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Toll-Like Receptor 2 Deficiency Is Associated with Enhanced Severity of Group B Streptococcal Disease[▽]

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Group B streptococcus (GBS) has been recognized as an ever-growing cause of serious invasive infections in nonpregnant adults, in particular, in association with severe underlying diseases. The most common manifestations include primary bacteremia, urinary tract infections, pneumonia, meningitis, peritonitis, and osteoarticular infections. Toll-like receptor-2 (TLR2) mediates host responses to gram-positive bacteria. TLR2 function was investigated in murine GBS-induced sepsis and arthritis in wild-type (wt) and TLR2-deficient ($TLR2^{-/-}$) mice. Mice were infected with different doses of GBS (10^7, 5×10^6, or 10^6 CFU per mouse). Mortality, appearance of arthritis, GBS growth in the organs, and local and systemic cytokine and chemokine production were examined. $TLR2^{-/-}$ mice showed earlier and higher mortality rates and increased incidence and severity of arthritis than wt mice at all the infecting doses employed. Histopathological analysis of the joints confirmed clinical observations. $TLR2^{-/-}$ mice exhibited a higher microbial load in blood, kidneys, and joints than wt animals. In vitro experiments performed with peritoneal polymorphonuclear cells and macrophages showed a significantly lower bactericidal ability of cells from $TLR2^{-/-}$ mice. Increased systemic and local levels of interleukin-1β (IL-1β), IL-6, tumor necrosis factor alpha, macrophage inflammatory protein-1α (MIP-1α), and MIP-2 accompanied the more severe development of sepsis and arthritis in $TLR2^{-/-}$ mice. In conclusion, the lack of TLR2 was associated with an impaired host resistance to GBS infection, likely due to a diminished bacterial clearing and a consequent enhanced inflammatory response.

Group B streptococci (GBS) have long been known as a leading cause of life-threatening infection in neonates, young infants, and pregnant women (16, 29, 31). In addition, these microorganisms have been recognized as an ever-growing cause of serious invasive infections in nonpregnant adults (9, 14, 23). The most common manifestations include primary bacteremia, skin and soft tissue infections, urinary tract infections, and pneumonia. Other relevant conditions are endocarditis, intravascular device infections, meningitis, peritonitis, endoophthalmitis, and osteoarticular infections. Although serious invasive GBS disease occurs in adults who are otherwise in good health, the majority of GBS disease occurs in those with significant underlying conditions (10, 30).

Septic GBS arthritis in nonpregnant adults was considered extremely rare (5, 18) until the early 1980s, when two independent studies stressed that its incidence seemed to be increasing (17, 34). This trend has been confirmed by recent studies in which GBS infection was found to account for 7 to 10% of all diagnosed cases of bacterial arthritis (7, 22). GBS usually reach the joints hematogenously from infections at other sites, and the most frequently affected joints are the hip, ankle, and wrist (4). We have previously described an experimental mouse model of type IV GBS infection with clinical features that closely resemble infection in humans (37, 38). This laboratory mouse model offers outstanding potential for GBS arthritis in that localization of arthritis seems to mimic the human situation. Mice given a single intravenous dose (ranging from 5×10^6 to 2×10^7 CFU) of live type IV GBS develop clinical signs of arthritis within 48 h, characterized by the evolution from acute exudative synovitis to permanent lesions with irreversible joint damage and ankylosis (38). Appearance of articular lesions is not dependent on the strain of mice or GBS serotype used since GBS serotypes II, III, V, VI, and VII are able to induce septic arthritis (40).

Toll-like receptors (TLRs) represent an evolutionarily conserved family of membrane proteins responsible for the recognition of diverse microbial products produced by bacteria, fungi, protozoa, and viruses (1, 2, 21, 41). Activation of TLRs results in upregulation of cytokines, costimulatory molecules, and antimicrobial responses known to be required for both the early control of pathogens by the innate immune system and the induction of an appropriate adaptative immune response. TLR2 is involved in cell activation by gram-positive bacterial cell wall and membrane components, such as peptidoglycan, lipoteichoic acid, and lipoproteins (3, 11, 32, 44).

The role of TLRs in the pathogenesis of arthritis is not completely understood. Analysis of synovial tissues of patients with rheumatoid arthritis revealed TLR2 expression in the synovial lining on fibroblasts as well as macrophages (33). By using an animal model, it has been shown that streptococcal cell wall (SCW) arthritis is dependent on the activation of the innate immune systems by TLR2 ligands (15).

In this study we compared GBS disease severity and outcome in TLR2-deficient ($TLR2^{-/-}$) and wild type (wt) mice in an adult mouse model of GBS-induced sepsis and arthritis.

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Mortality, incidence and severity of articular lesions, GBS growth in the organs, and cytokine production were analyzed.

MATERIALS AND METHODS

Mice. TLR2$^{-/-}$ mice were engineered as described previously on a 129Sv-C57BL/6 background and were backcrossed five times with C57BL/6J mice (36). Control wt C57BL/6J mice were purchased from Charles River Breeding Laboratories (Calco, Italy). Eight- to 10-week-old mice were used for experimental procedures.

