Acta Paediatrica Hungarica 29 (1—2), pp. 137—141 (1988—89)

Phagocytic cell function after administration of intravenous immunoglobulins in low birth weight neonates

M Xanthou, H Mandyla, S Photopoulos

B'Neonatal Intensive Care Unit and A'Paediatric Department of Athens University "AGHIA SOPHIA" Children's Hospital, Athens-Greece

It is well known that the deficiency in host defence mechanisms of the low birth weight neonates is partly due to their very low immunoglobulin levels at birth [2]. Until recently it has been possible to administer human antibodies to newborns only in the form of whole plasma or immune serum globulin given in small quantities by intramuscular or subcutaneous routes with inconclusive results [8]. However we now have preparations of immune globulins for intravenous use which can be given in much larger doses and which have been modified by either enzymatic or chemical methods in order to avoid systemic reactions [4]. The purpose of our study was to investigate the effect of administration of iv immunoglobulins on the polymorphonuclear (PMN) function of low-birth-weight neonates.

Neonates and Methods

We used an *in vitro* phagocytosis assay where the effect of immunoglobulins on phagocytosis of Pseudomonas aeruginosa by neonatal PMN neutrophils was assessed, using the technique of phagocytic chemiluminescence. With this technique we measure the metabolic activity of the cells which is associated with their phagocytic function [3].

The chemiluminescence responses of the neutrophils of 26 low-birth-weight neonates aged 1—10 days were studied. Fourteen of these neonates had a birth weight < 1500 gr and in 12 their b.w. ranged between 1500—2000 gr. All these babies were admitted to the Intensive Care Unit because of prematurity and/or mild respiratory distress. Ten healthy young adults were used as controls. In 10 neonates the chemiluminescence response of their neutrophils was studied using adult serum as a source of opsonins in addition to their own serum. Finally 12 neonates with a birthweight of 1500 gr received 0.5 g of Sandoglobulin and the chemiluminescence response of their neutrophils was studied before and immediately after the IV administration.

The Swiss Red Cross Immunoglobulin (Sandoglobulin) was used. The polyvalent immunoglobulins in this preparation have been extracted from a pool of 2000 donors and contain all the antibodies (mainly IgG) generally present in the normal population. A daily dose of 0.5 g of lyophilised concentrate of immunoglobulins, was dissolved in 15 ml of NaCl 0.9% and infused over 3 hr, daily.

Pseudomonas aeruginosa (serum resistant mucoid strain isolated from a neonate with septicaemia) was grown overnight in nutrient broth. The bacteria were washed

twice and resuspended in phosphate buffered saline (PBS) to a concentration of 2×10^8 colony forming units (CFU) per ml.

Polymorphonuclears were separated from 3 ml of blood after centrifugation on Ficoll-Triosill and dextran sedimentation. The cells were resuspended in PBS to provide a concentration of $3{,}5 \times 10^5/100$ μl. The neutrophils of each neonate were tested with bacteria opsonized: a) in their own serum b) in pooled AB serum from 5 healthy adults stored at -70 °C.

The bacteria were opsonized with 20% neonatal or adult serum in a shaking water bath at 37 °C for 30 min. They were subsequently washed twice and resuspended in PBS to provide a concentration of approximately $1 \times 10^7/100$ μl.

Chemiluminescence assay

Luminol-dependent phagocytic chemiluminescence (CL) was measured at 37 °C in a luminometer (Pico-Lite Luminometer Packard) according to the method of Easmon et al [3]. Three hundred microliters of 10^{-3} M Luminol and 100 μl cell suspension were added to a reaction vial which was transferred to the Luminometer chamber A suspension of opsonized bacteria (100 μl) was then added to the reaction vial and the light generated was rewarded every minute. Peak values were used for statistical evaluation which was done by the Mann-Whitney V-test.

Results

When neonatal neutrophils were stimulated with bacteria opsonized in their own serum the CL values were found to be very low when compared to those found in adults ($p < 0.001$) and more so in those neonates with a birth weight of < 1500 gr (Fig. 1).

When neonatal neutrophils were stimulated with bacteria opsonized in adult serum the CL responses increased significantly ($p < 0.01$) and reached adult values showing that the low CL values were mainly due to

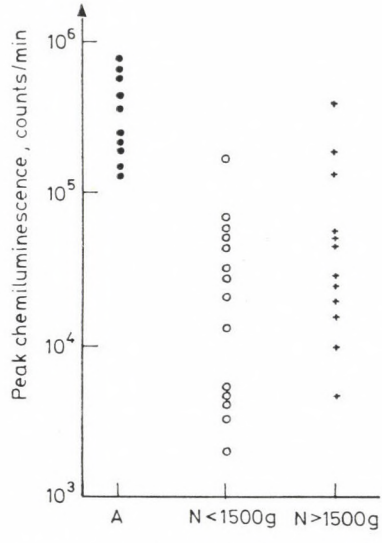

Fig. 1. Peak chemiluminescence values of adult and neonatal peripheral blood neutrophils, when bacteria were opsonized in their own serum

lack of opsonizing factors in neonatal serum (Fig. 2).

