Defective Production of IL-18 and IL-12 by Cord Blood Mononuclear Cells Influences the T Helper-1 Interferon Gamma Response to Group B Streptococci

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ABSTRACT

Human neonates are uniquely susceptible to group B streptococcal (GBS) infections. We have shown that neonatal mixed mononuclear cells have a deficiency in the production of the T helper-1 (Th-1) cytokine, interferon gamma (IFN- γ), and that incubation of neonatal neutrophils with recombinant IFN- γ corrects these neutrophil defects. IL-12 and the more recently described IL-18 are also Th-1 type cytokines that are able to induce the production of IFN- γ in the presence of bacteria and bacterial products. We examine the ability of GBS to induce the production of IFN- γ , IL-18, and IL-12 by cord blood mixed mononuclear cells and compared these results with the IFN- γ , IL-18, and IL-12 response of mixed mononuclear cells from adult blood. We demonstrate that cord blood mixed mononuclear cells produced significantly less IFN- γ , IL-18, and IL-12 in response to GBS compared with mixed mononuclear cells from adults. Cord blood mixed mononuclear cells' production of

IFN- γ is enhanced by added recombinant IL-18 and IL-12. The maximal cord blood cell production of IFN- γ , in response to GBS, is achieved by priming the cells with both IL-18 and IL-12. We conclude that neonatal mixed mononuclear cells exhibit deficiencies in three main Th-1 type cytokine responses, IFN- γ , IL-12, and IL-18. This combined Th-1 type cytokine deficiency may contribute to the enhanced susceptibility of the human neonate to GBS and other microbial infections. (*Pediatr Res* 54: 276–281, 2003)

Abbreviations

GBS, group B streptococci
Th-1, T helper-1
IFN- γ , interferon gamma
PMNL, polymorphonuclear leukocytes

IL-18 is a recently described member of the IL-1 cytokine family, which was initially defined as IFN- γ -inducing factor (1). IL-18 gene expression and/or protein secretion has been observed in macrophages (2), dendritic cells (3), mononuclear cells (4), keratinocytes (5), chondrocytes (6), pituitary and adrenal cells (7), astrocytes and microglia (8), and intestinal epithelial cells (9). Studies have elucidated a broad array of effector functions implicating IL-18 as an important regulator of both innate and acquired immune responses (10, 11). In animals, IL-18 contributes to protective immunity against a

variety of pathogens, including *Cryptococcus*, *Leishmania*, *Staphylococcus*, *Salmonella*, and *Mycobacteria* (12–15).

IL-12 is also an integral immune regulator, which promotes Th-1 responses while suppressing Th-2 responses (16, 17). IL-12 is primarily produced by macrophages and dendritic cells and has been shown to induce the production of IFN- γ by T cells and natural killer (NK) cells (18). Recent studies have focused on the interaction between IL-18 and IL-12 in certain inflammatory responses. In Th-1 immune responses, IL-18 and IL-12 are important cytokines that may synergistically stimulate IFN- γ production and enhance NK and T cell-mediated cytotoxicity (19). Recent studies implicate the interaction of these Th-1 cytokines in the development of autoimmune diseases and suggest that regulating their function may be therapeutically beneficial (20, 21).

Early-onset GBS infections in neonates often lead to sepsis and severe septic shock, with an approximate 5–15% mortality rate (22–24). Abnormalities observed in the neonatal immune

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system, likely predisposing neonates to GBS infection include type-specific opsonic antibody deficiency (25) and defective PMNL chemotaxis (26). The depressed production of the Th-1 cytokine, IFN- γ , by human neonates probably contributes to their increased susceptibility to bacterial infections (27–29). We have also shown that recombinant human interferon gamma (rIFN- γ) can correct these defects and enhance the activation and movement of neonatal PMNL (30). In this study, we examine the ability of cord and adult blood mixed mononuclear cells to produce the Th-1 cytokines IFN- γ and IL-18 in response to GBS. We also describe the effect of IL-18 and IL-12 on GBS-stimulated production of IFN- γ with and without MAb to IL-18 or IL-12 to determine their combined influence, if any, on the IFN- γ response to GBS.

