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Experimental Model of Type IV *Streptococcus agalactiae* (Group B Streptococcus) Infection in Mice with Early Development of Septic Arthritis

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We have established an experimental murine model to gain insight into the pathogenicity and clinical features of type IV group B streptococcus (GBS) infections. Adult CD-1 mice were challenged intravenously with 10^7 type IV GBS cells, inducing systemic invasion. Most of the animals were able to clear the infection from the blood, brain, and lungs within 2 weeks and from the spleen and liver within 1 month. However, the animals were unable to clear the microorganism from the joints and kidneys during the 60-day observation period. About 80% of the mice challenged intravenously with type IV GBS manifested early septic arthritis, which evolved from an acute exudative synovitis to permanent lesions characterized by irreversible joint damage and ankylosis. Induction of persistent septic arthritis was dependent on the number and viability of microorganisms inoculated and was unrelated to the strain of type IV GBS and the growth phase of the inoculum. Type-specific antibodies of both immunoglobulin M and G classes could be detected by agglutination and enzyme-linked immunosorbent assay from days 7 and 14, respectively; immunoglobulin G antibodies persisted for more than 40 days. Complexes of antibodies and group- and type-specific antigens were detected in mouse sera 24 h after infection and persisted up to day 22. These results were obtained an experimental model of type IV GBS chronic infection with early development of septic arthritis, which could be useful in future studies of pathogenicity and immune mechanisms involved in the host resistance to this microorganism.

Group B streptococci (GBS) are important human pathogens which cause serious neonatal diseases (1, 16). All GBS serotypes cause early-onset infections, and type III is associated with the majority of late-onset infections, (2–13). Recently, two new GBS serotypes, type IV and provisional type V, have been reported (17–19, 24, 26). Type IV GBS is characterized by a serologically specific type polysaccharide localized on the bacterial surface (23, 35). However, little is known about type IV GBS pathogenicity and the immune mechanisms of resistance to infection (23–25), due, in part, to lack of a suitable experimental model. Relevant results for a better understanding of human diseases have been obtained by studying type I, II, and III GBS infections in adult mice (6, 9, 12, 14, 27, 34) as well as infant and adult rats (11, 30).

The purpose of this investigation was to establish an experimental murine model of infection to evaluate pathogenicity and clinical features of type IV GBS infection. In particular, we demonstrate that systemic challenge of mice with type IV GBS results in chronic infection with a high incidence of early septic arthritis and that this phenomenon is not strain specific.

MATERIALS AND METHODS

Animals. Outbred CD-1 mice of both sexes, 8 to 10 weeks of age, were obtained from Charles River Breeding Laboratories, Calco, Milan, Italy.

Microorganisms. Type IV GBS, reference strain GBS 1/82, was kindly supplied by J. Jelínková, Prague, Czechoslovakia. Two additional type IV GBS strains (designated laboratory names, 1606 and 1613) were isolated from urine samples from diabetic patients. The microorganisms were grown at 37°C in Todd-Hewitt broth (Oxoid Ltd., London, United Kingdom), and samples were stored at −70°C until use. For experimental infections, microorganisms in log or stationary phase of growth (overnight culture) were used. GBS were washed and diluted in serum-free RPMI 1640 medium (Flow Laboratories, McLean, Va.). The inoculum size was estimated turbidimetrically at 540 nm in a Beckman DU-68 spectrophotometer (Beckman Instruments, Fullerton, Calif.). The number of live bacterial cells was confirmed by enumeration of the CFU on Islam agar (Oxoid Ltd.) plates containing 5% inactivated horse serum and incubated under anaerobic conditions. The desired numbers of bacteria diluted in RPMI medium were injected intravenously (i.v.) via the tail vein in a volume of 0.5 ml per mouse.

Inactivation of GBS and preparation of cell sonic extracts. GBS 1/82 was grown overnight, washed three times with RPMI medium, suspended at concentrations ranging from 2×10^7 to 2×10^9/ml, and killed by heating at 60°C for 30 min. Microorganism inactivation was confirmed by culturing 0.1 ml of each dilution on Islam agar plates for 48 h.

