THE PLATELET OF THE NEWBORN INFANT: AGGREGATION AND ADENINE NUCLEOTIDE RELEASE: J.M.Whaun, V.Sochor, P.Lievaert. (Intr. by M.Delivoria-Papadopoulos) Div. Ped., Univ. Calgary, Calgary

Platelet-rich plasma (PRP) was prepared from cord blood collected in 1/12 volume 0.11 M sodium citrate and from adult blood in 1/9 volume citrate. PRP of newborn infants, mothers and normal adults was tested for aggregation with epinephrine, collagen and adenosine diphosphate (ADP). Total adenine nucleotide pools and adenine nucleotide metabolites were studied on ³H-adenine labelled PRP before and after exposure to collagen and epinephrine. Collagen and ADP-induced aggregation was similar in all 3 groups. Epinephrine, which induces 2-wave aggregation in adults, did not aggregate platelets of any infant, regardless of dose. Washed infant platelets, resuspended in group-compatible adult plasma, did not respond to epinephrine. Washed adult platelets, resuspended in compatible infant plasma showed 2-wave response. In unstimulated platelets of neonates, total adenine nucleotide pools were similar to those of adults or mothers. After collagen stimulation, neonatal platelets showed similar release of adenine nucleotides compared to the other 2 groups. Adenine nucleotide labelling patterns were similar in all groups. With epinephrine induction, platelets of neonates neither released adenine nucleotides into the plasma nor showed decreased nucleotides. There was no change in nucleotide labelling patterns. These studies suggest neonatal platelets have different epinephrine receptor sites from those of adults and there may be, in addition, an intrinsic structural membrane difference.

A NEW FORM OF CONGENITAL HEMOLYTIC ANEMIA WITH EXTREME MICROCYTOSIS AND CALCIUM LEAK. James S. Wiley and Frances M. Gill (Intro. by Elias Schwartz) Dept. of Med. and Ped., Univ. of Pa., Philadelphia, Pa.

A 4 year old male child with congenital hemolytic anemia and bizarre red cell morphology has been studied. Many cells showed blunt projections plus fragmentation and there were numerous microspherocytes. Splenomegaly was present and splenectomy at the age of 21 months resulted in an increase in hemoglobin from 4 to 10 gm% and a fall in reticulocytes from 23 to 3%. However, the abnormal red cell morphology persisted. Microcytosis remained extreme with MCV 42 μ^3 and the MCHC was 38-44 gm%. Osmotic fragility was greatly increased. Tests for usual red cell abnormalities and unstable hemoglobin were negative while the intracellular concentrations of Na⁺ and K⁺ were normal. Moreover both active and passive components of Na⁺ and K⁺ fluxes were normal. Inward Ca²⁺ movement was measured in cells in which the activity of the outwardly-directed Ca²⁺ pump was inhibited. When cells were incubated in 1.5 mM Ca²⁺, the entry of Ca²⁺ was increased more than 10-fold, from 64.0 nanomole/ml cells/4 hr. for the patient compared with 5.3 $\pm$ 1.6 nanomole/ml cells/4 hr. for normals. Measurement of Ca²⁺ pump activity in the patient's red cells was normal. The results suggest that a selective inward Ca²⁺ leak may be responsible for the red cell fragmentation and microcytosis in this syndrome.

IMMUNOLOGY

THE ROLE OF SUPEROXIDE ANION (O₂⁻) IN OXIDATIVE METABOLISM OF NORMAL AND CHRONIC GRANULOMATOUS DISEASE (CGD) POLYMORPHONUCLEAR LEUKOCYTES (PMN). Baehner, R.L., Murrmann, S.K. and Johnston, R.B., Jr., Indiana Univ., Dept. of Ped., Indianapolis and Univ. of Alabama, Dept. of Ped., Birmingham.

O₂⁻, a highly reactive, unstable free radical formed by hydrogen peroxide (H₂O₂) producing oxidases, has recently been identified in PMN and may play a role in phagocytic bactericidal activity. The physiologic control of O₂⁻ appears to depend on the enzyme superoxide dismutase (SOD), which catalyzes the reaction O₂⁻ + O₂⁻ + 2H⁺ → H₂O₂ + O₂. Superoxide can be identified by the inhibition of O₂ mediated reduction of cytochrome C (Cyto C) or nitroblue tetrazolium (NBT) by SOD. SOD completely inhibited phagocytic bactericidal activity and the reduction of Cyto C by phagocytizing PMN but increased all H₂O₂ related metabolic reactions: H₂O₂ production (¹⁴C-formate → ¹⁴CO₂) by 30%, hexose monophosphate shunt activity (Glucose-1-¹⁴C → ¹⁴CO₂) 3-fold, and ¹²⁵I fixation to ingested particles 2-fold. PMN from 4 CGD patients (3 with x-linked and 1 female with the autosomal recessive form), which lack these H₂O₂ related metabolic reactions, failed to reduce NBT and Cyto C. These studies confirm that O₂⁻ is generated by normal PMN and suggest that H₂O₂ production by PMN is mediated via O₂⁻ generating oxidase. Increased iodination yet decreased phagocytic killing in the presence of SOD suggests that iodination of ingested microorganisms is not in itself responsible for their death. The failure of CGD PMN to generate O₂⁻ supports the contention that H₂O₂ producing oxidase activity is diminished in these cells.

