Immunity to mycoplasma infections of the respiratory tract: a review[1]

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The pathogenesis of mycoplasma respiratory infections of different animal species appears to be very similar. For this reason most investigations of immunity to mycoplasma infections of the respiratory tract have been carried out using small laboratory animals. Although much information has been provided by these studies it must be remembered that each infectious disease is a distinct entity with its own particular features. Thus, events important in the resistance of a particular animal species to one mycoplasma may not necessarily be the same for others.

After the first exposure of an animal to mycoplasmas, the organisms often persist in the respiratory tract for prolonged periods. For example, carriage of *M. pneumoniae* in the upper respiratory tract of man may persist for several weeks (Grayston *et al.* 1967), while in mice inoculated intranasally with *M. pulmonis*, both the pneumonic lesions and the mycoplasmas persist for several months (G Taylor, unpublished observations). The prolonged nature of many mycoplasma infections suggests that the host's immune response may be inefficient. However, persistently infected animals are resistant to a subsequent intranasal challenge with the same organism. Thus, mice inoculated intranasally with small numbers of *M. pulmonis* organisms do not develop lung lesions, although mycoplasmas can be isolated from their lungs for at least six weeks after inoculation. Following intranasal challenge with a large dose of *M. pulmonis*, which produces severe pneumonia in control animals, mice previously inoculated with small numbers of mycoplasmas develop much less severe pneumonia and fewer organisms are isolated from their lungs compared with controls (Taylor *et al.* 1977). Thus, recovery from a naturally occurring or experimentally induced mycoplasma respiratory disease results in immunity. Although reinfection of previously infected animals can occur, such animals are generally protected against the development of a subsequent mycoplasma respiratory disease.

The factors involved in resistance to mycoplasma infections of the respiratory tract are still not clearly understood. Resistance to an infectious agent usually consists of the interactions of several components such as nonspecific factors, various classes of immunoglobulin, different cell types and complement components. The importance of some of these factors in relation to resistance to mycoplasma infections of the respiratory tract are discussed below.

Nonspecific defence mechanisms

(1) *Mucociliary blanket:* One of the first barriers that an invading organism meets in the respiratory tract is the various inhibitory substances present in mucus. Howard and coworkers (1975) have shown that a heat-stable, dialysable fraction of bovine nasal secretions is inhibitory for a variety of mycoplasma species *in vitro*. Nevertheless, mycoplasmas are able to penetrate the mucus blanket in some way, and attach firmly to the bronchial epithelial cells (Collier & Clyde 1971, Organick *et al.* 1966). In this position, between the cilia, the mycoplasmas are probably protected from the mucociliary clearance mechanisms.

(2) *Alveolar macrophages:* These phagocytic cells play an important part in the clearance of particles from the bronchoalveolar spaces of the lower respiratory tract. However, in the absence of specific antibody, the phagocytosis of mycoplasmas by alveolar macrophages *in vitro* is not very efficient. In fact the organisms attach to the surface of the macrophage where

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they replicate (Howard *et al.* 1976). This initial interaction of mycoplasmas and macrophages appears to be important in determining the extent of pulmonary disease. Cassell and colleagues (1973) compared the pulmonary clearance of *M. pulmonis* organisms in rats and mice and showed that, 48 hours after intranasal inoculation of mice, few phagocytic cells in the lung contained mycoplasmas; large numbers of mycoplasmas persisted in the alveoli for at least seven days. In contrast, most of the mycoplasmas in rat lungs were in or associated with alveolar macrophages from 10 minutes to 48 hours after inoculation; the organisms had cleared from the alveoli by day 7.

(3) *Interferon:* Several mycoplasma species induce the production of interferon by lymphocytes (Cole *et al.* 1976). However, the role (if any) of interferon in resistance to mycoplasma infections is not known.

(4) *Complement: M. pneumoniae* organisms can activate the alternate pathway of complement and this mycoplasma and *M. pulmonis* are susceptible to the products of complement activation (Bredt & Bitter-Suermann 1975, Taylor-Robinson *et al.* 1978). However, the importance of complement in resistance to mycoplasma respiratory infection is not known.