Whole-cell sonic extracts were obtained as follows. A 5.1-g portion of lyophilized GBS 1/82 was suspended in 25 ml of phosphate-buffered saline and sonicated for 90 min in an ultrasonic disintegrator at 8 amplitude microns of power (MSE Sonuprep 150). The disrupted material was centri-

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fuged at 3,000 × g for 20 min, and the supernatant was then filtered through 5- and 0.8-μm (pore sizes) membrane filters (Millipore Corp., Bedford, Mass.). Sterility was tested by culturing 0.1 ml of the filtered supernatant on an Islam agar plate for 48 h. After lyophilization, the whole-cell sonic extract, diluted to a concentration of 1 mg/ml, was tested by immunodiffusion against anti-type IV and anti-group B rabbit polyclonal antiserum.

The rhamnose content of whole-cell sonic extracts was about 22.0 μg/mg of dry cell weight and was determined by the method of Dishe and Shettles (8). Sialic acid content of the type-specific polysaccharide of GBS 1/82 cells, in both log and stationary phases of growth, was determined by the method of Shigeoka et al. (31).

Virulence determination. To evaluate the virulence of the different strains of type IV GBS, groups of 20 CD-1 mice were inoculated i.v. with 10^6 to 10^9 CFU per mouse and mortality was recorded at 24-h intervals for 60 days. The 50% lethal dose (LD_{50}), calculated by the method of Reed and Muench (29), represents the mean of three experiments.

Clinical and histological studies. Mice challenged i.v. with 10^7 type IV GBS of each strain were examined two or more times during day 1 after inoculation and then daily for 2 months to evaluate the clinical features of the disease, in particular, the presence of joint inflammation. Time of onset, number of joints involved, incidence, duration of arthritis, and occurrence of ankylosis were recorded. To evaluate the histopathological features of the disease, mice inoculated i.v. with 10^7 type IV GBS 1/82 were sacrificed every 2 days after infection. Specimens from the spleen, liver, kidneys, brain, lungs, and joints were fixed in 10% Formalin for 24 h. The joint specimens were successively decalcified in 5% trichloroacetic acid for 7 days. All specimens were dehydrated, embedded in paraffin, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin.

GBS growth in organs of infected mice. Blood and organ infections were determined by CFU evaluation at different times after type IV GBS 1/82 i.v. inoculation. Spleen, liver, kidneys, lungs, and brain were aseptically removed and placed in a tissue homogenizer with 3 ml of sterile RPMI medium. Blood samples were obtained by retro-orbital sinus bleeding before sacrifice. After organ grinding, appropriate 10-fold dilutions were made in sterile RPMI medium and 0.2 ml of tissue suspensions or blood was plated in triplicate on Islam agar plates. The plates were incubated under anaerobiosis at 37°C for 48 h, CFU were enumerated, and the results were expressed as the number of CFU per milliliter of blood or per whole organ. For the other strains of type IV GBS, the growth of microorganisms was evaluated only in the blood and kidneys.

Samples were also recovered from the infected joints by gentle needle aspiration. These fluids were examined for the presence of type IV GBS by plating on Islam agar, and the group and type of the isolated microorganisms were determined.

Preparation of antisera. Group- and type-specific antisera were prepared in New Zealand White rabbits by the method of Lancefield (21). Group-specific antisera were obtained by using the group B reference strain; type IV specific antisera were prepared by i.v. inoculation of type IV GBS 1/82. Sera were collected after 6 weeks and tested for group- and type-specific antibodies by agar immunodiffusion. The sera were stored at −70°C.

Enzyme-linked immunosorbent assay. Specific immunoglobulin G (IgG) or IgM antibodies against GBS type IV polysaccharide were measured by enzyme-linked immunosorbent assay. GBS type IV polysaccharide antigen was extracted by the method of Wilkinson and Jones (36) and purified by wheatgerm agglutinin affinity chromatography (15). The antigen was checked for specificity in immunodiffusion against type IV and group B immune rabbit sera.

Flat-bottom plates (Microelisa; Dynatech, Alessandria, Italy) were coated overnight at room temperature with 1 μg of the antigen, suspended in 0.1 M carbonate buffer, pH 9.6. Plates were then washed with phosphate-buffered saline–Tween 20 (0.05%, vol/vol), and mouse serum samples diluted 1:100 in phosphate-buffered saline–Tween 20 plus 0.01% gelatin were added. After 2 h of incubation at room temperature and three washings, 200 μl of goat anti-mouse IgG or anti-mouse IgM alkaline phosphatase-conjugated antiserum (Sigma Chemical Co., St. Louis, Mo.) diluted 1:1,000 in phosphate-buffered saline–Tween-gelatin was added and plates were incubated for 2 h at room temperature. After washing, 200 μl of a 1-mg/ml concentration of *p*-nitrophenylphosphate (Sigma) diluted in diethanolamine buffer was added. After 1 h, the optical density was read at 405 nm with a Titertek Multiscan (Skatron, Oslo, Norway). Results were expressed as change in optical density at 405 nm, calculated for each serum sample by subtracting the absorbance of the serum in the absence of the coating antigen.

