721 THE BREASTMILK MACROPHAGE: A POTENTIAL VEHICLE FOR IMMUNOGLOBULIN TRANSPORT. William B. Pittard,III, Stephen Polmar,Avroy Fanaroff.CWRU,Ped.Dept.Cleve.,O.

When lymphocytes and macrophages from human milk were co-cultured, significantly greater amounts of IgA and IgM were found than when lymphocytes were cultured alone. To determine whether the breastmilk macrophage was regulating lymphocyte synthesis or itself releasing immunoglobulin, we cultured macrophages and lymphocytes from the fresh milk of 33 healthy mothers. The cellular components were isolated by centrifugation and the lymphocytes separated from the glass adherent macrophages by overnight incubation in glass flasks. The release of IgA,IgM,and IgG into 1 ml of culture media by $2x10^6$ lymphocytes or macrophages was quantitated using double antibody radioimmunoassays. In 7 day lymphocyte cultures mean IgA, IgM, and IgG released was 358, 46 and 11 ng/ml respectively. Mean IgA, IgM, and IgG released in macrophage cultures was 9089, 319, and 9 ng/ml respectively. To determine whether the immunoglobulin present in macrophages from 6 mothers were both cultured for 7 days and on day one sonicated. Sonicates were ultracentrifuged and immunoglobulins in the supernatant and pellet were measured. The immunoglobulin content of the supernatant following sonication of macrophages was significantly greater than both that bound to the cell pellet ($p < .05$) and that of the 7 day culture($p < .05$). These data indicate that the breastmilk macrophage releases significantly more immunoblobulin than does the lymphocyte ($p < .02$) and that it may serve as a transport vehicle capable of delayed immunoglobulin release.

722 TISSUE ADENOSINE DEAMINASE ACTIVITY IN AN ADENOSINE DEFICIENT-COMBINED IMMUNODEFICIENCY DISEASE PATIENT. Bernard Pollara, William P. Schrader, and Hilaire J. Meuwissen. (SPON. BY Richard J. Pickering). Albany Medical College, Dept. of Pediatrics & NYS Kidney Disease Inst., Albany, NY.

Patients with combined immunodeficiency (CID) and adenosine deaminase (ADA) deficiency may have low or absent ADA activity in various tissues. Hirschhorn et al. have shown that the activity in patient fibroblasts is a mutant form of ADA (PNAS 73: 213, 1976). Others have suggested that the deficiency reflects an inhibition of ADA (Trotta et al., PNAS 73: 104, 1976). We have isolated and in part characterized properties of the residual ADA from spleen of one patient. Radioimmunoassay for ADA protein in erythrocytes and spleen extracts of the patient showed no cross-reacting protein. Chromatography of ADA-CID spleen extracts produced a fraction with adenosine deaminating activity which could not be adsorbed on affinity or anti-ADA columns. The deaminating activity could not be inhibited with erythro-9-(2-hydroxyl-3-nonyl) adenosine. The pH optimum activity curve and Km differed from normal spleen ADA. A similar fraction, representing about 1% of the total ADA activity, was isolated from normal spleen extracts. We conclude that the adenosine deaminating activity observed in patients with ADA-CID is not ADA. The activity may be due to an enzyme whose principal substrate may be another metabolite. The presence of this non-ADA deaminating activity in normal spleen suggests that the observed activity in ADA-CID tissue is not due to a mutant form of ADA and that the inhibited enzyme activity reported by Trotta et al. may be due to an amplification of this non-ADA deaminating enzyme.

723 IMMUNOPHARMACOLOGIC STUDIES OF ADENOSINE DEAMINASE DEFICIENT LYMPHOCYTES by Stephen H. Polmar, Erica M. Wetzler and Robert C. Stern, Case Western Reserve University, Departments of Pediatrics and Microbiology, Cleveland,OH

Deficiency of adenosine deaminase (ADA) is associated with a form of severe combined immunodeficiency disease (SCID). Enzyme replacement therapy using red blood cells as a source of ADA restores the ability of ADA deficient SCID lymphocytes to respond to mitogens in vitro but does not increase ADA activity within these cells. The availability of ADA deficient lymphocytes capable of responding to mitogens permitted us to study the effects of various pharmacological agents in order to gain a better understanding of the metabolic basis of this immunodeficiency disease.

Phytohemagglutinin (PHA)-induced proliferation of lymphocytes from an ADA deficient SCID patient were significantly more sensitive to inhibition by theophylline ($p < .01$), norepinephrine ($p < .05$) and prostaglandins E_1 and E_2 ($p < .01$ and $< .0005$, respectively) than lymphocytes from individuals with normal ADA activity. In contrast, there was no significant difference ($p > .4$) in sensitivity to inhibition by adenosine at concentrations less than 100 μ molar. Since theophylline, norepinephrine and prostaglandins E_1 and E_2 elevate intracellular cAMP concentrations, which inhibit many lymphocyte functions, these data suggest that defective lymphocyte proliferation in ADA deficiency may be due to an increased potential for excessive cAMP synthesis. Drugs capable of inhibiting cAMP synthesis or enhancing its catabolism may be useful in the therapy of this disorder. Extracellular adenosine probably plays a minor role, if any, in causing this immunodeficiency.

