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Intranasal inoculation of *Mycoplasma pulmonis* in mice with severe combined immunodeficiency (SCID) causes a wasting disease with grave arthritis

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SUMMARY

Mycoplasma pulmonis or *Myc. pneumoniae* were inoculated intranasally to C.B-17 *scid/scid* mice (severe combined immunodeficient (SCID) mice). Immunocompetent C.B-17 mice were inoculated as controls. During the observation period of 5 weeks the mice were killed and necropsied. *Mycoplasma pulmonis* was recovered from all of the inoculated mice, and dissemination to various tissues increased with time. SCID mice, unlike immunocompetent mice, did not show lung lesions but exhibited severe inflammatory changes of the joints. *Mycoplasma pulmonis*, however, was isolated both from the lungs and the articular lesions. In addition, SCID mice infected for more than 3 weeks suffered from a pronounced loss of weight and emaciation. In the experiment with *Myc. pneumoniae* the agent could be reisolated, but lesions were not found in any of the infected mice. *Mycoplasma pulmonis* infection in SCID mice may be useful as a model of arthritis in immunodeficient humans.

Keywords *scid* mice arthritis *Mycoplasma pulmonis* *Mycoplasma pneumoniae*

INTRODUCTION

Mycoplasmas are ubiquitous organisms that may infect animals and man, and can contaminate cell cultures. The course of mycoplasmal infections can be influenced by various factors, a major one being the immune system of the host. In immunodeficient patients mycoplasmas can act as opportunists. Mycoplasmas generally occurring in the urogenital or respiratory tract have been found to cause arthritis and sometimes osteomyelitis in patients with hypogammaglobulinaemia [1–4].

The murine infection with *Mycoplasma pulmonis* resembles in many respects the human infection with *Myc. pneumoniae* [5,6]. *Mycoplasma pulmonis* is a rodent pathogen with an affinity for the mucosal cell surfaces. *Mycoplasma pulmonis*-infected animals frequently suffer from lesions in the respiratory tract, but the reproductive organs, joints, and brain can also be affected [7]. The natural course of rodent mycoplasmosis depends on the immune status of the animal, reflected by age, species, and strain. The disease can be subclinical as well as overt and sometimes lethal. In terminal stages there is weight loss, roughened hair coat, nasal discharges, and dyspnoea. Experimental infection with *Myc. pulmonis* in mice produces rhinitis, otitis, laryngotracheitis and an acute alveolar pneumonia with a rich infiltration of neutrophils and a high mortality. Surviving animals may develop a chronic disease. Lesions in the lungs are characterized by chronic inflammation with lymphoid hyperplasia [8,6]. If *Myc. pulmonis* is inoculated intravenously arthritis can occur [9,10].

Nude animals, i.e. mice and rats with T cell deficiency, have been used to investigate the immune response to mycoplasma organisms. Nude mice intranasally infected with *Myc. pulmonis* developed arthritis [10] and parenteral infection with *Myc. pulmonis* resulted in more severe arthritis in athymic nude mice than in normal mice. Furthermore, in athymic animals larger numbers of *Myc. pulmonis* organisms could be isolated from joints and spleens and the infection persisted for a longer time [11].

The mutation severe combined immunodeficiency (*scid*) is characterized by the virtual absence of functional B and T lymphocytes, due to a defect in the mechanisms responsible for the rearrangement of antigen receptor genes [12,13]. The aim of this investigation was to describe the lesions caused by *Myc. pulmonis* in *scid* mice. Although mice are not natural hosts of *Myc. pneumoniae* [14], infections with *Myc. pneumoniae* were also performed, to evaluate the susceptibility of the *scid* mouse

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to this organism, and to explore the possibility of developing a murine model of the infection.

MATERIALS AND METHODS

Mice

Scid mice, males, 7 weeks old, were obtained from a breeding colony at Stockholm University and from Bommice (Ry, Denmark). The animals from the colony at Stockholm University were intermittently given prophylactic treatment with trimethoprim and sulfadoxin in the drinking water to protect against *Pneumocystis carinii* infection. Animals from Bommice were not treated with antibiotics, and remained free from *P. carinii* throughout the study. During the experiment the mice were housed in sterilized plastic boxes provided with high efficiency air filters (environmental conditions in the isolator: temperature 22°C, relative humidity 60%, 12 h light:12 h dark schedule), and had free access to sterilized pellets and water.

