715

DECREASED HYPOCALCEMIC AND HYPOMAGNESEMIC BLEBBING OF NEWBORN NEUTROPHILS. Alan D. Mease, Doris T. Burgess, Gerald W. Fischer and Frederick B. Ruymann.

Department of Pediatrics, Uniformed Services University School of Medicine, Bethesda and Walter Reed Army Medical Center, Washington, DC.

Although chemotactic differences between newborn (NB) and adult (AD) neutrophils (PMNs) are well documented, the cellular basis of this difference is not known and morphologic differences have not been described. PMNs were separated from anticoagulated venous blood of 10 AD controls and the cord blood of 10 term NBs. The PMNs were suspended in protein free balanced salt solutions with and without Ca and Mg and cytocentrifuge preparations were made. The percentages of PMNs with ≥ 3 membrane projections were determined by counting 200-300 PMNs and these PMNs were considered blebbed cells.

CONDITION	NO OF EXPERIMENTAL PAIRS	% $\pm$ SD BLEBBED PMNs	
		AD	NB
Absence of Ca & Mg	6	54.7 $\pm$ 27.4	11.8 $\pm$ 5.4
Presence of Ca & Mg	4	8.5 $\pm$ 5.7	7.5 $\pm$ 3.9

In the absence of Ca and Mg the mean percentage of blebbed NB PMNs was significantly less than that of AD PMNs ($p < .005$). Ca and Mg reduced blebbing in AD PMNs to NB levels. These findings suggest that NB PMNs have an inability to undergo conformational membrane changes in response to alterations in Ca and Mg balance. Since membrane regulation of Ca and Mg flux is important in chemotaxis, a NB PMN membrane abnormality affecting this flux may be the cellular basis of the NB PMN chemotactic defect.

716

IDENTIFICATION OF A NEUTROPHIL MEMBRANE DIFFERENCE BETWEEN ADULT AND NEWBORN CELLS USING A LECTIN-INDUCED ASSAY. Alan D. Mease, Gerald W. Fischer, Askold D. Mosijczuk, Frederick B. Ruymann, and Dale D. Landis.

Department of Pediatrics, Uniformed Services University School of Medicine, Bethesda and Walter Reed Army Medical Center, Washington, DC.

Intrinsic membrane differences between newborn (NB) and adult (AD) neutrophils (PMNs) have been suggested as the mechanism of decreased NB PMN chemotaxis. Using lectin-induced aggregation (AGGR) a rapid turbidometric assay was developed and demonstrated a difference between NB and AD PMN membrane characteristics. PMNs were separated from anticoagulated venous blood of 13 AD controls and cord blood of 8 normal term NBs. With constant temperature and PMN concentration, PHA caused reproducible AGGR of both NB and AD PMNs. The rate (slope) of PMN AGGR was dependent on PHA concentration. At the optimal PHA concentration the mean percentage ($\pm$ S.E.M.) of NB PMN AGGR was significantly less than AD PMN AGGR (NB%AGGR:45.7 $\pm$ 5.4 vs. AD%AGGR 62.5 $\pm$ 1.7, $p < .0025$). The difference between the mean rate of NB and AD PMN AGGR was highly significant (NB slope:12.0 $\pm$ 1.2 vs. AD slope 17.3 $\pm$ 0.7, $p < .005$). Con A did not cause measurable AGGR of either NB or AD PMNs. Preexposure to NB plasma did not alter AD PMN AGGR. These studies using PHA-induced AGGR have identified a NB PMN membrane abnormality when compared to AD PMNs. This technique may be useful in studying other disorders with neutrophil membrane abnormalities.

717

MARROW TRANSPLANTATION (MTP) IN WISKOTT-ALDRICH SYNDROME (WAS): T CELL ENGRAFTMENT WITH CYCLOPHOSPHAMIDE (CY), COMPLETE ENGRAFTMENT WITH TOTAL BODY IRRADIATION. H.J. Meuwissen, M.A. Kieserman, E.G. Taft, B. Pollara, R. J. Pickering. Albany Med. Coll., Birth Defects Inst. and Kidney Disease Inst., N.Y.S. Dept. of Health, Albany, N.Y. 12208.

We studied two patients with WAS who had MTP with marrow from HLA-identical siblings. The first patient received 200 mg/kg CY over 4 days (Bach et al., Lancet 1:1364, 1968). We have followed this patient for 7 years. He has petechiae, bleeding in joints, and severe thrombocytopenia. IgM has remained low; antibody production to some antigens, lymphocyte response to mitogens and in vitro B cell functions are defective. Growth and development have been normal. The second patient received cytosine arabinoside 5 mg/kg/day x7, 6-thioguanine 4 mg/kg/day x7, and CY 50 mg/kg/day x4 prior to MTP. In both patients after MTP, megakaryocytes, red cells, neutrophils, macrophages, and most B cells remained of recipient type while most T cells were of donor type. The second patient was retransplanted after preparation with anti-lymphocyte globulin, 2 ml IV daily x4, Procarbazine, 12.5 mg/kg/day x 3, and 800 R total body irradiation. Ninety days after the 2nd transplant, all nucleated marrow and blood cells, including B and T cells were of donor type; WBC 12,500, platelets 132,000/ mm^3 , hemoglobin 9.8 gms % and reticulocytes 3.5%. No graft-versus-host disease was observed. We conclude that in marrow transplantation for WAS, 200 mg/kg CY may be inadequate preparation. In WAS, T cells may more readily be engrafted than other blood cells. Supported by USPHS GCRC #MO-1-R00749-05.

