ-treated mice infected 16 days earlier was done. Counts of IFU per lung were 10, 20, 15, 8, and 13 in NGS-treated lungs and 18, 5, 0, 70, and 0 in anti-μ-treated lungs; the respective means (± standard deviation) were 13.2 ± 4.7 and 18.6 ± 29.7 ($P > 0.30$ by Student's t test). With the minor exception of one anti-μ-treated mouse (70 IFU per lung), both groups had controlled the infection equally well by this time. There had been no mortality in either group given this low dose of 10^3 IFU by the time of sacrifice (day 16). These mice were checked for antibody status at sacrifice, and again no anti-μ-treated mouse had produced serum antibody against MoPn while all control mice were antibody positive.

DISCUSSION

These data show that this primary invasive chlamydial infection was not significantly influenced by a failure to generate either local or systemic antibody in this model of MoPn pneumonia in superclean mice. Both NGS- and anti-μ-treated mice recovered from infection. Unrestricted multiplication of MoPn did not occur in B-cell-deficient mice. In our initial experiment with this model, commercial anti-μ serum (containing azide) was used. In that study, anti-μ-treated mice were somewhat more susceptible by mortality criteria than were NGS controls ($P = 0.049$ by the Wilcoxon test) but were more resistant than *nu/nu* mice ($P < 0.03$). Unrestricted replication of MoPn did not occur (data not shown). It is not clear whether the statistically significant increase in mortality in this initial experiment was related to the azide, but we avoided using azide in the experiments reported in this paper. All our data, however, are consistent with the concept that primary invasive MoPn infection in our model is controlled in the absence of antibody. Because anti-μ treatment can have subtle effects on T-cell function (3), it is reassuring to note that delayed-type hypersensitivity measured by SES (a T-cell-directed function in this model [12]) is intact in anti-μ-treated mice and that ConA stimulation is normal. Because of possible subtle changes in T-cell function in anti-μ-treated mice, however, these data are probably more convincing than they might be if a positive effect induced by anti-μ therapy were shown.

We deliberately performed experiments using low doses of MoPn with consequent late and relatively modest mortality. A higher-dose experiment (Table 4, experiment 3) also showed no significant difference in mortality between the two groups, but this is probably a less valuable model for this purpose because significant antibody is not seen in control mice until about day 7 (12, 13, 15). A model with significant very early mortality would have mice dying at a time when inhibition of antibody would be expected to play a minor role since significant antibody would not have been present in control mice either.

These data do not indicate, however, that antibody plays no role in host defense against *C. trachomatis*. A reinfection model, with antibody present at the time of infection, is a model more likely to show a significant effect of antibody on an intracellular infection than a model in which infection is well established at the time antibody is first generated. Although technically difficult to perform with mice in sufficient numbers because of the very large numbers of anti-μ treatments needed during at least 12 weeks, it may be possible to use survivors of anti-μ treatment experiments to test for resistance to subsequent later rechallenge to see whether the protective local antibody and opsonization results (15) can be confirmed in that model.

Our earlier data have shown that host resistance in our model is strongly T cell dependent (12–15). Both antibody and cell-mediated immunities in this model are similarly T cell dependent (12–15). The data presented here strongly suggest that antibody is not the dominant T-cell-dependent host defense mechanism in primary invasive infection with MoPn. Because gamma interferon can inhibit replication of MoPn in vitro (G. I. Byrne, B. Grubbs, T. J. Dickey, J. Schachter, and D. M. Williams, unpublished data), we plan further studies of the role of this and other T-cell-dependent cytokines in host defense against MoPn.

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