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Role of antibody in the protection of mice from arthritis induced by *Mycoplasma pulmonis*

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SUMMARY

C_3H and $C_{57}B1_{10}$ mice exhibit a differential susceptibility to arthritis induced by *Mycoplasma pulmonis*. Resistant $C_{57}B1_{10}$ mice consistently demonstrated higher complement-fixing antibody titres than susceptible C_3H mice throughout the development of the acute arthritis. A strong correlation was demonstrated between the levels of anti-mycoplasma antibody and resistance to acute arthritis in six strains of mice. To test the hypothesis that the difference in arthritis resistance of C_3H and $C_{57}B1_{10}$ mice was caused by a different susceptibility of their lymphocytes to mitogenic stimulation by *M. pulmonis*, we investigated the generation of non-specific immunoglobulin *in vivo* and *in vitro*. *M. pulmonis* acted as a polyclonal mitogen, but stimulated approximately equal responses in lymphocytes of both strains.

INTRODUCTION

Arthritis induced by *Mycoplasma pulmonis* in mice provides a murine model of chronic arthritis with features resembling rheumatoid arthritis (Barden & Tully, 1969). Although the pathological features of the model are well defined, there is limited information on the immune factors determining disease susceptibility.

The rôle of antibody resistance to an *M. pulmonis* infection is unclear. Harwick *et al.* demonstrated that disease was at its nadir when titres of antibody to *M. pulmonis* were at their highest suggesting that the humoral host response suppressed the microorganism (Harwick *et al.*, 1976). The possibility that the humoral response was actually promoting disease was suggested by the development of antibody which cross-reacted with synovial tissue (Harwick *et al.*, 1976). More recently, Taylor and Taylor-Robinson demonstrated that convalescent-phase serum was effective in conferring resistance to acute arthritis in syngeneic recipient mice (Taylor & Taylor-Robinson, 1977). However, in this study, the protective capacity of the serum could not be correlated with the presence of complement-fixing antibody.

Another approach to determining the rôle of antibody in resistance to infection by *M. pulmonis* in mice is investigation of the humoral response in mice with a differential susceptibility to the organism. Preliminary studies revealed that two strains of mice, namely C_3H and $C_{57}B1_{10}$, exhibited a markedly different susceptibility to *M. pulmonis*-induced arthritis (Keystone *et al.*, 1978). The

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present study was undertaken to determine the kinetics of the antibody response in these and other mouse strains with a differential susceptibility to the arthritis.

MATERIALS AND METHODS

M. pulmonis (J.B. strain) was obtained as a broth culture from Dr Taylor-Robinson. The strain was originally provided by Dr J.G. Tully (NIH, Bethesda, Maryland, USA). Growth medium for *M. pulmonis* consisted of trypticase soy broth (80 ml), heat-inactivated horse serum (Flow Laboratories, Toronto, Canada) (20 ml), 0·002% phenol red, 0·05% thallium acetate and penicillin G (1000 units ml^{-1}). The pH of the medium was adjusted to 7·8.

Organisms used for mouse inoculation were grown in thallium-acetate-free medium and stored at −70°C.

Mice

Adult mice, 5–6 weeks old, of strains C_3H/Hej and $C_{57}B1_{10}$/Sn (Bar Harbour, USA) weighing 20–30 g were used. They were free of detectable mycoplasmas as determined by cultures of nasopharynx swabs and complement-fixing antibody to *M. pulmonis*.

Induction of arthritis and assessment of severity. Mice were inoculated by the intravenous route with 200 μl of the mycoplasma stock culture containing 5×10^8 colour-changing units (ccu). Controls consisted of mice inoculated with mycoplasma medium.

The severity of the arthritis was assessed by scoring the swelling of each joint on a scale from 0 to 3. Ankle, metacarpal, metatarsal and digit joints were assessed and a total score was obtained for each mouse. The mean arthritis score was derived by dividing the total score for all the mice in a group by the number of mice within the group.

Mycoplasma isolation procedures. At the termination of a study, mice were anesthetized by an intraperitoneal injection of sodium pentobarbitone and exsanguinated by severing the axillary vessels. Bilateral ankle and wrist joints were removed in sterile fashion from each mouse and homogenized in Ten-Broek grinders with mycoplasma medium to give 10% (w/v) suspensions. An estimation of the numbers of mycoplasmas isolated was carried out as described previously (Keystone *et al.*, 1978). Mycoplasma organisms were identified by the micromethod modification of the metabolism-inhibition test (Taylor-Robinson *et al.*, 1966).

