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Defective Opsonophagocyte Function in Newborns Studied by Luminol Dependent Chemiluminescence

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The ability of polymorphonuclear cell (PMNC) to mount a normal respiratory

burst is dependent upon both humoral (opsonic) and cellular (oxidative) metabolic factors and defects in either of these

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parameters are frequently associated with increased susceptibility of infection. The importance of normal serum opsonic and PMN metabolic function in host defence against infection promoted the development of *in vitro* clinical methods designed to assess phagocytosis. During the process of phagocytosis, PMNC undergo remarkable alteration in oxidative metabolism(1). One such change is a generation of chemiluminescence as originally described by Allen *et al.*(1). Recently, many investigators have employed the compound luminol, luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione) as a method of amplifying the chemiluminescence response(2). The luminol dependent chemiluminescence (LDCA) permits use of far lower number of cells and has become the method of choice for screening PMNs for defects in oxidative metabolism. Improper opsonization of particle is documented in patients with clinically defective immunoglobulin, complement component and natural tuftsin disorders, resulting in increased susceptibility to infection(3).

From our previous studies on newborn infections, we found that absolute PMNC count was a good parameter for predicting the outcome of disease, *i.e.*, survival or death. Hence, we decided to explore the defect, whether it is in the neutrophil function or opsonic activities in normal newborns.

Material and Methods

Blood was collected from the cord of newborns for the studies. Neutrophil function was studied by chemiluminescence in 10 normal full term newborns and compared with 8 normal adults.

Chemicals: Zymosan A (Sigma) is a preparation from *Saccharomyces cerevisiae* cell wall. A stock solution of 500 mg of

zymosan was prepared in phosphate buffered saline (PBS) (pH 7.4), kept in water-bath for one hour and centrifuged at 1000 rpm. It was resuspended in fresh PBS to a final concentration of 50 mg/ml. The stock solution was kept at 4°C until use.

Opsonized Zymosan: Zymosan was opsonized with normal serum. To one part of stock solution three parts of normal or patient serum was added, incubated for 15 minutes at 37°C with intermittent shaking and centrifuged at 1000 g. The supernatant was decanted and the pellet resuspended in PBS to the original four part volume. This was kept in dark at 4°C till use.

Luminol: (5-Amino-2, 3-Dihydro-1, 4-phthalazinedione): A concentrated solution was prepared by dissolving 10 mg of luminol and 1 ml of dimethylsulphoxide (DMSO) in 19 ml of PBS to make a concentration of 0.5 mg/ml. One ml aliquots were frozen at -20 °C. These were enclosed in a foil to prevent light exposure.

The blood samples were collected into heparinized tubes. Red cells were lysed by 0.87% ammonium chloride (NH₄Cl) for 15 minutes. Residual WBCs were washed twice with PBS. Polymorphs separated by Ficoll were counted and resuspended in PBS to desired concentration of 0.5 X 10⁶ cells. Glass scintillation vials were prepared containing 0.4 ml (5 mg) opsonized dark adapted zymosan with 5.1 ml of PBS. These vials were covered with foil and 40 µl of luminol from stock solution was added. This was placed in /? counter. The vials were allowed to equilibrate to ambient temperature of 20°C and dark adopted for 20 minutes. The background counts were taken such as empty vial, vial with luminol and vial with luminol plus cells. After this, under red illumination leucocytes 0.5 x 10⁶ cells of 1 ml was added to each vial at 0 time.

Mixture was agitated for 30 seconds and each vial was counted for one minute at four minutes interval. Chemiluminescence activity was thus determined. Four combinations were chosen for the assay to determine where exactly the defect lies, (i) newborn cells (NBC) and newborn serum (NBS), (ii) newborn cells with adult serum (ADS), (iii) adult cells (ADC) with newborn serum, and (iv) adult cells with adult serum.

The results of the study are depicted in Table I. The peak count of newborn PMN was 1288 ± 919 adult PMN was 154777.5 ± 74339 in autologous sera. This shows newborn PMN cells are poorer in function compared to adult which is statistically significant ($p < 0.05$). The peak adult PMN count in autologous sera was 154777.5 ± 74339 , while in the presence of newborn sera was 82375 ± 50299 . This shows newborn sera is poorer in opsonic function as compared to adult sera though statistically not significant.

The peak count of adult PMN (154777.5 ± 74339) was significantly higher than newborn PMN (1396 ± 717) in adult sera. Similarly, adult PMN was more active than newborn PMN in the presence of newborn sera.

TABLE I-Peak PMN Count

	Peak PMN count	
	Mean	(SE)
1. NBC + NBS	1288	(919)
2. NBC + ADS	1396	(711)
3. ADC + NBS	82375	(50299)
4. ADC + ADS	154777.5	(74339)
Comparison	1 & 4	
	2 & 4	$p < 0.05$
	1 & 3	

Discussion

The phenomenon of PMN generating light or chemiluminescence (CL) was first reported by Allen(1,3,4) and has been linked with increased glucose oxidation via hexose monophosphatase shunt of leukocytes undergoing phagocytosis specifically(3,4). As CL is a byproduct of complex cellular metabolic activity of PMN during phagocytosis, it has been utilized to detect intracellular abnormalities of PMN function as well as extracellular opsonic deficiencies in various conditions(5,6,7). The application of the luminol dependent CL assay as a clinical test in terms and preterm infants is possible due to minimal requirement of PMN concentration.

We have studied the PMN metabolic activity as well as opsonic activity of newborns. We observed marked defect in phagocytic function of PMN and marginally poor opsonic activity.

LDCA analysis holds several advantages over existing clinical tests for assessing phagocytic function like (i) Relative ease with which it can be performed; (ii) Improved sensitivity requiring fewer number of cells; (iii) Ability of LDCA to be performed using a standard liquid scintillation counter; and (iv) Requirement of least amount of chemicals.

Our study suggests that one of the factors for the poor ability to fight infection in newborn is due to poor opsonophagocytic function. The opsonic activity can be aided by fresh plasma or intravenous gammaglobulins. The phagocytic function can be aided with irradiated granulocytes.

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Anthropometric Data in Term Newborns

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Many anthropometric studies have been conducted with a view to establish the criteria for prematurity. We report some anthropo-

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metric data measured on 1000 consecutive term normal birth weight neonates.

Material and Methods

The measurements made with the measuring tape included total arm length from tip of the acromian to the tip of middle finger, upper arm length from tip of acromian to the tip of olecranon with elbow bent at 90°, lower arm length from tip of olecranon to the styloid process, leg length from lateral femoral condyle to the lateral malleolus with leg flexed at popliteal angle of 90° and thigh circumference at the level of maximum projection of medial surface below the gluteal sulcus and at right angle to the long axis of thigh. The measurements with the Vernier callipers included hand length from distal wrist crease to the tip of middle finger, hand breadth from the root of the thumb along a horizontal line across the palm, little finger length from the first phalanx to the tip, elbow width as distance between the most lateral point of lateral