# The Capability of Neonatal Leukocytes to Produce IL-6 on Stimulation Assessed by Whole Blood Culture

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ABSTRACT. IL-6 is a cytokine with a wide variety of influences on the cells involved in immune and inflammatory responses. Defective production of IL-6 may be partly responsible for the impaired immune defense and inflammatory response often observed in the neonatal period. In our study, we used whole blood culture to examine the capacity of neonatal leukocytes to produce IL-6 in response to various stimuli. IL-6 activity was evaluated by growth promoting assay using an IL-6-dependent murine hybridoma clone. IL-6 activity was undetectable in fresh or unstimulated blood obtained from both newborns and adults. In contrast, incubation of whole blood with lipopolysaccharide or concanavalin A resulted in marked IL-6 activity. After stimulation, IL-6 activity was induced as early as 2 h after culture and increased with time, reaching a plateau at around 12 h. Comparative examinations suggested that the IL-6 activity induced in neonatal blood on stimulation was similar to that seen in stimulated adult blood. Neutralization experiments with anti-IL-6 antiserum confirmed the presence of IL-6 proteins in the stimulated blood, and induction of cellular IL-6 mRNA was demonstrated in the stimulated blood as well. In addition, immunocytochemical observations suggested that the major IL-6 producing cells in the stimulated blood may be monocytes. The results suggest that the production of IL-6 in response to specified stimuli is normal at birth. (Pediatr Res 27: 227-233, 1990)

## Abbreviations

MNC, mononuclear cell

LPS, lipopolysaccharide

Con A, concanavalin A

APP, acute phase proteins

BSF-2, B cell stimulatory factor 2

Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide

TBS, Tris buffered saline

TNF, tumor necrosis factor

IL-6 was first identified as T cell-derived BSF-2 that causes the terminal differentiation of B cells into Ig-producing cells (1).

After cDNA for BSF-2 was cloned, the molecule was found to be identical to 26-kD protein, IFN-$\beta_2$, and hybridoma growth factor, all of which had been described previously (2). It has been established that IL-6 has many biologic functions, which participate in the growth and differentiation of B cells, T cells, myeloma-plasmacytoma and hematopoietic cells (3-6). Recently, Gauldie et al. (7) have shown that IL-6 is functionally and immunologically identical to hepatocyte stimulating factor that can elicit the de novo production of APP by hepatocytes. Using recombinant preparations of IL-6, its induction of hepatocyte-derived APP has been demonstrated in vivo (8) as well as in vitro (9). Additionally, IL-6 appears to play an important role in mediating the pyrogenic response to tissue damage (10). Increased serum levels of IL-6 activity have been observed in patients with febrile illnesses such as bacterial and viral infections and autoimmune disorders (11, 12). Extremely high titers of IL-6 have been found in body fluids, such as cerebrospinal fluids, urine and synovial fluids, obtained from various disease conditions (13-15). Thus, IL-6 has now emerged as a major component mediating the host response to inflammatory stimuli such as infection and tissue damage.

The neonatal immune system is relatively immature compared to that of the adult, with significant differences present with regard to the Ig-producing ability of B cells and regulation of B cell differentiation by T cells (16, 17). In addition, the neonatal period is characterized by a diminished febrile response during bacterial infections (18). This diminished febrile response of newborn babies has been, in part, attributed to the deficient production of endogenous pyrogens or decreased hypothalamic thermoregulator sensitivity to pyrogens (19, 20). The limited production of IL-6 in response to many pathogens may be partly responsible for the inadequate febrile response and lymphocyte dysfunction in the neonatal period. In our work, we used the culture method using whole blood, in an attempt to mimick in vivo conditions, to examine whether the ability to produce IL-6 in response to various stimuli is well developed at birth or not.

## MATERIALS AND METHODS

Samples. Heparinized venous blood was obtained from healthy adult volunteers aged 25 to 35 y. Neonatal cord blood samples were collected from the placental end of the umbilical cord immediately after the birth of full-term newborn babies. The MNC were separated freshly or after culture from the whole blood by Ficoll-Hypaque gradient centrifugation after dextran sedimentation (21).

