Characteristics and Functional Capacities of Human Cord Blood Granulocytes and Monocytes

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ABSTRACT. Cord blood phagocytic cells were characterized with respect to cytochemical activities, Fcγ and C3b receptors, and capacity to phagocytose and kill various species of bacteria.

The percentages of peroxidase-positive granulocytes and monocytes from neonates and adults were comparable; the percentage of esterase-positive cord-blood monocytes was about two-thirds of that of adults' blood monocytes. The numbers of cord blood and adults' monocytes with Fcγ and C3b receptors were similar.

Phagocytic and intracellular killing capacities of cord blood granulocytes and monocytes were investigated for *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, and group B *Streptococcus* type III. The rates of phagocytosis and intracellular killing by granulocytes from newborns proved to be comparable to the corresponding values for granulocytes from adults.

Cord blood monocytes phagocytosed *E. coli*, *S. aureus*, and *S. pyogenes* at a normal rate, and killed ingested *E. coli* and *S. pyogenes* intracellularly at the same rate as adults' monocytes did. However, *S. aureus* was killed at a much lower rate by cord blood monocytes than by monocytes from adults. Phagocytic activity for group B *Streptococcus* was impaired and killing of these bacteria by cord blood monocytes was virtually nil. The latter finding might partially explain the frequent streptococcal infections in newborns. (*Pediatr Res* 18:1127-1131, 1984)

Abbreviation

HBSS, Hanks' balanced salt solution

Despite improved diagnostic techniques and medical care, bacterial infections still play an important role in neonatal morbidity and mortality. A wide variety of microorganisms, including those considered to have relatively low virulence and to be susceptible to commonly used antibiotics, can lead to overwhelming septicemia in neonates. The frequency of infections in the newborn suggests a defective host defense mechanism, and various attempts have been made to investigate this point. Particular attention has been paid to the functioning of phagocytic cells. Defects in the chemotactic motility of granulocytes and monocytes (reviewed in Ref. 18) and impaired opsonization of

Gram-negative microorganisms by sera of newborns have been reported (7). The phagocytic function of newborn's granulocytes is normal, and bactericidal killing by these cells has been reported to be normal or decreased (4, 5, 8, 20, 22, 25). Studies on monocytes from newborns have been hampered because the available techniques required too much blood. Recently, we developed a micromethod (19) which permits detailed investigation of the functional activities of newborns' granulocytes and monocytes.

The present paper describes the cytochemical characteristics, the metabolic activity, and the phagocytic and microbicidal activities of human cord blood granulocytes and monocytes as well as the occurrence of Fcγ and C3b receptors on these cells.

MATERIALS AND METHODS

Blood samples. Five to 10 ml blood (anticoagulated with 20 units/ml heparin) was collected aseptically from the placental end of the cut umbilical cord of 45 healthy full term infants (21 males and 24 females) at normal delivery in the Department of Obstetrics and Gynaecology of the Leiden University Hospital. For control studies, 10 to 15 ml blood was obtained by venipuncture from 60 healthy adult donors.

Isolation of monocytes and granulocytes. Monocytes were collected by differential centrifugation of 5 ml heparinized blood on Ficoll-Hypaque gradients; granulocytes were obtained from the Ficoll-Hypaque pellet by dextran sedimentation (19). Monocytes and granulocytes were suspended in HBSS with 0.1% gelatin to a concentration of 10^7 cells/ml. Cell counts were done in a Bürker hemocytometer; differential counts were made in Giemsa-stained cytocentrifuge preparations (9).

Cytochemical stainings. The percentage of peroxidase-positive cells was determined in cytocentrifuge preparations according to Kaplow (12) with benzidine dihydrochloride (Fluka, Buchs, Switzerland) as substrate (pH 6.0) and hydrogen peroxide at a concentration of 0.002% (vol/vol).

Esterase activity was investigated in cytocentrifuge preparations according to Ornstein (10) with α -naphthyl butyrate (Sigma Chemical Co., St. Louis, MO) as substrate (pH 6.1) and incubation at room temperature for 25 min.

Serum. Serum was obtained from healthy AB blood group donors and stored in small aliquots at -70°C .

Microorganisms. *Staphylococcus aureus* (type 42D), *Escherichia coli* (054), *Streptococcus pyogenes*, and a clinical isolate of group B *Streptococcus* type III (*Streptococcus agalactiae*) (kindly provided by Dr. D. G. Groothuis, Rijksinstituut voor de Volksgezondheid, Bilthoven) were cultured overnight at 37°C in Nutrient Broth No. 2 (Oxoid Ltd., London), harvested by centrifugation at $1500 \times g$ for 10 min, washed twice with phosphate-buffered saline and finally suspended in gelatin-HBSS to a concentration of 10^7 bacteria/ml. All bacteria were serum resistant,

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i.e. incubation of 5×10^6 bacteria/ml in 90% serum for 2 h at 37° C did not decrease the number of viable bacteria.

