0031-3998/02/5106-0670
PEDIATRIC RESEARCH
Vol. 51, No. 6, 2002
Printed in U.S.A.

ARTICLES

Extracellular Release of Bactericidal/Permeability-Increasing Protein in Newborn Infants

IRMELI NUPPONEN, RIIKKA TURUNEN, TIMO NEVALAINEN, HEIKKI PEURAVUORI, MAIJA POHJAVUORI, HEIKKI REPO, AND STURE ANDERSSON

Hospital for Children and Adolescents [I.N., R.T., M.P., S.A.], Department of Medicine, Division of Infectious Diseases [H.R.], Department of Obstetrics and Gynecology [S.A.], FIN-00029 University of Helsinki, Finland; Department of Bacteriology and Immunology, Haartman Insitute, FIN-00014 University of Helsinki, Finland [I.N., R.T., H.R.]; Department of Pathology, University of Turku, Finland FIN-20014 [T.N., H.P.]

ABSTRACT

Upon activation, polymorphonuclear leucocytes (PMN) release bactericidal/permability-increasing protein, (BPI) from their azurophil granules. BPI selectively binds to the lipopolysaccharide (LPS) on gram-negative bacteria and induces their death. This study examined plasma BPI concentration levels in healthy newborns and in newborns with clinical sepsis, and the ability of PMN from preterm and term infants to release BPI. We also studied the release of myeloperoxidase (MPO), and the surface expression of adhesion molecule CD11b on PMN. In infants with clinical sepsis, plasma BPI concentration was higher, 27.8 μg/L [8.6–883; median (range)] ($n = 11$), than in healthy term infants 8.9 μg/L (3.9–179) (n = 17), and in adults 7.3 μg/L (0.7–18.4) ($n = 15$); $p = 0.014$, Kruskal-Wallis. In preterm infants ($n = 8$), the ability of PMN to release BPI *in vitro* after stimulation with PMA was 8.8, in term infants it was 15.9 ($n = 29$; $p > 0.05$ vs. preterm infants) and in adults 23.4 ng/10^6 PMN ($n = 15$; $p = 0.024$ and $p > 0.05$ vs. preterm and term infants, respectively). The corresponding values of MPO were 20.0 ng/10^6 (11.3–46.7) in preterms, 19.0 ng/10^6 (2.2–223.7) in terms, and 27.8 ng/10^6 (9.1–80.7) in adults; $p = 0.67$ between groups. In infants with clinical sepsis, CD11b level was higher, 292 RFU (234–403) than the basal CD11b expression levels in healthy newborn infants, 116 RFU (76–145); $P = 0.0001$. FMLP-stimulated PMN CD11b expressions in preterm cord blood, 1071 RFU (552–1286) and in term cord blood, 918 (567–1472) were on the same level, but lower than that in adult blood, 1592 (973–1946); $p < 0.001$, ANOVA. Our findings suggest that in preterm infants the ability to release BPI is lower than in adults and term infants. These findings suggest that premature neonates have an impaired ability to mobilize BPI, possibly contributing to their marked susceptibility to infections with Gram-negative bacteria. **(*Pediatr Res* 51: 670–674, 2002)**

Abbreviations

BPI, bactericidal/permeability-increasing protein
LBP, lipopolysaccharide-binding protein
LPS, lipopolysaccharide
MPO, myeloperoxidase
PMA, phorbol myristate acetate
PMN, polymorphonuclear leukocyte

Newborn infants, especially those born prematurely, are at risk for overwhelming bacterial infections. A number of immaturities in the host defense system have been reported in newborn infants. Among the immune mechanisms that apparently function suboptimally are the PMN (1). PMN contain multiple antibiotic proteins such as BPI, which is found in human PMN and eosinophils. BPI selectively binds to the LPS on the outer membrane of Gram-negative bacteria, causing immediate growth arrest followed by irreversible damage and then the death of the bacterium (2). BPI also blocks the endotoxic effects of LPS and promotes phagocytosis of BPI-coated bacteria (3, 4).

