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Role of bacterial DNA in macrophage activation by group B streptococci☆

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Abstract

Bacterial DNA (CpG DNA) induces macrophage activation and the production of inflammatory mediators, including tumor necrosis factor (TNF) and nitric oxide (NO) by these cells. However, the role of bacterial DNA in the macrophage response to whole bacteria is unknown. We used overlapping strategies to estimate the relative contribution of bacterial DNA to the upregulation of TNF and NO production in macrophages stimulated with antibiotic-treated group B streptococci (GBS). Selective inhibitors of the bacterial DNA/TLR9 pathway (chloroquine, an inhibitory oligonucleotide, and DNase I) consistently inhibited GBS-induced TNF secretion by 35–50% in RAW 264.7 macrophages and murine splenic macrophages, but had no effect on inducible nitric oxide synthase (iNOS) accumulation or NO secretion. Similarly, splenic and peritoneal macrophages from mice lacking TLR9 expression secreted 40% less TNF than macrophages from control mice after GBS challenge but accumulated comparable amounts of iNOS protein. Finally, studies in both RAW 264.7 cells and macrophages from TLR9−/− mice implicated GBS DNA in the upregulation of interleukins 6 (IL-6) and 12 (IL-12) but not interferon-beta (IFNβ), a key intermediary in macrophage production of iNOS/NO. Our data suggest that the bacterial DNA/TLR9 pathway plays an important role in stimulating TNF rather than NO production in macrophages exposed to antibiotic-treated GBS, and that TLR9-independent upregulation of IFNβ production by whole GBS may account for this difference.

Keywords: Bacterial; Macrophages; Cytokines; Nitric oxide; Toll-like receptor

1. Introduction

The group B streptococcus (GBS) is an important cause of neonatal sepsis and meningitis [1]. The host inflammatory responses to sepsis caused by Gram-positive and Gram-negative bacteria are similar but not identical [2]. Extensive studies of one highly pro-inflammatory component of Gram-negative bacteria, lipopolysaccharide (LPS), have resulted in important breakthroughs in our understanding of innate immunity, including the discovery of the key role of toll-like receptors (TLRs) [3]. However, the long search for a Gram-positive bacterial equivalent of LPS has been largely unsuccessful. Instead, it appears that multiple components of Gram-positive bacteria are capable of being recognized by TLRs [3] and other innate immune receptors, triggering the production of host inflammatory mediators, including tumor necrosis factor (TNF) and, via the inducible nitric oxide synthase (iNOS), nitric oxide (NO).

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The systemic signs and symptoms related to GBS sepsis are primarily mediated by TNF, NO and other host inflammatory mediators produced by macrophages [4–6]. This dysregulated host inflammatory response may lead to multi-organ dysfunction and death. Others and we have previously shown that GBS potently induces the secretion of TNF and production of iNOS/NO by macrophages [4,6,7]. Many GBS components have been reported to stimulate macrophage activation, including lipoteichoic acid (LTA), peptidoglycan, capsular polysaccharide, beta-hemolysin, and at least one soluble secreted or released factor [4,8–10]. However, the contribution of bacterial DNA in triggering the host immune response to whole GBS has not been examined.

TLR4 recognizes LPS from Gram-negative bacteria, while TLR2 plays a major role in recognizing LTA and other components of the cell walls of many Gram-positive bacteria [3,11]. Interestingly, recognition of GBS by the innate immune system appears to be more complicated than the recognition of many other Gram-positive pathogens. Golenbock, Teti and colleagues [8,12–14] have reported that TLR2 is involved in the macrophage cytokine response to extracellular components of GBS but not in the response to whole bacteria or to GBS cell wall preparations. On the other hand, these investigators have found that MyD88 (a key adaptor protein involved in signaling downstream from all TLRs except TLR3) is required for all macrophage cytokine responses to GBS [5]. These findings suggest that TLRs other than TLR2 play a relatively greater role in macrophage responses to GBS (compared with other Gram-positive bacteria).