MATERIALS AND METHODS

Leukocyte preparation. Umbilical cord blood, obtained after normal term deliveries, and whole blood obtained from healthy adult volunteers was collected in tubes containing acid citrate dextrose solution A (Becton Dickinson, Franklin Lakes, NJ, U.S.A.), and processed within 18 h of delivery. Blood was diluted with Hanks balanced salt solution (1 vol/2 vol blood) (HBSS; BioWhittaker, Walkersville, MD, U.S.A.), and mixed mononuclear cells were separated by density gradient centrifugation on Ficoll-Paque Plus separation media (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.). The mononuclear cell layer was removed, washed twice with HBSS, and adjusted to a concentration of $1.0 \times 10^6/\text{mL}$ in RPMI 1640 medium with 2 mM L-glutamine, 0.35 mM HEPES buffer, 0.05 mg/mL gentamicin (all from BioWhittaker), and 10% heat-inactivated normal human serum (North American Biologic Institute, Miami, FL, U.S.A.). Cells were incubated at 37°C with added 5% CO₂ with IL-12 and IL-18 (R & D Systems, Minneapolis, MN,

U.S.A.) both individually and combined for 24, 48, and 120 h with GBS. In the priming studies, the mixed mononuclear cells were preincubated with IL-12 and/or IL-18 for 30 min at 37°C. GBS was then added along with IL-12 and/or IL-18 for an additional 24 h.

Bacterial strains. COH-1, an encapsulated type III GBS strain, isolated from a patient with GBS infection (provided by Craig Rubens, University of Washington, Seattle, WA, U.S.A.) was grown overnight in Todd Hewitt broth (Difco, Detroit, MI, U.S.A.) at 37°C. Bacteria were washed with PBS and the concentration was adjusted to an OD of 0.9 at 620 nm wavelength ($\sim 5.0 \times 10^8$ cfu/mL). Aliquots of bacteria were heat inactivated at 56°C for 1 h and stored at -70°C .

ELISA

The tissue culture supernatants were assayed for IFN- γ , IL-12, and IL-18 protein by ELISA (Immunotech, Westbrook, ME, U.S.A.). Duplicate determinations were done in each assay. Data were analyzed by unpaired *t* test.

Institutional review board approval was obtained for the proposed studies and appropriate informed consent was obtained.

RESULTS

GBS in concentrations of 1×10^6 to $1 \times 10^7/\text{mL}$ induced IL-18 production by cord blood mixed mononuclear cells and adult blood cells after 24 h incubation (Fig. 1). This response peaked at a GBS concentration of $1 \times 10^6/\text{mL}$. At this concentration, there was a significant difference in IL-18 production between cord and adult blood mixed mononuclear cells ($p < 0.007$). The time course of appearance of IL-18 after exposure of mixed mononuclear cells from cord and adult blood to

Figure 1. Dose response effect of GBS on adult and cord blood mixed mononuclear cell production of IL-18 after 24 h incubation. Significant differences in IL-18 production between cord and adult blood cell preparations are observed at GBS concentrations of 1×10^5 cfu/mL and 1×10^6 cfu/mL ($p < 0.07$).

Figure 2. Time course production of IL-18 by cord and adult blood mixed mononuclear cells in response to GBS ($10^6/\text{mL}$). Significant differences between cord and adult mixed mononuclear cell production of IL-18 in response to GBS are observed at 18, 24, and 48 h ($p < 0.03$).

$1 \times 10^6/\text{mL}$ GBS is shown in Figure 2. The response was detectable after 4 h and reached a peak at 24 h, declining somewhat thereafter, especially in cord blood mixed mononuclear cells (Fig. 2). There was a statistically significant difference between IL-18 production by cord and adult blood mixed mononuclear cells observed at 18, 24, and 48 h ($p < 0.03$). All additional experiments were carried out after 24 h incubation of the mononuclear cells with GBS with, and without, added cytokines.

Examining the effects of IL-18 (100 ng/mL) with and without anti-IL-18 MAb on the IFN- γ response to GBS with mixed mononuclear cells from adult blood, we found that IL-18 (100 ng/mL) alone had no effect on mixed mononuclear cell production of IFN- γ at 24 h (Fig. 3). It did, however, significantly

increase IFN- γ production by mixed mononuclear cells in response to GBS ($p < 0.01$). The addition of anti-IL-18 MAb completely blocked the added IL-18 enhanced response to GBS and actually lowered the total IFN- γ production below that seen with GBS alone. This suggests that the antibody may be blocking an effect of endogenous IL-18 production as well as the added IL-18. Addition of anti-IL-18 antibody alone had no effect on mononuclear cell production of IFN- γ (data not shown).