Microorganisms. Type IV GBS, ATCC reference strain GBS 3139 (Czech National Collection of Type Cultures strain CNCTC 1/82), supplied by J. Jelinkova (Prague, Czech Republic), was used throughout the study. For experimental infection, the microorganisms were grown overnight at 37°C in Todd-Hewitt broth (Oxoid Ltd., Basingstoke, Hampshire, England) and then washed and diluted in RPMI 1640 medium (Gibco-Life Technologies, Milan, Italy). The inoculum size was estimated turbidimetrically, and viability counts were performed by plating on tryptic soy agar–5% sheep blood agar (blood agar) followed by overnight incubation under anaerobic conditions at 37°C. A bacterial suspension was prepared in RPMI 1640 medium. Mice were inoculated intravenously via the tail vein with different doses of GBS in a volume of 0.5 ml. Control mice were injected by the same route with 0.5 ml of RPMI 1640 medium.

Clinical evaluation of arthritis and mortality. GBS-infected mice were evaluated for signs of arthritis and for mortality. Mortality was recorded at 24-h intervals for 20 days. After challenge, mice were examined daily by two independent observers (L. Tissi and M. Puliti) for 20 days to evaluate the presence of joint inflammation, and scores for arthritis severity (macroscopic score) were given as previously described (26, 39). Arthritis was defined as visible erythema and/or swelling of at least one joint. Clinical severity of arthritis was graded on a scale of 0 to 3 for each paw, according to changes in erythema and swelling (0, no change; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling, erythema, and/or ankylosis). Thus, a mouse could have a maximum score of 12. The arthritis index (mean ± standard deviation [SD]) was determined by dividing the total score (cumulative value of all paws) by the number of animals used in each experimental group.

Histological assessment. GBS-infected mice were examined 6 days after infection for histopathological features of arthritis. Arthritic hind paws (1 per mouse) were removed aseptically and fixed in 10% (vol/vol) formalin for 24 h; they were then decalcified in 5% (vol/vol) trichloroacetic acid for 7 days, dehydrated, embedded in paraffin, sectioned at 3 to 4 μm, and stained with hematoxylin and eosin. Samples were examined under blinded conditions. Tibiatarseal, tarsus-metatarseal, and metatarsus-phalangeal joints were examined, and a histologic score was assigned to each joint based on the extent of infiltrate (presence of inflammatory cells in the subcutaneous and/or periarticular tissues), exudate (presence of inflammatory cells in the articular cavity), cartilage damage, bone erosion, and loss of joint architecture. Arthritis severity was classified as mild (minimal infiltrate), moderate (presence of infiltrate, minimal exudate, and integrity of joint architecture), and severe (presence of massive infiltrate/exudate, cartilage and bone erosion, and disrupted joint architecture).

GBS growth in blood, kidneys, and joints. Blood, kidney, and joint bacterial load in GBS-infected mice was determined by CFU evaluation at different times after inoculation. Blood samples were obtained by retroorbital sinus bleeding before the mice were killed. Tenfold dilutions were prepared in RPMI 1640 medium, and 0.1 ml of each dilution was plated in triplicate on blood agar and incubated under anaerobic conditions for 24 h. The number of CFU was determined, and the results were expressed as the number of CFU per ml of blood. Kidneys were aseptically removed and homogenized with 3 ml of sterile RPMI 1640 medium. All wrist and ankle joints from each mouse were removed, weighed, and homogenized in toto in sterile RPMI 1640 medium (1 ml/100 mg of joint weight). After homogenization, all tissue samples were diluted and plated in triplicate on blood agar, and the results were expressed as the number of CFU per whole organ or per ml of joint homogenate.

Killing of GBS by TLR2$^{-/-}$ or wt peritoneal PMN or macrophages. TLR2$^{-/-}$ or C57BL/6 mice were injected intraperitoneally with 1 ml of 10% thioglycolate (Sigma, St. Louis, MO). After 1 or 3 days, peritoneal exudate cells were harvested by lavage with RPMI 1640 medium in order to harvest polymorphonuclear cells ([PMN] day 1) and macrophages (day 3). The cells were washed with medium supplemented with 10% fetal bovine serum and 50 μg of gentamicin/ml (complete medium), counted in a hemocytometer, and plated in 24-well plates (Falcon, Becton Dickinson, NJ) at a density of 1×10^6 cells/ml. Macrophages were obtained by removing nonadherent cells after 2 h of incubation. GBS cells were washed in PBS and then incubated for 30 min at 37°C in 10% serum from rabbits immunized with GBS type IV, as previously described (28), as a source of specific streptococcal antibodies plus 50% human serum as a source of complement. GBS cells were then washed with PBS, suspended in RPMI 1640 medium, and coincubated with phagocytic cells at an effector-to-target ratio of 1:2. Bactericidal activity of PMN and macrophages was measured as previously described (6, 19) at different times postinfection (0, 30, or 60 min). Cells were lysed, and serial dilutions of lysates were plated on blood agar. The number of CFU was determined after overnight incubation at 37°C. The percentage of killing was calculated by using the number of GBS CFU associated with cells at the zero time point as 100%.

Sample preparation for cytokine assessment. Blood samples from the different experimental groups were obtained by retroorbital sinus bleeding at different times after infection before the mice were killed. Serum was stored at −80°C until analyzed. Joint tissues were prepared as previously described (37). Briefly, all wrist and ankle joints from each mouse were removed and then homogenized in toto in 1 ml of lysis medium (RPMI 1640 medium containing 2 mM phenylmethylsulfonyl fluoride and a 1 μg/ml final concentration of aprotinin, leupeptin, and pepstatin A) per 100 mg of joint weight. The homogenized tissues were then centrifuged at $2,000 \times g$ for 10 min, and supernatants were sterilized using a Millipore filter (pore size, 0.45 μm) and stored at −80°C until analyzed.