BPI has been localized to azurophil granules, where its hydrophobic C-terminal end is believed to anchor it to the granule membrane. Studies of PMN conducted *in vitro* suggest that BPI is capable of acting against Gram-negative bacteria in the intracellular environment. Data on isolated cells stimulated to secrete granule contents *in vitro* and on plasma derived from animals and humans with sepsis *in vivo* have demonstrated that a portion of cellular BPI stores is released from stimulated PMN (5–9). Recent clinical studies of recombinant BPI as a

Received October 24, 2000; accepted January 17, 2002.
Correspondence: Irmeli Nupponen, Department of Bacteriology and Immunology, Haartman Institute, P.O. Box 21 (Haartmaninkatu 3), FIN-00014 University of Helsinki, Finland; e-mail: irmeli.nupponen@kolumbus.fi
Supported by grants from the Foundation for Pediatric Research, Helsinki, Finland; the Helsinki University Central Hospital Research Funds, Helsinki, Finland; and the Paulo Foundation, Helsinki, Finland.

DOI: 10.1023/01.PDR.0000017477.64723.9D

therapeutic agent in the treatment of severe meningococcemia have suggested a beneficial effect (10, 11).

Another component of the azurophilic granules of neutrophils is MPO, an enzyme that uses H_2O_2 to oxidize chloride, bromide, iodide, and thiocyanate to their respective hypohalous acids, contributing to microbicidal activity. MPO, a heme protein that is a major component of azurophil granules of PMN and monocytes, is necessary for the effects of the oxygen-dependent microbicidal system (12, 13).

Neutrophil activation is characterized by an increase in CD11b/CD18 complex (Mac-1, $\alpha_M\beta_2$, CR3) density on neutrophils (14). CD11b/CD18 derives from translocation of the intracellular storage granules, *i.e.* secretory vesicles and specific granules including gelatinase granules to the cell surface (15).

In nonstimulated neutrophils, only 5% of the total cell content of CD11b/CD18 complexes is expressed on the cell surface, whereas 75% is expressed as membrane components of specific granules and 20% as membrane components of secretory vesicles (16). A strong neutrophil agonist such as *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) promotes exocytosis of secretory vesicles and also, in part, of specific granules (16).

The ability of the PMN to release BPI has not yet, to our knowledge, been studied in the newborn. Recently, it has been shown that intracellular BPI content is three- to four-fold higher in adult neutrophils than in newborn neutrophils (17). This may contribute to the increased incidence of Gram-negative sepsis among newborn infants.

The aim of this study was to investigate the ability of PMN of preterm and term infants to release BPI. To determine whether diminished BPI release reflected a generally diminished ability of preterm neutrophils to release azurophil granule contents, we compared release of MPO, another azurophil granule–derived protein. To determine whether mobilization of additional granule compartments differed between our study populations, we measured surface expression of CD11b.

MATERIALS AND METHODS

The study protocol was approved by the institutional review board, and informed consent was obtained from subjects or their parents. The study was carried out between September 1998 and July 1999 and included healthy term newborn infants ($n = 46$), prematurely born infants ($n = 21$), term newborn infants with clinical early-onset sepsis ($n = 11$), and healthy adult volunteers ($n = 15$). Cord blood samples were obtained from 21 preterm infants (12 males, 9 females) at a mean gestational age of 29.5 ± 2.1 wk (mean ± SD), birth weight 1129 ± 334 g, and from 29 term infants (14 males, 15 female), gestational age 39.8 ± 1.2 wk, birth weight 3640 ± 567 g. Ten of the 21 preterm infants and 15 of the 29 term infants were born by cesarean section.

Postnatal samples were obtained from 11 infants with clinical sepsis, and from 17 healthy term infants, born after uncomplicated pregnancy and delivery, who had mild physiologic hyperbilirubinemia and did not receive phototherapy. For clinical characteristics, see Table 1.

Table 1. *Clinical characteristics of the study populations*

	Healthy Term Infants ($n = 17$)	Term Infants, with Clinical Sepsis ($n = 11$)
Gestational age (wk), mean ± SD	38 ± 2	38 ± 4
Birth weight (g), mean ± SD	3307 ± 554	3279 ± 1109

The diagnosis of clinical early-onset sepsis was made on the basis of clinical signs consistent with infection, concentration of plasma C-reactive protein >50 mg/L during the first 3 d of life, and no other diseases present. Clinical signs were divided into five categories: temperature instability (hypothermia, hyperthermia); respiratory distress (grunting, intercostal retractions, apnea, cyanosis); cardiovascular (tachycardia, bradycardia, poor perfusion, shock); neurologic (hypotonia, lethargy); and gastrointestinal (feeding intolerance, distension). A blood sample for bacterial culture requested by the clinician (not involved in this study) was obtained from each neonate in this group. The symptoms were recorded by nursing staff members in the neonatal unit. Blood leukocyte count, platelet count, and serum C-reactive protein concentration were determined at the request of the clinicians at the initial evaluation and, during the subsequent 3 d, on the basis of the clinical picture.

