SHORT ARTICLE

Failure of *Mycoplasma pneumoniae* infection to confer protection against *Mycoplasma genitalium*: observations from a mouse model

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Mycoplasma pneumoniae and *M. genitalium* are genomically distinct but share antigens that induce some serological cross-reactivity. Therefore, the possibility that *M. pneumoniae* infection of the human respiratory tract might provide immunity to *M. genitalium* infection of the genital tract was considered. Because of the difficulty of assessing this proposition in man, it was evaluated experimentally in a mouse model. Female BALB/c mice were susceptible to infection of the vagina with *M. pneumoniae*, whereas those infected previously in the oropharynx with *M. pneumoniae* were completely immune to infection of the vagina with this mycoplasma. However, all mice with such a respiratory tract infection were susceptible to infection of the vagina with *M. genitalium*. The findings suggest that an *M. pneumoniae* infection of the human respiratory tract is unlikely to influence infection of the genital tract by *M. genitalium*.

Introduction

Mycoplasma pneumoniae is a well-known human pathogen that infects early in life, often without causing overt disease at this time [1]. Re-infection during adolescence or in later life results in a spectrum of respiratory disease, sometimes culminating in pneumonia [1]. *M. genitalium*, discovered two decades ago [2], is strongly associated with non-gonococcal urethritis, in a manner largely independent of *Chlamydia trachomatis* [3]. Although this mycoplasma has been recovered from the respiratory tract, together with *M. pneumoniae* [4], its predilection is apparently for the genital tract. *M. pneumoniae* and *M. genitalium* are genomically distinct, but they have a similar morphology, similar metabolic features and share various antigens that induce some serological cross-reactivity [5]. Therefore, it is plausible that resistance to genital tract infection with *M. genitalium* might occur in man as a consequence of previous respiratory infection with *M. pneumoniae*, particularly as the latter infection is very likely to occur first.

Earlier studies in a murine model showed that experimental infection of the oropharynx with *M. pneumoniae* protects partially against re-infection of

the oropharynx and, more strikingly, protects completely against subsequent attempted infection of the genital tract with this mycoplasma [6]. It is also possible to infect the genital tract of mice with *M. genitalium* [7], so that this small animal model can be used to determine whether a respiratory tract infection with *M. pneumoniae* might protect the genital tract against infection with *M. genitalium*.

Materials and methods

Mice

Female BALB/c mice (6–8 weeks old) were screened for, and considered to be free of, indigenous mycoplasmas before being caged in groups of five. They were inoculated subcutaneously with progesterone (Depo-Provera; Upjohn Ltd) 2.5 mg in a 0.1-ml volume on four occasions, at weekly intervals [6].

Media

The glucose-containing medium used for the cultivation of *M. pneumoniae* or its isolation from murine specimens has been described previously [8], as has the medium (SP4) for the cultivation of *M. genitalium* [9].

Experimental procedures

Mice, anaesthetised by an intraperitoneal injection of a mixture of Hypnorm and Hypnovel, were inoculated

intranasally with 50 μ l of medium containing 2.5×10^7 colour-changing units (ccu) of *M. pneumoniae* strain MY12763 [6]. Unanaesthetised mice were inoculated intravaginally, immediately after the second inoculation of hormone, with 50 μ l of medium containing the same strain of *M. pneumoniae* (2.5×10^7 ccu) or *M. genitalium* strain G37 (2.5×10^6 ccu).

Throat and vaginal specimens were collected as described previously [6], with a nasopharyngeal swab which was rotated to abrade epithelial cells, and the contents were expressed in 1.8 ml of glucose-containing mycoplasmal broth medium. *M. pneumoniae* organisms were detected and their number estimated as described previously [6] and *M. genitalium* organisms were detected by means of a specific PCR assay, also as described previously [10].

Results and discussion

All of 10 hitherto uninoculated mice became infected in the vagina after intravaginal challenge with *M. pneumoniae*, the organisms persisting in large numbers (10^3 – 10^7 ccu) for at least 35 days. Another 10 mice became infected in the oropharynx with *M. pneumoniae* after intranasal inoculation, the organisms persisting in small numbers (10^1 – 10^3 ccu) for at least 14 days; however, none of these mice was infected in the vagina after intravaginal challenge with this mycoplasma 35 days later. In another experiment, all of 10 hitherto uninoculated mice were infected in the vagina after intravaginal challenge with *M. genitalium*, the organisms being detected up to 21 days later. Furthermore, after intranasal inoculation, 10 other mice were infected in the oropharynx with *M. pneumoniae*, in the same manner as described above. However, despite this, all of them became infected in the vagina with *M. genitalium* after intravaginal challenge with this mycoplasma 35 days later. It is not known whether there might have been a reduction in the number of *M. genitalium* organisms in the vagina of the latter mice compared to those not given *M. pneumoniae*, because the PCR assay was qualitative and not quantitative.

Mycoplasma-positive vaginal swabs probably represent infection of the cervix, as it has been shown previously by detailed examination that this site, rather than the vagina, became infected following intravaginal inoculation of *M. pulmonis* or *M. hominis* [11]. The current experiments were performed in female mice because of the difficulty of inoculating and obtaining repeated specimens from the urethra of male mice. Furthermore, the difficulty of infecting the murine respiratory tract with *M. genitalium* precluded determination of whether such infection would prevent infection of the genital tract with *M. genitalium*. However, perhaps this is an

irrelevant issue in view of the apparently rare occurrence of *M. genitalium* in the human respiratory tract. Far more relevant is the fact that in the current study, and in a previous one [6], complete protection against *M. pneumoniae* infection of the murine genital tract was shown to occur as a consequence of a prior infection of the respiratory tract with *M. pneumoniae*. Immunity induced in this way is a plausible reason for *M. pneumoniae* being detected only rarely in the human genital tract [12], despite the opportunity for such infection to occur. Infection of the human respiratory tract with *M. pneumoniae* is far more common, so that the question of whether such infection might protect the genital tract against *M. genitalium* is germane. It is a question that would be difficult to resolve from an epidemiological viewpoint. Although the extent to which the findings of a study in a murine model are relevant to the human situation is debatable, the result of the current study does suggest that the induction of cross-protective immunity is unlikely.

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