Serum antibody

Mycoplasma-induced pneumonic lesions are characterized by peribronchiolar and perivascular accumulations of lymphoid cells as shown in Figure 1. Many of these cells appear to be producing specific antibody to the mycoplasma and it has been suggested that the lung and draining lymph nodes are the main sites of antibody production in mycoplasma respiratory diseases (Cassell *et al.* 1974). Antibody may be transported from these sites into serum and respiratory secretions.

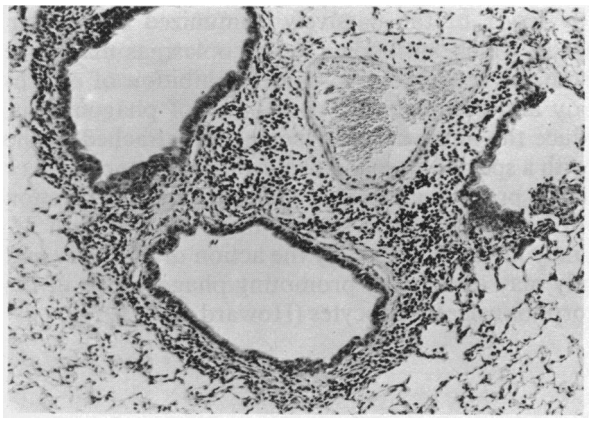

Figure 1. Vertical section of lung from a hamster inoculated intranasally 2 weeks previously with *M. pneumoniae*, showing mononuclear cell accumulations in the peribronchiolar and perivascular areas. (× 88)

Serum antibodies to mycoplasmas can be measured by a variety of techniques such as complement fixation, indirect haemagglutination, metabolism inhibition and mycoplasmacidal tests. However, there is often a poor correlation between the presence of such antibodies and resistance to reinfection with mycoplasmas (*see* Whittlestone 1976). This has led to the suggestion that serum antibodies to mycoplasmas may be solely a reflection of previous infection and not the actual determinants of resistance. A way of examining whether serum antibody plays a part in resistance to mycoplasma respiratory disease is to determine whether immunity can be conferred on recipient animals by the passive transfer of serum. A number of investigations of this kind have been successfully undertaken. For example, resistance of cattle to *M. mycoides* var. *mycoides* (Masiga *et al.* 1975) of pigs to *M. hyorhinis* (Goiš *et al.* 1974)

and to *M. hyopneumoniae* (Lam & Switzer 1971), and of mice to *M. pulmonis* (Taylor & Taylor-Robinson 1976) has been transferred with convalescent serum. Although passive transfer of immune serum suppressed the development of pneumonia in mice, there were no significant differences in the numbers of mycoplasmas isolated from the lungs of the passively immunized and control animals examined 14 days after challenge with *M. pulmonis* (Taylor & Taylor-Robinson 1976). The way in which the transferred serum prevented the development of pneumonic lesions, despite the presence of large numbers of mycoplasmas in the respiratory tract, is not clear. It is known that passively administered antibody can suppress the immune response by competing with receptors on lymphocytes for the antigen (Dixon *et al.* 1967). The serum antibody response of passively immunized pigs to *M. hyorhinis* and of passively immunized mice to *M. pulmonis* was suppressed when compared with that of control animals (Goiš *et al.* 1974, Taylor & Taylor-Robinson 1976). Thus, one explanation is that the transferred serum prevented the sensitization of lymphocytes by the mycoplasma, so that the accumulation of lymphoid cells in the peribronchiolar and perivascular areas of the lung was suppressed. Another explanation may be that the protective antibodies are not directed against the mycoplasma itself, but against some product of the organism that is essential for the development of pneumonia. However, the presence of such a factor has not been demonstrated.