Following the I.V. administration of 0.5 g Sandoglobulin the CL responses of neonatal neutrophils, when bacteria were opsonized in their immunoglobulin-enriched serum increased significantly ($p < 0.01$) demon-

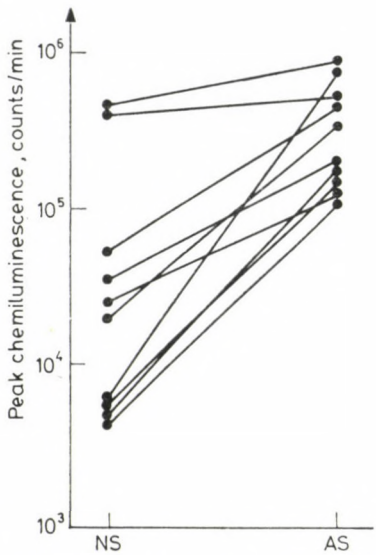

FIG. 2. Peak chemiluminescence values of neonatal neutrophils when bacteria were opsonized in their own serum (N. S) and in adult serum (A. S.)

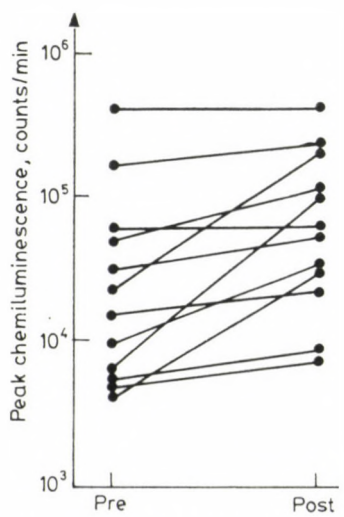

FIG. 3. Peak chemiluminescence values of neonatal peripheral blood neutrophils when bacteria were opsonized with serum before (pre) and immediately after (post) the I. V. administration of 0.5 gr Sandoglobulin

strating that high doses of I.V. immunoglobulins increase neonatal serum opsonic activity (Fig. 3). However the CL values remained significantly lower than those found when neonatal neutrophils were stimulated with bacteria opsonized in adult serum. (Figs 2, 3).

No adverse reactions were observed on the clinical condition of the infants.

Discussion

It is well known that Pseudomonas aeruginosa is a significant cause of nosocomial infections, frequently encountered in immunologically supressed patients and in neonates in intensive care units [5]. Antibiotics have been only partially successful in controlling the morbidity and mortality associated with Pseudomonas infections [1]. Normal adult serum contains all the antibody and complement necessary for phagocytosis of prevalent antigenic types of P. aeruginosa. Low-birth-weight neonates born prematurely do not have the opportunity to aquire these antibodies from their mother [2].

The low phagocytic CL found in our babies can be attributed mainly to lack of specific antibodies since neonatal leucocytes responded well when stimulated with bacteria opsonized in adult serum. The well known deficit of complement components in LBW neonates [6] may have contributed to their low CL response. However it has been found that sera with high titers of specific antibody are able to increase phagocytosis of pseudomonas in the absence of complement [9].

We found that the administration of 0.5 g of I.V. immunoglobulins improved the phagocytic capacity of neonatal neutrophils. However, the phagocytosis was lower than that elicited when adult serum was used. This could be due to the still low antibody levels after only one dose of 0.5 g of Sandoglobulin. It has been shown that 6 daily doses of 0.5 g immunoglobulin should be given to LBW neonates before their levels reach those found in fullterms [7].

From using findings it can be seen that the administration of I.V. immunoglobulins improves opsonic activity of neonatal serum and increases phagocytic chemiluminescence against Pseudomonas aeruginosa.

We think that immunoprophylaxis and immunotherapy will prove to be major weapons in our fight against serious neonatal infections.

References

1. Andriole VT: Pseudomonas bacteremia: can antibiotic therapy improve survival? J Lab Clin Med 94: 196, 1979
2. Conway SP, Dear PRF, and Smith I: Immunoglobulin profile of the preterm baby. Arch Dis Child 60, 608, 1985
3. Easmon CSF, Cole PJ, Williams AJ et al: The measurement of opsonic and phagocytic function by luminoldependent chemiluminescence. Immunology 41: 67, 1980
4. Finlayson JS: "History of immunoglobulin uses" in B. M. Alving and J. S. Finlayson (ed). Immunoglobulins: Characteristics and uses of intravenous preparations. U. S. Department of Health and Human Services publication no FDA 80—9005. Government Printing Office, Washington, DC 1980

5. Flick MR and Cluff LE: Pseudomonas bacteremia: review of 108 cases. Im J Med 60: 501, 1976
6. Johnston RB, Jr, Altenburger KM, Atkinson AW, Jr, and Curry RH. Complement in the Newborn Infant Pediatrics 64: 781, 1979
7. Muralt G. and Sidiropoulos D: Experience with intravenous immunoglobulin treatment in neonates and pregnant women Vox Sang 51: suppl. 2, pp 22, 1986
8. Stiehm ER: Standard and Special human immunoserum globulins and therapeutic agents. Pediatrics 63: 301, 1979
9. Young SL, and Armstrong D: Human Immunity to Pseudomonas aeruginosa. I. In vitro Interaction of Bacteria Polymorphonuclear Leucocyte, and Serum Factors. J Inf Dis 126: 257, 1972