Mycoplasma strains

Mycoplasma pulmonis, M 195/82, was isolated from a mouse with pneumonia and had been passaged four times before being stored at −70°C. *Mycoplasma pneumoniae*, M28/92, was isolated from a patient sputum sample and had been passaged three times before being stored at −70°C.

Mycoplasma cultivation

Broth for cultivation was composed of 300 ml Hanks' balanced salt solution (HBSS), 450 ml glass-distilled water, 4·9 g Bacto-Brain Heart Infusion (Difco, Detroit, MI), 5·2 g Bacto-PPLO Broth w/o CV (Difco), 35 ml fresh yeast extract, 100 mg ampicillin (Astra, Södertälje, Sweden), 2·8 ml 0·5% phenol red, 2·0 ml 5% thallium acetate, and 185 ml heat-inactivated horse serum. The pH was adjusted to 7·4. For solid medium 0·6% agarose (Marine Colloids, Rockland, MN) was added. Pure inoculates were cultured without thallium acetate and ampicillin. Tissue samples, 0·1 g, were ground in a mortar in 2·7 ml broth. The cotton wire swabs were immersed in 2·7 ml broth and left for 1 h. From these and from the tracheobronchial washings 0·01 ml was streaked to plates. For each animal, pooled sample suspensions were inoculated to broth in 10-fold serial dilutions to 10^{-4}. Plates were incubated 7 days in an atmosphere with 5% CO_2. The broth samples were incubated for 21 days or until they showed an unequivocal change in pH, when they were subcultured on agar. From one of the highest dilutions showing pH change subculture was also made in broth. After detection of mycoplasma colonies on the plates the isolates were identified by immunofluorescence on unfixed agar colonies, using antisera against *Myc. pulmonis* and *Myc. pneumoniae*, respectively.

Histological preparation

Tissue specimens were fixed by immersion in 10% buffered formalin pH 7·4 for 24 h, approximately. The skull and bones were decalcified by immersion in Christensen's fluid until softened. Tissue specimens were processed routinely and stained with haematoxylin and eosin. Sections of the nasal mucosa, lung, and joints were also stained with the Giemsa stain. In addition, selected sections from lung tissues were stained with the Warthin–Starry (silver) stain, to exclude the presence of the ciliae-associated respiratory (CAR) bacillus.

Preparation of inoculate

The strains were thawed and cultured until the acid pH shift appeared in the broth after 1–3 days. The numbers of colony-forming units (CFU)/ml broth were determined. Before inoculation the cultures were appropriately diluted with PBS.

Experimental design

In the first experiment 14 *scid* mice and three C.B-17 mice were inoculated through the nose with *Myc. pulmonis*. Additionally, two uninfected *scid* mice were killed as controls. The *scid* mice were randomly allocated into three groups, exposed to 10^5 CFU, 10^4 CFU and 10^2 CFU (five, four and five *scid* mice were allocated in each group, respectively). One mouse from each group given 10^5 CFU and 10^2 CFU was then killed at post-infection days 6, 13, 20, 24 and 35. The *scid* mice infected with 10^4 CFU were killed at day 30, except one who died at day 25. The C.B-17 mice were killed at post-infection day 38.

In the other experiment 12 *scid* mice and five C.B-17 mice were given *Myc. pneumoniae* intranasally. Six of the *scid* mice and three of the C.B-17 mice received 10^5 CFU, and the other mice 10^3 CFU. Five *scid* mice and two C.B-17 mice served as uninfected controls. Infected animals were killed after 6, 13, 20, 27 and 34 days, respectively.

In both experiments the mice were necropsied immediately after euthanasia by CO_2 inhalation. Samples for histopathological examination and mycoplasma isolation were obtained from the nasal mucosa, trachea, bronchi, lung, spleen, and the coxofemoral and the knee joints. Cultural samples from the nasal mucosa and the articular surfaces were collected by thorough swabbing. The trachea and bronchi were sampled by washing the mucosa with culture media injected into the lumen. The samples from the other tissues consisted of cut tissue blocks. Gross lesions found in other tissues were also examined histologically and cultured.