IL-6 induction in vitro. The whole blood cultures were prepared in polypropylene tubes (Falcon no. 2005; Becton Dickinson and Co., Lincoln Park, NJ) at 37°C in a humidified atmosphere of 5% $CO_2$ in air. Two milliliters of whole blood were incubated for 12 h with various concentrations of LPS (0.01 to 10 μg/mL) (lot. no. 026:B6; Difco Laboratories, Detroit, MI) or

Con A (0.08 to 50 μg/mL) (Difco). For the kinetics study, the whole blood was cultured with 1 μg/mL LPS or 10 μg/mL Con A for various time periods up to 48 h. Unless otherwise indicated, the whole blood cultures were carried out for 12 h in the presence or absence of 1 μg/mL LPS or 10 μg/mL Con A. In some experiments, the whole blood was stimulated with a streptococcal preparation, OK432 (10 μg/mL) (Chugai Pharmaceuticals, Tokyo, Japan). After culture, the plasma was separated from the cultured blood by centrifugation at 500 × g for 10 min, heat-inactivated at 56°C for 30 min, filtered through 0.45 μm millipore filters, and provided for IL-6 assay as described below.

Freshly isolated MNC were suspended at 1 × 10⁶ cells/mL in RPMI 1640 medium supplemented with 25 mM Hepes, 0.3 mg/mL L-glutamine, 200 U/mL penicillin, and 10 μg/mL gentamicin. The cells were incubated in polypropylene tubes with or without LPS or Con A for 12 h at 5% $CO_2$ in air. Two different sources of FCS (Mitsubishi Chemical Industries Ltd., Tokyo, Japan and M. A. Bioproducts, Walkersville, MD) were added to some of the cell cultures. It was confirmed by a standard assay using the Limulus test that these FCS preparations and Con A contained less than 50 pg/mL endotoxin.

*IL-6 assay.* IL-6 activity in the plasma from cultured blood or supernatants from MNC cultures was evaluated by a colorimetric assay using a murine IL-6-dependent hybridoma clone, MH60.BSF2 (provided by Drs. T. Hirano and T. Kishimoto, Osaka University, Osaka, Japan) (11, 22). The hybridoma cells were maintained in the presence of human rIL-6 (provided by Drs. T. Hirano and T. Kishimoto) in RPMI 1640 medium containing 10% FCS (Gibco, Grand Island, NY), 25 mM Hepes, 0.3 mg/mL L-glutamine, 200 U/mL penicillin, 10 μg/mL gentamicin, and 60 μg/mL tylocine (assay medium). The cells at logarithmic growth were washed three times in assay medium and preincubated for 6 h without IL-6 at 37°C in an attempt to remove the cell-bound IL-6. After this starvation period, the cells were spun down and resuspended in the assay medium. These cells (10⁴) were added to 96-well flat bottom microtiter plates (Corning Glass Works, Corning, NY) at a volume of 0.1 mL/well. Serial dilutions of the samples (100 μL) were added to each well. The cells were cultured for 48 h at 37°C in a humidified atmosphere of 5% $CO_2$ in air. After 44 h of culture, MTT (Sigma Chemical Co., St. Louis, MO) was freshly prepared, and added to each well at a concentration of 0.5 mg/mL. After further incubation for 4 h at 37°C, the plates were spun down at 200 × g for 5 min. The supernatants were discarded by inverting the plates, and the excess dye and the medium was removed by paper towels. Acidified propylalcohol (0.04 N HCl in isopropanol) was added to each well (100 μL/well). After leaving the plates for 20 min at room temperature, the contents were gently mixed by a plate mixer. A detergent solution (3% SDS in $H_2O$) was added to each well (20 μL/well) to prevent the interference of the optical measurement by protein precipitates induced by the alcohol. The OD of the MTT formazan was read at 550 nm by an automatic microplate sepctrophotometer. The IL-6 activities were calculated using a standard curve obtained for rIL-6. Human rIL-6 used in this work had a specific activity of 5.2 × 10⁶ U/mg of protein determined by the augmentation of IgM secretion by a EBV-transformed cell line, SKW-CL14.

*Neutralization of IL-6 activity by anti-IL-6 antiserum.* The specificity of IL-6 activity found in the plasma from cultured blood was confirmed by the neutralization experiments using a rabbit antiserum raised against rIL-6 (a gift from Drs. T. Hirano and T. Kishimoto). The samples were mixed with various concentration of the antiserum or the nonimmune rabbit serum and incubated at 37°C for 1 h, after which the neutralized samples were provided for IL-6 assay as above. Recombinant IL-6 preparation (1 U/mL) was used as a control.

*Immunocytochemical staining of IL-6-producing cells.* The cells with cytoplasmic IL-6 proteins were examined by the immunocytochemical method using the rabbit anti-rIL-6 antiserum (kindly provided by Dr. L. T. May, Rockefeller University) (23).

