1020 INTRAVENOUS IMMUNE SERUM GLOBULIN FOR HYPOGAMMAGLOBULINEMIA: A COMPARISON OF OPSONIZING CAPACITY IN RECIPIENT SERA. Russell W. Steele, R. Ann Augustine, A. Susan Tannenbaum, and Daniel J. Marmer. Department of Pediatrics, University of Arkansas for Medical Sciences/Arkansas Children's Hospital. Little Rock, AR.

Twelve severely hypogammaglobulinemic patients received infusions of alkylated immune globulin and two other non-alkylated products. Administration was separated by an interval of 3 weeks. Serum was obtained prior to and at 24 hours and 3 weeks after each infusion for measurement of total IgG, specific and opsonizing antibodies. The latter was accomplished against S. pneumoniae types 5, 12F and 14 and zymosan using chemiluminescence methodology.

Changes in total IgG concentrations were comparable for the three products. Prior to enrollment, IgG levels averaged 115±72 mg/dl, increasing to 779±399 at 24 hours postinfusion, and were 337±200 after 3 weeks. No differences among the products was seen in their ability to produce antibodies against Herpes simplex virus types 1 and 2, rubella, toxoplasma, cytomegalovirus or tetanus. However, differences in opsonizing antibody were observed between alkylated and native IgG preparations. Peak chemiluminescence responses of neutrophils following opsonization of S. pneumoniae with native immune globulin were significantly higher than with alkylated IgG, indicating greater functional capacity. These studies suggest that native immune serum globulin provides a greater potential for augmenting host defense mechanisms against pneumococcal infection in hypogammaglobulinemic patients.

1023 AN ENZYME-LINKED IMMUNOSORBANT ASSAY FOR ANTI-HERPES SIMPLEX VIRUS (HSV) AND ANTI-VARICELLA ZOSTER VIRUS (VZV) ANTIBODY (AB). Patrick A. Tellez, Joseph Portanova and Anthony R. Hayward, University of Colorado School of Medicine, Department of Pediatrics, Denver 80262.

The human newborn and immunocompromised host are particularly susceptible to the devastating effects of disseminated VZV and HSV infection. To further investigate the relevant immune parameters, we developed an ELISA for the detection of specific AB. Plastic microtiter plates (Dynatech)) were the solid phase for the antigen (Ag) (VZV/HSV antigen - Flow Labs), 100 ul/well of a 1:20 dilution in PBS. Following overnight (O/N) incubation Ag is replaced with PBS-gelatin (1 mg/ml) and incubated O/N 4°C. After washing thrice with PBS-tween 20 (.05%), the index sera or culture supernates are incubated 100 ul/well O/N 4C. Wells are washed as above and goat anti-human peroxidase conjugated Ab 1:2000 in PBS-gel-Tween-bovine -globulin (5 mg/ml) is incubated 2 hrs. at room temperature. After washing, substrate 100 ul/ml (ABTS-sigma, 1 mg/ml in phosphate buffer + .15% H_2O_2) is allowed to react at room temperature and read at 2 hrs. at 405 nm (Titertek Multiscan). We compared anti-VZV ELISA titers with those determined by FAMA technique:

FAMA:	1:2048	1:512	1:64	1:16	NEG1	NEG2	NEG3
ELISA:	1:104,00	1:25,600	1:6400	1:200	NEG	NEG	NEG

The sensitivity of the method has been useful in the detection of Ab production by mononuclear cells in vitro.

●1021 LIPOPOLYSACCHARIDE (LPS) STIMULATES HUMAN MACROPHAGES (MO) TO PRODUCE INCREASED AMOUNTS OF THE THIRD COMPONENT OF COMPLEMENT (C3). Robert C. Strunk, Kathleen S. Kunke, and F.Sessions Cole, Dept. of Pediatrics, Nat'l. Jewish Hosp. and Res. Ctr., Denver, and Children's Hosp. Med. Ctr., Boston.

MO synthesize and secrete small amounts of C3. Studies were designed to determine if C3 production could be stimulated by LPS, a product present at sites of inflammation. Monocytes separated from normal human peripheral blood were cultured for 7 days in 5% human serum to promote maturation into $MO_{3,5}$. C3 production was assessed by hemolytic assay and incorporation of ^{35}S-methionine into C3 protein. MO cultured in LPS (E.coli 0111: B4-Westphal extraction), 500 ng/ml, produced more C3 than controls: in 6 experiments increases were 15.1-fold for intracellular pro-C3 protein (range 5.4-32.6), 11.4-fold for extracellular native C3 protein (10.5-13.1), and 10.4-fold for extracellular C3 hemolytic activity (5.1-31.8). In contrast, LPS did not increase production of C2, lysozyme, lactate dehydrogenase, or total protein. Stimulation of C3 production was maximal after 24 hrs exposure to LPS. C3 production was increased by a polysaccharide-free mutant of LPS, but not by alkali-treated LPS, indicating that lipid A was responsible for stimulation. C3 production in human breast milk and bronchoalveolar MO was stimulated by LPS, but production in freshly adherent blood monocytes was not affected. The LPS-stimulated increase in C3 production was cycloheximide inhibitable, suggesting LPS increased translation of C3 protein stimulation. There was no alteration in post-translational processing. This potential for increased C3 synthesis in localized sites of inflammation may be important in resolution of the inflammation process.