RESULTS

Mycoplasma pulmonis *infection in* scid *mice*

The findings in mice infected with *Myc. pulmonis* are summarized in Table 1. Body weights of infected *scid* mice were decreased. During the first 20 days after infection this was expressed as a retardation of growth, and later on as a pronounced loss of weight and emaciation. *Mycoplasma pulmonis* was recovered from all of the inoculated mice. In C.B-17 mice *Myc. pulmonis* was solely reisolated from the respiratory organs, which was also the site affected by lesions. In *scid* mice dissemination of *Myc. pulmonis* increased with time. *Mycoplasma pulmonis* was recovered from the nasal mucosa, trachea, lung, joints, spleen, kidney, and middle ear. The animals treated with trimethoprim and sulfadoxin (*scid* L217–330) showed the same pattern as untreated animals. Despite widespread colonization of tissues by *Myc. pulmonis*, the main lesions were found in the joints. Lesions in the lungs were not observed. However, the nasal mucosa showed pathological signs. Gross changes were not evident, but histological examination revealed focal degeneration of the nasal epithelium, mucusal oedema, and a discrete infiltration of the nasal mucosa with neutrophils and monocytes. In other tissues changes were not noticed. Mice killed at post-infection day 13 did not show lesions. Moderate lesions of rhinitis in *scid* mice were also found histologically at 20, 24, and 30 days post-infection. One *scid* mouse found dead

Table 1. Experimental infection in mice with *Mycoplasma pulmonis*

Mouse	Dose of infection (CFU)	Body weight* (g)	*Myc. pulmonis* culture						Histological examination		
			Days†	Nose	Trachea	Lung	Joints	Spleen	Other organs	Nasal mucosa	Bronchi, trachea, lung	Joints
scid L248	10^5	25	6	+ + +	+ + +	–	NE	NE	NE	Moderate rhinitis	Lung: negative	NE
scid L249	10^2	24	6	+	+	–	NE	NE	NE	Negative	Lung: negative	NE
scid L280	10^5	23	13	+ +	+	+	NE	–	NE	Negative	Negative	NE
scid L279	10^2	24	13	+ + +	+	+	NE	–	NE	Negative	Lung: perivascular monocyte infiltrates	NE
scid L282	10^5	21	20	+ +	+	NE	NE	–	NE	Severe rhinitis	Negative	NE
scid L284	10^2	26	20	+ +	+ +	+ +	NE	–	Middle ear + + + +	Moderate rhinitis	Negative	NE
scid L303	10^5	17	24	+ + +	+ + +	+ +	NE	+ +	Ear + + + +	Moderate rhinitis	Lung: negative	NE
scid L304	10^2	20	24	+ + +	+	+	NE	+ + +	NE	Moderate rhinitis	Negative	NE
scid L610	10^4	10–12	25	+	NE	+ +	+	NE	NE	NE	Lung: negative	Severe arthritis
scid L626	10^4	10–12	30	+	NE	+ +	+	NE	NE	Mild rhinitis	Lung: negative	Severe arthritis
scid L627	10^4	10–12	30	+ +	NE	+ +	+	–	NE	Severe rhinitis	Lung: negative	Mild arthritis
scid L628	10^4	10–12	30	+ +	NE	+ + +	+ +	+ +	NE	Moderate rhinitis	Lung: negative	Moderate arthritis
scid L328	10^5	18	35	+ + + +	+	+ + +	+ +	+ + +	Kidney + +	Negative	Lung: negative	Severe arthritis
scid L330	10^2	18	35	+ + +	+ + +	+	+ + +	+ + +	NE	Mild tracheitis	Lung: negative	Severe arthritis
C.B-17L629	10^4	22–23	38	+ +	NE	+ +	–	–	NE	Mild rhinitis	Severe bronchitis Severe pneumonia	Mild arthritis
C.B-17L630	10^4	22–23	38	+ +	NE	+ +	–	–	NE	Moderate rhinitis	Mild bronchiolitis	Negative
C.B-17L631	10^4	22–23	38	+	NE	–	–	–	NE	Moderate rhinitis	Mild bronchiolitis Pneomonia	Negative
Controls												
scid L217	0	23	0	–	–	–	NE	–	NE	Negative	Lung: negative	NE
scid L218	0	23	0	–	–	–	NE	–	NE	Negative	Negative	NE
scid L559	0	21	0	–	–	–	–	–	NE	Negative	Lung: negative	Negative
scid L560	0	21	0	–	–	–	–	–	NE	Negative	Lung: negative	Negative
C.B-17L561	0	21	0	–	–	–	–	–	NE	Negative	Lung: negative	Negative
C.B-17L562	0	21	0	–	–	–	–	–	NE	Negative	Lung: negative	Negative

* Body weight at euthanasia.
† Time elapsed between infection date and euthanasia.
NE, Not examined.