The samples for plasma BPI determination were collected concurrently with clinical samples at onset of symptoms and always before administration of antimicrobials. All the newborns with clinical sepsis received antimicrobials for 6–7 d, and none received corticosteroids.

Blood samples. Each cord blood sample was aspirated from the umbilical cord vein immediately after delivery. The postnatal samples and samples from 15 adults were drawn by venipuncture. To study basal plasma BPI and neutrophil CD11b expression, two portions of blood sample were placed into a pyrogen-free tube containing citrate phosphate dextrose (Baxter Health Care Ltd., Norfolk, U.K.; 0.14 mL/mL blood), which was immediately placed in an ice-water bath to minimize neutrophil activation *ex vivo*. Within 30 min, the plasma was separated by centrifugation at 4°C and stored at −70°C until analysis. Because the time of blood storage and its handling strongly influences BPI release, each blood sample was handled identically.

Stimulation of neutrophils with PMA and fMLP. To study the ability of PMN to release BPI and MPO, two portions of 1 mL blood were anticoagulated and rapidly placed into a 37°C water bath and immediately incubated with PMA (final concentration 500 ng/mL). After incubation, the sample was cooled at 0°C. The supernatant was then separated and stored as described above. The whole process from the collection through the storing of the sample took 20–30 min. To study the ability of PMN to up-regulate CD11b expression, an aliquot of working solution of fMLP (2.5 μL, final concentration 10^{-6} M, from Sigma Chemical, St. Louis, MO, U.S.A.) was added to a 25-μL aliquot of blood sample kept at 37°C and further incubated at 37°C for 10 min. After incubation, the sample was kept at 0°C until staining for flow cytometry.

Because the minimum whole blood volume of 2.5 mL was required to determine concurrent basal plasma and supernatant

BPI and MPO, a stimulation assay was performed only on cord blood and adult blood samples. The calculation of total white blood cell counts was automated, whereas, for neutrophil counts, differentials were performed manually. Plasma BPI was measured by time-resolved fluoroimmunoassay (18), and extracellular immunoreactive MPO was quantitated using commercial ELISA (R & D Systems, Oxford, U.K.). The amount of BPI and MPO detected in the supernatant are expressed as BPI/PMN and MPO/PMN ratio (ng/10^6 PMN).

To test the analytical recovery of the assay, 49.3 μg/L of BPI in human plasma were added to 17 PMA-stimulated and nonstimulated plasma samples, 5 from preterm infants, 6 from healthy term infants, and 6 from adult donors.

Determination of CD11b expression by flow cytometry. Neutrophil CD11b expression was assessed as described previously (19, 20). fMLP-stimulated and nonstimulated samples, both at 0°C, were processed for flow cytometry within 1 h. Neutrophils were labeled with saturating concentrations of the R-phycoerythrin conjugate of mouse anti-CD11b IgG1 antibody, clone 2LPM19c, and its control antibody *Aspergillus niger* glucose oxidase IgG1–R-phycoerythrin, clone DAK-GO1, purchased from DAKO (Glostrup, Denmark). After labeling, contaminating erythrocytes were lysed by addition of 2 mL of a 1/10 diluted ice-cold fluorescence-activated cell sorter (FACS) lysing solution (BD Biosciences, San Jose, CA, U.S.A.). After a 3-min incubation on ice, the leukocytes were centrifuged for 5 min at 4°C at 1400 $\times$ *g*, and a second incubation with 2 mL of FACS lysing solution was performed for 5 min at room temperature. After centrifugation, leukocytes were resuspended in 1% formalin at 0°C. A FACScan flow cytometer (BD Biosciences) and CellQuest analysis software (BD Biosciences) were used for the acquisition and analysis of the data. Neutrophils were identified on the basis of their light-scattering properties. For each sample, 5000 events were recorded. CD11b expression is reported in relative fluorescence units (RFU), *i.e.* as the mean channel of the positive fluorescing cell population.

