Endocarditis Caused by a Group B Streptococcus Strain, Type III, in a Nonencapsulated Phase

MATS SELLIN, MATS LINDERHOLM, MARI NORGREEN, AND STELLAN HÅKANSSON

Departments of Clinical Bacteriology, Infectious Diseases, and Pediatrics, University of Umeå, S-901 87 Umeå, Sweden

Received 4 March 1992/Accepted 30 May 1992

A nontypeable blood isolate of group B streptococci (GBS) from a patient with endocarditis is suggested to be the nonencapsulated phase of a GBS strain, type III. From the original high-density isolate, a low-density, encapsulated phase was selected by Percoll gradient centrifugation. This phenomenon should be considered before a GBS strain is classified as truly nontypeable.

Group B streptococci (GBS) are now well established as the predominant cause of neonatal septicemia and meningitis (1). Reports on invasive GBS disease in adults emphasize the opportunistic nature of infection. Several predisposing conditions are known such as diabetes mellitus, malignant diseases, and alcoholism (2, 6, 20). GBS have also been recognized as a cause of endocarditis (7, 16, 17).

The major virulence factor of GBS is the antiphagocytic polysaccharide capsule (10, 18). In vitro observations of a phenomenon which resembles phase variation of capsule expression have previously been published (9, 12). The present description of a case of endocarditis caused by a nontypeable GBS isolate with the potential for phase variation suggests that this phenomenon is relevant in vivo.

A 38-year-old, previously healthy woman was admitted to the hospital with a 6-week history of recurrent episodes of fever, chills, and arthralgias. On admission, the patient was febrile but in good general condition. A low-grade parasternal systolic murmur was noted. Laboratory findings included a moderate anemia of 104 g/liter (normal value [N], 115 g/liter), an elevated sedimentation rate (104 mm [N < 10 mm]), and an elevated C-reactive protein concentration (260 mg/liter [N < 10 mg/liter]). In four of four blood cultures, recovered on two consecutive days, GBS were isolated. A chest roentgenogram disclosed a slight, general enlargement of the heart, but no abnormalities in the lung. Apart from sinus tachycardia, electrocardiography was normal. Echocardiography revealed a moderate tricuspid insufficiency and a slight pericardial effusion (3 to 4 mm). No valvular vegetations were observed. Endocarditis was suspected, and the patient was treated for 4 weeks with intravenously administered penicillin G, initially combined with netilmicin. Treatment was successful, although the penicillin had to be changed to vancomycin for the last 10 days of treatment because of a probable allergic reaction. The patient did not have apparent predisposing factors for either GBS infection or endocarditis. Even though GBS infection more often occurs in patients with predisposing conditions, there are other reports of exceptions (16). Irrespective of possible ambiguities raised in the interpretation of the clinical picture, with persistent positive blood cultures and a new tricuspid valve insufficiency, the patient did fulfill clinical criteria for probable endocarditis (24). In addition, on follow-up 6 months later, echocardiography showed highly echogenic lesions on the tricuspid valve, suggesting valvular damage. No other focus for the GBS infection could be established.

The isolated GBS strain, subcultured once after primary recovery from the blood culture, was verified by CAMP reaction (3) and latex agglutination (Streptex; Wellcome Diagnostics, Täby, Sweden). The strain did not coagglutinate (11) with antibodies against type antigen Ia, Ib, II, III, IV, or V. Immunoprecipitation (14) with anti-type III antibodies proved negative. An overnight culture of the isolated strain in trypticase-yeast broth (13) was pelleted and resuspended in Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden). The suspension was applied to the bottom of a sterile hypotonic Percoll gradient with a cannula, through the bottom rubber stopper. After centrifugation in a swing-out rotor (4,000 × g, 4 h, 4°C), samples were aspirated from

FIG. 1. Double-immunodiffusion gel. The center well was filled with rabbit anti-type III serum. The outer wells were filled with hydrochloric acid extracts from the original strain (OS); the first selected subpopulation (1); the second selected subpopulation (2); and the third selected subpopulation (3).

* Corresponding author.
the top of the gradient and used for inoculation of TY broth and blood agar plates. This cycle was repeated three times. After each cycle, serotyping, buoyant density assessment (12), and estimation of relative sialic acid content (22) were performed. For immunoelectron microscopy, whole bacteria applied to Formvar-coated copper grids were incubated with rabbit anti-type III serum. Labeling with goat anti-rabbit immunoglobulin G-gold conjugate (Biocell Research Laboratories, Juniper Ultra Micro, Stockholm, Sweden) was performed according to the protocol of the manufacturer. For electron microscopy, a JEOL 100 B electron microscope was used.

After the first gradient centrifugation cycle, type III polysaccharide could already be identified by both coagglutination and immunoprecipitation (Fig. 1). The laser scanning pattern of the gradient-selected populations changed toward lower densities as the procedure was repeated (Fig. 2). In contrast, without gradient selection, the scanning pattern remained unchanged even after five passages of the original isolate in broth. The population isolated after three cycles produced a 10-fold-higher amount of sialic acid than the original isolate. In immunoelectron microscopy, a pronounced increase in immunogold labeling was noticed in the selected population (Fig. 3b). The buoyant density of the selected low-density population remained unchanged through subcultures in broth without gradient selection. This makes a rapid in vitro shift from an in vivo-encapsulated isolate less probable.

Published reports of invasive GBS infections in which strains have been serotyped (2, 5, 15, 16) reveal that the predominant number of isolates are typeable and thus encapsulated. The herein-described GBS isolate must by standard measures be regarded as nonencapsulated in the originally isolated state. However, the isolate did not lack the ability to express polysaccharide since an encapsulated phase of the strain could be recovered after Percoll gradient centrifugation. This ability to regulate capsule expression in a phase-shift-like manner may be important. Adhesion of GBS to epithelial cells seems to be inversely proportional to the degree of encapsulation (8), an observation also made with other encapsulated bacteria (4, 21, 23). Since bacterial adherence is an important factor in the pathogenesis of endocarditis (19), the ability to phase shift may be of particular interest in GBS endocarditis. However, further investigation is needed to elucidate this matter. The described phase-shift phenomenon should be considered before classifying a GBS strain as truly nontypeable.

This work was supported by grants from the Swedish Medical Research Council to S.H. (B-92-16X-08675-04), from the Research Foundation for Cooperation in the North of Sweden to M.N., and from the J. C. Kempe Foundation to M.S.

Leonor Johansson and Rolf Sjöberg are acknowledged for valuable assistance with the electron microscopy.

REFERENCES

Submitted for publication.