PARTIAL RECONSTITUTION OF A PATIENT WITH CONGENITAL THYMIC DYSPLASIA BY FETAL TISSUE AND TRANSFER FACTOR. Linda T. Cahill, Sara Kaffé, Photini S. Papageorgiou, Carol S. Petigrow, and Philip R. Glade. Mt. Sinai School of Medicine, Dept. of Peds., New York, N.Y. 10029.

Attempts at immunologic reconstitution of patients with congenital thymic dysplasia have been disappointing. We have studied an 18 month old male with chronic history of mucocutaneous candidiasis (CMC), diarrhea, repeated infections and failure to thrive. He had a marked deficiency of T-cell functions with a decrease in circulating T-cells, absent cutaneous reactivity to skin test antigens and absent PHA responsiveness *in vivo* and *in vitro*. Although lymphopenic he had adequate numbers of circulating B-cells and plasma cells in his bone marrow aspirates. B-cell functions including levels of serum immunoglobulins, isohemagglutinins and pokeweed mitogen responsiveness were within the normal range. Implanted tissues from a female abortion of 16 weeks gestation restored *in vivo* and *in vitro* PHA responsiveness within 24 hours. Karyotypic and HL-A analyses demonstrated that chimerism had not occurred. In an attempt to augment persistently negative responses to candida antigen, multiple doses of transfer factor (2.4 x 10⁸ lymphocytes/dose) were administered. Peripheral T-cell rosettes increased from 5% to 40% without change in his response to candida antigen or his clinical CMC. After eight months of continuing transfer factor therapy the patient has tripled his weight and remains clinically stable.

PREVENTION OF HOMOCYTOTROPIC ANTIBODY RESPONSE IN RATS Mirla F. David-Faridy (Intr. by J.C. Haworth), Univ. of Manitoba, Dept. of Ped., Winnipeg, Manitoba

The prevention of the triggering effect of Bordetella pertussis vaccine on the induction of homocytotropic antibody to ragweed and horse serum was studied in inbred DA and random bred Sprague Dawley rats.

Rats received one of the following treatments: I- pertussis vaccine intraperitoneally (IP) (0.01 ml/g body weight. Vaccine contained 10x10⁹ phase I organisms/ml); II- pertussis IP and antigen (1:10 giant ragweed extract, 0.005 ml/g BW or horse serum 1 ml/rat) in the footpads; or III- antigen (giant ragweed extract or horse serum) in the footpads. Rats were bled 10 days later and serum collected. The presence of homocytotropic antibody against ragweed or horse serum was tested by 48 hour passive cutaneous anaphylaxis on normal adult recipients. Only rats which received antigen simultaneously with pertussis vaccine (II) formed homocytotropic antibody which was specific for the antigen used. If, however, the rats (II) were fed antigen, for 5-7 days, 2 weeks prior to sensitization, they failed to produce homocytotropic antibody to the antigen which they were fed with.

These results suggest that the induction of homocytotropic antibody formation to an antigen can be prevented by pre-feeding the antigen.

IMMUNOLOGIC RESPONSIVENESS DURING PREGNANCY. Mark Diamond and Jean F. Kenny. Children's Hospital of Pittsburgh, Pittsburgh.

Female sex hormones significantly increase immunologic responsiveness by stimulating the proliferation of immunocompetent cells. To determine whether the immune response is altered during pregnancy and lactation, Swiss mice (gestation 19-21d) were studied during early, mid and late pregnancy and in the early postpartum (PP) period. Groups of pregnant (PR) and nursing mice along with equal numbers of nonpregnant female littermate controls (C) were injected with 2x10⁶ heat-killed *E. coli* 0127 and sacrificed 4 days later for enumeration of splenic anti-*E. coli* plaque-forming cells (PFC). Total PFC/10⁸ spleen cells were analyzed by rank and comparison of results from test and control littermate pairs.

Experimental Group	Range PFC/10 ⁸ Spleen Cells	Group Median	% PR > Group Median	% PR of pairs > C	p.
4-10d PR+C(62)	0-103,000	3,632	71%	79%	<.01
14-17d PR+C(46)	3925- 27,750	10,400	74%	100%	<.001
19-21d PR+C(28)	0- 19,140	828	29%	30%	<.05
7- 9d PP+C(40)	1855- 57,059	10,965	50%	44%	>.1

Results indicate that primary immunologic responsiveness is increased during early and mid pregnancy, depressed immediately prior to delivery and normal during lactation. The marked variations observed may be secondary to changes in the balance of female sex hormones and adrenocorticosteroids which occur as pregnancy progresses.