Statistical analysis. The significance of difference between the groups was determined by Kruskal-Wallis ANOVA. Two-group comparisons were done by the Mann-Whitney *U* test, and the *p* values were corrected by use of the Bonferroni test, if not otherwise indicated. Probabilities were regarded as statistically significant at the 0.05 level. Results are expressed as medians (ranges). To examine the correlation between PMN count and BPI, Spearman's rank correlation was used. All calculations were done with StatView 4.1 (Abacus Concepts, Berkeley, CA, U.S.A.).

RESULTS

BPI concentrations in plasma. The results in Figure 1*A* show that the BPI concentration of infants with clinical sepsis was significantly higher than that of healthy term infants (n = 11, p = 0.014). The plasma BPI concentrations were as follows: 5.5 μg/L (1.4–38.6 μg/L) in cord blood of preterm infants (n = 21), 5.9 μg/L (1.2–35.2 μg/L) in cord blood of term infants (n = 29), 8.9 μg/L (3.9–179.0 μg/L) in healthy term infants at the age of 2–5 d (n = 17), 7.3 μg/L (0.7–18.4 μg/L) in healthy adults (n = 15), and 27.8 μg/L (8.6–883 μg/L) in infants with clinical sepsis (n = 11). The difference between these groups was significant (p = 0.014, Kruskal-Wallis ANOVA). The diagnosis of clinical early-onset sepsis was made by a clinician not involved in this study. There were no blood culture positive infections among the infants with clinical sepsis. White blood cell count was 10.0 (4–17) 10^9/L in the sepsis group, and no differential was performed.

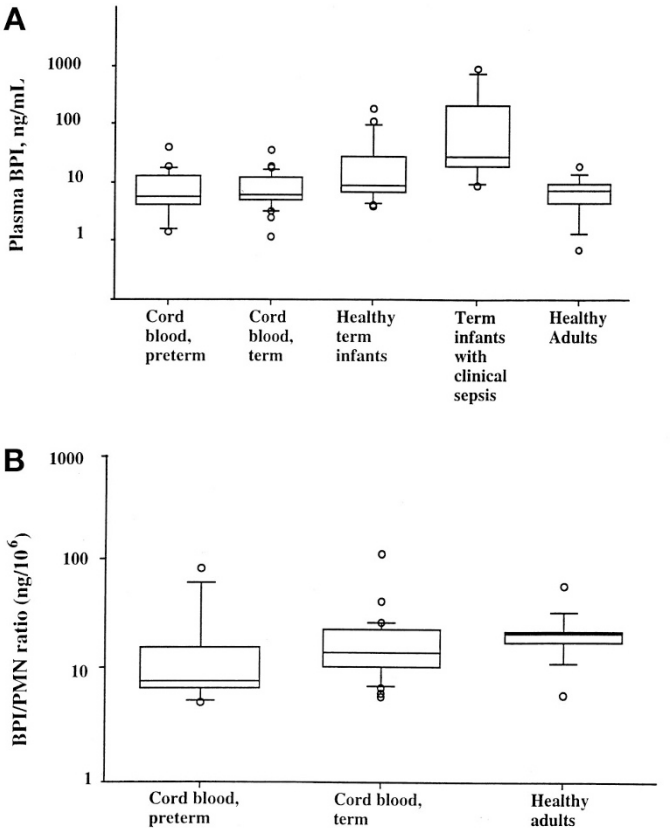

Figure 1. Box and whiskers plots of BPI concentrations in plasma (*A*) and extracellular BPI/PMN ratio in culture of whole blood supplemented with PMA (*B*). The box indicates 75th and 25th percentiles, with the central line the median. Whiskers represent the range, with outliers indicated by open circles. (*A*) Cord samples of preterm infants (n = 21) and term infants (n = 29), and samples from healthy term infants aged 2–5 d (n = 17), term infants with clinical sepsis (n = 11), and healthy adults (n = 15); p = 0.014 between the groups (ANOVA); sepsis *vs* healthy term infants, p = 0.014 (without Bonferroni correction for multiple comparisons). (*B*) Cord samples from preterm infants (n = 8) and term infants (n = 29), and samples from healthy adults (n = 15); p = 0.043 between the groups; p = 0.024, preterm cord *vs* adults.