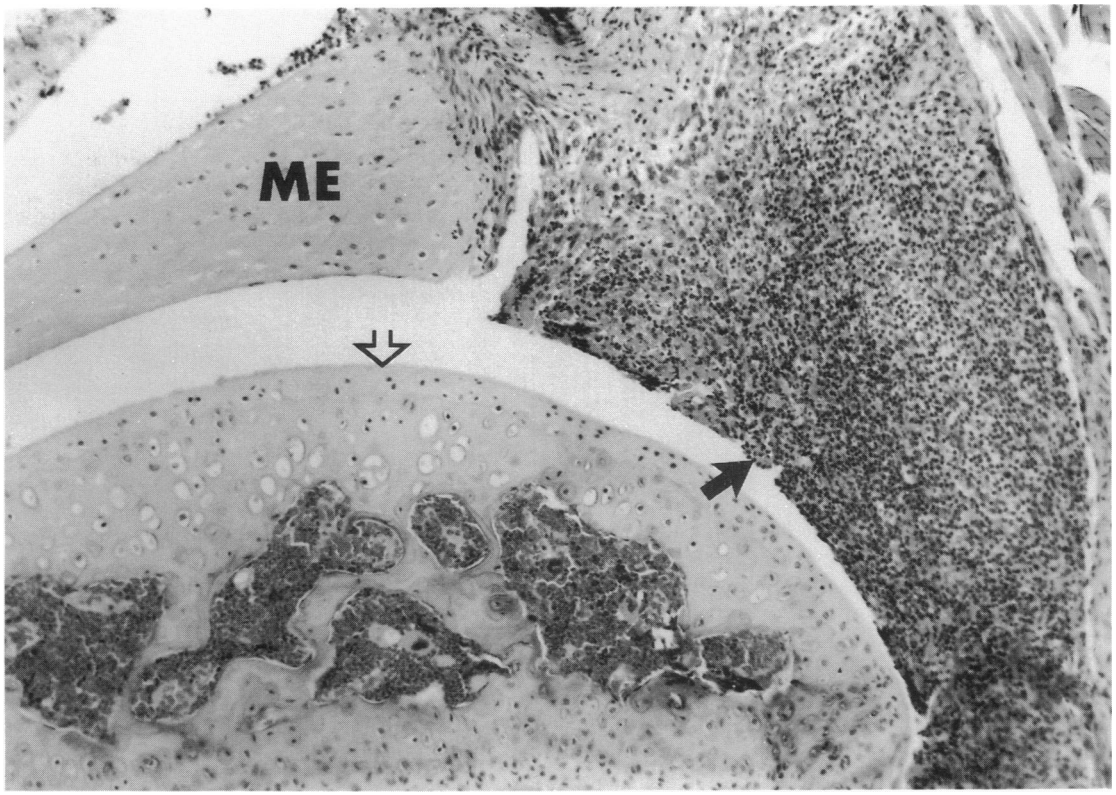

Fig. 1. Arthritis in the femorotibial joint of a *scid* mouse infected with *Mycoplasma pulmonis*. Necrosis and severe leucocytic infiltration of the synovial membrane (arrow) and the articular capsule. The articular cartilage is preserved (arrowhead). (ME: Meniscus ×36.)

at 25 days post-infection and five *scid* mice necropsied at 30 and 35 days post-infection exhibited synovitis and polyarthritis. In these mice the joints in the pelvic limbs appeared swollen and oedematous. Histologically, the hallmark of the lesions examined was destruction of the intimal cells of the synovial membranes.

