Severity of Group B Streptococcal Arthritis in Selected Strains of Laboratory Mice

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Group B streptococci (GBS) are a leading cause of life-threatening infection in neonates and young infants (1), and invasive infections caused by GBS have been increasingly recognized in adult populations (2–4). Different susceptibilities to GBS infections among the races have been shown, with the prevalence of GBS colonization during pregnancy greatest in Hispanics of Caribbean origin, followed by, in descending order for prevalence, blacks, whites, and other Hispanics (predominantly Mexicans) (5, 7). More recently, it has been reported that GBS disease rates for infants born to black, Hispanic, and Asian mothers are higher than those for infants born to white mothers (17). Similar differences in susceptibility to GBS infection have also been observed in adults (3).

Septic arthritis has been described as a clinical manifestation of late-onset GBS infection in neonates (1). In adults, GBS arthritis is often associated with advanced age and other risk factors, including cancer, diabetes mellitus, cardiovascular disease, chronic renal insufficiency, alcoholism, human immunodeficiency virus infection, neurological disease, and cirrhosis (8–12).

A previous study described an experimental mouse model of type IV GBS systemic infection with clinical features that closely resemble those of infection in humans, in particular, the appearance of multifocal septic arthritis (14).

The aim of this study was to ascertain whether the genetic disparity among different mouse strains influenced the response to GBS infections. In particular, the appearance and severity of arthritis and the cytokine profile during infection were monitored for each mouse strain.

Inbred BALB/c (H-2b), C57BL/6N (H-2b), and C3H/HeN (H-2a) mice, 8 weeks old, were obtained from Charles River Breeding Laboratories (Calco, Milan, Italy).

Type IV GBS reference strain GBS 1/82 (GBS IV) was used throughout the study. Microorganisms were grown overnight at 37°C in Todd-Hewitt broth (Oxoid Ltd., Basingstoke, Hampshire, England). The bacterial suspension for experimental infection was prepared in RPMI 1640 medium (GIBCO, Life Technologies, Milan, Italy) as previously described (14). Mice were inoculated intravenously via the tail vein with 1 × 107 or 5 × 107 GBS IV CFU/mouse in a volume of 0.5 ml. Control mice were injected in the same way with 0.5 ml of RPMI 1640 medium. Ten animals per mouse strain were used in each experimental group. Mortality was recorded at 24-h intervals for 30 days. Mice were examined daily by two independent observers for 2 months to evaluate joint inflammation. Arthritis was defined as visible erythema or swelling of at least one joint. To evaluate the intensity of arthritis, clinical scoring (arthritic index) was used for each limb, with points assigned as follows (maximum score, 12 points): 0, normal; 1 point, mild swelling and erythema; 2 points, moderate swelling and erythema; 3 points, marked swelling, erythema, and/or ankylosis. The arthritic index was constructed by dividing the total score by the number of animals used in each experimental group. To confirm clinical signs of arthritis, histological studies were performed as previously described (14). Joints were examined for the presence of articular damage. Blood and joint infections in mice infected with 107 GBS IV CFU were determined by CFU evaluation as previously described (14). Blood and articular samples from mice injected with 107 GBS IV CFU and from uninfected controls were obtained as previously described (15), and interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor alpha concentrations were measured with commercial enzyme-linked immunosorbence assay kits (Amersham Pharmacia Biotech Ltd, Amersham, United Kingdom) according to the manufacturer’s instructions.

Upon injection with 1 × 107 GBS IV CFU/mouse, no mortality was observed in C57BL/6N mice, while 40% of C3H/HeN and 30% of BALB/c mice died (Fig. 1A). C3H/HeN mice developed severe arthritis (mean arthritic index ± standard deviation, 3.9 ± 0.2); 90% of the animals had articular lesions on day 10 after infection, whereas only 10% of BALB/c mice developed mild arthritis (Fig. 1B and C). No articular lesions were evident in C57BL/6N mice. With an inoculum size of 5 × 107 GBS IV CFU/mouse, mortality increased to 100% in C3H/
HeN and in BALB/c mice, while only 20% of C57BL/6N mice died (data not shown). Using the increased inoculum size, 30% of BALB/c mice developed arthritis, while none of the C57BL/6N mice manifested any clinical sign of arthritis (data not shown). Quantitative monitoring of bacteremia and GBS growth in the joints, after injection of $1 \times 10^7$ GBS CFU/mouse, showed that the number of CFU was always higher in C3H/HeN mice than in C57BL/6N or BALB/c mice (Fig. 2); the lowest rate of GBS growth in the joints was observed in C57BL/6N mice.

