Deficient Serum Opsonic Activity for Macrophage Function in Newborn Infants[1] (37454)

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Phagocytosis by the fixed macrophages of the reticuloendothelial system (RES) in the liver and spleen represents an important systemic host resistance mechanism relative to the clearance of foreign particulate material from the blood stream (1). The critical importance of RES clearance function *in vivo* relative to host immunity is clearly reflected in the altered state of immunity and increased susceptibility of the splenectomized animal to septicemia (2, 3). Recent studies demonstrate that optimal hepatic and splenic macrophage phagocytosis is dependent on the presence of a specific plasma protein opsonic factor (1, 4–6) which is a heat-labile alpha-2-globulin (5). Decreased levels of this opsonic protein in the blood results in decreased RES phagocytosis whereas replacement of opsonic protein leads to a return of phagocytic capacity (1). In contrast to the abundant information on the interrelationships between opsonic and phagocytic activity in the adult animal and human experimental model, little information exists as to the functional opsonic or phagocytosis promoting capacity of serum in the newborn infant. The present study was designed to evaluate the phagocytosis promoting or opsonic activity of serum in the newborn human relative to hepatic Kupffer cell population.

Materials and Methods. In the present study, twenty-nine full-term, normally delivered infants (17 females and 12 males) between one and nine days of life were evaluated for opsonic activity, and twenty-three healthy adults (15 males and 7 females) with a mean age of 28.4 years served as donors of adult control serum for comparison.

Blood was collected (1–2 ml) from the infants by the heel stick technique, allowed to clot and subsequently centrifuged at 1500 rpm for five minutes for serum collection. Control blood was obtained by antecubital venipuncture in the adults and treated in the same fashion. Babies with serum bilirubin concentrations above 10 mg/100 ml were excluded. Coded sera were maintained at 4° and assayed for opsonic activity within two hours.

Circulating serum opsonic activity was assayed utilizing triplicate determinations in an isotopic *in vitro,* liver slice micro-bioassay. This basic procedure has been previously employed to assay circulating serum opsonic activity in dogs, rats, and mice as well as humans (1, 6). In this technique, 0.2 ml of test serum was combined with 1.3 ml of a buffered Krebs–Ringer phosphate solution (pH 7.4), 50 USP units of heparin, and 1000 μg of a radiolabelled gelatinized R.E. test lipid emulsion. To the assay mixture was added a liver slice weighing approximately 100–150 mg, prepared by a Stadie Riggs tissue slicer employing adult male Holtzman rats (250–300 g). The reaction mixture was incubated under a gas phase of 95% O_2–5% CO_2 at 37° for 30 min with oscillation. Following incubation, the liver slices were removed, washed in cold isotonic saline, and counted in an autogamma deep well scintillation counter (Nuclear Chicago, Des Plaines, Ill.) for determination of phagocytosis of the

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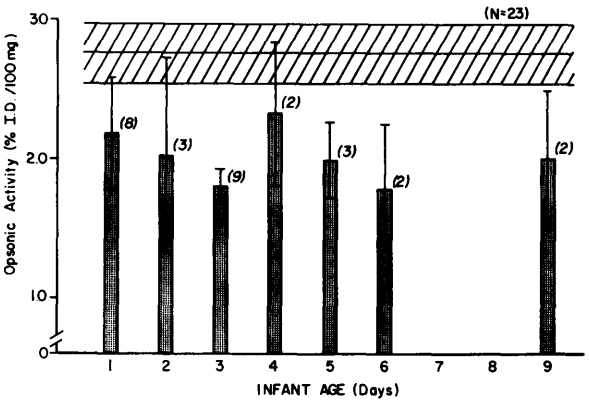

FIG. 1. Circulating serum opsonic activity for fixed RE cells in 1–9 day old newborn infants as compared to adult control levels. Control values are shown as mean ± SEM and the number of infants at each time is indicated. Triplicate determinations were done on each infant. □ = Adult control; ■ = newborn infant.

test colloid by the liver slice as previously described (5, 6).