The cytocentrifuged preparations of MNC isolated from cultured blood were stained for cytoplasmic IL-6 immunocytochemically in the following procedure. The slides were fixed in 5% acetic acid and 95% ethanol for 30 min at −20°C. They were then washed three times in 0.05 M TBS pH 7.6 and stained with the anti-IL-6 antiserum diluted at 1:100 in TBS for 60 min. The control slides were stained with normal rabbit serum diluted at 1:100 in TBS. Subsequently, they were incubated with 1:20 dilution of alkaline phosphatase-conjugated goat anti-rabbit IgG (Tago Inc., Burlingame, CA) for 30 min, and next with 1:40 dilution of alkaline phosphatase-conjugated swine anti-goat IgG (Tago) for 30 min. All reactions were performed at room temperature. Each step was followed by three 3-min washes in TBS. They were finally stained by incubation in the alkaline phosphatase substrate solution containing naphtol AS-MX and Fast Red TR salt (Sigma) (24). This substrate contained 1 mM levamizole to block endogenous neutrophil alkaline phosphatase activity. After 10 to 20 min, the reactions were terminated by rinsing the slides in distilled water. The slides were counterstained with Carrazi's hematoxylin and mounted in glycerol gelatin.

*RNA dot blot hybridization.* The cytoplasmic RNA was isolated by the method of Chomczynski and Sacchi (25). The RNA was denatured with 2.2 M formaldehyde, 50% formamide, 0.04 M 4-morpholine propane sulfonic acid, 10 mM sodium acetate, 1 mM EDTA at 65°C for 15 min. For dot blot analysis, each aliquot of 5 μg/mL RNA and its 2-fold dilutions were blotted onto nylon membrane filters using a BIO-DOT apparatus (Bio-Rad Lab., Richmond, CA) as recommended by the manufacturer. These filters were air-dried and baked at 80°C for 1 h, prehybridized in 50% formamide, 5 × SCC, 5 × Denhardt's solution, 50 mM NaPO₄, pH 7.0, 0.1% SDS, 0.2 mg/mL sheared salmon sperm DNA for 24 h at 42°C. The hybridization buffer was the same as the prehybridization buffer except that it contained a 3 × 10⁶ cpm/mL ³²P-labeled probe. The *TaqI-XbaI* fragment of pBSF2.38.1 (IL-6 cDNA) (provided by Drs. T. Hirano and T. Kishimoto) was radiolabeled with a random-primed DNA labeling system (Amersham International plc., Amersham, UK). The hybridization was carried out for 16 h at 65°C and the filters were exposed at 80°C for 48 h to RX x-ray film (Fuji Photo Film Co. Ltd., Kanagawa, Japan) with an intensifying screen.

## RESULTS

*Whole blood culture system for IL-6 induction.* In our work, IL-6 activity was evaluated by the ability of the samples to promote the growth of a murine IL-6-dependent hybridoma clone, MH60.BSF2. First, we investigated the appropriate culture conditions for IL-6 induction by various stimuli. Table 1 shows

Table 1. *IL-6 induction in different culture conditions on stimulation**

| | IL-6 activity (U/mL) | | | |
| | Separated MNC | | | |
| Stimulants | None | FCS I | FCS II | Whole blood |
|---|---|---|---|---|
| None | 8.1 (2.61)† | 192.5 (1.62)‡ | 62.3 (1.46)‡ | 0.1 (1.48) |
| LPS | 107.1 (1.62) | 352.2 (1.21) | 298.7 (1.03) | 200.1 (1.44) |
| Con A | 222.8 (1.10)‡ | 275.9 (1.32) | 304.2 (1.50)† | 63.0 (1.07) |

* Freshly isolated MNC from adult donors were suspended in culture medium with or without different FCS preparations. The whole blood or MNC were incubated either alone or with LPS (1 μg/mL) or Con A (10 μg/mL) for 12 h and the supernatants from the MNC cultures or the plasma from the whole blood cultures were examined for IL-6 activity. The results represent the geometric means $\gtrless$ geometric SEM of three separate experiments; statistical significance was determined by *t* test. Values were compared with whole blood data.

† $p < 0.05$.

‡ $p < 0.001$.

the results of IL-6 induction in the cultures using isolated MNC or whole blood from the adult subjects. In all culture conditions, the cultures of MNC contained detectable levels of IL-6 activity (10–200 U/mL) even when cultured without stimulants. This spontaneous induction of IL-6 activity in MNC cultures was marked when FCS was added to the culture. Although a relatively low activity of IL-6 was induced in the serum-free medium (8.1 U/mL), the background responses seen in the MNC cultures made it difficult to evaluate the IL-6 enhancement in response to appropriate stimuli. In distinct contrast to the MNC cultures, little IL-6 activity (less than 0.1 U/mL) was induced when the whole blood was cultured for the same period of time. As shown in Table 1, the addition of LPS or Con A resulted in the induction of appreciable IL-6 activity in the plasma of the cultured blood.

