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Phagocytosis of microspheric hydrophile particles by peripheral blood glass-adherent phagocytes of healthy and high risk neonates

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The human neonate is uniquely susceptible to severe and overwhelming bacterial infections [8, 20]. The incidence of infection during the neonatal period is inversely proportional to gestational age and birth weight of the infant. Morbidity and mortality from sepsis neonatorum and meningitis is highest in very low birth weight infants [22].

The increased frequency of infection is multifactorial, but is principally due to developmental deficiencies of the neonatal immune system [15]. While a number of abnormalities have been described in the host defense system of newborn infants [24], one of the most important appears to be in the function of the polymorphonuclear leucocyte (PMN) [9]. However, some results of previous investigations of phagocytosis, oxidative metabolism, and bactericidal capacity during the neonatal period are still contradictory. In addition, although numerous investigators have reported subtle functional abnormalities in the PMN of term newborns, only little information is available regarding the preterm newborn. This paper presents the results of our studies on the phagocytic activity of peripheral blood glass-adherent leucocytes of preterm newborns and small- for-date newborns in comparison with the cells of healthy term newborns.

Materials and Methods

Patients

Phagocytosis was studied in 97 preterm newborns ("appropriate for gestational age", mean 33,1 weeks, range 27—36 weeks), 35 small-for-date newborns ("small for gestational age", mean 38,2 weeks, range 37—41 weeks) and 46 term newborns (control group, mean gestational age 40,1 weeks, range 39—41 weeks; see Tab. I). The mean birthweight was 1885 g (range 740—2470 g) for preterm, 2180 g (range 1790—2480 g) for small-for-date and 3527 g (range 3040—4230 g) for term newborns. All of the neonates were clinically normal and no infant was on antibiotics when studied.

Phagocytic assay

Blood samples were obtained by heel-skin puncture within the first week of life. The phagocytic test, employed in the pre-sent study, was described in detail previously [13, 14]. Briefly, few drops (approximately 0,2 ml) of fresh blood were placed on a glass microscopic slide. Blood

TABLE I

Groups of neonates

Group 1: Healthy newborns (control group)	n = 46
Group 2: Preterm infants (birth weight 2001—2500 g)	n = 28
Group 3: Preterm infants (birth weight 1501—2000 g)	n = 28
Group 4: Preterm infants (birth weight 1001—1500 g)	n = 25
Group 5: Preterm infants (birth weight $\leq$1000 g)	n = 16
Group 6: Small-for-date newborns	n = 35

was allowed to clot during the 30 min incubation at 37 °C, the blood clot was then removed and the slide flushed with tissue culture medium (MEM, SIFIN, Berlin); the glass-adherent cells were immediately used for the phagocytic test. Few drops of opsonized microspheric hydrophile particles (MSHP, Institute for Research, Production and Apllication of Radioisotopes, Prague), were applied over the monolayer of adherent cells and the slides were incubated in a moist chamber for 20 min at 37 °C. After incubation, the slides were flushed with MEM, the cells were fixed with methanol and stained with Giemsa (diluted 1 : 5) for 20 min. 100 cells were examined microscopically, the percentage of phagocytosing leucocytes as well as the number of engulfed MSHP was determined.

Statistical analysis of results

For the description of experimental data, basic statistical characteristics of parameters x, y and z of cell activity, i.e. means ($\bar{x}$), variances (S^2), standard errors (SE) and 95% confidence intervals were calculated (Tab. II). For statistical analysis the Bartlett test, the Scheffe's method and the Welch approximation of the t-test were performed.

RESULTS

The phagocytic activity of glass-adherent peripheral blood leucocytes of preterm and small-for-date newborns was compared with the activity of cells from healthy term neonates. Three parameters of cell phagocytic activity were used: percent of active phagocytosing cells (x), mean number of phagocytosed particles per 1 cell (y) and mean number of engulfed particles per 1 active, phagocytosing cell (z). The results, summarized in Table II and Figures 1, 2 and 3

TABLE II

Phagocytosis of MSHP by glass-adherent leucocytes of healthy term, preterm and small-for-date newborns. Statistical characteristics of individual experimental groups

Group	1		
	x	y	z
n	46	46	46
$\bar{x}$	27.87	0.9439	3.3902
S^2	6.69	0.0108	0.0672
SE	0.38	0.0153	0.0382
95% c. int.	27.1— 28.64	0.9129— 0.9749	3.313— 3.4675

(Table II. cont.)

Group	2		
	x	y	z
n	28	28	28
$\bar{x}$	24 04	0.7343	3.0451
S^2	6.92	0.0134	0.0517
SE	0.5	0.0219	0.043
95% c. int.	23.02— 25.06	0.6893— 0.7793	2.9569— 3.1333

Group	3		
	x	y	z
n	28	28	28
$\bar{x}$	22.68	0.6175	2.7253
S^2	6.82	0.0052	0.0168
SE	0.49	0.0136	0.0245
95% c. int.	21.67— 23.69	0.5896— 0.6454	2.6749— 2.7756

Group	4		
	x	y	z
n	25	25	25
$\bar{x}$	21.04	0.5264	2.4973
S^2	9.37	0.0083	0.0229
SE	0.61	0.0182	0.0303
95% c. int.	19.78— 22.3	0.4888— 0.564	2.4348— 2.5578

Group	5		
	x	y	z
n	16	16	16
$\bar{x}$	20.31	0.4063	1.9869
S^2	10.63	0.0073	0.0341
SE	0.82	0.0213	0.0462
95% c. int.	18.57— 22.05	0.3609— 0.4517	1.8886— 2.0853

Group	6		
	x	y	z
n	35	35	35
$\bar{x}$	22.11	1.1523	5.1989
S^2	5.34	0.0322	0.2041
SE	0.34	0.0303	0.0764
95% c. int.	21,32— 22.9	1.0407— 1.2139	5.0439— 5.3539

x = percent of active phagocytosing cells. y = mean number of phagocytosed particles per 1 cell. z = mean number of engulfed particles per 1 active cell.