There were no significant differences in the numbers of mycoplasmas isolated from the lungs of the passively immunized and control animals 14 days after intranasal challenge with *M. pulmonis*; but when these animals were examined at intervals for 6 weeks after challenge, there appeared to be an increased clearance of mycoplasmas from the lungs of passively immunized mice when compared with controls (Taylor & Taylor-Robinson 1976). In addition, 14 days after challenge with a small number of *M. pulmonis* organisms, mycoplasmas were isolated from the respiratory tract of significantly fewer of the passively immunized mice when compared with control animals. The ways in which antibodies to mycoplasmas may have brought about this increased clearance from the respiratory tract are: inhibition of attachment; growth inhibition; lysis by antibody and complement; promotion of phagocytosis. Antibody in the bronchial lumen may reduce the numbers of mycoplasmas attached to the bronchial epithelium either by combining with a specific attachment site on the mycoplasma or by agglutinating them. Although some animals produce antibodies that inhibit the replication of mycoplasmas, such antibodies have not been detected in sera of mice infected with *M. pulmonis* (Cole *et al.* 1970). Mycoplasmas may have been lysed by the action of antibody and complement (Cole & Ward 1973). Antibody may also act by promoting phagocytosis of the mycoplasmas by macrophages and polymorphonuclear leukocytes (Howard *et al.* 1976).

Cell-mediated immunity

Until recently the significance of cell-mediated immunity (CMI) in mycoplasma infections has received less attention than that of antibody. Various *in vitro* correlates of CMI reactions, such as delayed skin hypersensitivity reactions, lymphocyte transformation and macrophage migration inhibition tests, have been demonstrated in a number of mycoplasma respiratory infections (*see* Taylor & Taylor-Robinson 1975). However, it is becoming increasingly apparent that such tests are not exclusive for thymus-dependent lymphocytes (T cells). For example, following antigenic stimulation human B and T cells produce similar quantities of macrophage inhibition factor (Rocklin *et al.* 1974). Thus, taken on their own, these tests do not provide direct evidence for a major role of CMI in resistance to mycoplasma infections, although they do indicate the presence of antigen-reactive lymphocytes. It may be that a test such as lymphocyte transformation provides a better indicator of previous exposure to mycoplasmas than does the presence of serum antibody. Care must be taken in the interpretation of results from lymphocyte transformation tests, since some mycoplasmas are mitogenic (Cole *et al.* 1975, Biberfeld & Gronowicz 1976).

In order to determine whether CMI is important in resistance, it is necessary to show that immunity can be conferred on recipient animals by the passive transfer of sensitized

lymphocytes. There have been few reports of such experiments in relation to mycoplasma infections because of the requirement for inbred animals. However, spleen cells obtained from mice at various intervals after intravenous inoculation of *M. pulmonis* failed to confer resistance to respiratory disease on recipient animals (Taylor & Taylor-Robinson 1976).

In infections where CMI plays an important part in resistance, it is possible to demonstrate effector cells *in vitro*. For example after antigen stimulation, T cells produce factors that 'activate' macrophages thereby increasing their phagocytic and microbicidal capacities (Mackaness 1964). Figure 2 shows the inactivation of *M. pulmonis* by peritoneal exudate cells obtained from infected and control mice, in the presence of either normal or convalescent mouse serum. It appears that there are no significant differences in the inactivation of *M. pulmonis* by cells obtained from either group of animals.

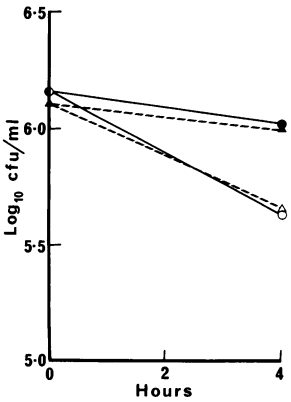

Figure 2. Interaction of *M. pulmonis* with peritoneal exudate cells obtained from normal mice and incubated with normal mouse serum (●—●) or with convalescent mouse serum (○—○), or obtained from infected mice and incubated with normal mouse serum (▲---▲) or with convalescent mouse serum (△---△). All sera at a final dilution of 1/50

Sensitized lymphocytes or phytohaemagglutinin (PHA) transformed lymphocytes can be directly cytotoxic for virus-infected cells (Blanden 1974). The effect of lymphocytes on mycoplasmas has not been studied extensively. However, a preliminary experiment to examine the effect of normal and PHA transformed human peripheral blood lymphocytes on *M. pneumoniae* indicated that these cells had no effect on the viability of the mycoplasmas (Taylor & Taylor-Robinson, unpublished observations).