Figure 1 shows the kinetics of IL-6 induction in LPS- or Con A-stimulated blood from an adult donor. When the whole blood was incubated with either LPS or Con A, IL-6 activity was detectable in the plasma as early as 2 h after stimulation, and increased with time, reaching a plateau at around 12 h after the culture. As shown in Figure 2, as little as 10 pg/mL of LPS could induce detectable levels of IL-6 activity in this system. Ten ng/mL of LPS appeared to be sufficient to achieve maximum IL-6 induction in the cultured blood. However, in the Con A-stimulated blood, higher doses were required for maximum induction of IL-6 activity, which was less than that seen in the LPS-stimulated cultures.

*IL-6 production by stimulated neonatal cord blood and adult peripheral blood.* Based on the preliminary experiments above, we here used the whole blood culture system to evaluate the capability of neonatal leukocytes to produce IL-6 in response to various stimuli. Neonatal cord blood and adult peripheral blood were incubated in the presence of LPS, Con A, or OK432, and the plasma from each stimulated blood sample was tested for IL-6 activity. IL-6 activity was undetectable in both the unstimulated neonatal blood and adult blood, but after any stimulation, IL-6 activity was detectable in the neonatal blood as well as adult blood at 2–4 h, and continued to increase thereafter, peaking at around 12 h. The dose-responses of IL-6 activity induced in the cord blood to different stimuli were similar to those in the adult blood (data not shown). Results from separate experiments from the cord blood and adult blood are summarized in Table 2. With respect to LPS and Con A stimulation, it should be noted that the IL-6 activity induced in neonatal blood was similar to that of adult subjects. Stimulation with OK432, a bacterial prepara-

tion, seemed to induce somewhat higher levels of IL-6 activity in neonatal blood than in adult blood ($p < 0.05$).

To examine whether the MH60.BSF2 growth promoting activity induced in the LPS- or Con A-stimulated blood from the neonatal and adult samples was indeed due to the secretion of IL-6 molecules, neutralization experiments using rabbit anti-rIL-6 antiserum were performed. As shown in Figure 3, the hybridoma clone growth promoting activity in the LPS-stimulated blood samples from newborns as well as adults was almost completely abolished by the rabbit anti-rIL-6 serum, but not by normal rabbit serum. Similarly, IL-6 activity of Con A-stimulated blood was eliminated by anti-rIL-6 antibody (data not shown). We found that this hybridoma clone could proliferate in response to only rIL-6, but not to recombinant preparations of other cytokines such as IL-1, IL-2, IL-3, IL-4, interferons ($\alpha$, $\beta$, $\gamma$) and TNF. These observations confirmed that IL-6 proteins can actually be induced in the plasma of neonatal blood and adult blood by LPS or Con A stimulation.

*Expression of IL-6 mRNA in stimulated neonatal cord blood and adult peripheral blood.* We next examined the expression of cellular IL-6 mRNA in stimulated blood. MNC were isolated from LPS-stimulated neonatal and adult blood, and analyzed for IL-6 mRNA content by the dot blot method. Cultures of freshly isolated MNC were also examined as controls for the expression of IL-6 mRNA. As shown in Figure 4, no detectable IL-6 mRNA was induced in the whole blood cultures in the absence of stimuli, whereas measurable amounts of transcripts for IL-6 were present in unstimulated cultures of separated MNC. Incubation of the whole blood with LPS induced significant levels of IL-6 mRNA. Corresponding to the IL-6 induction above, the expression of cellular IL-6 mRNA in the LPS-stimulated neonatal blood was similar to that of adult blood.