Pre-opsonization of bacteria was performed by incubating 5×10^6 bacteria/ml with 10% (vol/vol) AB serum for 30 min at 37° C at 4 rotations/min, next, the suspension was cooled to 4° C and the excess serum removed by centrifugation and two washes with ice-cold HBSS. The bacteria were then resuspended in gelatin-HBSS to a concentration of 10^7 /ml.

Micromethod to determine phagocytosis. Phagocytosis by monocytes and granulocytes was measured as the decrease in the number of viable extracellular bacteria during incubation of bacteria and phagocytes in the presence of serum. In short, 10^6 phagocytic cells were incubated with 10^6 bacteria in a total volume of 200 μ l in the presence of 20 μ l serum, at 37° C at 4 rotations/min. After various intervals, 50- μ l aliquots were taken and added to 450 μ l ice-cold HBSS, after which the cells were centrifuged for 6 min at $75 \times g$ and the number of viable extracellular bacteria was determined by plating serial 10-fold dilutions on DST agar plates (Diagnostic Sensitivity Medium Agar, Oxoid Ltd., London). The plates were incubated at 37° C for 24 h, after which the number of viable bacteria/ml was calculated from the colony counts (19).

Phagocytosis at a given time point was expressed as the percentage decrease of the initial number of the viable extracellular bacteria.

Micromethod to assess intracellular killing. Intracellular killing of microorganisms was measured as the decrease in the number of viable intracellular bacteria during incubation of phagocytes containing ingested bacteria (19). In brief, 10^6 pre-opsonized bacteria were incubated with 10^6 monocytes or granulocytes in a suspension of 200 μ l for 3 min at 37° C under at 4 rotations/min. Phagocytosis was stopped by rapid chilling to 4° C in ice, and the noningested bacteria were removed by differential centrifugation and two washes with ice-cold HBSS. Next, the phagocytic cells containing ingested bacteria were resuspended to a concentration of 5×10^6 /ml (volume of 200 μ l) and incubated at 37° C under 4 rotations/min in the presence of 10% AB serum. At various time points, a 50- μ l sample was taken added to 450 μ l distilled water to disrupt granulocytes. Lysis of monocytes was achieved by three cycles of freezing and thawing in liquid nitrogen. Next, serial 10-fold dilutions of the lysate were made and the number of viable bacteria was determined by colony counts as described under the phagocytosis assay.

Intracellular killing at a given time point was expressed as the percentage decrease in the initial number of the viable intracellular bacteria.

Fc γ and C3b receptors. The presence of Fc γ and C3b receptors on cord blood monocytes was investigated in cytochrome preparations with IgG-coated sheep erythrocytes and IgM- and complement-coated sheep erythrocytes, as described elsewhere (10).

Oxygen (O₂) consumption by cord blood phagocytes. The consumption of O₂ by cord blood granulocytes and monocytes was assessed as the decrease in the O₂ concentration of the extracellular medium in suspension, measured with a Clark-type oxygen electrode (30) (Oxygen Monitor, Yellow Springs Instruments, Yellow Springs, OH).

Hydrogen peroxide (H₂O₂) production by cord blood phagocytes. H₂O₂ production was measured as the oxidation of fluorescent scopoletin to its nonfluorescent product as described by Root *et al.* (28). 2.5×10^6 phagocytic cells in 3 ml HBSS without phenyl red were incubated in the presence of 10 nM scopoletin (Sigma Chemical Co.), 1 mM NaN₃, and 30 units horseradish peroxidase (Sigma) at 37° C; fluorescence was recorded with a Perkin-Elmer 3000 spectrophotometer (Perkin-Elmer, Beaconsfield, England) at 460 nm when activated at 360 nm. H₂O₂ production was induced by the addition of 10^9 opsonized heat-killed *S. aureus*.

Calculations. All results are means and standard deviations of at least five experiments performed with five different cord blood

samples on different days. Data on cells from healthy adults, i.e. control cells, are means and standard deviations of at least 10 experiments performed on different days. The results of the various kinds of experiment, i.e. the percentage cells with receptors, the percentage phagocytosis, and the percentage of intracellular killing obtained for cells of healthy adults, followed a log normal distribution. Statistical analysis to compare data of cord cells with cells of healthy adults was performed with a two-tailed Student's *t* test for unpaired observations after logarithmic transformation of the results.

The results of the experiments on O₂ consumption and H₂O₂ production experiments by cord blood cells were compared with the results of similar experiments with adult cells on the same day. Statistical analysis of these results was performed on a *t* test for comparison of means for paired samples.