In addition to necrosis and ulceration of the synovial surface, pronounced leucocytic infiltrations in the articular tissues were also observed (Fig. 1). Neutrophils, monocytes, and macrophages were abundant. Mononuclear leucocytes with a rather large cytoplasm were also noticed, but these cells could not be accurately identified. Low numbers of mast cells, displaying a typical appearance, occurred in a perivascular location and also irregularly distributed in the inflamed tissues. The synovial space contained leucocytes, necrotic cells, amorphous debris, and fibrin, whereas the synovial membranes and the joint capsules appeared infiltrated by granulation tissue. Degeneration and necrosis of the articular cartilages was observed in most of the joints examined (Fig. 2). In some of these joints the bones showed extensive necrotic zones, involving the whole depth of the epiphysis. There were also horizontal splits, haemorrhages between the epiphysis and the physis, and reparative changes, in the form of a highly cellular fibrocartilagenous tissue proliferating from the articular capsule towards the edges of the necrotic areas (Fig. 2). Mononuclear cells and polykaryons resembling osteoclasts appeared on, or in the vicinity of the osseous surfaces, mixed with the inflammatory exudate. In addition, lesions of arteriolitis with severe necrosis of the tunica media and tunica adventitia were found in isolated arterioles of the joints, periarticular tissues, brain, spleen, liver, and kidney, but not in the lungs. Other changes observed in the periarticular tissues were tendinitis, myositis, and regeneration of muscle fibres.

Mycoplasma pulmonis *infection in immunocompetent C.B-17 mice*

The immunocompetent C.B-17 mice exhibited inflammatory reactions in the respiratory organs. At necropsy, one mouse displayed grey and red areas suggestive of lung consolidation. Histology confirmed the macroscopical findings, and revealed changes also in the other two mice examined. The nasal mucosa, bronchi, and the peribronchial lung tissues were heavily infiltrated by monocytes, macrophages, and lymphocytes, and the bronchial lumen contained numerous neutrophils, mixed with mucus, desquamated epithelial cells, necrotic cells, and debris. Other lesions found in the lung tissues were haemorrhages, necrosis of the alveolar epithelium, and perivascular infiltrations of mononuclear leucocytes, mostly lymphocytes, monocytes, and plasma cells. In the peripheral areas of the lung there were accumulations of large cells with a foamy cytoplasm that resembled histiocytes. Mild arthritis was observed in one C.B-17 mouse. However, as we were unable to isolate *Myc. pulmonis* from the joint the cause of the arthritis is not clear.