*Immunocytochemical identification of IL-6 producing cells in stimulated blood.* Using immunocytochemical technique to identify the cells producing IL-6 protein in stimulated blood, MNC from the whole blood cultures, which had been incubated in the presence of LPS or Con A, were deposited on glass slides and fixed in 5% acetic acid and 95% ethanol. Figure 5 represents a typical result obtained from neonatal blood. When the cells were stained by an immunoalkaline phosphatase method using rabbit anti-rIL-6 antiserum, intense granular staining was observed in a proportion of cells from the LPS- and Con A-stimulated blood. It was found that positive, but faint, staining appeared as early as 6 h after stimulation with LPS or Con A, and the cells with the most intense staining were found around 10 to 12 h after the
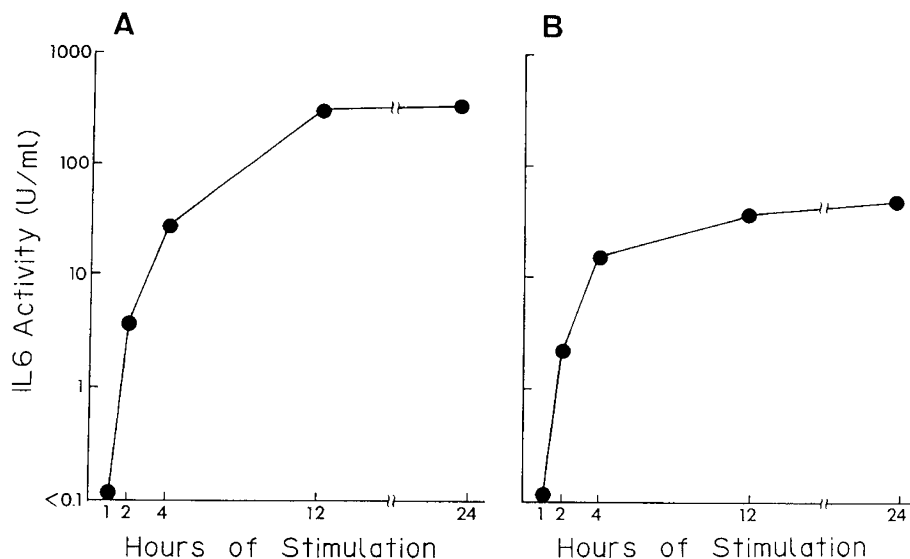


Fig. 1. Kinetics of induction of IL-6 activity in stimulated blood. Adult blood was stimulated with 1 µg/mL of LPS (*A*) or 10 µg/mL of Con A (*B*). The data represent the geometric means of five separate experiments.
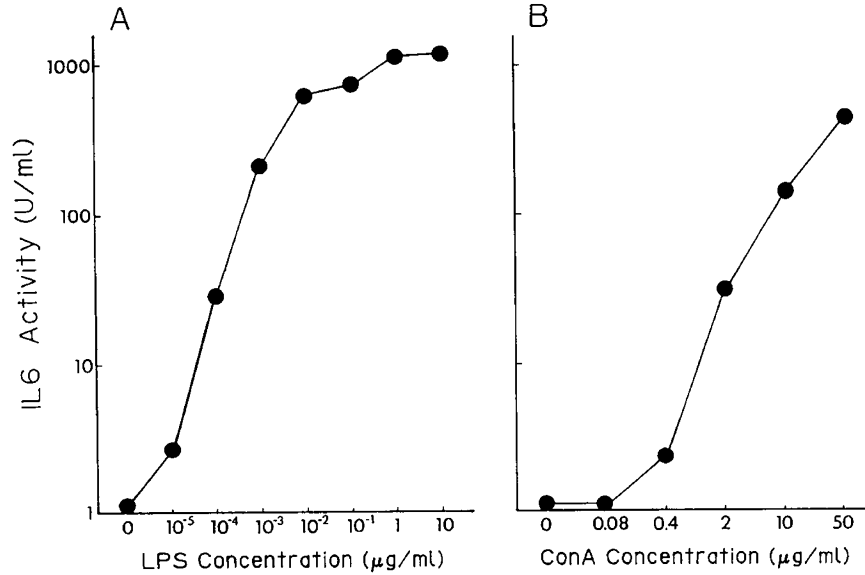
Fig. 2. Dose responses of IL-6 activity induction to LPS and Con A. Adult blood was stimulated with various doses of LPS ($A$) or Con A ($B$) for 12 h. The data represent the geometric means of five separate experiments.

Table 2. *Induction of IL-6 activity in neonatal cord blood and adult peripheral blood on stimulation**

| | IL-6 activity (U/mL) | |
| --- | --- | --- |
| Stimulants | Neonate ($n = 7$) | Adult ($n = 6$) |
| None | 0.1 | 0.1 |
| LPS (1 $\mu$g/mL) | 1193 (1.17) | 1419 (1.23) |
| Con A (10 $\mu$g/mL) | 217 (1.18) | 176 (1.49) |
| OK432 (10 $\mu$g/mL) | 228 (1.08)† | 51 (1.69) |

* Neonatal cord blood and adult peripheral blood were cultured for 12 h in the presence of various stimulants. The results represent the geometric means $\overset{\times}{\div}$ geometric SEM; statistical significance was determined by $t$ test.

