



Group B Streptococci Induce Interleukin 8 Production in Human Cervical Epithelial Cell Cultures: The Role of Capsule Polysaccharide

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ABSTRACT

Objective: Group B streptococci (GBS) are the major cause of pneumonia, sepsis, and meningitis in neonates and adults. Epithelial invasion and early cytokine response of female genital tract considered to be important in the pathogenesis of GBS infection. In this study, we studied the IL-8 induction in cervical epithelial cells in response to stimulus with encapsulated (COH1) and unencapsulated (COH1-13) strains of group B streptococci.

Methods: Human cervical epithelial cancer cell (HeLa) cultures were stimulated with different concentrations (10^6 CFU/ml and 10^8 CFU/ml) of two GBS strains. *E.coli* LPS was used as positive control and at specified time points (4, 8 and 24 hour) cell culture supernatant samples were collected. IL-8 level in samples was quantified by using ELISA assay.

Results: Both GBS strains caused an equal IL-8 response in HeLa cells in a time-dependent manner. In addition, cytokine levels triggered by different bacterial concentrations were similar and comparable with LPS.

Conclusion: Our study showed that GBS induce proinflammatory IL-8 levels in cervix epithelial cells. This induction seems to be independent from capsule polysaccharides and suggesting that other bacterial components are involved in IL-8 stimulation.

Keywords: Streptococcus Group B, bacterial capsule, epithelial cells, IL-8

1. INTRODUCTION

Group B Streptococci (GBS) or *Streptococcus agalactiae* are among the members of the microbiota residing in the human gastrointestinal, respiratory and genitourinary systems (1). This pathogen is an important cause of pneumonia, sepsis, and meningitis of newborns and also associated with invasive infections in pregnancy, immunocompromised people and elderly population (2).

Since the GBS infections in babies as well as in mothers in the postnatal period express high morbidity and mortality, GBS colonization in female genital tract attract remarkable scientific interest. There are numerous studies investigating the colonization rates of this pathogen in child-bearing age. Vaginal and/or rectal carriage rates of GBS vary between 15-35% in pregnant women globally (3). GBS colonization rates were found between 1-16% in Turkey based studies and differences in detection rates mainly related with methodological approaches (4). Several strategies are suggested to eradicate the pathogen in antenatal period to prevent the neonatal infections.

GBS produce several virulence factors that contribute the bacterial invasiveness and defense. A group of GBS virulence factors are well defined such as fibrinogen-binding proteins,

β -hemolysin/cytolysin, and polysaccharide capsule which have a distinct role in host cell adhesion, cellular invasion and immune evasion, respectively (5).

Most of the clinical GBS isolates carry a polysaccharide capsule demonstrating unique structure with sialic acid residues. Sialic acid renders the bacterial cell wall to mimic the mammalian cell surface expressing the glycans from the same family. So, the host immune system can not recognize the encapsulated GBS as an invader (6). Several studies are used the encapsulated and unencapsulated GBS strains to investigate the effects of capsule production on the pathogenesis of GBS infection (7).

Colonization of the female genital tract is the first step of GBS related diseases. After bacterial adhesion to epithelium; there are several pathways triggering the host immune response and the production of the bacterial virulence factors (8). From the host sight, PAMPs (Pathogen-Associated Molecular Patterns) from pathogenic bacteria interact with PRRs (Pattern Recognition Receptors) on the host cell to induce the production of proinflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-alpha), interleukin-1 β (IL-1 β), IL-6 and IL-8 as a triggering step of inflammation-infection cascade (9).

IL-8 is a member of CXC or alpha-chemokine family and secreted from monocytes, macrophages, and also from several non-immunological cells like fibroblasts, endothelial and epithelial cells (10). IL-8 has powerful chemoattractant capacity for neutrophils and some T lymphocyte subsets, and induces neutrophil activation and degranulation (11).

In this study, we focused on GBS interaction with cervix epithelial cells and investigated the effects of capsule production and the inoculum size on IL-8 response of epithelial cells.

2. METHODS

2.1. Bacterial strains

We used two GBS strains in this study, encapsulated one (COH1) and unencapsulated isogenic mutant of parent strain (COH1-13). COH1-13 strain was derived by transposon insertion mutagenesis (7). These strains were kindly provided by Dr. C.E. Rubens (University of Washington, Seattle, WA, USA).

2.2. Cell culture

The HeLa cell line was derived from cervical cancer cells and has been used for cytotoxicity and cytokine release experiments. Cells were cultured in minimal essential medium (MEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibco, Invitrogen, Carlsbad, CA, USA), 2 mM L-glutamine (Invitrogen) and 1% antibiotic and antimycotic solution (Invitrogen) at 37°C with 5% CO₂. Cell viability was determined by 0.4% trypan blue staining and counting Thoma chamber. Before stimulation experiments, HeLa cells suspended in 0.5 mL of culture medium with 10% fetal calf serum were seeded into 6-well culture plates (Greiner Bio-One, Kremsmuenster, Austria) and incubated for 2 days at 37°C with 5% CO₂ to obtain monolayers. Nonadherent cells were removed by washing with MEM twice.