724 CHRONIC GRANULOMATOUS DISEASE (CGD) IN A BLACK FAMILY WITH ATYPICAL GENETIC TRANSMISSION. Bruce E. Ponce, Catherine U. Kyong, Charles P. Darby, Lapsley G. Hope and H. Hugh Fudenburg. (Spon. by Mitchell I. Rubin). Dept. of Immunology & Pediatrics, Medical University of South Carolina, Charleston, South Carolina.

We studied CGD in a Black family with three children, including a pair of identical twin sisters (10 yr.), DJ and SJ, and brother (13 yr.), BJ. SJ has had recurrent lung, liver abscesses, DJ recurrent pulmonary infections. BJ has always been in good health. DJ, SJ and BJ could not reduce NBT (.04,.03,.03 O.D.). Father (.16) and mother (.12) were normal (.14). Neutrophil superoxide production was absent in twins (.00, .03, O.D.) and brother (.02). Mother (.13) was intermediate between control (.39), father (.34), and children. HMP activity for twins (31, 101 CPM) and brother (85 CPM) were well below control (1350), mother (1307) and father (1350). Neutrophils of twins and brother killed only 23%, 32% and 16% Staph. aureus after 2 hours (control 83%). Mother and father were normal. All had normal neutrophil MPO staining. Phagocytic neutrophil O_2 consumption expressed in nanomoles/min/$12x10^6$ cells was DJ (.00), SJ (.01), and BJ (.00). Father (7.3) was normal (5.5). Mother (.77) was very low. Since children of both sex are affected, the mode of inheritance of CGD is not sex-linked. If the mode is autosomal recessive, the apparent absence of neutrophil dysfunction in the father suggests an undetectable carrier state. The unusual severity and frequency of infection in the female twins and the total absence of infection in the brother, a classic CGD, suggest a new variant of this syndrome.

725 CELL-MEDIATED IMMUNITY (CMI) TO RESPIRATORY SYNCYTIAL VIRUS (RSV) IN CHILDREN. Clare Purcell, Michael Mizen and Victoria Schauf, (Spon: by Ira M. Rosenthal). Abraham Lincoln Sch. of Med., Dept. of Ped., Univ. of Ill.Chicago

We have previously shown that CMI to RSV is common in adults and absent in susceptible neonates. To further define immunologic responses to RSV, we studied 24 children hospitalized from 7-12/76 and 34 healthy adults. Heparinized whole blood was diluted with RPMI-1640 and incubated for 6 d. with antigens prepared from RSV infected Vero cells, from uninfected control cells, with no antigen and with phytohemagglutinin (PHA). Triplicate cultures were labelled with ^3H-thymidine. Stimulation indices (SI = mean DPM RSV culture/mean DPM control culture) were positive if over 2.0 and if the means were significantly different. Ten children had respiratory illnesses. Nasopharyngeal cultures for RSV were negative. Lymphocyte reactivity occurred as shown:

Age (Yr.)	No. Positive/Total	RSV SI	PHA SI
1	0/9	-	7-35
1-4	3/7	2.9-5.9	8-104
5-15	1/8	2.1	10-130
Adult	18/34	2.1-16.3	11-710

Children were intermediate between infants and adults in frequency of reactivity to RSV antigens. The higher frequency of reactivity in adults may result from more RSV exposures. The absence of reactivity to RSV in 9 infants under age 1 yr agrees with our previous observations in neonates. We speculate that lack of CMI may be associated with the susceptibility of this group to lower respiratory tract infection by RSV.

726 SUPPRESSION OF MYELOPOIESIS IN THE SOFT AGAR CULTURE SYSTEM IN A PATIENT WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) AND APLASTIC ANEMIA (AA). Norma K.C. Ramsay, William Krivit, Mark E. Nesbit, Peter F. Coccia and John H. Kersey*; University of Minnesota School of Medicine and Hospitals, Departments of Pediatrics and *Laboratory Medicine/Pathology, Minneapolis.

The etiology for the AA associated with PNH is unknown. A report by Ascensão (Lancet 1:699, 1976) demonstrated in vitro suppression of myelopoiesis in idiopathic AA. A 15 year old female with PNH and AA was studied for the presence of in vitro suppression using the soft agar system for assay of colony forming cells in culture (CFU-C). Bone marrow from the patient and a normal donor was separated on a ficoll hypaque gradient and was cultured on two occasions. After incubation for 18 hours, bone marrow from the patient yielded no colonies, whereas bone marrow from the normal donor had 25.8 ± 1.7 (1SD) and 20.67 ± 2.08 (1SD) CFU-C/2 x 10^5 nucleated cells plated. When equal numbers of marrow cells from the patient and donor were co-cultured, only 28% and 36% of expected colonies were found in two separate determinations. The patient's peripheral blood lymphocytes added to a normal bone marrow also significantly reduced the number of CFU-C. Serum from the patient added to normal marrow did not inhibit colony formation more than normal serum. Suppression of normal myelopoiesis has not been demonstrated in 5 other patients with idiopathic AA using marrow in co-culture technique. These findings indicate that this patient with PNH and AA has an immunological mechanism responsible for the AA, similar to that described in some patients with idiopathic AA.