A well was considered positive for the presence of specific antibodies when its absorbance was at least twice the absorbance of the wells coated only with the antigen. Specific antibody titers were determined in pooled sera from three animals for each determination point.

Agglutination reaction. Slide agglutination tests were performed by mixing 10 μl of type IV GBS 1/82 cells from a thick suspension in phosphate-buffered saline with undiluted or a 1:5, 1:10, or 1:100 dilution of mouse sera collected at different times after bacterial challenge.

Antigenemia detection. The presence of free circulating type- and group-specific antigens was examined by immunodiffusion of sera of i.v. infected mice against anti-type IV and -group B rabbit immune sera. A commercial latex agglutination rapid test (Oxoid Ltd.) was also used to detect antibody-linked group B antigen. Latex agglutination and immunodiffusion tests were repeated with sera of infected mice pretreated with pronase (0.1 mg/ml) for 24 h and then boiled for 15 min and centrifuged at 3,000 rpm for 15 min. Irrelevant anti-type IV serum was used as a specificity control.

RESULTS

Effect of inoculum size on mouse mortality after challenge with different strains of type IV GBS. The capacity of the

TABLE 1. Effect of inoculum size of different strains of type IV GBS on mortality of CD-1 mice

Inoculum size (CFU/mouse)[a]	Type IV GBS strain					
	GBS 1/82		GBS 1606		GBS 1613	
	Median survival time (days)	Dead mice/total[b]	Median survival time (days)	Dead mice/total	Median survival time (days)	Dead mice/total
10^9	1	20/20	1	20/20	1	20/20
10^8	3.5	20/20	3	20/20	3.5	20/20
10^7	>60	6/20	>60	7/20	>60	6/20
10^6	>60	0/20	>60	0/20	>60	0/20

[a] GBS were injected by i.v. route after overnight culture.
[b] Dead mice at day 60 and total animals tested.

TABLE 2. Effect of growth phase of different strains of type IV GBS on virulence in CD-1 mice

Type IV GBS strain	LD_{50}[a]	
	Log phase[b]	Stationary phase[c]
GBS 1/82	1.88×10^7	1.94×10^7
GBS 1606	1.62×10^7	1.77×10^7
GBS 1613	2.18×10^7	1.96×10^7

[a] LD_{50} values were calculated for the i.v. route of GBS infection. Standard errors, usually <10%, have been omitted.
[b] GBS were inoculated in log phase of growth (~3 h).
[c] GBS were inoculated after overnight culture.

different strains of type IV GBS in the stationary phase of growth to produce lethal infection was examined by i.v. inoculation of mice with different numbers of microorganisms. The results (Table 1) show similar mortality rates for mice inoculated with the three strains of type IV GBS with low pathogenicity; inoculation of 10^6 GBS did not cause death. LD_{50}s of the three GBS strains grown to the log or stationary phases were also evaluated. In Table 2 similar LD_{50}s were recorded regardless of the phase of growth of the inoculated microorganism. The sialic acid content of the GBS strains, evaluated at various times during 24-h culture, ranged from 0.74 to 0.89% (dry weight) of whole microbial cells. Experiments on in vivo growth of the three strains of type IV GBS were carried out by quantitative monitoring of bacteremia and GBS growth in the kidneys 3 and 10 days after systemic infection. The persistence of bacteremia and bacterial burden in the kidneys was dependent on inoculum size but not on the strain of GBS (Table 3). In other experiments, the growth kinetics of GBS 1/82 in various organs of mice injected i.v. with 10^7 microorganisms was examined. All organs, except the brain, were colonized 2 h after challenge (Fig. 1), but after 24 h GBS 1/82 was present also in the brain and with increased numbers in all other organs examined. Subsequently, the number of microorganisms in the spleen, liver, lungs, and brain decreased while progressively increasing in the kidneys. In this organ, an apparent plateau of about 10^{10} GBS was maintained from 15 to 20 days, and an elevated number of CFUs was still present 1 month after challenge (Fig. 1). Some 50 days after infection, GBS 1/82 could still be isolated from the kidneys of about 11% of the animals (data not shown).