RESULTS

Number of phagocytic cells in cord blood. The absolute numbers of granulocytes and monocytes in heparinized cord-blood were calculated on the basis of the total number of white cells and the differential count and amounted to 5.6×10^9 /liter (range, $2-8 \times 10^9$) and 0.8×10^9 /liter (range, $0.2-1.3 \times 10^9$), respectively. After differential centrifugation of cord blood on Ficoll-Hypaque, approximately $60 \pm 15\%$ of the granulocytes and $90 \pm 13\%$ of the cord monocytes were recovered.

Cytochemical characteristics of cord blood phagocytes. Esterase staining revealed that only 58% of the cord blood monocytes were esterase positive, which is significantly lower ($p < 0.001$) than the corresponding percentage of adults' monocytes (Table 1).

Peroxidase staining revealed similar percentages of monocytes containing peroxidase-positive granules for newborn and adults ($p > 0.1$; Table 1). Almost all of the cord blood granulocytes were peroxidase positive (data not shown).

Fc γ and C3b receptors on cord blood monocytes. The percentage of blood monocytes with Fc γ and C3b receptors did not differ between adults and newborns ($p > 0.1$; Table 1).

Phagocytosis of microorganisms by cord blood granulocytes. Phagocytosis of *E. coli*, *S. aureus*, *S. pyogenes*, and group B streptococci by cord blood and adults' granulocytes at a bacteria-to-cell ratio of 1:1 at 37° C was equally effective ($p > 0.1$; Fig. 1). A 10-fold increase in the number of bacteria relative to the number of granulocytes did not alter either the rate or degree of phagocytosis over a period of 120 min (data not shown). In control experiments in which the various bacteria and granulocytes were incubated in the absence of serum or in the presence of serum but without rotation, no phagocytosis occurred.

Intracellular killing activity of cord blood granulocytes. After incubation of cord granulocytes containing *E. coli*, *S. aureus*, *S. pyogenes*, or group B streptococci, for 120 min, the number of viable intracellular bacteria was decreased to a comparable degree as found for adults' granulocytes (for all bacteria species, $p > 0.1$; Fig. 2).

Phagocytosis of microorganisms by cord blood monocytes. Incubation of cord and adults' monocytes with *E. coli*, *S. aureus*,

Table 1. Cytochemical characteristics and surface receptors of blood monocytes from neonates and adults*

	Cord blood monocytes (%)	Adult blood monocytes (%)	<i>p</i>
Esterase activity	58.0 ± 13.0	95.0 ± 3.0	<0.001
Peroxidase activity	90.4 ± 1.7	87.2 ± 6.4	NS
IgG receptors	89.8 ± 13.6	96.7 ± 2.6	NS
C3b receptors	84.0 ± 9.0	82.7 ± 5.5	NS

* Values are mean (percentage) and standard deviations of 10 samples. NS, not statistically significant ($p > 0.1$).

Fig. 1. Phagocytosis of microorganisms by cord blood (●) and adults' (○) granulocytes. The results are the means of at least five experiments with maximal standard deviations of 5%.

Fig. 2. Intracellular killing of microorganisms by cord blood (●) and adults' (○) granulocytes. The results are the means of at least five experiments with a standard deviation of maximally 9%.

or *S. pyogenes* gave a comparable decrease in the number of viable extracellular bacteria (for all three species: $p > 0.1$; Fig. 3). When the bacteria-to-monocyte ratio was 10:1 instead of 1:1, there was no change in the rate of ingestion of bacteria by cord monocytes (data not shown).

Incubation of cord and adults' monocytes with group B streptococci at a ratio of 1:1 showed a lower rate of phagocytosis in the former ($p < 0.01$) (Fig. 3). At a bacteria-to-monocyte ratio of 10:1, the ingestion of group B streptococci was also decreased (data not shown).

Intracellular killing activity of cord blood monocytes. Incuba-

tion of cord and adults' monocytes containing ingested *E. coli* or *S. pyogenes* revealed no divergence in the rate of intracellular killing of these microorganisms ($p > 0.1$; Fig. 4). However, no intracellular killing of *S. aureus* by newborns' monocytes was observed, whereas the intracellular killing of group B streptococci by cord blood monocytes ($58 \pm 10\%$, $n = 5$ at 120 min) was suboptimal compared with the killing by adults' blood monocytes (97 ± 2 , $n = 10$ at 120 min) (Fig. 4).

O₂ consumption and H₂O₂ production by cord blood phagocytes. Determination of the metabolic burst activity of cord blood granulocytes and monocytes, as reflected by O₂ consumption

Fig. 3. Phagocytosis of microorganisms by cord blood (●) and adults' (○) monocytes. The results are the means of at least five experiments with a maximal standard deviation of 7%.