Neutrophil counts. The PMN count in the cord blood of preterm infants, 1.1 (0.7–3.1) 10^9/L, was significantly lower than in term infants, 5.1 (2.0–16.5) 10^9/L, $p < 0.001$, and also lower than in adults, 2.0 (0.7–6.3) 10^9/L, p = 0.036. The difference between these groups was significant ($p < 0.001$, ANOVA).

Supernatant BPI. After stimulation with PMA, the BPI concentrations of the culture supernatants were 11.1 μg/L (6.5–66.7 μg/L) in preterm cord samples, 81.0 μg/L (35.0–377.0 μg/L) in term cord samples, and 41.4 μg/L (28.6–82.1 μg/L) in adult samples. The difference between these groups was significant (p = 0.001, ANOVA; preterm cord *versus* term cord, p = 0.001; term *versus* adult, p = 0.006). A positive correlation appeared between concentration of BPI and PMN count (p = 0.0001, r = 0.60).

The BPI/PMN ratio (ng/10^6 PMN) for preterm cord blood was significantly lower than that for healthy adults (8.8 *versus* 23.4) (Fig. 1*B*).

Analytical recovery. The analytical recovery of the BPI assay was 97.2 ± 11.2% (mean ± SD). The recoveries for stimulated and nonstimulated plasma did not differ between the groups. Values of the preterm infants' samples were 95.2 ± 3.7% for PMN-stimulated and 86.3 ± 3.8% for nonstimulated plasma. For healthy term infants, the values were 116.2 ± 7.4% and 91.0 ± 9.5%, and for adults 99.8 ± 5.2% and 92.6 ± 1.7%, respectively.

To determine whether diminished BPI release reflected a generally diminished ability of preterm neutrophils to release azurophil granule contents, we compared release of MPO, another azurophil granule–derived protein. To determine whether mobilization of additional granule compartments differed between our study populations, we measured surface expression of CD11b.

Supernatant MPO. After stimulation with PMA, the concentrations in the culture supernatants were 28.5 μg/L (12.0–42.0 μg/L) in preterm cord samples ($n = 21$), 147.5 μg/L (20.0–783.0 μg/L) in term cord samples ($n = 29$), and 43.0 μg/L (20.0–248.0 μg/L) in adult samples ($n = 15$, $p = 0.0002$, Kruskal-Wallis; preterm cord *versus* term cord, $p = 0.0002$; term *versus* adult, $p = 0.018$; preterm *versus* adult, $p = 0.013$).

MPO/PMN ratio. After stimulation with PMA, MPO/PMN ratios (ng/10^6 PMN) for preterm 20.0 (11.3–46.7), for term 19.0 (2.2–223.7), and for adult blood samples 27.8 (9.1–80.7), were comparable ($p = 0.67$).

Basal and fMLP-stimulated neutrophil CD11b expression levels. Basal neutrophil CD11b expression levels were comparable in the cord blood of preterm infants, 123 RFU (81–173 RFU), in the cord blood of term infants, 107 RFU (55–235 RFU), in healthy term infants at the age of 2–5 d, 116 RFU (76–145 RFU), and in adults, 114 RFU (57–237 RFU). In infants with clinical sepsis, neutrophil CD11b level was higher, 292 RFU (234–403 RFU) than the basal CD11b expression levels in healthy term infants ($p = 0.0001$).

fMLP-stimulated PMN CD11b expression in preterm cord blood, 1071 RFU (552–1286 RFU), and in term cord blood, 918 RFU (567–1472 RFU), were on the same level, but significantly lower than that in adult blood, 1592 RFU (973–1946 RFU); $p < 0.001$, Kruskal-Wallis.