Clinical observations. Mice injected i.v. with 10^7 GBS of the three strains were examined for signs and symptoms of disease throughout the 60-day observation period. All animals manifested one or more of the following clinical signs: neurological disorders (circling, incoordinate movements, holding the head to one side) and panophthalmitis. In particular, the majority of mice manifested articular swelling. The kinetics of appearance and the incidence of this inflammation are reported in Table 4. Clinical onset of articular lesions occurred with all GBS strains about 2 days after infection. The most involved joints were the ankle, wrist, and coxaphemoral joint, and, less frequently, tarsophalangeal, carpophalangeal, and interphalangeal joints. On day 8 after infection 77% of the animals showed persistent articular inflammation. Complete remission occurred only in 20% of the affected mice within 20 days. On day 60, animals with permanent lesions showed irreversible joint damage with marked ankylosis. Type IV GBS was constantly isolated from articular lesions during the whole course of infection with the three GBS strains.

Induction of joint arthritis was dependent on both the number of GBS inoculated and the viability of the microorganisms, because no clinical signs of inflammation were evident in mice infected i.v. with 10^6 microorganisms or inoculated with inactivated cells or whole-cell sonic extracts. The development of arthritis was similar with all strains of GBS (Table 4).

Histopathology. Because the kidneys and joints are the target organs for chronic replication of the three type IV GBS strains, we evaluated the major histopathological changes occurring in these tissues at various stages of systemic infection with 10^7 GBS 1/82. The main kidney lesions, detected on day 4, consisted of isolated suppurative foci in the cortex with neutrophilic infiltrates surrounding a central necrotic area. On day 8 many of these inflammatory foci, often confluent, were present in the cortical and occasionally in the medullary zone. The suppurative lesions became chronic 20 days after infection, and many proliferative fibroblastic areas in the cortical and medullary zones progressively replaced the suppurative necrotized areas, producing tissue sclerosis.

The major histopathological changes in the affected joints were the presence of an acute exudative synovitis, starting as early as 48 h after infection, and a polymorphonuclear leukocyte-monocyte infiltrate of the subsynovium and periarticular connective tissues. One week later, articular cavities of involved joints were filled with purulent exudate (Fig. 2). In mice with persistent articular inflammation, joint destruction progressed rapidly, with loss of cartilage and proliferation of granulation tissue. Fibrous ankylosis was observed on day 60.

Antibody response and antigenemia. Kinetics of specific IgG and IgM antibody responses of mice inoculated i.v. with 10^7 GBS 1/82 was assessed by agglutination and enzyme-

TABLE 3. Growth of different strains of type IV GBS in blood and kidneys of CD-1 mice

Type IV GBS strain[a]	Inoculum size (CFU/mouse)	CFU recovered ± SE[b]			
		Day 3		Day 10	
		Blood	Kidneys	Blood	Kidneys
GBS 1/82	10^8	$2.8 \times 10^5 \pm 0.1 \times 10^5$	$1.4 \times 10^{11} \pm 0.1 \times 10^{11}$	NT[c]	NT
GBS 1/82	10^7	$4.1 \times 10^3 \pm 0.1 \times 10^3$	$1.2 \times 10^8 \pm 0.1 \times 10^8$	$7.9 \times 10^2 \pm 0.2 \times 10^2$	$8.2 \times 10^9 \pm 0.3 \times 10^9$
GBS 1/82	10^6	$1.4 \times 10^3 \pm 0.1 \times 10^3$	$6.4 \times 10^7 \pm 0.4 \times 10^7$	0	$5.9 \times 10^6 \pm 0.4 \times 10^6$
GBS 1606	10^7	$3.1 \times 10^3 \pm 0.2 \times 10^3$	$2.1 \times 10^8 \pm 0.2 \times 10^8$	$1.9 \times 10^3 \pm 0.2 \times 10^3$	$1.6 \times 10^{10} \pm 0.2 \times 10^{10}$
GBS 1613	10^7	$2.5 \times 10^3 \pm 0.2 \times 10^3$	$4.5 \times 10^8 \pm 0.5 \times 10^8$	$5.7 \times 10^2 \pm 0.5 \times 10^2$	$1.8 \times 10^{10} \pm 0.2 \times 10^{10}$

[a] GBS strains were cultured overnight before i.v. inoculation.
[b] Values represent the means of three separate experiments. Eight mice per group were sacrificed at each time. Number of CFU per milliliter of blood or for both kidneys are reported.
[c] NT, Not tested (dead mice).