Tissue culture and plaque assays. Spleen lymphocytes used in tissue culture for induction of plaque-forming cell responses were harvested aseptically in Hanks' balanced salt solution supplemented with 5% fetal calf serum (FCS) and washed three times. Cells were resuspended in alpha minimal essential medium (MEM) containing 10% FCS. Cultures were established in a concentration of 10^6 cells ml^{-1} in MEM with 5×10^{-5} M 2-mercaptoethanol and 10% FCS. Plastic trays or tubes containing 200 or 250 μl of cells, respectively, were used. *Escherichia coli* lipopolysaccharide (LPS) (Difco, Michigan, USA) and washed mycoplasma antigen (in growth medium) was added at a concentration of 20 and 160 μg ml^{-1}, respectively. Mycoplasma growth medium served as a control.

Sheep–trinitrophenyl (S–TNP). Sheep red-blood-cells (SRBC) were coupled with the trinitrophenyl (TNP) hapten using the method of Rittenberg & Pratt (1969) and utilized in the plaque-forming cell (PFC) assay as described previously (Cunningham & Szenberg, 1968).

Reverse-PFC. The reverse-PFC which detects Ig-releasing cells, regardless of their antibody specificity, was performed according to the method of Molinaro & Dray (1974). Briefly, SRBC were coated with sheep anti-mouse Ig using chromic chloride, and PFC were allowed to develop in the presence of rabbit anti-mouse Ig serum.

RESULTS

The previous studies with C_3H-CRC and $C_{57}B1_{10}$-CRC mice demonstrated that C_3H mice were highly susceptible to induction of *M. pulmonis* arthritis while $C_{57}B1_{10}$ mice were relatively resistant (Colt & Ward, 1979). Confirmation of a similar differential susceptibility was achieved with

C_3H/Hej and $C_{57}Bl$/sn mice (Fig. 1). That the differential susceptibility to induction of arthritis reflects a differential susceptibility to *M. pulmonis* growth within the joints was demonstrated by the isolation of *M. pulmonis* from fewer joints of the $C_{57}Bl_{10}$ mice as compared with C_3H mice (Table 1).

After establishing the differential susceptibility of the C_3H and $C_{57}Bl_{10}$ strains, we first determined the antibody titres at the time of the peak arthritis score. In four experiments, the antibody titre in $C_{57}Bl_{10}$ mice was consistently higher than that of the C_3H mice (Table 2).

One explanation for the differential antibody response to *M. pulmonis* mice might be differences in the time kinetics of antibody formation. Thus, kinetics studies were carried out to evaluate this possibility. However, the results of these studies revealed similar kinetics in both strains with antibody titres in $C_{57}Bl_{10}$ mice exceeding those of the C_3H mice throughout the period of observation (Fig. 2).

Since we demonstrated a differential antibody response inversely related to the severity of arthritis in C_3H and $C_{57}Bl_{10}$ mice, we were interested to determine whether a similar inverse relationship could be observed in other strains of mice with a differential susceptibility to *M. pulmonis*. We therefore inoculated several additional mouse strains with variable susceptibility to *M. pulmonis* including AbySn and A/J, 129 SV and $B_{10}A$ mice. The results demonstrated that C_3H,

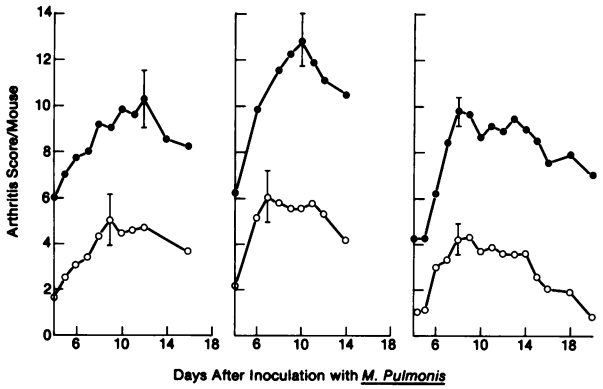

Fig. 1. Arthritis response of (•) C_3H and (o) $C_{57}Bl_{10}$ mice to 5×10^8 ccu *M. pulmonis* inoculated intravenously. Each point represents the mean arthritis score per mouse from a group of at least five mice and the error bars represent ± 1 s.e. Each panel represents a separate experiment.