Great differences in systemic and local cytokine production were evident among the mouse strains after injection of $1 \times 10^7$ GBS CFU/mouse (Fig. 3). IL-1β reached detectable levels in the sera of each strain, with a maximal value on day 5 after infection; the highest concentration was detected in C3H/HeN mice ($P < 0.001$). No IL-1β production was observed in the joints of C57BL/6N or BALB/c mice, while in C3H/HeN mice the IL-1β joint concentration began to increase 5 days after GBS injection and reached a value of about 500 pg/ml on day 10. High levels of IL-6 were detected in the sera of all mouse
strains, with peak values on day 1 after infection, followed by a progressive decrease. As for IL-1β, the highest IL-6 production was observed in the sera of C3H/HeN mice. Moreover, the IL-6 concentration progressively increased from day 1 to day 10 in the joints of these mice, while in the joints of C57BL/6N and BALB/c mice IL-6 levels never exceeded a value of 50 pg/ml. No tumor necrosis factor alpha production was evident in the sera and joints of any mouse strain (data not shown).

The high severity of arthritis in C3H/HeN mice was confirmed by histopathological studies, showing articular cavities filled with purulent exudate 1 week after infection and joint destruction within 20 days (data not shown). There was much less inflammatory involvement in the joints of BALB/c mice, and no signs of inflammation were observed in C57BL/6N mice (data not shown).

The laboratory mouse offers outstanding potential as a model for GBS arthritis. Septic arthritis observed in late-onset GBS disease is hematogenously acquired, and the more frequently affected joints are hip, ankle, and wrist (1). In our murine model, bacteremia is present for more than 10 days after GBS infection, and the localization of articular lesions is similar to that observed in humans (13). In this study, various genotypes appeared to be differently susceptible to GBS infection. The appearance of arthritis is undoubtedly the by-product of a multifactorial process. As previously reported (14, 16), the number of microorganisms that reach the joints is a determinant for the establishment of permanent arthritis. BALB/c and particularly C57BL/6N mice appeared to efficiently control GBS levels at this site, thus accounting for the appearance of only mild articular lesions (or the complete absence of such lesions) in these two strains. On the contrary, the severe arthritis induced by GBS IV in C3H/HeN mice correlated with the high number of bacteria recovered from the joints. Recent evidence that proinflammatory cytokines, such as IL-6 and IL-1β, may participate in the pathogenesis of GBS disease (14) is confirmed by the present study. In fact, the highest concentrations of these cytokines in the sera and joints of C3H/HeN mice correlated with the highest mortality and the most severe articular lesions. It is worth noting that BALB/c mice, although showing mild arthritis, had a mortality rate similar to that of C3H/HeN mice. IL-1β and IL-6 serum levels in these mice appeared to account for their respective susceptibility to GBS infection.

In conclusion, our results indicate that experimental infection of different strains of inbred mice with GBS IV results in articular lesions whose severity appears to be based on a genetically determined host immune response which controls the bacterial burden and inflammatory response. A better understanding of the host factors that influence the different resistance to arthritis of mice infected with GBS may have implications for humans.
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FIG. 3. Kinetics of appearance and cytokine concentrations in sera and joints of C3H/HeN (■), C57BL/6N (●), and BALB/c (▲) mice infected with $1 \times 10^7$ CFU of GBS IV per mouse. The data are the means ± standard deviations of three separate experiments. Three mice per strain were sacrificed at each time point. P values of $\geq 0.001$ have been omitted. *, $P < 0.001$ (C3H/HeN versus C57BL/6N and BALB/c mice); ●, $P < 0.001$ (C3H/HeN versus C57BL/6N mice); †, $P < 0.001$ (BALB/c versus C57BL/6N mice) according to Student’s t test.

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