2.3. Bacterial inoculum preparation

COH1 and COH1-13 strains were grown in 20 mL fresh Todd-Hewitt broth (Oxoid, London, England) at 37°C overnight. Bacterial cultures were washed twice with sterile phosphate – buffered saline (PBS, pH 7.4) and suspended in PBS at cell density of 3.0x10⁹ CFU/mL by using spectrometry at 600 nm wavelength. Bacteria were heat killed by incubating the organisms at 56°C for 1 h, and samples subcultured on blood agar plates and incubated at 37°C for 48 h to ensure the killing process. Aliquots were stored at – 70°C for later use in the experiments.

2.4. Epithelial cell stimulation

HeLa cell monolayers were stimulated with heat killed COH1 and COH1-13 GBS strains at 10⁶ CFU/mL and 10⁸ CFU/mL cell densities. HeLa cells with medium alone served as negative control. Purified *Escherichia coli* 055:B5 LPS (Sigma-Aldrich, St. Louis, MO, USA) at 100 ng/mL final concentration was

added to wells as positive control. Culture supernatants were collected from study and control wells at selected incubation periods (4, 8, and 24 h), centrifuged, and stored at –20°C until IL-8 determination assay.

2.5. Determination of IL 8 levels

IL-8 levels secreted into the culture supernatants were detected with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) by following the manufacturer's instructions. This kit had 7.5 pg/mL sensitivity and assay range between 31.2-2000 pg/mL for human interleukin-8. All measurements were performed in duplicate.

2.6. Statistical analysis

IL-8 values are expressed as mean ± standard deviation of values. Differences between groups were analyzed with Microsoft Excel 2013 software (Microsoft, Redmond, WA, USA) for significance by using Student's unpaired t test (two-tailed P value). P value of 0.05 was considered to indicate statistical significance.

3. RESULTS

3.1. HeLa cells are stimulated by GBS to secrete IL-8

The induction of IL-8 production by cervix epithelial cells (HeLa) was evaluated after stimulation with heat-killed (56°C for 30 min) GBS strains. There was no difference in IL-8 production by HeLa cells exposed to the encapsulated (COH1, 10⁸ CFU/mL) or the isogenic unencapsulated mutant strain (COH1-13, 10⁸ CFU/mL) (mean IL-8 levels at 24 h, 440.9 vs 473.3 pg/mL, respectively; P>0.05). Cell culture supernatant from medium alone was used as negative control, and purified *E. coli* LPS (100 ng/mL) was used as positive control. Both strains were yielded responses similar to LPS in inducing IL-8 secretion (mean 364.7 pg/mL; P>0.05) and caused significantly higher cytokine production compared to negative control (72.9 pg/mL; P<0.05) (Figure 1).

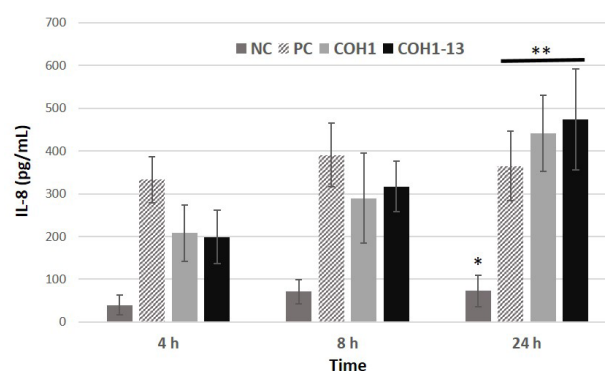


Figure 1. IL-8 production measured by ELISA in the supernatant of HeLa cells incubated with heat killed encapsulated (COH1) or unencapsulated (COH1-13) GBS (10⁸ cfu/mL). Media alone (unstimulated) was used as negative control (NC) and LPS from *E. coli* served as positive control (PC). Data are duplicate assays for each experiment (*: P<0.05; **: P>0.05).

3.2. Time course of epithelial cell IL-8 production in response to GBS

For the determination of the changes in IL-8 secretion after GBS stimulation, HeLa cells were incubated for up to 24 h with both GBS strains. The IL-8 level was significantly increased within 4 h (mean 223.2 pg/mL with COH1 and 222.8 pg/mL with COH1-13), and its concentration increased steadily over time. At 24 h, the mean IL-8 level was 4.4 and 5.0 fold greater than negative control for COH1 and COH1-13 strains, respectively (Figure 2).

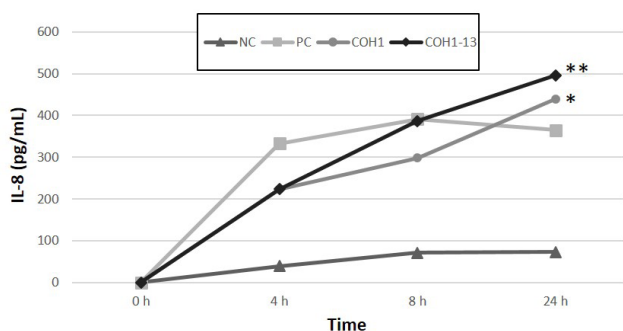


Figure 2. Time curves of IL-8 responses by HeLa cells after stimulation with encapsulated (COH1) or unencapsulated (COH1-13) GBS and with LPS (100 ng/mL) from *E. coli*. Media alone (unstimulated) was used as negative control (NC) and LPS from *E. coli* served as positive control (PC). Data are duplicate assays for each experiment (*: 4.4 fold increase versus NC; **: 5.0 fold increase versus NC).

