Role of Bacteriophage MAV1 as a Mycoplasmal Virulence Factor for the Development of Arthritis in Mice and Rats

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The lysogenic bacteriophage MAV1 has been shown to be a virulence factor for the development of arthritis in rats infected with *Mycoplasma arthritidis*. In the present study, arthritis was evaluated by histopathologic examination to demonstrate that MAV1 is a virulence factor not only in the rat but also in the mouse. Specifically, the MAV1 lysogen 158L3-1 was more virulent than the nonlysogenic strain 158 in DBA/2NCr, C3H/HeNCr, C3H/HeJ, and C3Smn.CB17-Prkdc<sup>−−</sup>/J mice, as well as in LEW rats.

*Mycoplasma arthritidis* causes acute polyarthritis in rats and chronic proliferative arthritis in mice [1, 2]. *M. arthritidis*-induced arthritis has been studied as a model for arthritis caused by infectious agents and as a model to be used in examining the role of the superantigen MAM (*M. arthritidis* T cell mitogen) in the development of autoimmunity [3]. MAV1 is a lysogenic bacteriophage isolated from *M. arthritidis*. During lysogenization, the double-stranded DNA genome of MAV1 is integrated into the *M. arthritidis* chromosome at any of numerous sites at the sequence TATTTTT [4]. MAV1 lysogens are more arthritogenic than nonlysogens in rats, regardless of the particular chromosomal site into which MAV1 DNA has been inserted [5].

Sequence analysis of the 16-kb MAV1 genome [6] reveals 15 open-reading frames (ORFs). One gene, vir, is located on the minus strand of the phage genome, and the other 14 ORFs are on the plus strand. Only 2 MAV1 genes are thought to be transcribed in MAV1 lysogens [6]. It is predicted that one of the transcribed genes, imm, will code for a phage repressor. The other transcribed gene is vir. Because it is predicted that vir will code for a lipoprotein that is associated with the mycoplasmal membrane [6], vir is a candidate for being the virulence determinant responsible for the enhanced arthritogenicity of MAV1 lysogens.

It has been shown, on the basis of an assessment of joint diameters and weight loss in infected animals, that MAV1 lysogens are more virulent than nonlysogens in rats [5]. Whether MAV1 is also a virulence factor in mice has not, to our knowledge, been examined. Rats and mice differ substantially in their response to experimental infection with *M. arthritidis*. In rats, the disease is an acute, self-limiting arthritis, whereas in mice, the disease is chronic and can persist for at least 200 days after inoculation [7]. Rats are less susceptible to toxicity and death induced by *M. arthritidis* than are mice [8]. Some strains of *M. arthritidis*, such as 158p10, are arthritogenic in rats but much less so in mice [1]. The relative resistance of mice to the development of arthritis after infection with 158p10 suggests that MAV1 might not be a virulence factor in the mouse, because 158p10 is a MAV1 lysogen [5].

The major goal of the present study was to determine whether MAV1 is a virulence factor for the development of arthritis in infected mice, in which case the power of mouse genetics could be used to study the role of MAV1 in disease pathogenesis. We find that the lysogenization of *M. arthritidis* strain 158 with MAV1 increased the arthritogenicity of the mycoplasma in DBA/2NCr, C3H/HeJ, C3H/HeNCr, and C3Smn.CB17-Prkdc<sup>−−</sup>/J (SCID) mice, as assessed by histopathologic examination of the joints. In addition, we confirm that MAV1 is a virulence factor in LEW rats and causes arthritic lesions similar to those observed in mice.

Materials and Methods

*Mycoplasmas*. Two strains of *M. arthritidis* were used in this study. Strain 158 lacks MAV1. Strain 158L3-1 is a MAV1 lysogen obtained by infection of strain 158 [5]. Mycoplasmas were grown overnight at 37°C in EB medium, as described elsewhere [6]. In preparation for infection of mice and rats, cells were harvested by centrifugation, suspended in fresh EB medium containing 15% glycerol, and stored at −70°C. Before the animals were infected, an aliquot was thawed, serially diluted, and assayed for colony-forming units to determine viability.