Although there is no evidence that CMI reactions play an important role in resistance to mycoplasma respiratory infections, studies on the development of mycoplasma pneumonia in immunosuppressed animals indicate that T cells are involved in the production of antibody to mycoplasmas and in the development of pneumonic lesions (Denny *et al.* 1972, Taylor & Taylor-Robinson 1975). In addition, Fernald and co-workers (1972) reported that there is a rapid accumulation of lymphoid cells, which do not appear to be producing immunoglobulin, in the peribronchiolar and perivascular areas of the lungs of previously infected hamsters after intranasal challenge with *M. pneumoniae*. The time course of the appearance of these cells is reminiscent of a delayed-type hypersensitivity reaction and is associated with a marked outpouring of phagocytic cells into the bronchial lumen. It was suggested that this accumulation of cells may represent the appearance of memory cells which possibly initiates a rapid mobilization of the processes involved in recovery from infection. The occurrence of a similar response in other animals has not been reported.

Local immunity

Various studies have suggested that local immune mechanisms may play a greater role in resistance to mycoplasma infections of the respiratory tract than do systemic immune mechanisms (Fernald & Clyde 1970, Taylor *et al.* 1977). For example, stimulation of high levels of serum antibody by systemic vaccination with mycoplasmas does not always provide very good protection against disease (*see* Taylor & Taylor-Robinson 1975). Further, studies comparing the ability of various vaccination routes to induce resistance indicate the importance of stimulation of the respiratory tract in the development of immunity (Greenberg, *et al.* 1977, Taylor *et al.* 1977, Howard *et al.* 1977). Greenberg *et al.* (1977) demonstrated that hamsters vaccinated intranasally on three occasions with formalin-inactivated *M. pneumoniae* organisms were resistant one month later to intranasal challenge with live organisms.

Using formalin-inactivated *M. pulmonis* organisms in mice we compared the latter regime with that of a subcutaneous followed by intranasal vaccination regime described previously (Taylor *et al.* 1977). As shown in Figure 3, when animals were challenged seven days after

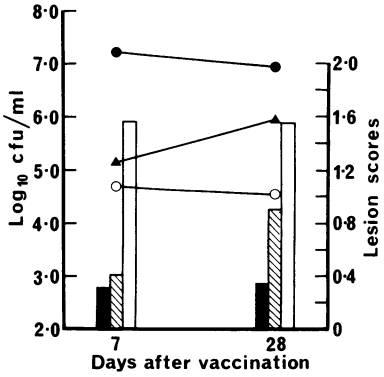

Figure 3. Severity of respiratory disease 14 days after intranasal (i.n.) challenge with 5×10^5 colony forming units (cfu) of *M. pulmonis* in mice vaccinated with formalin-inactivated mycoplasmas. Number of mycoplasmas isolated from lungs of: control mice (●—●); mice vaccinated subcutaneously (s.c.) and i.n. (▲—▲); mice vaccinated i.n. 3 × (○—○). Severity of lung lesions in control mice (□), mice vaccinated s.c. and i.n. (▨) and mice vaccinated i.n. 3 × (■)

vaccination, the numbers of mycoplasmas isolated from the lungs of both groups of vaccinated mice were significantly less than those isolated from controls. However, when the challenge was delayed until one month after vaccination, the numbers of mycoplasmas isolated from the lungs of the mice vaccinated three times intranasally were significantly less than those isolated from the lungs of animals vaccinated with a systemic followed by local regime. Similarly, protection against pneumonic lesions appeared to persist longer in those mice vaccinated solely by the intranasal route than in those animals vaccinated subcutaneously and intranasally.