3.3. Bacterial concentration independent IL-8 release

Both tested bacterial cell densities (10^6 and 10^8 CFU/mL) were equivalent in inducing IL-8 production for both GBS strains used in this study (mean 478 pg/mL and 457 pg/mL; $P > 0.05$). These data suggest that bacterial factors capable of causing IL-8 secretion are sufficient in both concentrations and were similar with that of LPS (100 ng/mL) (Figure 3).

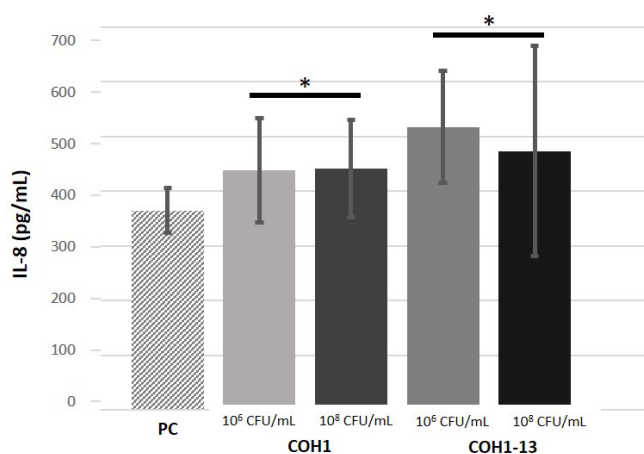


Figure 3. Dose-response effects of different concentrations (10^6 CFU/mL and 10^8 CFU/mL) of GBS strains on IL-8 secretion by HeLa cells. IL-8 was measured after 24 h of incubation at 37°C by ELISA assay (*: $P > 0.05$).

4. DISCUSSION

In this study, we demonstrated heat-killed Group B streptococci induced IL-8 secretion in human cervical epithelial cells *in vitro*. In addition, presence of bacterial capsule was not affected the induced IL-8 production (Figure 1). Furthermore, bacterial inoculum size was not related with the secreted IL-8 level from epithelial cells.

In general, capsule formation is considered a major virulence factor of GBS. The importance of the capsular polysaccharides in GBS virulence is maintained by the effect of protective type-specific anti-capsule antibodies and by the capability of encapsulated GBS to inhibit complement related opsonization, phagocytosis, and intracellular killing by human peripheral blood polymorphonuclear leukocytes (12). However, GBS with polysaccharide capsule (COH1) could induce IL-8 secretion in similar levels with the non-encapsulated isogenic strain (COH1-13). Concordant with our results, other studies have found similar findings for production of proinflammatory cytokines IL-6 and TNF- α from human monocytes using the same encapsulated and unencapsulated GBS strains used in our study (13, 14).

GBS induced cytokine release searching studies were also investigated the role of certain bacterial components, including capsular polysaccharide in triggering the cytokine response. Vallejo et al. studied the effects of whole cell GBS and also purified capsular polysaccharide, lipoteichoic acid and peptidoglycan isolated from of GBS cell wall, and group B polysaccharide on TNF- α release from neonatal monocytes. Their findings were highlighted significant TNF- α release with the induction by the group B polysaccharide or peptidoglycan. Additionally, they suggested any of the GBS components might have a synergistic role in the induction of cytokine release during infectious process (15).

Early cytokine responses have important roles in the host response to infection that is targeted for eradicating bacteria from the invaded body sites. IL-8 is a member of the chemokine family containing cytokines those have primary roles in attracting and activating immune effector cells such as neutrophils and macrophages. It plays a significant role in recruiting leukocytes at sites of acute inflammation (10). In this context, there is an increased level of IL-8 along with other proinflammatory cytokines such as TNF- α , IL-1 and IL-6 in the acute phase of GBS infection (16). On the other hand, there are some limitations of our study, since differentiated epithelial cells can show changed or unexpressed physiological cellular functions, extrapolation of the study results to physiological state may be limited. Over and above, our study does not allow us to establish the relationship between the IL-8 production and the responsible GBS components. Future studies aimed at defining the role of cellular factors of GBS in IL-8 response of epithelial cells would thus be of interest.

5.CONCLUSION

Our data suggest that GBS induced IL-8 release by cervical epithelial cells may be the initial trigger for the acute inflammatory reaction after bacterial invasion of mucosal tissue. Additionally, IL-8 production is not required that alive bacterial cells adhere or invade cervical epithelial cells. Moreover, the two strains of GBS examined here are also able to induce IL-8 by what appears to be a similar mechanism rather than capsule formation.

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