We next determined the effect of anti-IL-12 MAb on the IFN- γ response of adult mixed mononuclear cells to GBS because IL-18 and IL-12 may act in concert, or synergistically, to enhance IFN- γ production. This antibody alone had no effect on mononuclear cell production of IFN- γ (data not shown).

Figure 3. The effects of IL-18 (100 ng/mL) with and without anti-IL-18 MAb (1.0 $\mu\text{g}/\text{mL}$) on IFN- γ production after 24 h incubation with GBS ($10^6/\text{mL}$). IL-18 alone has no effect on IFN- γ production but significantly increases IFN- γ production in response to GBS. The addition of anti-IL-18 MAb (1.0 $\mu\text{g}/\text{mL}$) completely blocks the IL-18 response to GBS ($n = 9$). No effect on IFN- γ production was observed with anti-IL-18 antibody alone (data not shown).

The addition of anti-IL-12 depressed the IFN- γ response to IL-18 and GBS (Fig. 4). IL-12 has been reported to have an effect to up-regulate IL-18 receptors on mixed mononuclear cells so that endogenous concentrations of this cytokine are necessary to have an optimal IL-18 response (31). It is likely that the anti-IL-12 may have blocked endogenous IL-12 production by mixed mononuclear cells in response to GBS in this experiment, thereby lowering the response to the added IL-18. Similarly, we examined the effect of anti-IL-18 antibody on IFN- γ production by IL-12. The addition of anti-IL-18 antibody suppressed the GBS, IL-12, IFN- γ response, but the decrease was not statistically significant ($p = 0.06$; Fig. 5).

Figure 6 illustrates the effects of IL-18 and IL-12 alone on IFN- γ production by mixed mononuclear cells obtained from cord blood in response to GBS. As we have previously reported, the IFN- γ response to GBS is quite low by cord blood mixed mononuclear cells. Addition of both recombinant IL-12 (1.0 U/mL) and IL-18 (100 ng/mL) significantly increased the response to GBS ($p < 0.0001$).

Because IL-18 and IL-12 individually can enhance IFN- γ production by cord blood mixed mononuclear cells in response to GBS, we examined the combined effects of both cytokines together followed by priming of cord blood mixed mononuclear cells with either IL-18 and/or IL-12. Samples were primed for 30 min by incubation with either IL-18 and/or IL-12 before GBS exposure and then stimulated for 24 h with the organisms. As shown (Fig. 6), addition of either IL-12 and/or IL-18 to GBS-stimulated cord blood mixed mononuclear cell cultures enhanced IFN- γ production significantly above that of GBS-stimulated cells alone ($p < 0.0001$). Priming with either IL-18 or IL-12 alone 30 min before GBS exposure with added IL-18 or IL-12 does not induce IFN- γ production above that of unprimed cord blood mixed mononuclear cells exposed to both

cytokines simultaneously. In contrast, priming mixed mononuclear cells from cord blood with IL-18 in combination with IL-12 enhances the GBS-stimulated production of IFN- γ above that of mononuclear cells exposed to GBS in the presence of added IL-12 and IL-18 ($p < 0.001$). Thus, priming with both cytokines for 30 min before GBS exposure leads to optimal cord blood cell IFN- γ responses.

DISCUSSION

Mixed mononuclear cells from cord blood produced significantly smaller quantities of IFN- γ , IL-12 (29), and IL-18 in response to GBS compared with stimulated mixed mononuclear cells from adult blood. We acknowledge that the use of mixed mononuclear cells, which likely reflects a more physiologic milieu, may contain cellular elements or products that may influence the GBS induction of the Th-1 cytokines (IFN- γ , IL-12, and IL-18) between cord and adult cells. Nevertheless, our findings concerning GBS, an important neonatal pathogen, agree with those of Bryson and co-workers (27) and Wilson *et al.* (28), who reported neonatal deficiencies in IFN- γ production by mixed mononuclear cells in response to mitogens. This deficiency in neonatal IFN- γ production may be attributed, in part, to the developmental immaturity of neonatal T-lymphocyte populations. We suggest that, in addition to lymphocyte immaturity, other yet-to-be-described abnormalities in IL-18, IL-12, and IFN- γ synthesis and release may contribute to decreased neonatal host resistance.