*Animals*. C3H/HeNCr and DBA/2NCr mice were obtained...
Figure 1. Carpal joints, stained with hematoxylin-eosin. A, Normal intercarpal joints of sham-inoculated control rat. Bar, 332 μm. B, Intercarpal joints of a rat inoculated with Mycoplasma arthritidis strain 158L3-1, showing severe suppurative arthritis, synovitis, and periartthritis, with exudate of neutrophils and fibrin in the joint space (e) and inflammatory cells infiltrating the synovium, joint capsule, and periarticular tissues (i). Bar, 332 μm. C, Synovium of sham-inoculated control rat, showing normal synovial cells (arrows). Bar, 33 μm. D, Synovium of a rat inoculated with 158L3-1, showing severe suppurative synovitis with neutrophils, lymphocytes, and plasma cells infiltrating the synovium (i) and exudate of neutrophils and fibrin (e) overlying synovial cells (arrows). Bar, 33 μm. E, Normal carpal joint from a mouse inoculated with M. arthritidis strain 158. Bar, 332 μm. F, Carpal joint of a mouse inoculated with 158L3-1, showing severe suppurative arthritis, synovitis, and periartthritis, with exudate of neutrophils in the joint space (e) and inflammatory cells infiltrating the synovium, joint capsule, and periarticular tissues (i). Bar, 332 μm.

from the National Cancer Institute, Cancer Research and Development Center; C3H/HeJ and SCID mice, from Jackson Laboratories; and LEW (LEW/CrlBR) rats, from Charles River Laboratories. Animals were purchased from colonies free of rodent pathogens and maintained in a pathogen-free environment in microisolator cages within the animal facilities at the University of Alabama at Birmingham.

Induction and assessment of arthritis. Female mice (age range, 4–8 weeks; body weight, 10–20 g) were infected with *M. arthritidis* strain 158 or 158L3-1. Each mouse was injected intravenously with 200 μL of mycoplasma stock containing $1 \times 10^7$ cfu. Similar procedures were performed for infection of female LEW rats (age range, 34–37 weeks; body weight, 100–125 g), except that the inoculum received by each rat contained $1 \times 10^8$ cfu. Each treatment group included 5 animals. Animals were sacrificed at 3 weeks after inoculation, unless otherwise indicated below. The left wrist and ankle joints were prepared for histopathologic evaluation, and the right wrist and ankle joints were cultured for mycoplasmas. For cultures, each joint was placed in a sterile glass vial containing 1 mL of mycoplasma broth, manually minced, disrupted by sonication for 45 s at 100% power in a Vibra-cell ultrasonic processor (Cuphorn, model VC-250B; Sonics Materials), and assayed for colony-forming units. For histopathologic examinations, ankle and wrist joints were excised, fixed in 70% alcoholic formalin (for mouse joints) or 80% alcoholic formalin (for rat joints), demineralized, embedded in paraffin, sectioned at 5-μm thickness, and stained with hematoxylin-eosin for histopathologic evaluation. Histopathologic examination was done by the Comparative Pathology Laboratory.
at the University of Alabama at Birmingham. All experiments to assess the arthritogenicity of the mycoplasma strains were repeated at least once.

Statistical analysis. Severity of arthritis was quantified independently by 2 pathologists (J.R.L. and T.R.S.) who were “blinded” to experimental group. Joints were subjectively assigned a score from 0 (normal) to 5 (severe) for 5 characteristic histologic lesions of arthritis: inflammation, joint-capsule thickening, tendonitis, bone destruction/remodeling, and ankylosis. For each animal, an overall pathology score was obtained by adding the scores from the wrist and ankle joints. Data were analyzed using SigmaStat, version 2.03 (SPSS). Student’s t test was used to compare 2 groups, if data were normally distributed with equal variance. If data did not meet these criteria, the nonparametric Mann-Whitney rank sum test was used instead.