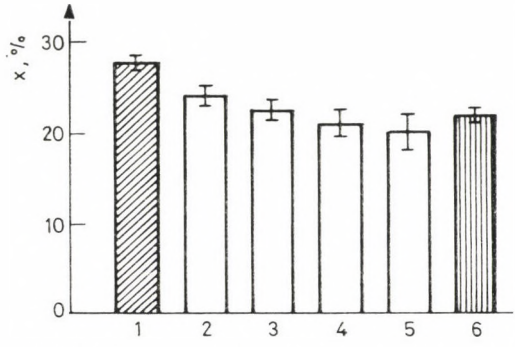

FIG. 1. Phagocytosis of MSHP by glass-adherent leucocytes of healthy term (1), preterm (2—5) and small-for-date (6) newborns. Percent of active phagocytosing cells (x)

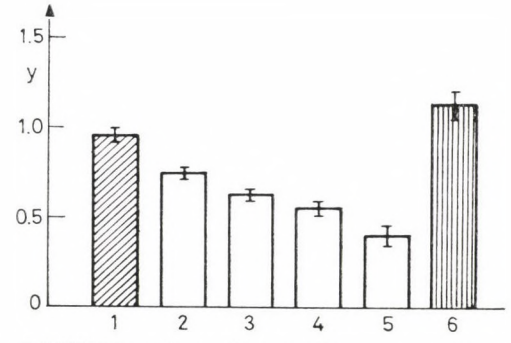

FIG. 2. Phagocytosis of MSHP by glass-adherent leucocytes of healthy term (1), preterm (2—5) and samall-for-date (6) newborns. Mean number of phagocytosed particles per 1 cell (y)

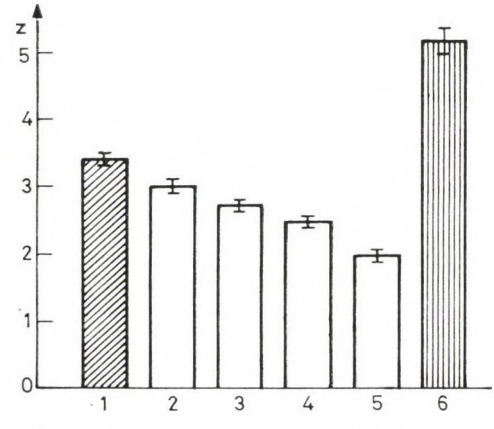

FIG. 3. Phagocytosis of MSHP by glass-adherent leucocytes of healthy term (1), preterm (2—5) and small-for-date (6) newborns. Mean number of engulfed particles per 1 active, phagocytosing cell (z)

showed that the percentage of active phagocytosing cells (x) was significantly lower in preterm newborns (group 2–5) and small-for-date newborns (group 6) compared with the control group (1) of term neonates. It was further shown, that there is a significant correlation between prematurity and impaired phagocytosis, i.e. the percentage of active phagocytosing cells (x) in very low birth weight infants (groups 4 and 5) is significantly lower than in group 2 of the preterm newborns. However, the group of small-for-date infants (group 6) did not differ significantly from preterm infants (Fig. 1). As regards the mean number of engulfed particles per 1 cell and per 1 active cell (parameters y and z) the statistically evaluation of our results has shown, that there is a significantly decrease together with decreasing birth weight, and, furthermore, that in small-for-date newborns these parameters are significantly higher (Fig. 2 and 3).

Discussion

The aim of the present study was to obtain deeper insight into the function of the neonate phagocytic system particularly in preterm and small-for-date newborns using a new phagocytic assay with microspheric hydrophile particles. Our results showed that the values of all parameters investigated for determining cell phagocytic activity were significantly lower in preterm newborns as compared with term neonates. It was further shown, that there is a significant correlation between prematurity and impaired phagocytosis; i.e. the phagocytic activity significantly decreases together with decreasing birth weight.

Comparable studies in the premature newborn are limited and the results still conflicting. In preterm newborns, both normal uptake of various bacteria e.g. staphylococci [5, 6, 17, 18], Ps. aeruginosa [4], C. albicans [25], streptococci [21] or non-living particles such as latex beads [23], and impaired phagocytosis of yeasts [1, 3, 16], carbon particles [7] and immunobeads [11], was described. Finally, increased uptake of colloid carbon particles of preterm infants was reported [19]. As regards other perinatal risk factors (besides prematurity) and their effect on PMN phagocytosis, only limited data are available also. In small-for-date infants the functional defect of phagocytic cells concerns chemotaxis [2], uptake of staphylococci [10] and NBT reduction [12]. In our study we have shown that small-for-date newborns have a significantly lower proportion of actively phagocytosing cells than healthy term newborns, but, in contrast, the mean number of ingested particles per cell was found to be higher than in term newborn cells.

On the basis of above mentioned data one may conclude that it is necessary for studies of phagocytosis in high risk neonates to have suitable, reproducible and sensitive phagocytic assays which are able to uncover such subtle defects.

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