† Values significantly different from adult, $p < 0.05$.

culture. Although the stained granules were usually seen throughout the cytoplasm, the most intense staining seemed to be localized in the Golgi area. In control experiments, there was no stai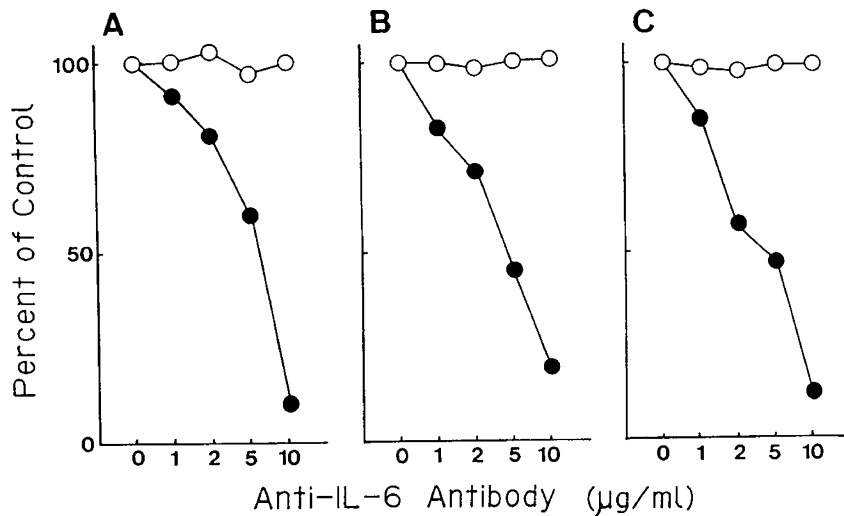ning when nonimmune serum was substituted for the specific antiserum. As can be seen in Figure 5, these positive cells were larger in size than other small lymphocytes, and had horseshoe-like nuclei with delicate nuclear chromatin, suggesting that they were morphologically monocytes. Only very few cells were stained by this technique in preparations from the unstimulated cultures. Essentially, adult cells stimulated with LPS or Con A were stained in the same manner as the neonatal cells. These results implied that monocytes might be the major cell type producing IL-6 molecules on stimulation with Con A as well as LPS in the whole blood cultures.

## DISCUSSION

We used a simple and reliable whole blood culture method to examine the IL-6 production by neonatal leukocytes in response to various stimuli in comparison with that of adult ones. In the conventional culture method using isolated MNC, much IL-6 activity was observed in the culture supernatants even in the absence of added stimulants. This phenomenon may be related to some of the culture conditions, including the activation of