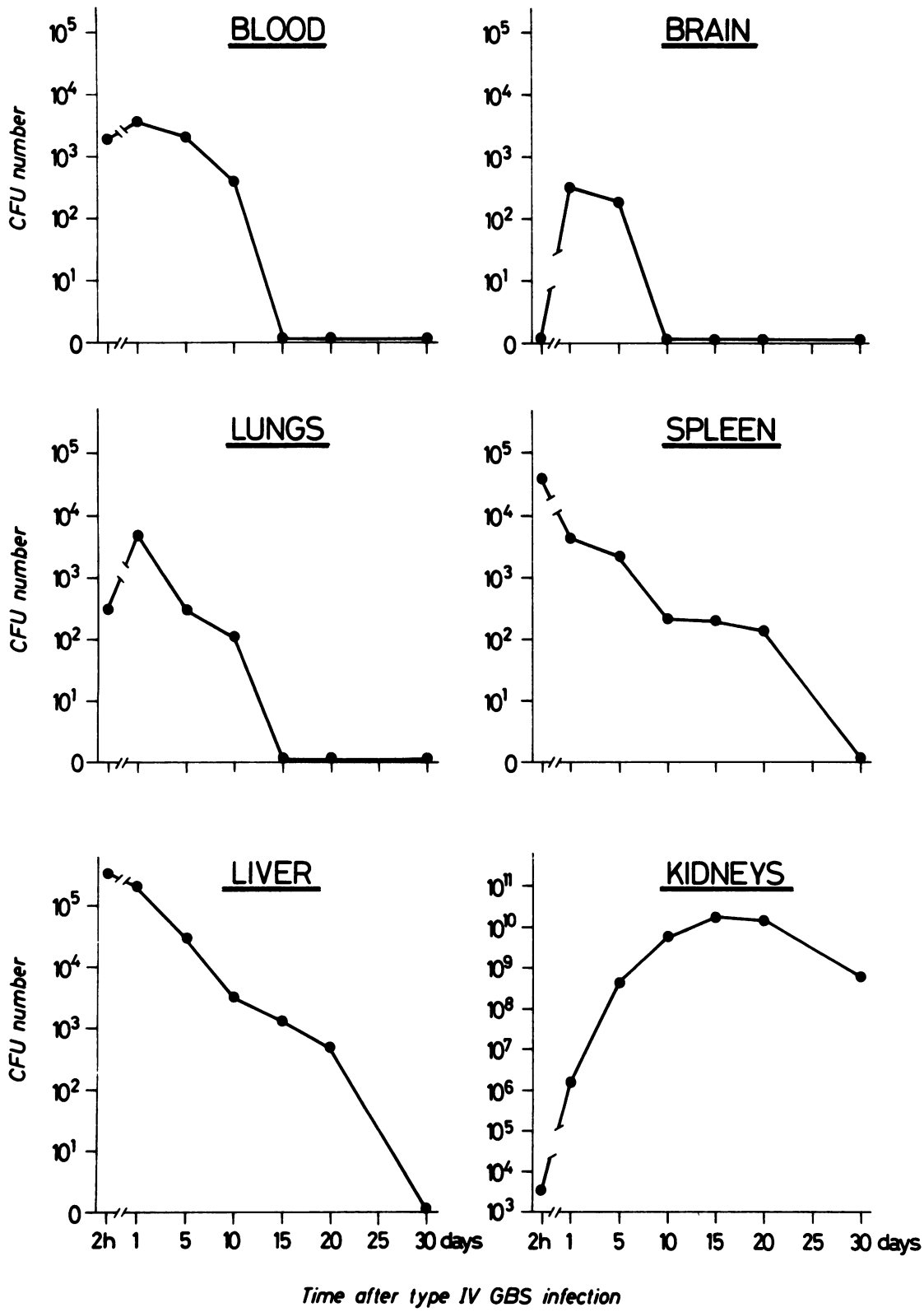

FIG. 1. Growth kinetics of type IV GBS 1/82 in various organs of CD-1 mice. Mice were inoculated i.v. with 10^7 microorganisms on day 0. Values reported are the mean CFUs recovered per milliliter of blood or whole organs of 10 mice. Standard errors, usually <10%, have been omitted.

