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ScienceDirect
Microbes and Infection 8 (2006) 2376–2383
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Exacerbation of group B streptococcal sepsis and arthritis in diabetic mice

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Received 22 November 2005; accepted 12 April 2006
Available online 30 June 2006

Abstract

Group B streptococci (GBS) have been recognised as an ever-growing cause of serious invasive infections in non-pregnant adults, in particular in association with severe underlying diseases such as diabetes mellitus. In the present study we used mice rendered diabetic to gain further insights into host–pathogen interaction during induced GBS sepsis and septic arthritis. Type I diabetes was induced in adult CD-1 mice by low-dose streptozotocin treatment. Mice were then infected with different doses of GBS, and mortality, appearance of arthritis, growth of microorganisms in the organs and cytokine and chemokine profile were assessed in diabetic and control animals. The LD_{50} was significantly lower in diabetics than in controls, while both incidence and severity of arthritis were higher. A significantly higher number of microorganisms were recovered from the organs of diabetic mice than in controls. The worsening of sepsis and arthritis was associated with a significant increase in systemic and local production of IL-6, IL-1 β, TNF-α, IL-10, macrophage inflammatory protein 1 α (MIP-1α), and MIP-2 and with a decrease in IFN-γ production. Taken together, our results indicate an impaired host resistance to GBS infection in diabetics, likely due to a dysregulation of the cytokine network and prolonged local inflammatory response.

Keywords: Group B streptococci; Diabetes; Septic arthritis; Inflammation

1. Introduction

Group B streptococci (GBS) have long been known as a leading cause of life-threatening infection in neonates, young infants and pregnant women [1]. Recently these microorganisms have been recognised as an ever-growing cause of serious invasive infections in non-pregnant adults [2]. GBS are responsible for a wide spectrum of clinical manifestations. The most common entities include primary bacteraemia, 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 [3]. Diabetes mellitus is the most common comorbid condition, typically present in 20–25% of non-pregnant adults with GBS disease. Patients with diabetes mellitus show a higher predisposition to infections, which appears to be due to a combination of angiopathy, neuropathy and hyperglycemia [4]. Impaired host defence mechanisms such as impaired wound healing, impaired granulocyte functions, decreased cellular immunity and impaired complement functions may be influenced by glycemic control [5]. However, many questions remain, and additional studies are needed to define other immune defects that predispose diabetics to GBS infection.

Abbreviations: GBS, group B streptococci; STZ, streptozotocin; CFU, colony-forming units.

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doi:10.1016/j.micinf.2006.04.021

Septic GBS arthritis in non-pregnant adults was considered extremely rare until the early 1980s, when two independent studies stressed that its incidence seemed to be increasing [6,7]. This trend has been confirmed by recent studies in which GBS account for 7 to 10% of all diagnosed cases of bacterial arthritis [8,9]. Interestingly, GBS were typically associated with involvement of multiple joints in patients with diabetes mellitus or cancer [8].

We have previously described an experimental mouse model of GBS infection with clinical features that closely resemble infection in humans [10]. Mice given a single intravenous dose of GBS develop clinical signs of arthritis within 48 h. Appearance and severity of GBS arthritis are the by-product of a multifactorial process. Viability and number of microorganisms injected and bacterial factors (i.e. presence and amount of capsule, amount of sialic acid in the capsular polysaccharide, β-haemolysin production) have been shown to influence the development of articular lesions [11,12]. Nevertheless, a crucial role in the pathogenesis of GBS arthritis is played by inflammatory cells (granulocytes and monocytes) that reach the joints [13] and by the production of proinflammatory cytokines, including IL-6, IL-1β and TNF-α [14].

In the present study, mice rendered diabetic by low-dose streptozotocin (STZ) administration were used for a better understanding of how hyperglycemia may alter host–pathogen interaction during GBS-induced sepsis and arthritis.

2. Materials and methods

2.1. Mice

Adult outbred male CD-1 mice were obtained from Charles River Breeding Laboratories (Calco, Italy). The animals were 6–8 weeks of age at the beginning of each experiment.

2.2. Induction of diabetes mellitus

Type I diabetes was induced by low-dose STZ (Sigma Chemical Co., St. Louis, Mo) injection as detailed by the NIDDK Consortium for Animal Models for Diabetic Complications' (AMDCC) protocol. Briefly, STZ was dissolved in 0.01 citrate buffer (pH 4.5) immediately before use and injected intraperitoneally for 5 consecutive days at a dose of 50 mg/kg. Mice were fasted for at least 4 h before injection. Control mice received the same volume of STZ-diluent buffer alone. Plasma glucose levels were measured by Ascensia ELITE™ Blood Glucose Meter system (Bayer, Bayer Corporation, Mishawaka, IN, USA). Mice showing serum glucose levels greater than 350 mg/dl 3 weeks after the last STZ treatment were considered diabetic and used for the study. The permanence of hyperglycemia was monitored periodically during experiments.

2.3. Microorganisms

Type IV GBS, reference strain NCTC 1/82 (ATCC 49446), originally isolated from a newborn sepsis case, was used throughout the study. Microorganisms were grown overnight at 37 °C in Todd-Hewitt broth (Oxoid Ltd., Basingstoke, England), 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), and overnight incubation at 37 °C under anaerobic conditions. Mice were inoculated intravenously via the tail vein with different infecting 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.

2.4. Clinical evaluation of arthritis and mortality

GBS-infected mice were evaluated for signs of arthritis and mortality. Mortality was recorded at 24-h intervals for 30 days. The 50% lethal dose (LD_{50}) was calculated by the method of Reed and Muench [15]. After challenge, mice were examined daily by two independent observers (L.T., M.P.) for 1 month to evaluate the presence of joint inflammation, and scores for arthritis severity (macroscopic score) were given as previously described [13]. Arthritis was defined as visible erythema and/or swelling of at least one joint. Clinical severity of arthritis was graded on a scale of 0–3 for each paw, according to changes in erythema and swelling (0 = no change; 1 point = mild swelling and/or erythema; 2 points = moderate swelling and erythema; 3 points = marked swelling, erythema, and/or ankylosis). Thus, a mouse could have a maximum score of 12. The arthritis index (mean ± SD) was constructed by dividing the total score (cumulative value of all paws) by the number of animals used in each experimental group.

2.5. Histological assessment

Groups of mice infected with GBS were examined 5 days after infection for histopathological features of arthritis. Arthritic paws (1 per mouse) were removed aseptically, fixed in formalin 10% v/v for 24 h and then decalcified in trichloroacetic acid 5% v/v for 7 days, dehydrated, embedded in paraffin, sectioned at 3–4 μm and stained with haematoxylin and eosin. Samples were examined under blinded conditions. Joints (3 per paw) were examined for synovitis (defined as synovial membrane thickness of >2 cell layers), 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 (mild synovial hypertrophy, minimal infiltrate), moderate (moderate synovial hypertrophy, presence of infiltrate, minimal exudate, integrity of joint architecture), and severe (marked synovial hypertrophy, presence of massive infiltrate/exudate, cartilage and bone erosion, and disrupted joint architecture).

2.6. GBS growth in blood, kidneys and joints

Blood, kidney and joint infections in GBS-infected mice were determined by evaluation of colony-forming-units