Cytokine assays. Interleukin-6 (IL-6), IL-1β, tumor necrosis factor alpha (TNF-α), macrophage inflammatory protein 1α, (MIP-1α), and MIP-2 concentrations in the biological samples were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations. Results were expressed as picograms per milliliter of serum or supernatant from joint homogenates or from macrophage cultures. The detection limits of the assays were 1.6 pg/ml for IL-6, 3 pg/ml for IL-1β, 1.5 pg/ml for MIP-1α, 1.5 pg/ml for MIP-2, and 5.1 pg/ml for TNF-α.

Statistical analysis. Differences in the arthritis index, number of CFU, killing activity, and cytokine concentrations between the groups of mice were analyzed by a Student's *t* test. A log rank test was used for data analyses of Kaplan-Meier survival curves. The incidence of arthritis and histologic data were analyzed by a Fisher's exact test. Each experiment was repeated three times. *P* values less of than 0.05 were considered significant.

RESULTS

Effect of TLR2 deficiency on GBS-induced sepsis and arthritis. To evaluate the in vivo role of TLR2 during GBS infection, wt and TLR2$^{-/-}$ mice were infected intravenously with different doses of bacteria (ranging from 5×10^7 to 1×10^6 CFU/mouse). The severity of the disease was monitored by assessing both the survival rates and the clinical arthritis score. With the highest dose employed (5×10^7 CFU/mouse), animals of both experimental groups died within 2 days (data not shown). As shown in Fig. 1A, infection with 10^7 CFU of GBS per mouse resulted in 100% mortality in TLR2$^{-/-}$ mice within 5 days, while only 30% of wt mice had died at the end of observation period. By lowering the infecting dose, TLR2$^{-/-}$ mice still showed higher mortality rates than controls. In fact, with an inoculation of 5×10^6 CFU per mouse, 100% mortality was found in TLR2$^{-/-}$ mice in comparison to 10% mortality in the control group. At an infecting dose of 10^6 CFU, 100% of wt mice survived, whereas 40% of TLR2$^{-/-}$ died.

The clinical signs of joint swelling were observed as early as 24 h after injection of 1×10^7 CFU of GBS in both groups of mice although the incidence of arthritis was significantly ($P < 0.01$) higher (45% versus 15%) in TLR2$^{-/-}$ than in wt mice (Fig. 2A). A rapid worsening of articular lesions was observed until death in TLR2$^{-/-}$ mice, which were 100% positive 5 days after infection and had an arthritis index of 2.5 ± 0.3. The incidence of arthritis increased also in wt mice although maximal levels of 65% with an arthritis index of 1.2 ± 0.4 were reached only 8 days after infection. Similarly, striking between-group differences were evident by lowering the infecting dose (Fig. 2B and C). In particular, with the lowest dose (10^6 CFU of GBS per mouse), none of the wt mice showed any clinical

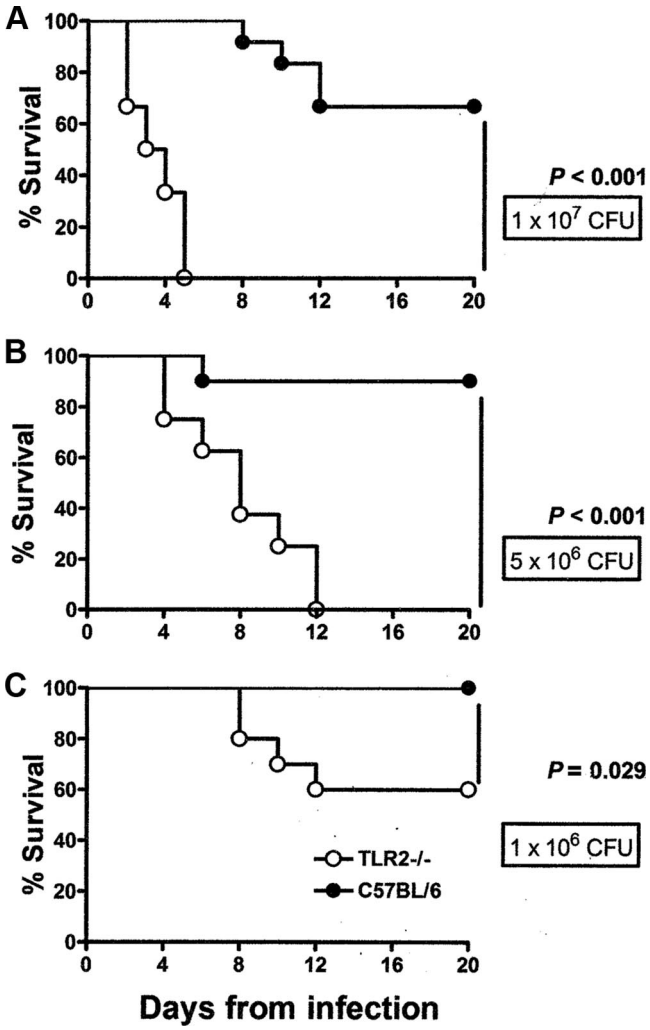

FIG. 1. Mortality rates in TLR2-deficient and wt mice infected with the indicated doses of GBS (CFU/mouse). Mortality was recorded at 24-h intervals for 20 days. The data are the cumulative results of three separate experiments, each with 10 animals per experimental group.