718

SPLIT PRODUCTS OF C3 IN RHEUMATIC DISEASES OF CHILDREN John J. Miller III, Richard B. Moss, Yao-Pi Hsu, and Angela Koken. Stanford University School of Medicine, Children's Hospital @ Stanford, Dept. of Pediatrics, Palo Alto

Arroyave's method for the detection of C3c and C3d by counter-immunoelectrophoresis (J. Immunol. Methods, 13:101, 1976) has been used to study plasma from children with various rheumatic diseases over a period of 18 mos. Eleven of 11 children with active SLE had (+) tests. Plasma from 2 of 5 children with clinically inactive SLE were (+). Variations with time confirmed Arroyave's finding that (+) tests are sensitive indicators of disease activity in SLE. Plasma from 1 child with MCTD was transiently (+). Consistently (-) tests were found in 7 children with dermatomyositis and 2 with scleroderma. On 1 occasion, 1 of 5 children with ankylosing spondylitis had a (+) test. Thirty of 74 children with JRA were (+) on one or more occasions. By mode of onset these were: 6 of 14 systemic, 13 of 27 polyarticular and 11 of 33 pauciarticular. Six of 8 latex fixation (+) patients had (+) tests for C3c and C3d, but only 9 of 24 ANA (+) patients had (+) tests. Correlations with sedimentation rates or other measures of disease activity were not clear. One traumatic knee effusion and synovial fluid from 2 children with pauciarticular, plasma (-) JRA had (-) tests for C3c and C3d. Synovial fluid and plasma from 1 child with Jacob's "Streaking Leukocyte Syndrome" were (+).

This data is consistent with other work indicating that some, but not all, children with JRA have antigen-antibody complexes in blood or elsewhere which are capable of activating complement.

719

CELL ELASTIMETRY IN THE CHARACTERIZATION OF DISORDERS OF NEUTROPHIL MOTILITY. Michael E. Miller, UCLA School of Medicine at Harbor General Hospital, Department of Pediatrics, Torrance, California 90509

Although clinically similar, disorders of neutrophil (PMN) movement are heterogeneous and involve a number of separable mechanisms. The characterization of these mechanisms has been limited by available research techniques. We recently described the application of cell elastimetry to the study of normal PMN motility. The assay, which measures amounts of negative pressure required to aspirate individual PMNs into micropipettes, provides an estimate of deformability of the PMN. In normal PMNs, deformability has similar Ca^{++} , energy (glycolysis), pH, temperature and contractile protein requirements to those of normal Boyden chamber chemotaxis. It was, therefore, suggested that the assay might provide an important probe in the study of abnormal PMN motility. We now report the first such studies. PMNs from subjects with recognized abnormalities of motility including normal neonates and children with lazy leukocyte syndrome, diabetes mellitus, familial chemotactic deficiencies, sporadic chemotactic deficiencies and hyper-IgE-eczema-infection syndrome were studied in 3 assays: chemotaxis (Boyden chamber); chemokinesis (capillary tube migration); and deformability (elastimetry). The data showed: 1) marked heterogeneity existed among PMN profiles obtained from the various subject groups; 2) a strong correlation existed between chemotaxis and deformability, but none between chemokinesis and deformability; 3) the only condition in which chemotaxis was consistently decreased but deformability normal was in 5 patients with the hyper-IgE syndrome. This strongly suggests a unique mechanism of abnormal PMN motility in that disorder. Since elastimetry is a single cell assay, it lends itself ideally to study of the individual steps of PMN movement in normal and abnormal states.

720

THE EFFECT OF VIRAL INFECTION ON THE ABILITY OF HUMAN LYMPHOCYTES TO DEVELOP ANTIBODY PRODUCING CELLS AFTER MITOGEN STIMULATION in vitro. James E. Nagel, F. Joseph Chrest, William H. Adler, and Mathuram Santosham. Gerontology Research Center, NIA, NIH, and Department of Pediatrics, Baltimore City Hospitals, Baltimore, MD.

Using a plaque forming cell (PFC) assay system the effects of clinically and laboratory diagnosed viral illness were determined on the ability of human peripheral blood lymphocytes to be stimulated with the mitogens phytohemagglutinin (PHA), pokeweed (PWM), staphylococcal protein A (SpA), lipopolysaccharide (LPS) and the antigens candida (C) and tetanus-diphtheria toxoids (Td). In contrast with control subjects who had a geometric mean 7 PFC's/ 10^6 cells, children with viral infections had a mean of 1188 PFC/ 10^6 cells for unstimulated cultures. PWM was found to be the most effective PFC stimulant in control subjects (geometric mean 1995 PFC/ 10^6 cells); however concurrent viral infection resulted in a reduced number (geometric mean 267 PFC/ 10^6 cells) of PWM induced PFC's when compared with background. Control subjects produced no PFC's in response to PHA, Con A, LPS, Td, SpA or C; however, children with rubella and disseminated herpes infection had PFC's with these mitogens, although again the absolute numbers were less than the unstimulated control cultures. These results suggest that viral infection produces dramatic shifts of peripheral blood lymphocyte subpopulation composition and function, reducing the effect of the mitogens on cellular differentiation. Alternatively, direct virus-B cell interaction may result in differentiation to immunoglobulin producing cells without a mitogen-T-B cell interaction.