Fig. 3. Neutralization of IL-6 activity in the whole blood culture with anti-rIL-6 antiserum. Recombinant IL-6 ($A$), neonatal blood ($B$), and adult blood ($C$) samples were incubated with various concentrations of rabbit anti-rIL-6 antiserum (●) or nonimmune serum (○) at 37°C for 1 h, and tested for IL-6 activity.
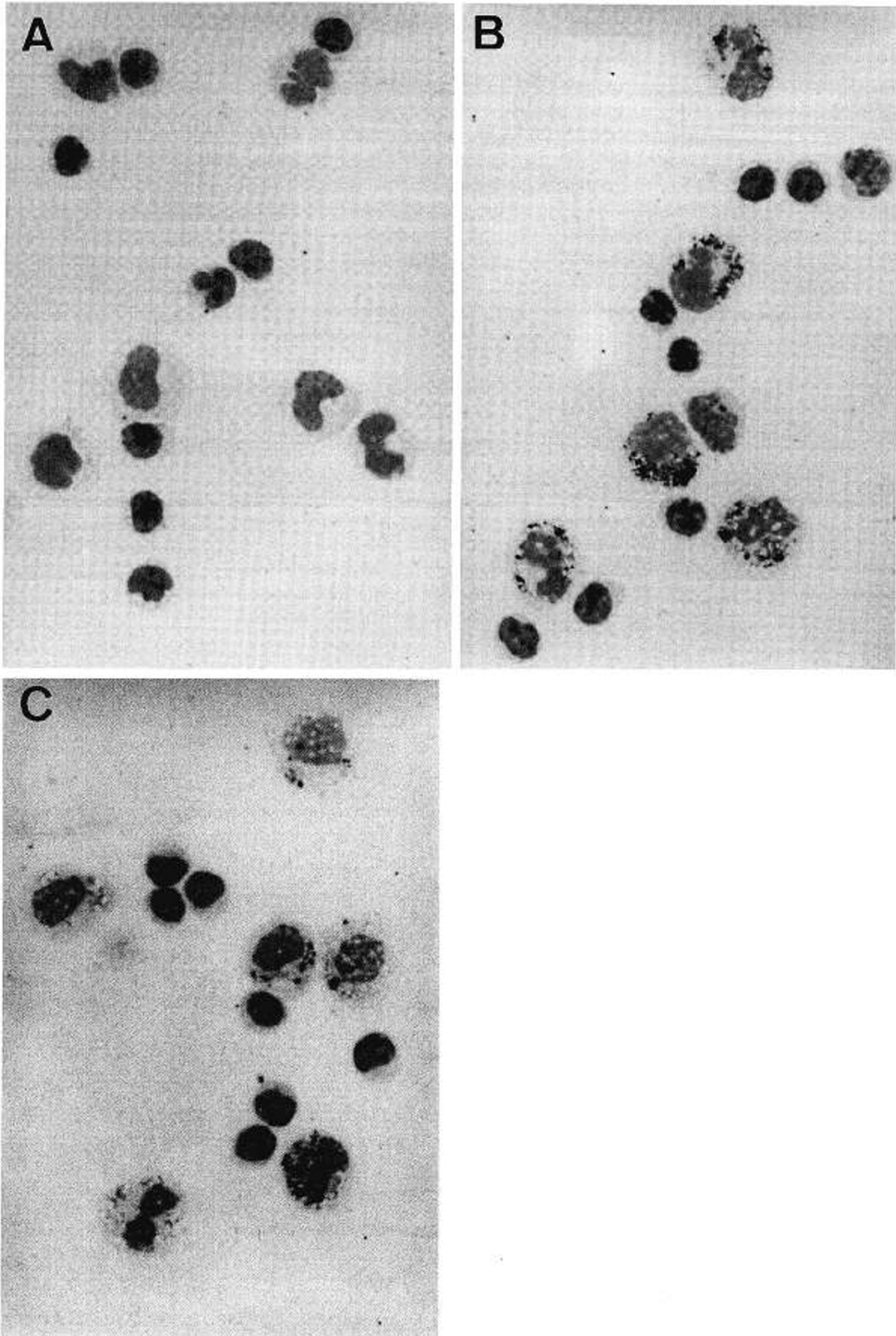
Fig. 4. Immunocytochemical detection of IL-6-containing cells in stimulated neonatal cord blood. MNC from neonatal blood, incubated either alone (*A*) or with LPS (*B*) or Con A (*C*) for 12 h, were deposited on glass slides, and stained for intracellular IL-6 proteins as described in Materials and Methods.
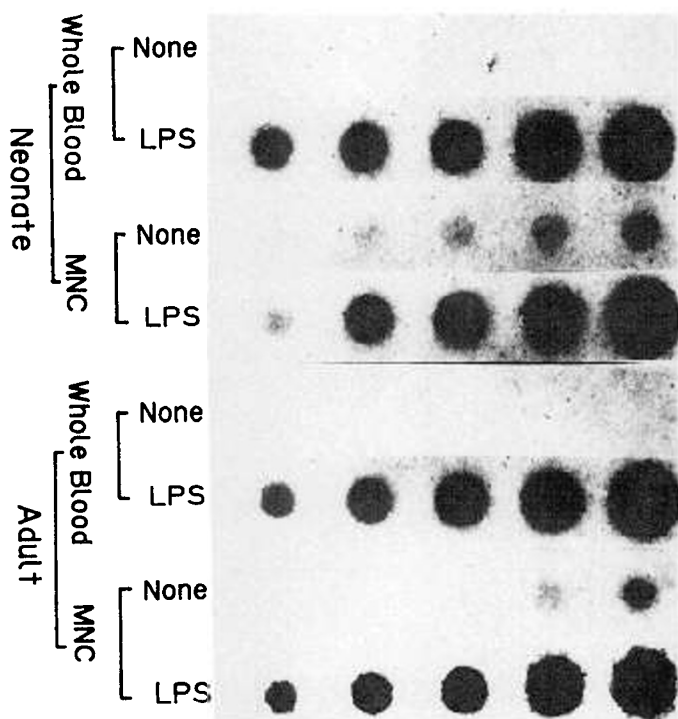
Fig. 5. Dot blot analysis for IL-6 mRNA. Total cytoplasmic RNA was obtained from MNC isolated from stimulated blood or cultured MNC, and hybridized with a random-primed cDNA probe for IL-6. Two-fold dilutions of 5 μg of RNA were blotted.

cells due to their adherence to the plastic tubes and the presence of foreign proteins in the culture. It has been reported that the attachment of monocytes to the surfaces of culture instruments can induce the transcription of mRNA for IL-6 as well as TNF (26).