The gelatinized R.E. test lipid emulsion used in the assay system consisted of a lipid base prepared by blendorizing soya lecithin, glycerol, and ^{131}I triolein (Mallinckrodt Nuclear, St. Louis, Mo.) in a ratio of 1:10:10 by weight, respectively. Prior to use, the lipid base was supplemented with a gelatin (0.1%) containing 5% dextrose and water solution adjusted to a pH 7.4. The gelatinized R.E. test lipid emulsion has previously been demonstrated to be selectively phagocytized by R.E. cells and possesses an average particle size of 1 μm (1, 5, 6).

To evaluate the effect of excess serum hemoglobin on the phagocytic uptake of the particles in the assay system, graded *in vitro* hemolysis was induced in the blood of six infants, to raise the serum hemoglobin levels prior to opsonic assay. Hemolysis was accomplished by agitation of blood before centrifugation and hemoglobin concentration was determined by the benzidine method using 0.02 ml of serum as described by Crosby and Furth (7).

Circulating serum opsonic activity was evaluated in terms of its ability to stimulate hepatic phagocytosis of the test colloid, expressed as the percent injected dose (1000 μg) phagocytized per 100 mg of tissue as previously described (1, 5, 6). Data analysis

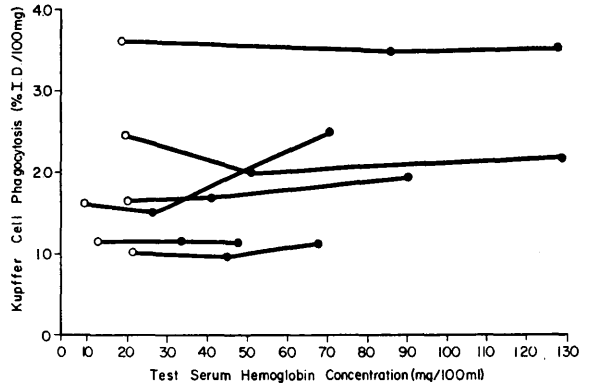

FIG. 2. Opsonin-mediated hepatic Kupffer cell phagocytosis prior to (○) and following (●) the addition of exogenous hemoglobin. Each continuous line represents data relative to the same control (○) value.

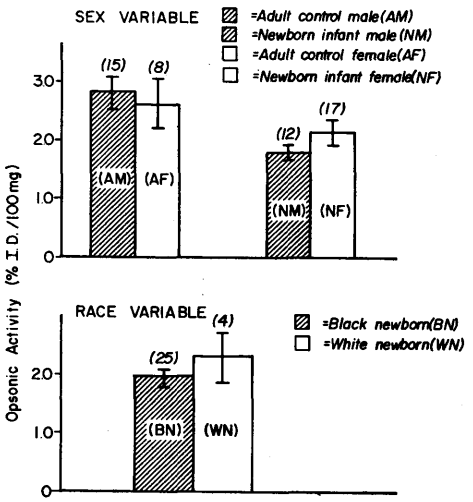

FIG. 3. (Upper) Serum opsonic activity in infant males (NM) and females (NF) and adult males (AM) and females (AF). (Lower) Serum opsonic activity in newborn black (BN) and white (WN) infants. The bar represents the mean ± SEM. No significant differences are noted with respect to race and sex.

was accomplished with the use of a PDP link-8-digital computer (Maynard, Mass.), and all data were subjected to a group t test utilizing a confidence limit of 95%.

Results. Evaluation of circulating opsonin levels in the infants over the first nine days of life revealed no set temporal pattern in the level of circulating serum opsonic activity (Fig. 1). The most distinct difference was in the opsonic activity of the three day old infants in which levels were 65% of adult control serum levels ($p < 0.01$). However, when viewed as a pooled, continuous pattern it can be seen that the opsonin levels appeared to remain consistently below normal range during the early days of the neonatal period.

Since the opsonic assay utilized here depended on phagocytosis of colloidal material, exogenous phagocytizable material, i.e., hemoglobin, in the assay medium might competitively inhibit the phagocytosis of the test lipid emulsion. As seen in Fig. 2, exogenous hemoglobin in the *in vitro* system produced no such effect. In this regard, concentration up to 130 mg% in the incubation media did not inhibit phagocytosis of the test colloid as compared to the control levels in the absence of exogenous hemoglobin.

The variables of sex and race on circulating opsonin levels in the newborn infant are presented in Fig. 3. There was no significant difference in opsonin activity among newborn infants based on race. In addition, it can be seen in Fig. 3, that evaluation of opsonic activity in male and female infants as well as adult controls revealed no significant sex differences for either group in terms of the phagocytosis promoting capacity of the serum.