TABLE 4. Time course of joint arthritis in CD-1 mice after i.v. inoculation of three live strains of type IV GBS or inactivated cells or whole-cell sonic extracts of type IV GBS 1/82

Type IV GBS strain[a]	Dose	No. (%) of mice with articular lesions at given time (days) after infection				
		2	4	6	8	>30
Live cells						
GBS 1/82	10^7	29 (44.6)	41 (63.0)	46 (70.7)	50 (76.9)	50 (76.9)
GBS 1606	10^7	20 (30.7)	33 (50.7)	36 (55.3)	43 (66.1)	43 (66.1)
GBS 1613	10^7	22 (33.8)	33 (50.7)	36 (55.3)	47 (72.3)	47 (72.3)
GBS 1/82	10^6	0	0	0	0	0
Inactivated cells						
GBS 1/82	10^7	0	0	0	0	0
GBS 1/82	3×10^9	0	0	0	0	0
Whole-cell sonic extract						
GBS 1/82	5[b]	0	0	0	0	0
GBS 1/82	15[b]	0	0	0	0	0

[a] Mice were injected i.v. on day 0. In each experiment 65 mice were used. Values are the means of three separate experiments. Standard errors, usually <10%, have been omitted.

[b] Micrograms of GBS rhamnose injected i.v. per gram per mouse.

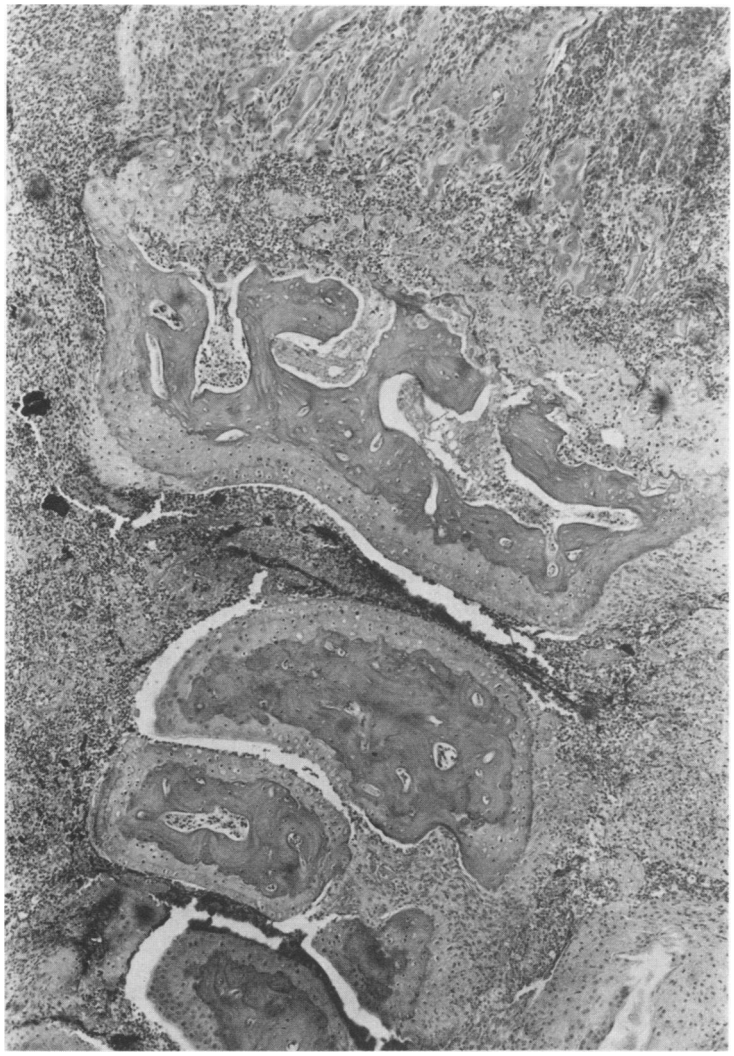

FIG. 2. Histology of wrist arthritis. On day 7 after type IV GBS 1/82 systemic infection, articular infiltration by purulent material was extended to periarticular tissues.