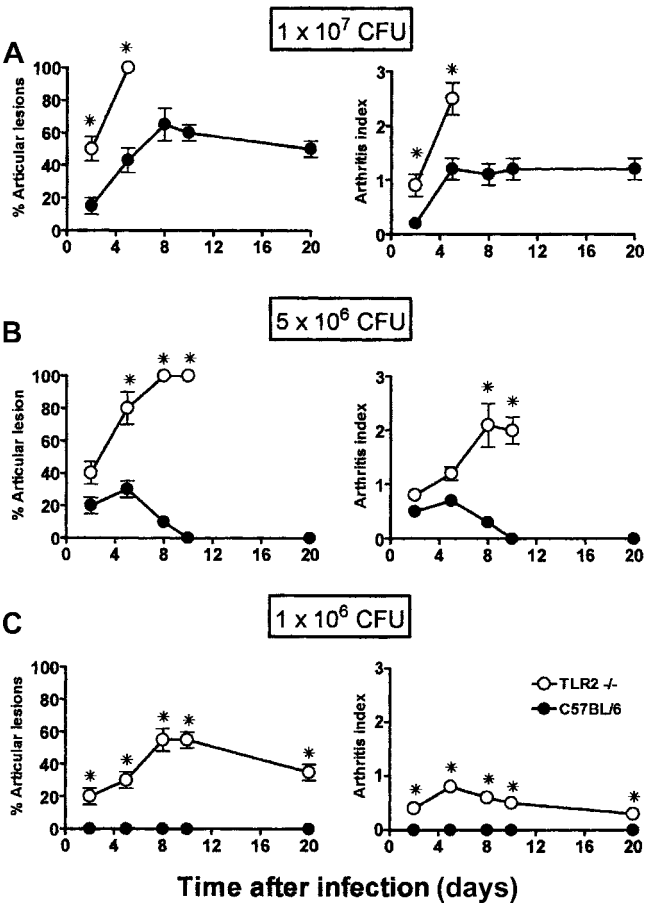

FIG. 2. Incidence and severity of arthritis in TLR2-deficient and wt mice infected with the indicated doses of GBS (CFU/mouse). Incidence of arthritis (percentage of mice with visible arthritis) and arthritis index (clinical severity of arthritis) were evaluated as described in Materials and Methods. The values represent the means ± SDs of three separate experiments, each with 10 animals per experimental group. *, $P < 0.01$ (deficient mice versus controls).

signs of arthritis while a significant ($P < 0.01$) number of TLR2$^{-/-}$ mice manifested articular lesions throughout the observation period (Fig. 2C).

Histopathological findings. Arthritic paws were removed for histological examination at day 6 after infection with 5×10^6 or 10^6 CFU per mouse and examined for histopathological features of arthritis. For each infecting dose employed, five arthritic paws from TLR2$^{-/-}$ mice and three arthritic paws from C57BL/6 mice were removed, and three joints for each paw were assessed for the histopathological score. Two separate experiments were performed. As shown in Fig. 3, at the infecting dose of 5×10^6 CFU of GBS per mouse, the majority of the joints from control animals did not show any sign of arthritis, and none of them could be classified as severely affected. In contrast, In TLR2$^{-/-}$ mice all the joints showed histological features of arthritis, and most of them were classified as severely (40%) or moderately (50%) affected. At the lowest infecting dose, all the joints assessed in wt mice were negative while 80% of the joints from TLR2$^{-/-}$ mice were positive (moderately and mildly affected).

Effect of TLR2 deficiency on GBS growth in blood, kidneys, and joints. In vivo GBS growth was assessed 3, 6, and 9 days after infection with 5×10^6 GBS per mouse by quantitative monitoring of bacteremia and bacterial growth in the kidneys and joints of TLR2$^{-/-}$ or wt mice. This infecting dose was chosen according to the above-described results. As shown in Fig. 4, rapid and progressive clearance of bacteria was observed in the blood of wt animals in contrast with the slight decrease in the number of bacteria observed in TLR2$^{-/-}$ animals. It is noteworthy that a consistent amount of GBS ($>10^4$ CFU/ml) was still present in the bloodstream of TLR2-deficient mice of at the end of observation period. GBS growth in the kidneys was significantly ($P < 0.01$) higher in TLR2$^{-/-}$ mice than in controls at all the time points assessed; however, in contrast to the results observed in the blood, wt mice were unable to clear bacteria from the kidneys. With regard to bacterial burden in the joints, opposite trends were found in the two experimental groups. In fact, the number of microor-

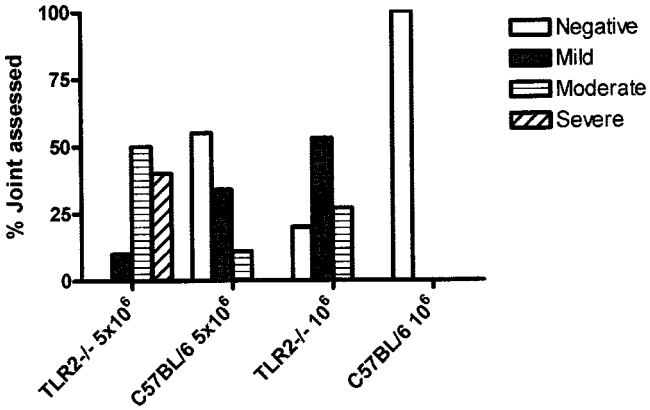

FIG. 3. Histopathological evaluation of arthritis in joints from TLR2-deficient and wt mice infected with GBS at 5×10^6 or 10^6 CFU/mouse. Histopathological severity of arthritis was assessed in $TLR2^{-/-}$ mice or controls 6 days after infection. Arthritis was defined as negative, mild, moderate, or severe as detailed in Materials and Methods. Cumulative results of two independent experiments are represented. For each infecting dose employed, five arthritic paws from $TLR2^{-/-}$ mice and three arthritic paws from C57BL/6 mice were removed, and three joints for each paw were assessed for histopathological score.

