

691 NEONATAL HOST DEFENSES AGAINST K-1 AND NON K-1 *E. COLI*. Lewis F. Gold, William F. Tuer and Russell W. Steele, Dept. of Ped., Brooke Army Med. Ctr., San Antonio, TX.

A subgroup of *E. coli* possessing a particular polysaccharide antigen designated 'K-1' is found in more than 75% of isolates of neonatal *E. coli* meningitis. Because of this observation, the present study was designed to evaluate those host factors which are important in defense against *E. coli*, primarily phagocytic ability, killing power of neutrophils, and serum opsonizing capacity of newborn infants. A comparison was made between responses to K-1 *E. coli* and non K-1 *E. coli*.

Assays of neutrophil phagocytosis and bacterial killing were accomplished using acridine orange as a vital stain thus enabling the distinction between live bacteria, which stain green, and killed bacteria, which are bright red color, under ultraviolet light.

The following results were noted:

	Killing Capacity of Neutrophils		
	bacteria	Killing capacity	Phagocytic index
Newborns (30)	<i>E. coli</i> K-1	83±5.6%	1.7±0.27
	<i>E. coli</i> non K-1	86±11.2%	1.8±0.19
Controls (30)	<i>E. coli</i> K-1	87±7.3%	1.8±0.24
	<i>E. coli</i> non K-1	91±14.5%	2.0±0.21

When autologous serum from newborns or controls was incubated with bacteria on neutrophil monolayers, killing capacity was increased to >95%. In this study, no statistically significant difference was noted between neonates and controls nor in results for K-1 vs non K-1 *E. coli*.

692 LYMPHOCYTE MOTILITY IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND X-LINKED HYPO- γ -GLOBULINEMIA.

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We have found that circulating human T lymphocytes are motile, whereas unstimulated B lymphocytes are not (RES, J. Reticuloendothel. Soc. 20:331, 1976). Since motility is a functional morphologic marker of normal circulating T cells, we have begun to examine whether motility is a marker for T cells in immunologic disorders.

Patients with CLL, a B cell malignancy, and x-linked hypo- γ -globulinemia, a genetic B cell deficiency, were studied. Motile lymphocytes were determined by interference contrast microscopy. Blood lymphocytes were examined in the absence of plasma or serum since these inhibit lymphocyte motility. Circulating T cells were quantitated by E-rosetting and B cells by EAC-rosetting or immunofluorescent detection of surface immunoglobulins.

E-rosetting lymphocytes were decreased in CLL and normal in hypo- γ -globulinemia. Immunoglobulin bearing lymphocytes were greatly increased in CLL and were decreased in hypo- γ -globulinemia. Motile lymphocytes (normal $\bar{x} \pm SD$, 45±5) were decreased in CLL (11±3), but were normal in hypo- γ -globulinemia (56±13). The number of motile lymphocytes in these two disorders correlated quantitatively with the number of T lymphocytes as determined by E-rosetting. Thus, it appears that lymphocyte motility may be an important marker of T lymphocytes in certain disease states as well as in healthy individuals.

693 CRYOGLOBULINS AND COLD INSOLUBLE IMMUNE COMPLEXES. William R. Griswold, and Gary A. Incaudo, University of California, San Diego, Department of Pediatrics, La Jolla, California and Clinical Investigative Center, Naval Regional Medical Center, San Diego.

Prior studies in our laboratory have established that a subpopulation of circulating immune complexes is cold insoluble and can be isolated from serum as a cryoprecipitate. This study was done to develop criteria which would differentiate non-specific trapping of proteins in a cryoprecipitate (NSC) from cold insoluble immune complexes (CIC). Acute serum sickness was produced in 12 rabbits using 125 I bovine serum albumin (BSA). Serial measurements of antigen concentration, cold insoluble protein, cold insoluble antigen and serum protein were performed. NSC occurred before immune catabolism of antigen. Total cryoprotein was 31 μ g/ml serum. Cold insoluble antigen was 0.13 μ g/ml serum or 0.4% of the cryoprotein. Only 0.028% of the antigen in serum was cold insoluble. Adding extra antigen to the serum *in vitro* before cold incubation increased the amount of cold insoluble antigen, suggesting non-specific trapping. CIC occurred during immune catabolism of antigen in 77% of animals. Total cryoprotein was 143 μ g/ml serum. Cold insoluble BSA was 12.2 μ g/ml serum or 9.8% of the cryoprotein. This was 139 times greater than the BSA concentration in serum (per milligram of protein). 43% of the antigen in the serum was cold insoluble. Addition of extra antigen *in vitro* before cold incubation decreased the amount of cold insoluble antigen by 50%. CIC occur at a critical antigen-antibody ratio in serum and can be differentiated from NSC.