This adherence mechanism is likely responsible for, at least in part, the spontaneous production of IL-6 activity in the cultures of MNC. Another possibility is contamination of the endotoxin in the FCS used for the culture medium. In fact, we encountered one lot of FCS containing a relatively large amount (200 pg/mL) of endotoxin. The use of this FCS resulted in the induction of a much higher level of IL-6 activity in the unstimulated cultures of isolated MNC, exceeding that of the stimulated cell cultures. We chose two lots of FCS containing less than 50 pg/mL of endotoxin to prepare the culture medium. Despite the use of these lots of FCS with less endotoxin, appreciable production of IL-6 activity was demonstrated in the cultures in the absence of stimuli, although spontaneous production of IL-6 activity was relatively modest in MNC cultures using FCS-free medium. In marked contrast, only a negligible amount of IL-6 activity was detected in the whole blood cultured without stimuli, whereas induction of moderate IL-6 activity was demonstrable after stimulation with LPS or Con A. Therefore, the whole blood culture system could overcome the artificial cellular activation seen in the standard culture method with isolated MNC, and thus appeared to allow proper evaluation of *de novo* induction of IL-6 activity. The culture method using whole blood seemed to resemble closely *in vivo* situations such as those during bacterial and viral infections. Recently, Genord *et al.* (27) used whole blood for the assessment of TNF gene expression after LPS stimulation.

The impairment of defense mechanisms in the newborn is associated with increased susceptibility to microorganism invasion or an elevated risk of tissue damage due to activation of the inflammatory process (28). Because IL-6 plays a complex role in immune and inflammatory responses, we were interested to learn whether or not IL-6 production is fully developed at birth. Our data clearly indicate that neonatal leukocytes do not differ from

adult ones in their ability to produce IL-6. When LPS or Con A was added to the neonatal blood, IL-6 activity was induced at levels comparable to those of adult blood. The neutralization experiments with anti-rIL-6 antiserum suggested that IL-6 activity found in the stimulated blood from both newborns and adults may reflect the secretion of true IL-6 molecules. We observed that stimulation with a streptococcal preparation, OK432, resulted in slightly higher levels of IL-6 activity in the plasma of neonatal blood when compared to adult blood, although there was only small statistical significance in IL-6 production between the two groups ($p < 0.05$).

The mechanisms by which IL-6 production is controlled in the host and the cell types that secrete IL-6 systemically or locally during infection or tissue damage remain to be elucidated. Recently, Jablons *et al.* (29) have demonstrated that *in vivo* administration of recombinant TNF results in the rapid appearance of IL-6 in the peripheral blood. Furthermore, the induction of IL-6 in the circulation has been found after injection of LPS (30). Although IL-6 is produced by a variety of cells including fibroblasts, monocytes, T cells and vascular endothelial cells *in vitro* (1), the cellular origin of IL-6 observed after *in vivo* administration of TNF or LPS is unknown. We demonstrated by immunocytochemical analysis that the major IL-6-producing cells appear to be monocytes when whole blood from newborns as well as adults is cultured with LPS or Con A. In another study combining the use of *in situ* hybridization and the corresponding MAb, we also confirmed that IL-6 is secreted largely by monocytes in the stimulated blood (31).

IL-1 and TNF are other monocyte-derived cytokines with multiple biologic activities, including immunologic effects, mediation of febrile responses, and acute phase response to infection. Consistent with our results, it has been reported that production of IL-1 by neonatal MNC is comparable to adult production (32). English *et al.* (33) have demonstrated that neonatal MNC produce substantial amounts of TNF in response to nonspecific stimuli such as PHA, although TNF production by neonatal MNC is somewhat less than production by adult cells. We demonstrated that the ability of monocytes to produce IL-6 is mature at birth. Taken together, it is unlikely that the limited production of these products by monocytes contributes to the diminished febrile response, increased susceptibility to severe infections, or lymphocyte immaturity of the human neonate.

It is well known that the etiologic characteristics of bacterial infections in the newborn are quite different from those in the older infant (34). It has been shown that the capability of monocytes to phagocytose microorganisms is almost normal in full-term newborns (35, 36). However, there is evidence that some monocyte functions are altered in the neonatal period, playing a role in the enhanced susceptibility to infectious agents. In particular, a lower expression of HLA-DR antigens, with an associated decrease of antigen presentation and of intracellular killing activity induced by some lymphokines, is noted (37–39). It remains to be determined whether the intracellular cytokine production process, such as that of IL-6 after phagocytosis of microorganisms, is impaired in neonatal monocytes. It is attractive to suppose that microorganism-mediated production of IL-6 by neonatal monocytes may differ according to the kind of infectious agent. To resolve this question, production of IL-6 in neonatal blood when incubated with various strains of live bacteria is currently under investigation.

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