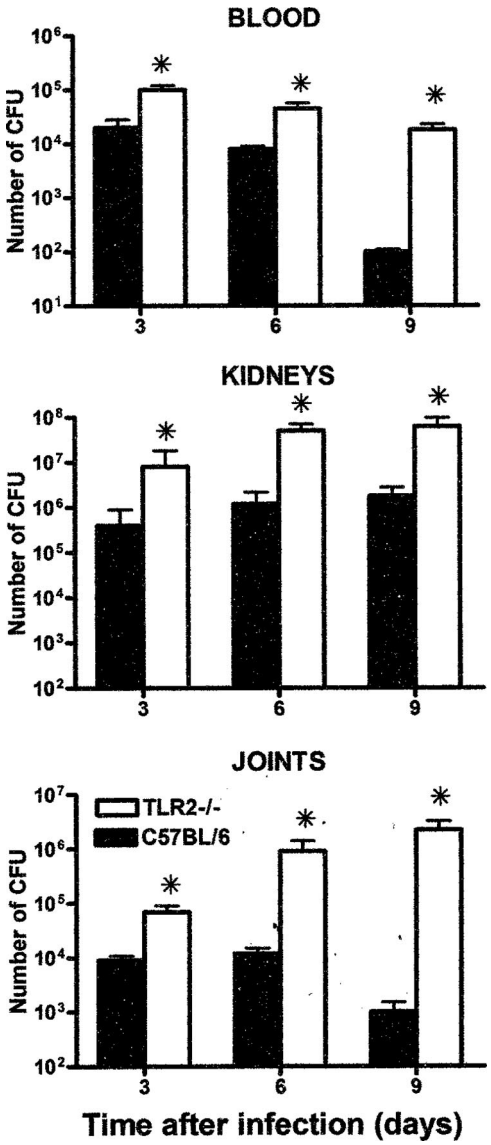

FIG. 4. Bacterial growth in blood, kidneys, and joints of TLR2-deficient and wt mice infected with GBS at 5×10^6 CFU/mouse. The values represent the means ± SDs of three separate experiments, each consisting of 18 $TLR2^{-/-}$ mice and 15 control mice. Three mice per group were sacrificed at each time point. Results are expressed as the number of CFU per ml of blood, per whole organ, or per ml of joint homogenate. *, $P < 0.01$ (deficient mice versus controls).

ganisms gradually increased in $TLR2^{-/-}$ mice while it dropped at day 9 after infection in control mice.

Effect of TLR2 deficiency on in vitro bactericidal activity of macrophages and PMN. In order to assess whether the differences observed in microbial load could be due to a weakened antimicrobial activity of TLR2-deficient phagocytes, bactericidal activity of wt and TLR2-deficient PMN and macrophages was investigated. Three independent experiments, each consisting of four replicates per experimental group, were performed for each cell population. As shown in Fig. 5, killing of both $TLR2^{-/-}$ PMN (Fig. 5 A) and macrophages (Fig. 5B) was significantly ($P < 0.01$) lower than killing by wt cells. In fact, 58% bactericidal activity was observed in wt PMN versus 29% found in $TLR2^{-/-}$ cells. When macrophage antibacterial activity was investigated, 44% killing was recorded in wt cells versus 20% in $TLR^{-/-}$ macrophages.

Effect of TLR2 deficiency on cytokine and monokine production. The role played by cytokines and monokines on the pathogenesis of GBS sepsis and arthritis is well defined (26, 39). Thus, local and systemic levels of IL-6, IL-1β, TNF-α, MIP-1α, and MIP-2 were compared between $TLR2^{-/-}$ and control mice at 3, 6, and 9 days after infection with 5×10^6 CFU of GBS per mouse. As depicted in Fig. 6, GBS infection in $TLR2^{-/-}$ mice resulted in high systemic levels of production of IL-6, IL-1 β, and TNF-α, with highly significant ($P < 0.01$) differences observed between TLR2-deficient and wt mice at all the time points assessed. Notably, in $TLR2^{-/-}$ mice TNF-α and IL-1β serum levels showed an increasing trend while a decrease was observed in IL-6 concentrations 6 days after infection. At joint level, dramatic increases in IL-6, IL-1β, and TNF-α concentrations were found in $TLR2^{-/-}$ mice at day 3 after infection, and sustained levels of the three cytokines were still present at the end of the detection period. A time-dependent increase in chemokine concentrations was also observed in the joints of TLR2-deficient mice. In particular, MIP-2 levels peaked at 759 ± 78 pg/ml on day 3 after bacterial inoculation while the MIP-1α concentration was 198 ± 37 pg/ml (Fig. 7). In contrast, barely detectable levels of both chemokines were found in the joints of control animals. In serum, MIP-1α concentrations showed a peak at day 3 after infection and then dropped in the subsequent days, with values similar to those observed in C57BL/6 mice. MIP-2 concentrations slightly increased until they reached plateau levels at days 6 to 9 after infection in $TLR2^{-/-}$ mice while steady levels were found throughout the observation period in wt animals.