Results

Effect of MAV1 lysogeny on the virulence of M. arthritidis. Arthritic lesions were commonly found in LEW rats and in DBA/2NCr, C3H/HeNCr, and C3H/HeJ mice after infection with 158L3-1 (figure 1). Some of the characteristics of the arthritic lesions in mice included the appearance of exudates in the articular space, extreme thickening of the synovial membranes, infiltration of granulocytes and macrophages into the synovium and periarticular tissues, and remodeling of bone along the periosteal membrane and endosteal surface. Ankylosis was not found. The presence of increased numbers of osteoclasts, osteoblasts, and fibroblasts indicated increased activities of inflammation and repair. Compared with strain 158, infection with strain 158L3-1 caused a significant increase in the median pathology score at 3 weeks after inoculation for the 3 mouse strains examined (i.e., DBA/2NCr, C3H/HeNCr, and C3H/HeJ; figure 2A–C). Thus, histopathologic evaluation showed that the MAV1 lysogen 158L3-1 was more virulent than the nonlysogen strain 158 in DBA/2NCr, C3H/HeNCr, and C3H/HeJ mice. As would be expected, because MAV1 has been shown to be a virulence factor in LEW rats [5], histopathologic analysis showed that strain 158L3-1 was more arthritogenic in LEW rats than was 158 (data not shown).

Infection of SCID mice. The arthritogenicity of the nonlysogen and lysogen strains of M. arthritidis was examined in SCID mice, which are B and T cell deficient [9]. SCID mice developed severe disease by 3 weeks after inoculation, regardless of whether the infecting agent was the lysogen or the nonlysogen M. arthritidis strain (figure 2D). Because SCID mice developed severe arthritis at 3 weeks after inoculation with strain 158, an earlier stage of the infectious process was examined to assess whether lysogenization with MAV1 would enhance the virulence of the mycoplasma: SCID mice were infected with 158 or 158L3-1 and subjected to histopathologic analysis at 2 weeks after inoculation. Under these conditions, the pathology score for mice infected with 158 was very low; the score for mice infected with 158L3-1 was significantly higher (figure 2E). Thus, MAV1 is a virulence factor in SCID mice. However, the compromised host defenses of SCID mice are such that severe arthritis develops by 3 weeks after infection, even in the absence of MAV1 lysogeny. Presumably, the severity of the arthritis is the result of a high load of M. arthritidis organisms in the joints. Attempts to culture mycoplasmas from the joints of C3H/HeJ mice at 3 weeks after inoculation were generally unsuccessful, whereas substantial mycoplasmal colonization was evident in the joints of the SCID mice. Usually, $10^5$–$10^6$ cfu were recovered from both the wrist and the ankle joints of SCID mice.

Discussion

Possible mechanisms by which MAV1 may enhance the virulence of M. arthritidis include altering the physiology of the mycoplasma, protecting the mycoplasma from host defenses, inducing a host autoimmune response, damaging host cells (e.g., by toxin production), and enhancing the adherence properties of the mycoplasma to host cells or tissues. The MAV1 lysogen (strain 158L3-1) was more virulent than the nonlysogen...
in C3H/HeJ mice. C3H/HeJ mice are hyposensitive to lipo-polysaccharide because of a missense mutation within the Tlr4 gene [10]. Because MAV1 is a virulence factor in C3H/HeJ mice, functional Toll-like receptor 4 is not required for MAV1 to exert its effect on virulence. Similarly, MAV1 lysogeny presumably does not affect virulence by altering interactions with B or T cells, because MAV1 is a virulence factor in SCID mice.

Few virulence factors have been identified in mycoplasmas, but evidence suggests that additional M. arthritidis factors exist that are important for the development of arthritis. The MAM superantigen likely contributes to arthritogenicity [11]. However, because all strains of M. arthritidis secrete MAM, regardless of a particular strain’s virulence, the role of MAM in disease pathogenesis has not yet been firmly established [12]. Other M. arthritidis proteins that are thought to be important for disease are the Maa cytadhesins [13, 14]. M. arthritidis strains 158p10p9 and 158p10 are MAV1 lysogens [5], but 158p10p9 is more arthritogenic in mice than is 158p10 [1]. Thus, 158p10p9 likely possesses an unknown virulence determinant that is distinct from MAV1, MAM, or Maa.

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References