DISCUSSION

These results show that newborn infants with clinical early-onset sepsis had significantly elevated plasma levels of BPI, levels nearly comparable to those in older children with sepsis syndrome (9) and in adults with bacteremia (7) or pneumonia (8). These findings are intriguing, because the total intracellular BPI content of term newborn PMN is lower than that of adult PMN (17). In accordance with this, the results in the present study showed that, after stimulation with PMA, the BPI/PMN ratio of preterm, but not of term, cord blood was significantly lower than that of adult blood. Taken together, our findings suggest that, in terms of the quantity of released BPI, term newborn infants' levels are not severely depressed, whereas preterm infants have lower capacity to release BPI. In preterm and term infants, on the other hand, the ability of PMN to release MPO, an oxygen-dependent bactericidal protein, seems not to differ from that of adults, suggesting that diminished BPI release is specific to this patient population and may reflect diminished intracellular BPI stores, rather than a global impairment in release of azurophil granule contents. Overall, our results, and those of Levy *et al.* (17) would suggest an age-dependent maturation in the ability of human neutrophils to mobilize BPI to sites of infection. Although the clinical importance of our findings requires further investigation, our study raises the possibility that diminished BPI release in preterm infants may contribute to their relatively high incidence of and morbidity and mortality due to Gram-negative bacterial sepsis.

The presence of an inhibitor of BPI detection in PMA-treated preterm cord blood was ruled out by a recovery test. To determine whether diminished BPI release reflected a generally diminished ability of preterm neutrophils to release azurophil granule contents, we compared it with release of MPO, another azurophil granule–derived protein. To determine whether mobilization of additional granule compartments differed between our study populations, we measured surface expression of CD11b. In addition to the finding of a lower cell content of BPI in newborn infants compared with that of adults, newborn infants have also lower total cell content of CD11b/CD18 complexes (21, 22). We observed that both preterm and term infants show a reduced ability to enhance CD11b expression on stimulation with fMLP. This is in harmony with previous findings of lower CD11b expression levels in these infants after fMLP stimulation (23–29).

BPI competes with LBP for endotoxin binding and functions as an antagonist of LBP-endotoxin interactions (30, 31). LBP is produced constitutively, and its large molar excess in normal plasma is considered to favor endotoxin-binding with LBP rather than with BPI (32). Plasma BPI concentrations are probably too low to neutralize LPS in some patients with sepsis; however, at infected sites, a molar excess of BPI is present, favoring neutralization of LPS (3). At least some patients with Gram-negative bacterial infection apparently benefit from treatment with recombinant BPI, as suggested by clinical studies of children with meningococcal sepsis (10, 11).

In this study, some infections were most likely caused by Gram-positive bacteria among the septic infants with increased plasma BPI concentrations, inasmuch as Gram-positive bacteria are the most common pathogens in early-onset neonatal sepsis (33). Increased plasma BPI levels in both Gram-negative and -positive sepsis can exist in children and in adults (7, 9, 34).

In neonates, bacterial infection is the most common cause of systemic inflammatory response syndrome. As a part of their systemic inflammatory reaction, neutrophils are activated and release a portion of their granule proteins into the plasma. Our neonates in the sepsis group had clinical sepsis; they showed no evidence of any additional noninfectious neonatal disease such as respiratory distress syndrome or aspiration syndrome. They all met the criteria of clinical sepsis. Although probably not specific for bacterial infection, the increased plasma BPI

may provide indirect evidence for the presence of infection in neonates with clinical symptoms of unknown cause.

Plasma BPI levels were elevated in our study at the time of the initial evaluation. This broad range of BPI elevation is consistent with previous studies indicating that plasma BPI is elevated during the first day of sepsis (9). In experimental endotoxemia, plasma BPI levels rose both at early (<2 h) and later (>5 h) times after administration of LPS and did not appear to fit the acute-phase response pattern (34). Furthermore, in septic adult patients, median plasma BPI levels either did (7, 34) or did not (35) differ from those of controls. The laboratory diagnosis of infection in newborn infants is difficult. The routine laboratory markers of infection show poor specificity and respond far too slowly to improve the diagnosis of infection in clinical practice (36). The possibility that BPI can serve as a marker of systemic inflammation and bacterial infection in the newborn infant warrants further study.

Our study suggests a diminished capacity of premature newborns to mobilize BPI to sites of infection. Recently, administration of recombinant BPI to newborn cord blood showed beneficial effects (37), raising the possibility that supplementing BPI may be of benefit to premature newborns with Gram-negative bacteremia, endotoxemia, or both.