TLR2 deficiency influences arthritis severity through impairment of bacterial clearance. To ascertain whether TLR2 deficiency directly influences the severity of arthritis, experi-

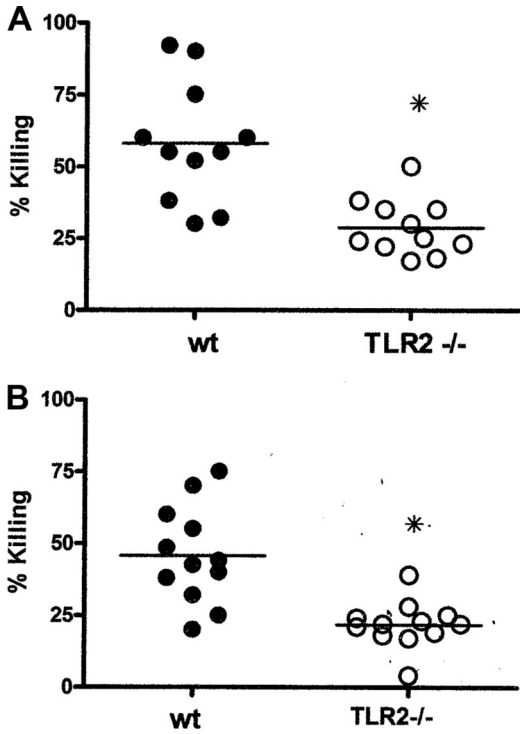

FIG. 5. Killing of GBS by peritoneal PMN (A) or macrophages (B) derived from TLR2-deficient or wt mice. Cells were recovered, and killing activity was determined as described in Materials and Methods. Data represent the values obtained in three separate experiments. *, $P < 0.01$ (deficient mice versus controls).

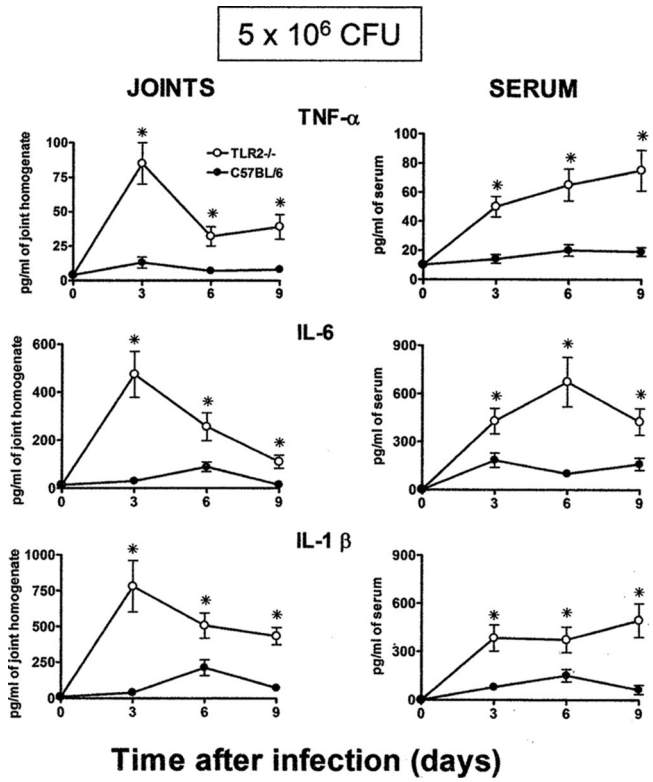

FIG. 6. Effect of TLR2 deficiency on cytokine production in joints and sera of mice infected with GBS at 5×10^6 CFU/mouse. Supernatants from joint homogenates or blood samples from TLR2-deficient or wt mice were collected at the indicated times after infection and assayed for TNF-α, IL-6, and IL-1 β by ELISA. Values are the means ± SDs of three separate experiments each consisting of 18 $TLR2^{-/-}$ mice and 15 control mice. Three mice per group were sacrificed at each time point. *, $P < 0.01$ (deficient mice versus controls).

ments were performed with wt and TLR2-deficient mice infected with different doses of GBS in order to obtain the same number of bacteria in the bloodstream. Seven wt mice were injected with 1×10^7 CFU/mouse, and the same number of $TLR2^{-/-}$ mice were injected with 2×10^6 CFU/mouse. At day 3 postinfection, five animals from each experimental group were sacrificed, and numbers of CFU in the blood and cytokine concentrations in the joints were assessed. The experiment was repeated twice. As shown in Fig. 8, at day 3 after infection similar numbers of GBS CFU were recovered from the blood of mice from both experimental groups. Under these conditions, no significant differences were observed in either the severity of arthritis or TNF-α production in the joints. Similar results were obtained by examining the IL-6 and IL-1β concentrations (data not shown).

DISCUSSION

The recognition of invading bacteria or bacterial products by innate immune mechanisms is the first step in a sequence of events leading to a host response intended to eradicate the pathogens. This function is performed by highly conserved structures on cells of the innate immune system, the TLRs.