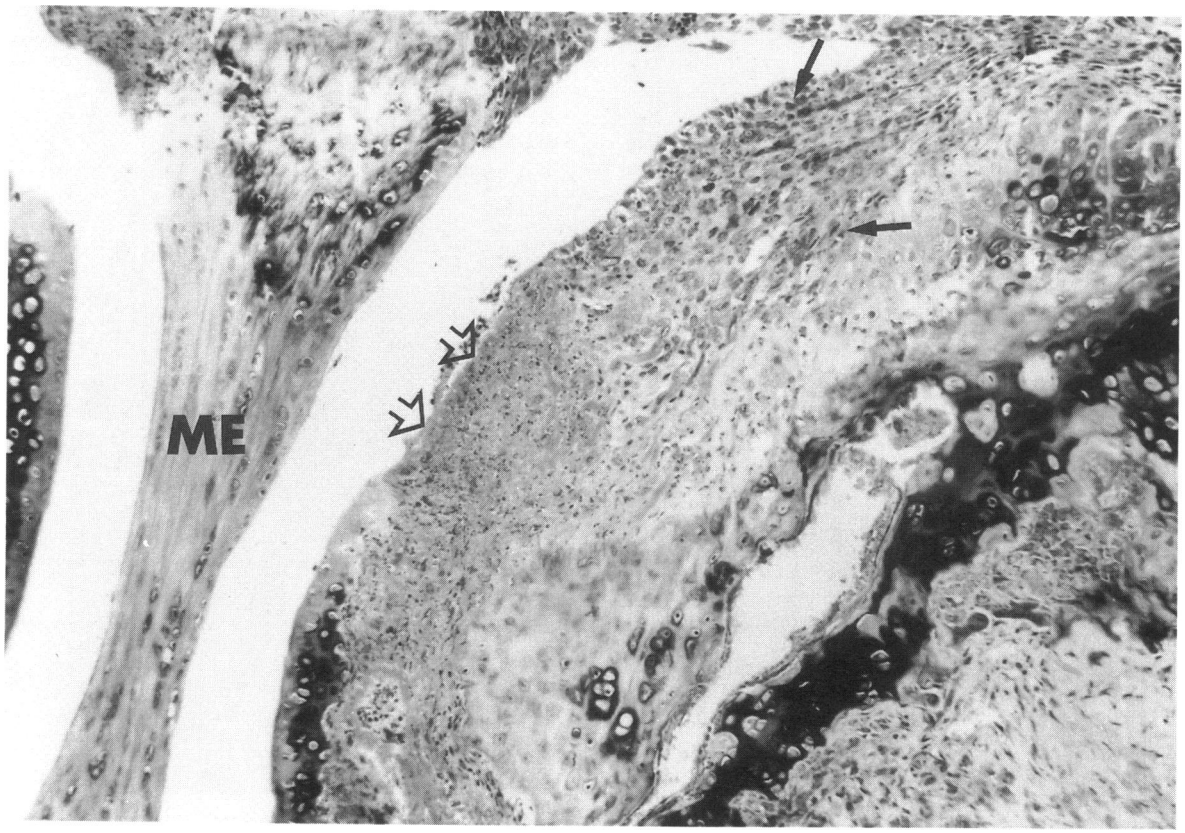

Fig. 2. Arthritis in the femorotibial joint of a *scid* mouse infected with *Mycoplasma pulmonis*. Severe necrotic changes in the articular and the epiphyseal cartilages cause ulceration of the articular surface (arrowheads). Ongoing reparative process is suggested by the highly cellular fibrocartilagenous tissue (arrows) proliferating from the periphery of the joint towards the edges of the necrotic zone. (ME: Meniscus ×144.)

Mycoplasma pneumoniae *infection in* scid *and in C.B-17 mice*
Results from infection with *Myc. pneumoniae* are presented in Table 2. *Mycoplasma pneumoniae* was recovered from the respiratory tract in 11 out of 12 infected *scid* mice and in three out of five infected C.B-17 mice. No *Myc. pneumoniae* was isolated from the spleen or joint samples. Significant gross or histological changes were not found in any of these mice.

Uninfected controls
In both of the experiments the uninfected control mice, *scid* as well as C.B-17, did not show growth of mycoplasmas or significant pathological changes.

DISCUSSION

Mycoplasma pulmonis produced a progressive disease in *scid* mice characterized by emaciation, arthritis, and lack of pulmonary pathology. The changes found in our immunocompetent mice are in keeping with the results of previous investigations where lesions of the respiratory organs are salient findings [6,8]. The disorder induced by *Myc. pulmonis* in *scid* mice also differs from mycoplasmosis as it has been described in other strains of immunodeficient mice. Davidson *et al.* [15] reported lung lesions in C3H/HeJ *scid* mice infected with *Myc. pulmonis*. The use of different *scid* mouse strains may be one explanation of this divergence in results. However, the lack of detailed information about the lesions makes a thorough comparison impossible. Arthritis has been noticed in athymic mice after intranasal inoculation [6,10,11], and also after intravenous injections of *Myc. pulmonis* in immunocompetent mice [10] and rats [16,17].

In the present study *Myc. pulmonis* was given intranasally. The lesions of arteriolitis in various tissues of the *scid* mice suggest that *Myc. pulmonis* disseminated to distant tissues via the blood stream. The *scid* mutation does not affect the differentiation of myeloid cells [18], but it is likely that the lack of specific immunity allowed *Myc. pulmonis* to gain access to, and to settle in the articular tissues. It has been demonstrated that anti-mycoplasma antibodies protect from arthritis induced by *Myc. pulmonis* in a variety of mouse strains [19]. In humans also the humoral immune system seems to give protection against mycoplasma arthritis, as these disorders are frequently found in patients with hypogammaglobulinaemia [1–4]. In mice, defects in the complement system have been reported to result in enhanced susceptibility to mycoplasma arthritis [20]. *Mycoplasma pulmonis* may cause direct damage to the synovial tissues, via diverse mechanisms, such as lesions to host cell membranes, production of toxic metabolites, forma-