REFERENCES

1. Hill HR 1987 Biochemical, structural and functional abnormalities of polymorphonuclear leukocytes in the neonate. Pediatr Res 22:375–382
2. Levy O 1996 Antibiotic proteins of polymorphonuclear leukocytes. Eur J Haematol 56:263–277
3. Opal SM, Palardy JE, Marra MN, Fisher Jr CJ, McKellington BM, Scott RW 1994 Relative concentrations of endotoxin-binding proteins in body fluids during infection. Lancet 344:429–431
4. Iovine NM, Elsbach P, Weiss J 1997 An opsonic function of the neutrophil bactericidal/permeability-increasing protein depends on both its N- and C-terminal domains. Proc Natl Acad Sci U S A 94:10973–10978
5. Weiss J, Victor M, Stendhal O, Elsbach P 1982 Killing of Gram-negative bacteria by polymorphonuclear leukocytes: role of an O_2-independent bactericidal system. J Clin Invest 69:959–970
6. Dentener MA, Francot GJ, Hiemstra PS, Tool AT, Verhoeven AJ, Vandenabeele P, Buurman WA 1997 Bactericidal/permeability-increasing protein release in whole blood *ex vivo*: strong induction by lipopolysaccharide and tumor necrosis factor-alpha. J Infect Dis 175:108–117
7. Froon AH, Dentener MA, Greve JW, Ramsay G, Buurman WA 1995 Lipopolysaccharide toxicity-regulating proteins in bacteremia. J Infect Dis 171:1250–1257
8. Froon AH, Bonten MJ, Gaillard CA, Greve JW, Dentener MA, de Leeuw PW, Drent M, Stobberingh EE, Buurman WA 1998 Prediction of clinical severity and outcome of ventilator-associated pneumonia. Am J Respir Crit Care Med 158:1026–1031
9. Wong HR, Doughty LA, Wedel N, White M, Nelson BJ, Havrilla N, Carcillo JA 1995 Plasma bactericidal/permeability-increasing protein concentrations in critically ill children with the sepsis syndrome. Pediatr Infect Dis J 14:1087–1091
10. Giroir GP, Quint PA, Barton P, Kirsch EA, Kitchen L, Goldstein B, Nelson BJ, Wedel NI, Carroll SF, Scannon PJ 1997 Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeability-increasing protein in children with severe meningococcal sepsis. Lancet 350:1439–1443
11. Levin M, Quint PA, Goldstein B, Barton P, Bradley JS, Shemie SD, Yeh T, Kim SS, Cafaro DP, Scannon PJ, Giroir BP 2000 Recombinant bactericidal/permeability-increasing protein (rBPI(21)) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial. Lancet 356:961–967
12. Nauseef WM 1998 Insights into myeloperoxidase biosynthesis from its inherited deficiency. J Mol Med 76:661–668
13. Gullberg U, Andersson E, Garwicz D, Lindmark A, Olsson I 1997 Biosynthesis, processing and sorting of neutrophil proteins: insight into neutrophil granule development. Eur J Haematol 58:137–153
14. Berger M, O'Shea J, Cross AS, Folks TM, Chused TM, Brown EJ, Frank MM 1984 Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. J Clin Invest 74:1566–1571
15. Borregaard N, Cowland JB 1997 Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 89:3503–3521
16. Sengeløv H, Kjedsen L, Diamond MS, Springer TA, Borregaard N 1993 Subcellular localization and dynamics of Mac-1 ($\alpha_{M\beta2}$) in human neutrophils. J Clin Invest 92:1467–1476
17. Levy O, Martin S, Eichenwald E, Ganz T, Valore E, Carroll SF, Lee K, Goldmann D, Thorne GM 1999 Impaired innate immunity in the newborn: newborn neutrophils are deficient in bactericidal/permeability-increasing protein. Pediatrics 104:1327–1333
18. Häggblom JO, Jokilammi-Siltanen AB, Peuravuori H, Nevalainen TJ 1996 Time-resolved fluoroimmunoassay for bactericidal/permeability-increasing protein. Mediators Inflamm 5:47–50
19. Repo H, Jansson S-E, Leirisalo-Repo M 1993 Flow cytometric determination of CD11b upregulation *in vivo*. J Immunol Methods 164:193–202