Previous in vitro studies indicated that TLR2 is involved in GBS-induced cell activation and cytokine production (12, 13). These studies implicated additional members of the TLR family, including TLR6 as well as at least one additional, and an as yet unidentified, TLR. Moreover, it has been demonstrated that interaction of GBS with TLR2 was beneficial in a low-dose GBS sepsis model, whereas it was detrimental in the high-dose model of septic shock (20). In the present study we demonstrated the in vivo pivotal role of TLR2 signaling not only in sepsis but also in arthritis induced by GBS infection. In contrast to the report by Mancuso et al. (20), we failed to observe that the role of TLR2 was affected by the infecting dose, probably because a different GBS serotype was used (type V in the previous study versus type IV in the present study). A similar involvement of TLR2 in resistance to infection has been pointed out in other experimental models using gram-positive bacteria, such as *Streptococcus pneumoniae* (8) or *Staphylococcus aureus* (35). Unlike results obtained with *S. aureus* as the infecting agent, in our model the failure of the host to recognize invading bacteria is manifested even with a lower number of microorganisms since significantly different mortality rates between TLR2-deficient and wt mice were still evident when a low dose (10^6 CFU/mouse) was administered to the animals. In our opinion, the main factor in the enhanced susceptibility to GBS infection observed in TLR2-deficient mice is the impairment in the clearance of the pathogen. In fact, a consistent and long-lasting presence of GBS was found in all the tissues examined (blood, joints, and kidneys) at three different time points (3, 6, and 9 days) following infection.

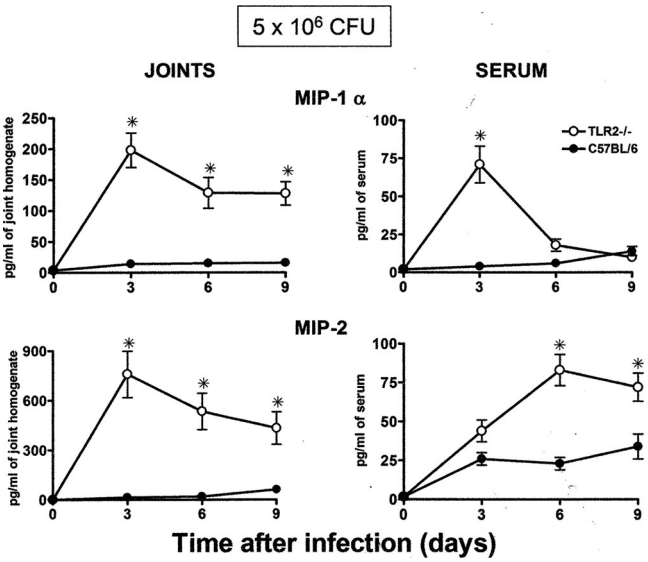

FIG. 7. Effect of TLR2 deficiency on chemokine production in joints and sera of mice infected with GBS at 5×10^6 CFU/mouse). Supernatants from joint homogenates or blood samples from TLR2-deficient or wt mice were collected at the indicated times after infection and assayed for MIP-1 α or MIP-2 by ELISA. Values are the means ± SDs of three separate experiments, each consisting of 18 $TLR2^{-/-}$ mice and 15 control mice. Three mice per group were sacrificed at each time point. *, $P < 0.01$ (deficient mice versus controls).

Moreover, when similar numbers of GBS were present in the blood of wt or $TLR2^{-/-}$ mice, no significant differences were observed in the severity of arthritis or cytokine production. Thus, we can speculate on an indirect effect of TLR2 deficiency on GBS-induced arthritis, mediated by impairment of antibacterial activity. It has been previously shown that deletion of TLR2 does not influence GBS uptake by peritoneal macrophages from knockout mice compared to wt controls (12). Moreover, opsonization of GBS appeared to be a prerequisite for the uptake of bacteria since no significant uptake of GBS was observed in the absence of complement. Letiembre et al. demonstrated that killing of *S. pneumoniae* by mouse PMN was impaired in the absence of TLR2 (19). The mechanism of defective killing in $TLR2^{-/-}$ mice was attributable to a failure in oxidative bactericidal activity and, in particular, to the weakened enzymatic activity of NADPH oxidase. Keeping in mind all these observations, we may hypothesize that, in our model, the observed defect in bacterial clearing seems to be due not to altered GBS uptake but to the weakened bactericidal activity of TLR2-deficient phagocytes.

Not only mortality but also worsening arthritis were associated with TLR2 deficiency since both the incidence and severity of articular lesions were more evident in $TLR2^{-/-}$ than in wt mice. As already observed for mortality, the phenomenon was evident at all the infecting doses used. The appearance and severity of GBS arthritis are the by-products of a multifactorial process. The viability and number of microorganisms injected and bacterial factors (i.e., presence and amount of capsule, amount of sialic acid in the capsular polysaccharide, and β-hemolysin production) have been shown to influence the development of articular lesions (25, 40). Nevertheless, a crucial role in the pathogenesis of GBS arthritis is played by inflammatory cells (granulocytes and monocytes) that reach the joints (26, 27) and by the production of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α (39). Here, the higher number of bacteria recovered from the joints of $TR2^{-/-}$ mice correlated with the greater severity of arthritis observed in these animals. These findings are in agreement with a report on experimental infection with *Borrelia burgdorferi* that show the underlying pivotal role of TLR2 in influencing host defense against the spirochete since TLR2-deficient mice harbored up to 100-fold more spirochetes in the tissues than controls and displayed greater joint pathology (43). In our model the rapid increase in bacterial growth in joints of $TLR2^{-/-}$ mice may, in turn, have contributed to the enhanced inflammation and to