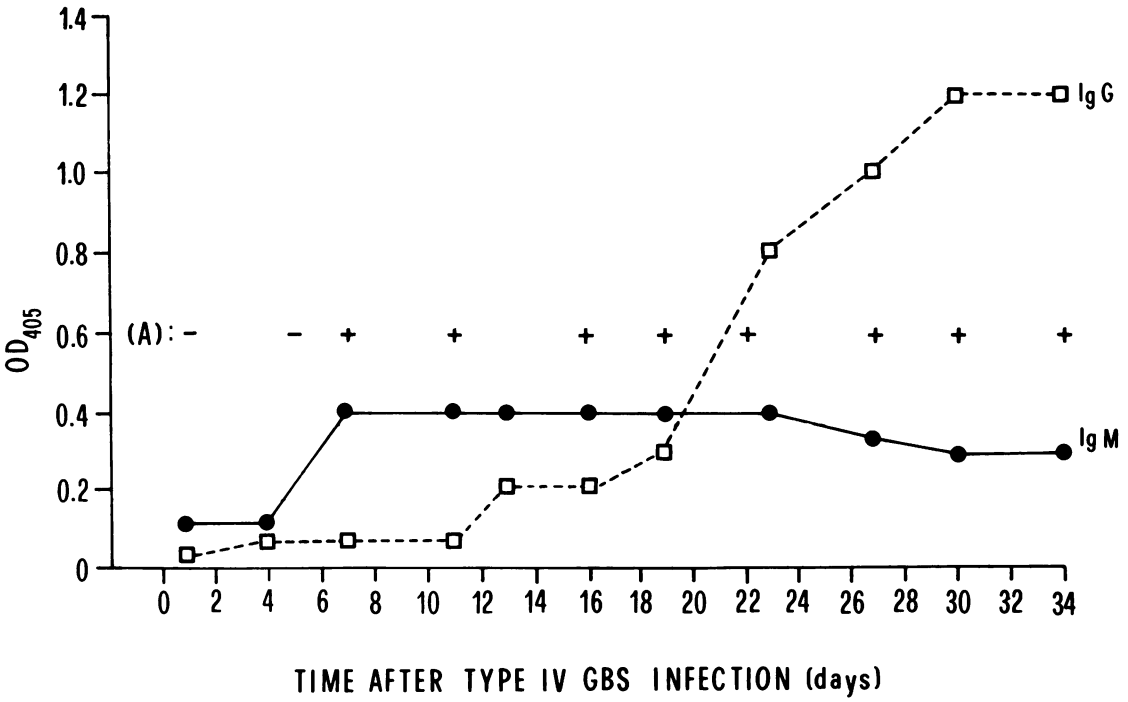

FIG. 3. Antibody response during the course of type IV GBS 1/82 systemic infection. CD-1 mice were injected i.v. with 10^7 microorganisms on day 0. Antibodies to type IV polysaccharide were detected by agglutination test with inactivated type IV GBS 1/82 cells (A). Specific IgG (□) and IgM (●) titers were evaluated by enzyme-linked immunosorbent assay and expressed as change in optical density (Δ OD) (mean of three separate experiments). The sera of normal control mice had OD values lower than 0.09 for IgG and 0.14 for IgM and were considered negative. In infected mice, only sera with OD of >0.18 for IgG and >0.3 for IgM were considered positive. Standard errors, usually <10%, have been omitted.

linked immunosorbent assay methods (Fig. 3). Agglutinating antibodies appeared on day 7 and persisted for at least 30 days. No prozone phenomenon was evident during week 1 of infection, as shown by the use of 1:5- to 1:100-diluted sera in the agglutination test. Enzyme-linked immunosorbent assay-determined IgM antibodies to type-specific polysaccharide were demonstrable between days 5 and 7 and persisted until week 3 of infection, while IgG antibodies were detected on day 13, increased until day 30, and persisted for >40 days (data not shown).

Serum samples from infected mice were also investigated for the presence of group- and type-specific GBS antigens. While no evidence for these antigens was found by immunodiffusion or latex agglutination in untreated sera, both antigens were detected in sera pretreated with pronase. Group-specific antigen was revealed by latex agglutination as early as 24 h after infection and was still detectable on day 22. Type IV antigen gave a light but discrete band in immunodiffusion against specific rabbit antiserum from days 5 to 16 after infection. Normal mice sera were negative for both group- and type-specific antigens.