Table 2. Experimental infection in mice with *Mycoplasma pneumoniae*

Mouse	Dose of infection (CFU)	Body weight* (g)	Days†	*Myc. pneumoniae* culture Nose, trachea, lung (pooled samples) (CFU recovered/ml sample)	*Myc. pneumoniae* culture Joints, spleen (pooled samples)	Histological examination Nasal mucosa, lung, joints
scid L388	10^3	25	6	0	0	Moderate rhinitis
scid L389	10^5	25	6	10^3	0	Negative
scid L392	10^3	26	13	$>10^4$	0	Negative
scid L393	10^5	25	13	$>10^4$	0	Negative
scid L400	10^5	27	20	10^2	0	Negative
scid L401	10^3	25	20	10^3	0	Negative
scid L411	10^3	24	27	10^2	0	Negative
scid L412	10^5	25	27	10^3	0	Negative
scid L413	10^3	27	34	10^3	0	Negative
scid L414	10^3	29	34	$>10^4$	0	Negative
scid L415	10^5	27	34	$>10^4$	0	Negative
scid L416	10^5	26	34	10^3	0	Negative
C.B-17L390	10^3	25	6	10^3	0	Negative
C.B-17L391	10^5	26	6	0	0	Negative
C.B-17L402	10^5	28	20	$>10^4$	0	Negative
C.B-17L417	10^3	28	34	10^3	0	Negative
C.B-17L418	10^5	27	34	0	0	Negative
Controls						
scid L378	0	25	0	0	0	Negative
scid L379	0	23	0	0	0	Negative
scid L440	0	28	41	0	0	Negative
scid L441	0	26	41	0	0	Negative
scid L442	0	28	41	0	0	Negative
C.B-17L377	0	25	0	0	0	Negative
C.B-17L439	0	27	41	0	0	Negative

* Body weight at euthanasia.
† Time elapsed between infection date and euthanasia.

tion of peroxides, and competition with the host cells for nutrients [6]. It can also induce release of hydrolytic enzymes from mouse macrophages [21].

In addition to the protective features of different parts of the immune system, others causing tissue damage must be considered. The absence of immunocompetent lymphocytes in *scid* mice argues against the occurrence of cross-reactions reported [22] between *Myc. pulmonis* and the synovial tissues of mice. In *scid* mice, natural killer (NK) cell activity is not affected [23]. NK cells have a potent cytotoxic and antibacterial activity which does not require previous sensitization or antigen presentation by accessory cells, and in histological sections the NK cell resembles a lymphocyte with a large cytoplasm [24]. Mononuclear leucocytes, resembling NK cells, were noticed in the articular lesions of our *scid* mice, but we could not identify these cells more accurately as NK cells. The role of the innate immune system for the production of joint lesions needs further investigation.

Immune factors seem to be important in the development of lung injury caused by mycoplasmas. The lack of lung pathology despite the heavy load of *Myc. pulmonis* in our *scid* mice, together with reports of lung lesions in mycoplasma-infected athymic mice [6] indicates that immunoglobulins contribute to the genesis of the pneumonia. *Mycoplasma pulmonis* has a mitogenic effect upon mouse [25] and rat lymphocytes, and a strong correlation between the combined T and B cell mitogenicity and lung pathogenicity has been reported [26]. *Scid* mice, lacking lymphocytes, consequently would be unable to respond to this mitogenicity. Growth of bacteria in the lung of *scid* mice in combination with lack of pulmonary pathology has been noticed previously. *Mycobacterium bovis* causes epithelioid granulomas in livers, spleens, and lungs of immunocompetent mice, but in *scid* mice the mycobacteria were located mostly in isolated alveolar macrophages, and granulomas were seen only in the spleen and liver [27].

A consequence of *Myc. pulmonis* infection in *scid* mice was a dramatic loss of weight. The reasons for this phenomenon are unknown, but factors like decreased food intake, competition for cell nutrients, and also release of cytokines, for instance tumour necrosis factor (TNF), may have been involved.

Mycoplasma pneumoniae infection of mice has been reported previously in immunocompetent mice [14] with absence, or only transient appearance, of lung lesions. In these studies, the colonization was poor and self-limiting, but in our study the recovery of *Myc. pneumoniae*, also in C.B-17 mice, was moderate and the organisms were present in the respiratory tract for at least 34 days. Yet the lack of pathological changes makes the model unsuitable.

The *Myc. pulmonis*-induced arthritis in *scid* mice can be further investigated by supplementing the animals with various

immune factors or cells. Alternatively, mice with other genetic defects can be studied to help to identify the components of the host response which are critical for protection, or the inflammation and destruction of the joint when infection is established.

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