The addition of recombinant IL-18 to mixed mononuclear cells from adults significantly enhanced IFN- γ production in response to GBS. The increased response to GBS induced by the added IL-18 was blocked by the addition of anti-IL-18 and partly by anti-IL-12 MAb, suggesting a role for both cytokines

Figure 4. The effects of IL-18 (100 ng/mL) with and without anti-IL-12 antibody (10 ng/mL) on the IFN- γ response of adult mixed mononuclear cells to GBS after 24 h incubation. The addition of anti-IL-12 antibody (10 ng/mL) depresses the IFN- γ response to IL-18 and GBS, indicating that IL-12 regulation of IL-18 influences the IFN- γ response to GBS. No effect on IFN- γ production was observed with recombinant IL-18 alone, as shown previously ($n = 10$), or with anti-IL-12 antibody alone (data not shown).

Figure 5. The effect of anti-IL-18 antibody (1.0 $\mu\text{g/mL}$) on IFN- γ production by IL-12 (1.0 U/mL). The addition of IL-18 antibody suppressed the GBS-induced IL-12 IFN- γ response, but this decrease was not statistically significant. No effect on IFN- γ production was observed with recombinant IL-12 alone or anti-IL-18 alone (data not shown) ($n = 7$). Incubations of the cells with GBS were carried out for 24 h.

Figure 6. The effect of IL-18 (100 ng/mL) and/or IL-12 (1.0 U/mL) on IFN- γ production by mixed mononuclear cells from cord blood with and without cytokine priming. The IFN- γ response of the cells primed for 30 min with both IL-12 and IL-18 was significantly higher than unprimed cells or cells primed with either cytokine alone ($p < 0.0001$). Priming was carried out for 30 min before addition of GBS. Incubations of the cells with GBS ($1 \times 10^6/\text{mL}$) were carried out for 24 h.

in the enhancement of GBS-induced IFN- γ responses by mixed mononuclear cells. Similarly, the addition of IL-18 to the mixed mononuclear cells obtained from cord blood enhanced the IFN- γ response to GBS. This suggests that IL-18 is involved in neonatal IFN- γ production, which has not been previously described to our knowledge.

Several recent studies have raised important questions about the relationship between IL-18 and IL-12. The combination of IL-18 and IL-12 is far more effective in the induction of IFN- γ production than either cytokine alone, and IL-18 induces IFN- γ only when its receptors are up-regulated by IL-12 (11, 31). In our studies, the treatment of adult mixed mononuclear cells exposed to GBS and recombinant IL-18 with anti-IL-12 significantly reduced IFN- γ production below that of IL-18 and GBS. Treatment of adult mixed mononuclear cells exposed to GBS and recombinant IL-12 with anti-IL-18, however, did not significantly decrease the production of IFN- γ below that of IL-12 and GBS. In addition, IFN- γ production by adult mixed mononuclear cells exposed to GBS is stimulated by both IL-12 and IL-18, but the higher production of IFN- γ induced by IL-18 seems to require the presence of IL-12. This suggests that IL-12 priming of IL-18-regulated IFN- γ production is necessary for optimal IFN- γ production in response to GBS. In our studies, priming the cord blood mixed mononuclear cells with both IL-12 and IL-18 enhances the IFN- γ response to GBS to levels significantly above that after incubation of the GBS-stimulated cells with either cytokines alone or both together.

We have demonstrated that cells from human neonates are deficient in the ability to produce IFN- γ in response to GBS. This deficiency may contribute to defective neonatal PMNL function and result in an increased susceptibility to infection. This IFN- γ deficiency may be attributed in part to lymphocyte immaturity as well as depressed production of both IL-12 and IL-18, as we show here. The decreased production of the three main Th-1 cytokines, IFN- γ , IL-12, and IL-18, likely contributes to the unique susceptibility of neonates to group B streptococcal as well as other bacterial infections.

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