Fig. 4. Intracellular killing of microorganisms by cord blood (●) and adults' (○) monocytes. The results are the means of five experiments with a maximal standard deviation of 13%.

and H_2O_2 production during extracellular membrane triggering by opsonized heat-killed *S. aureus*, revealed that cord blood granulocytes and monocytes are as active as granulocytes and monocytes from healthy adults (Table 2).

DISCUSSION

In the present study, the main differences between cord blood monocytes and monocytes from adults were a significantly decreased percentage of esterase-positive cord blood monocytes, a decreased capacity of these cells to phagocytose group B streptococci, and a decreased rate of intracellular killing of *S. aureus* and group B streptococci by cord blood monocytes. No differences were found between the various phagocytic and microbicidal activities of granulocytes isolated from newborns and adults.

A significantly lower percentage of esterase-positive monocytes in newborns' compared with adults' blood monocytes might be due to a difference in maturation between cells of both sources

(10, 26) or due to a diminished activity of the enzyme resulting in a decreased staining.

$Fc\gamma$ and C3b receptors were present in similar percentages on blood monocytes and granulocytes from newborns and adults. Since ingestion of bacteria is mainly (27) mediated via these receptors, this finding is consistent with the identical ingestion of most of the microorganisms by both kinds of granulocytes and monocytes. The results of the present study also confirmed earlier reports on the phagocytic capacities of cord blood phagocytes (7, 8, 20, 22, 25). Because of a similar opsonization of bacteria used in studies with adult and cord blood monocytes, the decreased rate and degree of ingestion of group B streptococci by cord blood monocytes, also found for another strain of these microorganisms (data not shown), was unexpected remains unexplained.

The rate of intracellular killing of the various species of microorganisms was similar for newborns' and adults' blood granulocytes, as well as for *E. coli* and *S. pyogenes* by monocytes. The main difference between the monocytes from the two sources

Table 2. O_2 consumption and H_2O_2 production by granulocytes and monocytes from cord blood and adults' blood*

	O_2 consumption (nmol/5 $\times 10^6$ cells/5 min)	n	H_2O_2 production (nmol/5 $\times 10^6$ cells/5 min)	n
Cord blood granulocytes				
(NS)				
Mean	35.6	10	19.7	10
Range	18.1–47.3		17.4–36.7	
Adults' blood granulocytes				
Mean	28.9	10	22.3	10
Range	12.1–38.3		9.6–34.1	
Cord blood monocytes				
Mean	38.0	10	8.1	10
Range	23.1–47.2		2.8–17.6	
Adults' blood monocytes				
Mean	44.2	10	8.2	10
Range	21.2–55.3		4.1–18.0	

* O_2 consumption and H_2O_2 production were measured during extra-cellular triggering with 10^9 heat-killed *S. aureus*. The age-dependent differences in mean O_2 consumption and H_2O_2 production by granulocytes were not statistically significant ($p > 0.1$).

was the decreased capacity of those from newborns to kill ingested *S. aureus* and group B streptococci. The reason for defective intracellular killing by cord blood monocytes could be immaturity of certain bactericidal systems in newborns' monocytes. However, the normal O_2 consumption and H_2O_2 production by newborns' phagocytes indicates normal functioning of the O_2 -dependent microbicidal systems of these cells. Therefore, another explanation must be sought for the differences seen in the intracellular killing of various microorganisms by cord blood monocytes. The results of the present study do not, however, point in any particular direction.

Identical bactericidal activities have been described for cord blood monocytes and adults' blood monocytes (3, 14, 24), but these results cannot be considered reliable because the concentrations of phagocytic cells used in the experiments were too low (at least 5×10^6 phagocytic cells/ml are required for reliable analysis of the results; (16, 17, 19) and because methods measuring phagocytosis and intracellular killing separately were not used (3, 14, 24, 31). Recent investigations in newborn animals showed deficient functioning of alveolar and peritoneal macrophages (2, 29).

The susceptibility of newborns to group B streptococcal infections may be partially due to malfunctioning of blood monocytes and macrophages. The fact that cord blood monocytes are unable to kill group B streptococci efficiently raises the question whether the microbicidal activity of the macrophages in the liver and spleen, which originate from blood monocytes (6; R. van Furth and M. M. C. Diesselhoff-den Dulk, unpublished observations) might also be impaired such that group B streptococci are not adequately cleared from the circulation.

Another source of problems which has not been taken sufficiently into account is that all too often only one species of microorganism is used in functional studies on phagocytes. The results of the present study show clearly that comparability of the bactericidal capacity of monocytes from newborn and adult monocytes for one or two species of microorganisms does not permit the conclusion that intracellular killing of other species will also be normal.

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