Table 1. Isolation of mycoplasmas from joints of C_3H and $C_{57}Bl_{10}$ mice infected with *M. pulmonis*

Experiment no.*	Mouse strain	Positive no. of isolations/ no. of isolations performed	No. of mice from which *M. pulmonis* isolated/no. of mice inoculated	No. of organisms isolated
1	C_3H	8/36	7/9	$3{\cdot}8 \dagger \pm 0{\cdot}4$
	$C_{57}Bl_{10}$	1/40‡	1/10‡	$4{\cdot}0 \pm 0{\cdot}0$
2	C_3H	18/20	5/5	$4{\cdot}5 \pm 0{\cdot}2$
	$C_{57}Bl_{10}$	11/20‡	3/5	$4{\cdot}4 \pm 0{\cdot}4$

* C_3H and $C_{57}Bl_{10}$ mice in groups of at least five each were inoculated with 5×10^8 ccu *M. pulmonis* intravenously. Just after the peak arthritis score was achieved the mice were killed and attempts were made to isolate *M. pulmonis* from the wrist and ankle joints of each mouse.

† Mean number of organisms isolated from culture-positive joints expressed as $\log_{10} \pm 1$ s.e.

‡ Significant difference between C_3H and $C_{57}Bl_{10}$ by χ^2 analysis with $P < 0{\cdot}05$.

A/J and AbySn mice were highly susceptible to *M. pulmonis* with peak arthritis scores (±s.e.) of 7·9±0·7, 6·8±0·5 and 8·0±0·9, respectively. In contrast, $C_{57}B1_{10}$ and $B_{10}A$ mice were relatively resistant to *M. pulmonis* with arthritis scores of 3·2±0·5 and 3·3±0·3, respectively. One hundred and twenty-nine SV mice exhibited an intermediate susceptibility with a score of 5·0±0·4. Serial antibody studies were performed on the six strains (Fig. 3) and the results confirmed an inverse relationship between susceptibility to *M. pulmonis*-induced arthritis and the antibody titre with $r=0·89$, $P<0·05$ (Fig. 4).

One possible mechanism for the differential antibody response to *M. pulmonis* might be a differential susceptibility to polyclonal B cell stimulation by mitogens of *M. pulmonis*. To address this question we first studied the effect of *M. pulmonis in vitro* in stimulating a polyclonal antibody

Table 2. Antibody response to *M. pulmonis* generated in C_3H and $C_{57}B1_{10}$ mice after intravenous inoculation with *M. pulmonis*

Experiment no.*	Antibody titre	
	C_3H	$C_{57}B1_{10}$
1	6·7†±0·1	7·3†±0·4‡
2	7·0±0·1	8·0±0·3‡
3	5·2±0·2	6·2±0·4‡
4	6·0±0·0	7·8±0·3‡

* In each experiment at least five mice of each strain were inoculated intravenously with 5×10^8 ccu *M. pulmonis* and the mice were killed just after the peak arthritis response was achieved. Serum was collected at the time of death for the antibody studies depicted.

† Mean antibody titre expressed as $\log_2\pm1$ s.e.

‡ Significant difference from C_3H mice at $P<0·05$.

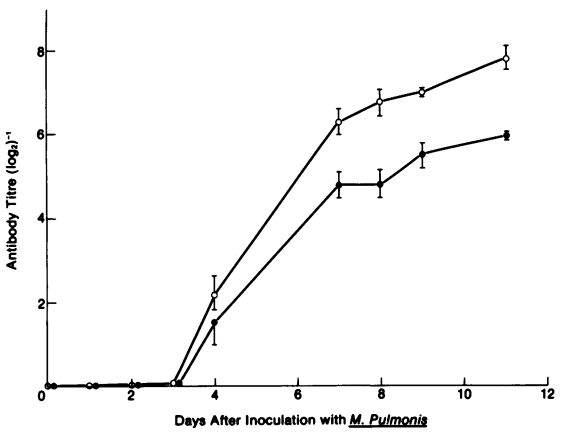

Fig. 2. Kinetics of the development of antibody to *M. pulmonis* in (●) C_3H and (○) $C_{57}B1_{10}$ mice after intravenous inoculation with 5×10^8 ccu of *M. pulmonis*. Each point represents the mean antibody titre (log 2)±1 s.e. from sera of five mice which were bled serially.