20. Repo H, Jansson SE, Leirisalo-Repo M 1995 Anticoagulant selection influences flow cytometric determination of CD11b upregulation *in vivo* and *ex vivo*. J Immunol Methods 185:65–79
21. McEvoy L, Zakem-Cloud H, Tosi MF 1996 Total cell content of CR3 (CD11b/CD18) and LFA-1 (CD11a/CD18) in neonatal neutrophils: relationship to gestational age. Blood 87:3929–3933
22. Abughali N, Berger M, Tosi MF 1994 Deficient total cell content of CR3 (CD11b) in neonatal neutrophils. Blood 83:1086–1092
23. Andersson DC, Becker Freeman KL, Heerdt B, Hughes BJ, Jack RM, Smith CW 1987 Abnormal stimulated adherence of neonatal granulocytes: impaired induction of surface MAC-1 by chemotactic factors or secretagogues. Blood 70:740–750
24. Andersson DC, Rothlein R, Marlin SD, Krater SS, Smith CW 1990 Impaired transendothelial migration by neonatal neutrophils: abnormalities of Mac-1 (CD11b/CD18)-dependent adherence reactions. Blood 76:2613–2621
25. Török C, Lundahl J, Hed J, Lagercrantz H 1993 Diversity in regulation of adhesion molecules (Mac-1 and L-selectin) in monocytes and neutrophils from neonates and adults. Arch Dis Child 68:561–565
26. Bruce MC, Baley JE, Medvik KA, Berger M 1987 Impaired surface membrane expression of C3bi but not C3b receptors on neonatal neutrophils. Pediatr Res 21:306–311
27. Smith JB, Campbell DE, Ludomirsky A, Polin RA, Douglas SD, Garty B-Z, Harris MC 1990 Expression of the complement receptors CR1 and CR3 and the Type III Fcγ receptor on neutrophils from newborn infants and from fetuses with Rh disease. Pediatr Res 28:120–126
28. Smith JB, Kunjummen RD, Raghavender BH 1991 Eosinophils and neutrophils of human neonates have similar impairments of quantitative up-regulation of Mac-1 (CD11b/CD18) expression *in vitro*. Pediatr Res 30:355–361
29. Carr R, Pumford D, Davies JM 1992 Neutrophil chemotaxis and adhesion in preterm babies. Arch Dis Child 67:813–817
30. Marra MN, Wilde CG, Collins NS, Snable JL, Scott RW 1990 Bactericidal/permeability-increasing protein has endotoxin neutralizing activity. J Immunol 144:622–666
31. Marra MN, Wilde CG, Collins NS, Snable JL, Thornton MB, Scott RW 1992 The role of bactericidal/permeability-increasing protein as a natural inhibitor of bacterial endotoxin. J Immunol 148:532–537
32. Tobias PS, Mathison JC, Ulevitch RC 1988 A family of lipopolysaccharide-binding proteins involved in responses to Gram-negative sepsis. J Biol Chem 263:13479–13480
33. Klein JO, Marcy SM 1990 Bacterial sepsis and meningitis. In: Remington JS, Klein JO (eds) Infectious Disease of the Fetus and Newborn Infant. WB Saunders, Philadelphia, pp 601–656
34. Von der Möhlen MAM, van der Poll, Jansen J, Levi M, van Deventer JH 1996 Release of bactericidal/permeability-increasing protein in experimental endotoxemia and clinical sepsis. J Immunol 156:4969–4973
35. Calvano SE, Thompson WA, Marra MN, Coyle SM, de Riesthal HF, Trousdale RK, Barie PS, Scott RW, Moldawer LL, Lowry SF 1994 Changes in polymorphonuclear leukocyte surface and plasma bactericidal/permeability-increasing protein and plasma lipopolysaccharide binding protein during endotoxemia or sepsis. Arch Surg 129:220–226
36. Da Silva O, Ohlsson A, Kenyon C 1995 Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J 14:3362–3666
37. Levy O, Sisson RB, Kenyon J, Eichenwald E, Macone AB, Goldmann D 2000 Enhancement of neonatal innate defense: effects of adding an N-terminal recombinant fragment of bactericidal/permeability-increasing protein on growth and tumor necrosis factor-inducing activity of Gram-negative bacteria tested in neonatal cord blood *ex vivo*. Infect Immun 68:5120–5125