Certain bacterial DNA sequences (unmethylated CpG dinucleotides in particular sequence contexts) are recognized as foreign by macrophages and other innate immune cells and are potent stimuli for macrophage production of TNF and iNOS/NO [15]. CpG DNA is endocytosed by macrophages and then recognized by endosomal TLR9, triggering a signal transduction pathway that requires MyD88 and results in the production of key inflammatory mediators. Selective inhibitors of this bacterial DNA/TLR9 signaling pathway include: (a) chloroquine (which blocks the endosomal acidification step that is required for TLR9 mediated signaling but not that for TLR2 and TLR4) [16]; (b) an inhibitory oligonucleotide, iCpG DNA, that acts as a competitive inhibitor of CpG DNA [17]; and (c) DNase I, which degrades bacterial DNA without affecting other bacterial components [18].

CpG DNA is the subject of intense study, but the relative contribution of bacterial DNA to the macrophage inflammatory response to whole bacteria is not known. In this study, we sought to determine whether bacterial DNA plays a significant role in the upregulation of macrophage TNF and NO production in response to challenge with whole, antibiotic-treated GBS. We also examined the role of bacterial DNA in the induction of macrophage interleukin 6 (IL-6), interleukin 12 (IL-12) and interferon-beta (IFNβ) production by whole GBS.

2. Experimental methods

2.1. Bacteria

A previously studied [7], GBS type 1a strain, initially isolated from the bloodstream of a neonate with sepsis was employed. Bacteria were routinely sub-cultured in TSB and late log phase bacteria were used after washing three times with PBS.

2.2. Reagents

Chloroquine phosphate and recombinant murine interferon-gamma (rIFNγ) were purchased from Sigma (St. Louis, MO). DNase I (turbo DNase) was obtained from Ambion (Austin, TX). Dulbecco's modified Eagle's Medium (DMEM) was purchased from Mediatech Inc. (Herndon, VA). Penicillin/streptomycin and L-glutamine were obtained from Lifetechnology (Grand Island, NY). Fetal bovine serum (FBS) was purchased from HyClone Laboratories (Logan, UT). Ultrapure LPS from *Escherichia coli* strain O111:B4 was purchased from List Biological Laboratories (Campbell, CA). Nuclease-resistant phosphorothioate oligodeoxynucleotides (S-ODN) were purchased from Operon (Valencia, CA): 5′-TCCATGA CGTTCCTGACGTT-3′ (CpG DNA: ODN 1826) and 5′-TCCTGGCGGGGAAGT-3′ (iCpG DNA: ODN 2088).

2.3. Macrophages

RAW 264.7 murine macrophages were obtained from ATCC and routinely cultured in DMEM supplemented with 10% heat-inactivated FBS and 2 mM L-glutamine. For studies of NO production, DMEM without phenol red was used.

2.4. Mice

BALB/cAnNCr mice at 4–5 weeks of age were obtained from The Frederick Cancer Research and Development Center, National Cancer Institute (Frederick, MD) and used within 3 weeks. TLR9 gene deficient mice in BALB/c background (TLR9−/−) were provided by Dr. A. Marshak-Rothstein (Boston Univ., Boston, MA) with permission of Dr. S. Akira (Osaka University, Osaka, Japan). MyD88 gene deficient mice (MyD88−/−) were provided by Dr. S.-C. Hong (Indiana Univ., Indianapolis, IN) with permission of Dr. S. Akira (Osaka University). All animal care and housing requirements set forth by the National Institutes of Health Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources were followed, and animal protocols were reviewed and approved by the University of Tennessee Animal Care and Use Committee.

2.5. Preparation of plastic-adherent splenocytes (splenic macrophages) and peritoneal cells (peritoneal macrophages)

Splenic and peritoneal macrophages were isolated from wild-type, MyD88−/−, or TLR9−/− mice. The mice were