Following intranasal inoculation of small laboratory animals most of the inoculum is distributed to the lower respiratory tract. This route is probably equivalent to an intratracheal or endobronchial inoculation in larger animals, and such a regime may therefore not be acceptable. However, these studies do suggest the importance of local immunity in the development of resistance to mycoplasma respiratory infections, although the relative importance of local CMI reactions and locally produced antibody is unclear. Several investigators have demonstrated a compartmentalization of the immune response, since sensitization of lymphocytes in the respiratory tract can occur in the absence of systemic sensitization, and *vice versa* (Waldman *et al.* 1972). Thus, the presence or absence of CMI reactions to mycoplasmas with systemically derived cells may not bear any relation to resistance at a mucosal surface. This requires further investigation.

Attempts to demonstrate specific antibody to mycoplasmas in various respiratory tract

secretions by growth-inhibition or complement-fixation tests have not been very successful (Davies 1969, Fernald & Clyde 1970). In contrast, application of the more sensitive technique of radioimmunoassay (RIA) has been more rewarding. Using this technique Brunner and colleages (1973) examined the response to experimental challenge with *M. pneumoniae* in volunteers with different levels of specific IgA antibody in nasal secretions. Their findings suggested that this antibody was related to resistance to *M. pneumoniae*. Using a solid-phase RIA test we have examined the antibody response to *M. pulmonis* in sera and lung washings obtained from vaccinated mice. We failed to demonstrate any IgA antibody to *M. pulmonis* in 10 × concentrated lung washings from mice vaccinated either three times intranasally, or subcutaneously and intranasally. In fact antibody to *M. pulmonis* in lung washings appeared to be predominantly of the IgG1 class (G Taylor, unpublished observations). The origin of this antibody is not known, although preliminary investigations indicate that it may be produced locally.

If IgG1 antibody to *M. pulmonis* in lung washings is involved in resistance, then its mode of action is unclear, since this class of immunoglobulin in mice does not fix complement very efficiently and does not promote phagocytosis (Spiegelberg 1974). We failed to detect any inhibitory activity *in vitro* of lung washings from vaccinated mice against *M. pulmonis*. Further, we have not been able to transfer resistance to *M. pulmonis* using such lung washings (Table 1).

Table 1. Effect of prior incubation of M. pulmonis *with lung washings or sera on infectivity for the respiratory tract*

M. pulmonis incubated with	Titre after incubation (37°C for 30 min)	No. of mycoplasmas isolated from lungs 24 h after intranasal inoculation
Phosphate buffered saline	6.0●	2.7●
Control lung wash	6.0	3.4
Vaccinated lung wash■	6.0	2.9
Normal mouse serum▲	6.1	3.9
Convalescent mouse serum▲	6.1	0

● $\log_{10}$ colony forming units per ml
■ Lung washings obtained 7 days after vaccination from mice inoculated subcutaneously and intranasally with formalin-inactivated *M. pulmonis*
▲ Serum used at 1/50 dilution in phosphate buffered saline

Thus, *M. pulmonis* organisms were incubated for 30 min at 37°C with: phosphate buffered saline (PBS); unheated lung washings obtained from control or vaccinated mice; heated (56°C for 30 min) normal mouse serum; or heated mouse serum obtained from previously infected animals. This procedure had no detectable effect on the viability of the organisms. Mice were inoculated intranasally with 0.05 ml of the various suspensions, after incubation, and examined 24 hours later. As shown in Table 1, only prior incubation of mycoplasmas with convalescent serum enhanced the clearance of mycoplasmas from the respiratory tract.

Conclusion

A possible sequence of events following challenge of previously infected animals with mycoplasmas may be that any IgA antibody present in the upper respiratory tract acts as a first line of defence by inhibiting the attachment of organisms to the respiratory epithelium. However, since the half-life of IgA is short, such antibody may be present in the respiratory tract for relatively short periods of time after either infection or vaccination. If this initial defence is absent or overwhelmed when mycoplasmas infect the respiratory tract, they may stimulate a local inflammatory reaction with consequent transudation of serum antibody into the bronchial lumen. This antibody, or locally produced antibody, may promote phagocytosis of the mycoplasmas or, with complement, cause lysis of the organisms and so enhance their clearance from the respiratory tract. Such a response may serve to limit the infection, resulting in a less severe disease.

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