694 IMMUNOGLOBULIN SYNTHESIS IN BURKITT'S LYMPHOMA. Samuel Gross, Rebecca Dunn, Joseph Levinsky. Case Western Reserve University, University Hospitals, Department of Pediatrics, Cleveland, Ohio.

American Burkitt's lymphoma has been identified as a monoclonal IgM (Kappa or Lambda chain) lymphoproliferative disorder. The nature of immunoglobulin synthesis in culture of tumor derived from 3 such patients (ages 5, 6, and 15 years) was investigated. Tissue with cytologic and morphologic Burkitt's criteria included mediastinal node, abdominal node and marrow. Cells were cultured as 1×10^6 cells/ml in leucine-deficient Eagle's media containing 10% fetal calf serum and 5 μ C 3 H-leucine in 5% CO₂ at 37°C for 72 hours. Ig's were measured by solid phase radioimmunoassay. Specific anti-Fab and anti-IgG, M, and A, following elution from insoluble immunoabsorbents, were conventionally linked to bromacetyl cellulose (BAC) and specificity was established on acrylamide gels. Synthesis was determined as degradation (DPM)/10⁶ cells: total protein - 1800 (640-2570), Ig-120 (20-280). Molecular weight of the Ig was constant with IgM and verified with BAC-anti-IgM. Neither IgG nor IgA were synthesized. The % of newly synthesized protein as IgM ranged from 2.5-12.5%. Total protein (TP) and total Ig synthesized from normal lymphocytes in culture averaged 3100 and 290 DPM/10⁶ cells, respectively (%TP as Ig-9%: IgG-5.5%, IgM-3%, IgA-1.5%). With this method, Ig quantitation clearly identified IgM as the sole Ig synthesized by this tumor. The patients were treated alike (prednisone, cyclophosphamide and vincristine initially). The longest survival to date has occurred in the patient with the highest level of IgM synthesis.

695 SUPPRESSION OF PLASMA CELL DIFFERENTIATION BY PRIMED T LYMPHOCYTES. Anthony R. Hayward (Spon. by Alexander R. Lawton), Department of Pediatrics, University of Alabama in Birmingham, Birmingham, Alabama.

Cord blood T lymphocytes inhibit the division of maternal T cells and can block plasma cell (PC) differentiation of unrelated B cells induced by pokeweed mitogen (PWM). These effects are variable in degree but could be important in regulating immune responses between mother and fetus. Contact with maternal HLA antigens not shared by the fetus is one possible stimulus to such suppression. This was investigated by using adult blood T lymphocytes since these are not normally suppressive but can be stimulated in one-way mixed lymphocyte culture (MLC). In 4 experiments, when 40,000 T cells harvested from control (X x X) cultures were added to autologous (donor X) PWM stimulated cultures the mean number of PC ($\times 10^{-3}$) per culture was 32.2, IgM and 17.2, IgG. The number fell to 6.3 IgM and 4.6 IgG (mean suppression 78%) when the added T cells had been primed in MLC (X x Y) to the cells tested for differentiation (donor Y). There was slight reduction in the numbers of PC obtained in cultures of lymphocytes from the same source as the primed T cells (X x Y added to X), giving a mean suppression of 35%, and a comparable reduction in the response of an unrelated control (X x Y added to Z). The results suggest that specific suppression, which might be mediated by cytotoxic cells, is of greater magnitude than non-specific suppression. They are compatible with the view that a histocompatibility stimulus to the fetus could induce the suppressive characteristics of human cord blood T lymphocytes. (Grant 1-354 from the National Foundation, March of Dimes)

696 PROLONGED SURVIVAL OF XENOGRAFTS DESPITE SENSITIZATION. Walter L. Henley, Zvi Aviner, Colette Severin. Mount Sinai School of Medicine of the City University of New York, New York, New York.

We have reported cell-mediated immunity to corneal protein antigens after corneal homotransplantation in man and after xenografts in animals. Patients were followed weekly with the Leucocyte Migration Inhibition (LMI) Test. Similar results were obtained in animals by direct and indirect MIF production.

When rabbit embryonic cartilage is placed around a guinea pig corneal transplant into a rabbit's eye, sensitization occurs as shown previously. The xenograft is rejected in the second week if no cartilage is added, or if it is surrounded by silicon strips. It is rejected in the third week when rabbit adult cartilage is used and not rejected for 4-7 weeks with embryonic cartilage implantation. Embryonic cartilage produces factors that inhibit neovascularization.

The experiments suggest that sensitization occurs in each instance but that expression of the efferent arc of the immune response is delayed by the inhibition of graft vascularization by the embryonic cartilage.