When the opsonic activity in the group of infants were pooled and compared to the collective levels of adult controls, the level of circulating opsonic activity in the newborn infants was significantly ($p < 0.005$) lower (Fig. 4). Consistent with prior work in the literature (1), phagocytic uptake of the particles by Kupffer cells in the serum devoid medium was minimal, and markedly stimulated by the presence of serum ($p < 0.001$) as presented in Fig. 4.

Discussion. While the precise mechanism of R.E. cell phagocytosis is not known, specific plasma proteins called recognition factors or opsonins, have been demonstrated to

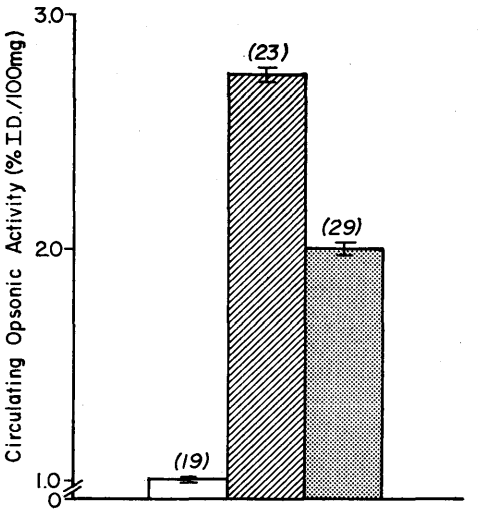

FIG. 4. Serum opsonic activity in buffer control (□) and newborn infants, aged 1–9 days, as a group (29) compared to adult control values (23) ($p < 0.005$). The data are presented as the mean ± SEM. The number of subjects tetsed is shown in parentheses.

be necessary for optimal phagocytosis by R.E. cells (1, 4, 9). In the present study, circulating opsonin levels for fixed macrophage activity during the early days of life were found to be lower in newborn infants than adult controls. This finding extends the previous studies in animals showing reduced opsonic activity in the freshly drawn blood of the neonatal rat especially at 21 days of age (6). While the present sample size is restricted after the third day making statistical inferences difficult on a day-by-day basis, opsonin levels appear to remain collectively low during the first nine days of life. Whether the presence of opsonic activity in the serum even 24 hr after birth reflects previous transplacental passage or endogenous early synthesis of opsonic protein in the infant remains to be clearly determined. However, a comparison of opsonin activity in cord blood with maternal levels at the time of delivery would be informative.

The sex and race of the evaluated subjects appeared to have no major significant effect on levels of circulating serum opsonin. However, throughout the series a tendency for a reduced opsonic activity in the male infant (Fig. 3) was suggested, which may relate to the tendency of male newborns to demonstrate an increased susceptibility to infection possibly due to decreased RES phagocytosis.

While the present study emphasizes the importance of serum factors in the newborn in the regulation of phagocytosis by fixed macrophages, previous studies have already documented a critical role for humoral factors in the phagocytic activity of polymorphonuclear leukocytes from newborn humans (10–13). However, the opsonic protein factor regulating RES phagocytosis as studied in the present investigation appears to differ from the major opsonic component governing polymorphonuclear leukocyte phagocytosis, namely specific antibody and complement (12, 14–16). In this regard, this factor has been recently isolated and identified as an apha-2-globulin which is heat-labile at 60° for 20 min and highly dependent on heparin for expression of its biologic activity (1, 7). Thus, while the deficiency of IgM opsonins in the serum of newborn infants has been implicated as an important factor leading to the increased susceptibility to gram-negative septicemia (12, 17), impaired RES function in terms of blood clearance in the newborn due to low levels of this humoral factor may be of importance in the pathogenesis of both gram-positive and gram-negative systemic infections.

Summary. Circulating serum opsonic or phagocytosis promoting activity relative to Kupffer cell function was deficient on a group comparison basis in newborn infants (1–9 days old) as compared to that manifested by serum from healthy adult controls. This early pattern of opsonin levels in the newborn was not significantly affected by sex or race, although a tendency for lower opsonin activity to be exhibited by serum from newborn males was noted. These findings suggest that hypoopsonemia in the newborn infant may mediate altered R.E. function and increased susceptibility to systemic infection.

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