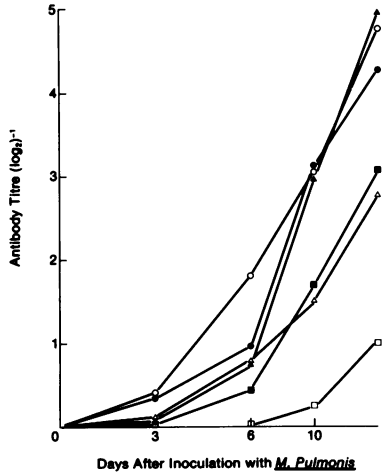

Fig. 3. Kinetics of the antibody response to *M. pulmonis* in a variety of mouse strains (▲) B10A, (○) $C_{57}B1_{10}$, (●) 129 SV, (■) C3H/HeJ, (△) AbySn, (□) A/J inoculated intravenously with 5×10^8 ccu *M. pulmonis*. Mice were inoculated in groups of at least 15 mice each. Mice were bled serially until just after the peak arthritis score for antibody levels. Each point represents the mean titre (log 2) for the particular mouse strain.

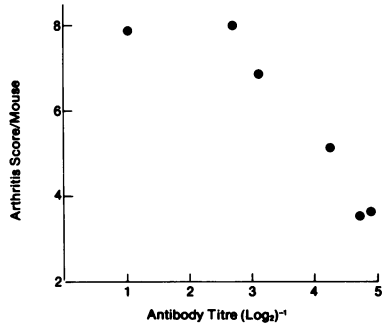

Fig. 4. Relationship between the clinical arthritis score and the antibody response to *M. pulmonis* (Fig. 3) in mice with a differential susceptibility to *M. pulmonis*.

Table 3. In-vitro PFC response to S–TNP and immunoglobulin production in C3H and $C_{57}B1_{10}$ mice in the presence of *M. pulmonis*, LPS, or medium alone

Experiment no.	PFC assay*	Mouse strain	Mycoplasma (PFC per 10^6 cells)	LPS (PFC per 10^6 cells)	Media (PFC per 10^6 cells)
1	S–TNP	C3H	69·2±8·0	72·6±5·8	21·2±3·5
		$C_{57}B1_{10}$	60·8±25·3	357·8±43·2†	14·4±0·9†
	Rev-PFC	C3H	137±24·4	110·6±10·6	76·8±6·3
		$C_{57}B1_{10}$	159·6±18·4	278·6±7·5†	72·4±7·3
2	S–TNP	C3H	50±1·2	11·0±0·6	7·0±0·6
		$C_{57}B1_{10}$	32·0±6·3†	119±5·8†	30·0±1·0†
	Rev-PFC	C3H	110·6±28·9	114±9·3	56±16·2
		$C_{57}B1_{10}$	177±37·6	429±131†	68±8·0

* In each experiment, spleen cells from five mice were cultured separately and the results expressed as the mean (±s.e.) PFC response. S–TNP PFC response was assayed on day 3 and reverse-PFC response was assayed on day 5.

† Significant difference between C3H and $C_{57}B1_{10}$ mice, $P < 0{\cdot}05$.

Table 4. *In-vivo* PFC response to S–TNP and immunoglobulin production in C_3H and $C_{57}Bl_{10}$ mice after intravenous inoculation with *M. pulmonis* or medium alone

PFC assays*	Mouse strain	Mycoplasma	Media
S–TNP	C_3H	46±3·5	17·3±1·3
	$C_{57}Bl_{10}$	34±6·1	8·0±3·2†
Rev-PFC	C_3H	1,450·7±197·0	426·7±121·9
	$C_{57}Bl_{10}$	1,472·0±37·0	95·3±15·9†

* Three C_3H and three $C_{57}Bl_{10}$ mice were inoculated intravenously with 5×10^8 ccu *M. pulmonis* or media and killed 4 days after inoculation. PFC responses were determined on the day of death with spleen cells from each mouse. The results are expressed as the mean (±s.e.) PFC response.

† Significant difference between C_3H and $C_{57}Bl_{10}$ mice, $P < 0{\cdot}05$.