DISCUSSION

Type IV GBS is a new serotype recently identified on the basis of the polysaccharide antigen. The original type IV GBS reference strain (GBS 1/82) was isolated from a case of neonatal sepsis (28). Strains carrying type IV polysaccharide have since been isolated from carriers and from pathologic specimens (17, 18, 24, 26). In the present study, the three type IV GBS strains used were poorly pathogenic in terms of acute lethality for mice. LD_{50}s after i.v. challenge with stationary-phase cells ranged from 1.77×10^7 (GBS 1606) to 1.94×10^7 (GBS 1613); similar LD_{50}s were found in mice challenged with GBS cells taken from the log phase of growth. Thus, LD_{50}s for the three strains of type IV GBS are similar to those for type III GBS in mice infected by the intraperitoneal or i.v. route (27, 34) and higher than those described by Baltimore et al. and Fleming for the same serotype (6, 12). LD_{50}s lower than those obtained with the type IV GBS strains have been reported for type I (3, 34) and II (9, 14) GBS. Overall, these results seem to indicate that type IV GBS strains are less virulent for mice with respect to other GBS serotypes.

As seen in experimental GBS infections (23), the high LD_{50}s of type IV GBS in murine models are possibly correlated with the relatively low sialic acid content of type IV polysaccharide as well as to the thinness of capsular material present on the bacterial surface (23). However, in our model all type IV GBS strains produced a long-lasting infection in mice when a relatively high number of microorganisms (10^7 GBS) was inoculated by the i.v. route. The joints and kidneys appeared to be main targets for chronic disease. In particular, there was a progressive increase of renal CFU during the first 2 weeks after GBS 1/82 infection, and a high number of GBS microorganisms were still present in the kidneys for at least 30 days after challenge. This contrasts with the relatively rapid clearance of GBS 1/82 from all other organs examined.

The most characteristic pathological feature of type IV GBS chronic infection was the development of a diffuse joint disease with inflammatory lesions (arthritis). This severe and prolonged arthritis had a very short latency (24 to 48 h after

challenge), and only a small percentage of animals recovered. Experimental septic arthritis was previously obtained with a number of bacterial species when a sufficient inoculum was given (22), with streptococci of groups A and D, or with GBS in rats. In such cases, articular lesions were also reproduced by inoculation of inactivated cells or bacterial sonic extracts containing high doses of rhamnose (7, 32). Polyarthritis was also produced in mice by i.v. injection of the cell wall or peptidoglycan fraction of group A streptococci (20). In our experimental model, septic arthritis was induced only by live type IV GBS and not with inactivated cells or cell sonic extracts containing group- and type-specific polysaccharides.

During infection, microorganisms were always isolated from the affected joints and the sera of arthritic mice contained type- and group-specific antigens for about 3 weeks after infection. A large number of viable type IV GBS were necessary to establish a chronic infection in mice and overcome host resistance. The early clinical manifestations of arthritis, the presence of purulent exudate, the long-lasting infection of type IV GBS from the affected joints, and the failure to produce arthritis with inactivated or sonicated cells all suggest a tropism of live type IV GBS for articular tissue. However, the exact mechanisms by which live GBS cells promote the disease remain to be clarified. The determination of antibody and leukocyte responses during the course of infection showed that both type IV-specific IgM and IgG were produced but were unable to eradicate infection in the organs of animals until a marked leukocytosis, mainly involving polymorphonuclear leukocytes and monocytes, was reached on day 15. A crucial role of phagocytic cells, with or without opsonizing antibodies, has been claimed for GBS types Ia and III (3–5, 10, 33). Our observations that type IV GBS infection cannot be eliminated by the host during the first days after challenge when antibodies are not present and phagocytic cells have not yet reached their maximum number have been confirmed by experiments which demonstrated that hyperimmune rabbit antisera, administered 6 h before GBS infection, greatly reduced the incidence of arthritis; in fact, only 15% of hyperimmune serum-treated mice had arthritis versus 65% of untreated mice. Furthermore, hyperimmune anti-GBS sera decreased the number of GBS in the various organs (data not shown).

In conclusion, systemic infection of CD-1 mice with appropriate numbers of different strains of type IV GBS resulted in a chronic infection with an early development and high incidence of septic arthritis. This may represent an interesting finding considering that no experimental model is available for studies on experimental pathogenicity, chemotherapy, and immune mechanisms of host resistance to type IV GBS infection.

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