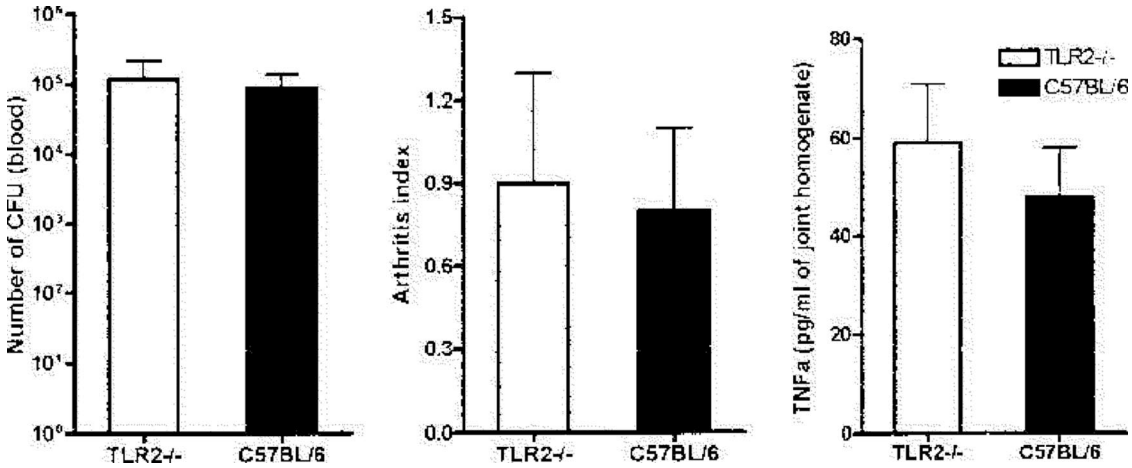

FIG. 8. Comparison of severity of arthritis and local TNF-α production between $TLR2^{-/-}$ and C57BL/6 mice in the presence of comparable numbers of GBS in the blood. TLR2-deficient mice were infected with 2×10^6 CFU/mouse, and C57BL/6 mice were infected with 1×10^7 CFU/mouse. The arthritis index (clinical severity of arthritis) was evaluated as described in Materials and Methods. The number of CFU in the blood, the arthritis index, and TNF-α production in the joints were determined 3 days after infection, as detailed in Materials and Methods. Values are the means ± SDs of two separate experiments, each consisting of seven $TLR2^{-/-}$ mice and seven control mice. Five animals for each experimental group were sacrificed.

the earlier aggravation of the symptoms in these animals. In fact, the high bacterial burden present in TLR2$^{-/-}$ mice in comparison to wt animals mice correlated with a dramatic increase in joint production of TNF-α, IL-1β, and IL-6, known to contribute directly to tissue damage through induction of the release of tissue-damaging enzymes from synovial cells and articular chondrocytes and through activation of osteoclasts (42). GBS expresses at least two pathogen-associated molecular patterns. The first is released by GBS and engages a receptor complex consisting of TLR2, TLR6, and CD14 and has recently been shown to consist of mature lipoproteins (11). The second is cell bound and potently engages an MyD88-dependent pathway (12, 13), but this factor has not been identified on the molecular level. Cytokine production in TLR2$^{-/-}$ mice is most likely triggered by the engagement of this latter receptor. Keeping in mind the high number of bacteria present in deficient mice in comparison to controls, this stronger bacterial stimulus may be responsible for the enhanced cytokine production observed in TLR2$^{-/-}$ mice. TLR2 signaling has been implicated also in joint inflammation induced by intra-articular injection of SCW fragment since TLR2-deficient mice were unable to develop either joint swelling or inhibition of cartilage matrix synthesis (15). Thus, in the SCW arthritis model, deletion of TLR2 seems to exert a positive role, whereas in our model it seems to be detrimental. This may reflect the differences between arthritis caused by tissue invasion of a living pathogen versus the inflammatory response directed to large concentrations of bacterial products. Our findings suggest that a pattern recognition receptor independent from TLR2, actively stimulated by the large number of GBS organisms accumulating in the joints, can contribute to GBS-induced lesions in vivo.

Finally, there appears to be a connection between inflammatory cell accumulation and joint destruction in septic arthritis since leukocytes produce cytokines and proteolytic enzymes that contribute to cartilage and bone destruction (24). Selective recruitment of activated leukocytes into inflammation sites is mediated by many factors, particularly chemokines. Actually, a higher production of MIP-1α and MIP-2 is observed in the joints of TLR2$^{-/-}$ mice than in controls at all the time points examined. The situation of TLR2-deficient mice suggests a complex interaction between inflammation and host defense, with the presence of extremely high numbers of bacteria in joints, resulting in recruitment of inflammatory cells that are unable to clear the high number of bacteria.

In conclusion, this study provides evidence of an impaired host resistance to GBS infection in TLR2$^{-/-}$ mice. Sepsis, clinical course of arthritis, number of bacteria, and systemic and local inflammation are aggravated in mice lacking TLR2. Taken together, these results indicate a contribution of TLR2 in the regulation of bacterial clearing and, consequently, in the inflammatory response during GBS-induced sepsis and arthritis.

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