('reverse') response in C_3H and $C_{57}Bl_{10}$ mice. The results demonstrated that C_3H mice exhibited a similar or greater polyclonal Ig response than $C_{57}Bl_{10}$ mice as assayed on S-TNP lawns (Table 3). Thus, the results did not support the hypothesis that the higher antibody response to *M. pulmonis* resulted from an augmented polyclonal response by $C_{57}Bl_{10}$ mice to mitogens in the organism.

To ensure that the in-vitro data had relevance in-vivo we studied the polyclonal antibody response to *M. pulmonis in vivo*. The results indicated that both strains exhibited a similar polyclonal response to *M. pulmonis* providing support for the in-vitro studies (Table 4).

DISCUSSION

The present study suggests a genetic influence on susceptibility of mice to *M. pulmonis*-induced arthritis. Data to support this observation has been provided by Hannan (1971) and Taylor, Taylor-Robinson & Slavin (1974). More frequent isolation of *M. pulmonis* from the more severely arthritic C_3H mice suggests that the differential susceptibility is based on the differential growth of *M. pulmonis* within the affected joints. This observation thus supports the notion that *M. pulmonis* arthritis is caused by the proliferation of viable mycoplasma organisms within the joint although a hypersensitivity mechanism cannot be excluded.

The present data suggests that antibody plays a major rôle in protection against *M. pulmonis*. Thus $C_{57}Bl_{10}$ mice with less severe arthritis and less frequent isolation of organisms from the joints consistently exhibited higher antibody titres at the peak clinical response than the more severely affected C_3H mice. This differential response was observed within several days after intravenous inoculation and persisted throughout the development of the arthritis. This inverse relationship between clinical arthritis and antibody titre was confirmed using a variety of mouse strains.

That antibody is important in the protection against *M. pulmonis* is supported by studies of Taylor & Taylor-Robinson who demonstrated the efficacy of passively administered convalescent serum in the protection of mice against *M. pulmonis* (Taylor & Taylor-Robinson, 1977). However, the protective effect of serum could not be adsorbed out with *M. pulmonis* antigen. That is, protection remained despite the removal of complement-fixing antibodies. This data suggests that other non-complement-fixing antibodies may be important but remain undetected by the usual serological methods. Indeed, studies by Taylor have demonstrated opsonizing antibodies in convalescent mouse serum (Cole & Ward, 1979). The present study suggests a rôle for complement-fixing antibody (or at least an antibody synthesized in association with complement-fixing antibody) as evidenced by the inverse correlation between the complement-fixing antibody titre and severity of arthritis.

The reason for the differential antibody response to *M. pulmonis* amongst the C_3H and $C_{57}Bl_{10}$ strains was unclear. One possible explanation might be a differential susceptibility to *M. pulmonis* as a polyclonal antibody stimulant. Thus, several investigators have demonstrated mycoplasmas to be capable of stimulating B cells in a polyclonal fashion (Biberfield & Gronowicz, 1976; Noat & Ginsburg, 1978). If *M. pulmonis* differentially activated B cells of the two strains, then differential antibody production might be explained. On this account, studies into the ability of *M. pulmonis* to act as a polyclonal antibody stimulant were undertaken.

The results demonstrated, for the first time, that *M. pulmonis* has the capability of activating lymphocytes *in vitro* to synthesize antibody in a polyclonal fashion (Table 3). This data is compatible with the observations of Naot, Siman-Tor & Ginsberg (1979) and Cole *et al.* (1975) who demonstrated the potential of *M. pulmonis* to cause non-specific proliferation of both B and T lymphocytes. Whether *M. pulmonis* activates B and/or T cells (and/or macrophages) to cause polyclonal antibody synthesis remains to be determined. Of interest, *M. pulmonis* appears to be a somewhat more effective stimulant *in vivo* than *in vitro*.

The ability of *M. pulmonis* to act on a polyclonal Ig stimulator was compared with the effects of a known stimulator, *E. coli* LPS, and with medium alone. The responses measured were PFC numbers, not only 'reverse'-PFC but also those active against S–TNP, an indicator which picks up a very broad range of antibody specificities. The results (Tables 3 and 4) showed that, in spite of a generally higher reaction by $C_{57}Bl_{10}$ cells to LPS, *M. pulmonis* stimulated approximately equal responses in lymphocytes of both strains *in vitro* and *in vivo*.

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