●1024 IN VITRO SENSITIZATION PRIOR TO FUSION GENERATES A HIGH FREQUENCY OF HUMAN-HUMAN HYBRIDOMAS BINDING TO GROUP B STREPTOCOCCI (GBS). Richard L. Wasserman and Thomas L. Kuhls (Spon. by Joseph B. Warshaw) Depts. of Ped. and Micro., U of TX, Southwestern Medical School, Dallas.

The production of useful human monoclonal antibodies has been limited by the inability to generate antigen specific B cells by in vivo immunization. We describe a procedure of in vitro stimulation and fusion which has produced significant numbers of antigen binding human-human hybridoma antibodies.

Twenty to thirty million mononuclear cells prepared by Ficoll-Hypaque separation of single-cell suspensions of human tonsil or spleen were cultured in the presence of GBS and pokeweed mitogen for 2 to 6 days and then fused with the HGPRT deficient human lymphoblastoid cell line LICR-LON-HMy2. Fused cells were cultured in selective medium for 2 weeks. Growth positive wells observed from the 10th to the 30th day following fusion were screened for the presence of antibody in an ELISA or RIA detecting human antibodies binding to GBS antigens. Results are as follows:

Patient	Fusions Performed	Growth Positive Wells	Anti-GBS Positive Wells
1	3	56/288 (19.4%)	4/288 (1.3%)
2	5	195/480 (40.6%)	14/480 (2.9%)
3	9	557/864 (64.4%)	120/864 (13.9%)
4	11	484/1056 (45.8%)	168/1056 (15.9%)

In vitro stimulation for the generation of human-human hybridomas is an effective means of producing useful human monoclonal antibodies.

●1022 IMMUNOREGULATORY DEFECTS AND EPSTEIN-BARR VIRUS (EBV) IN HEMOPHILIA. JL Sullivan, DB Brettler and P Levine (Spon. by James B. Hanshaw), Dept. Ped. and Med., Univ. of Mass. Med. Sch., Worcester, MA.

Hemophiliac patients are at risk for the development of acquired immune deficiency syndrome. Asymptomatic hemophiliacs demonstrate immunoregulatory defects. The etiology of immune defects in this population is not well understood. In a group of 137 consecutively chosen hemophiliacs, EBV serology was examined.

Antibody Status	Hemophiliacs	Healthy Blood Donors
Seronegative	46 (34%)	11 (27%)
Seropositive	91 (66%)	30 (73%)
Anti-VCA ≥ 1:320	45 (49%)	5 (17%)
Anti EA > 1:40	40 (44%)	3 (10%)
VCA ≥ 1:320 or EA ≥ 1:40	60 (66%) (p<.01)	7 (23%)

Age related acquisition of EBV did not differ from normal controls, however, 66% of hemophiliacs have serologic patterns consistent with persistent or reactivation EBV infection. In 60 patients, immune studies revealed significant immunoregulatory abnormalities (OKT.4/T.8 1.08±.68 vs. 1.98±.79 in normal controls). Functional studies revealed significant depression of Con A responses (mean $\log_{10}$ CPM 4.704±.341 vs. 5.074±.140 in normal controls). We hypothesize that chronic antigenic stimulation by Factor VIII concentrate results in immunoregulatory disturbances and increased reactivation of EBV infection. Reactivated EBV infection contributes further to the immunoregulatory defects in patients with hemophilia.

●1025 BACTERIAL AGENTS STIMULATE THE RELEASE OF SECRETORY IGA FROM HUMAN MILK LEUKOCYTES. Elizabeth A. Weaver, Charles P. Davis, Robert C. Fader, Randall M. Goldblum, and Armond S. Goldman. The University of Texas Medical Branch, The Departments of Pediatrics and Microbiology, Galveston, Texas.

Recently we reported that the secretion of secretory IgA (SIgA) from human milk leukocytes was stimulated by phagocytosis (J. Infect. Immun. 34: 498, 1981) and certain surface membrane stimuli including N-formyl-L-methionyl-L-phenylalanine (J. Immunol. In Press, 1984). Since that synthetic peptide is similar to ones secreted by certain strains of enteric bacteria, we questioned whether other bacterial products also trigger the release of that immunoglobulin from phagocytes in human milk. Supernatent fluids from cultures of Escherichia coli 07KL or Klebsiella pneumoniae obtained by passage through 0.22μ filters lead to the release of 40-50% of the SIgA found in unfractionated human colostral leukocytes ($p<0.01$). A similar degree and temporal pattern of SIgA release occurred when those leukocytes were exposed to heat-treated filtrates from those bacterial cultures, purified lipopolysaccharide from E. coli or type 1 pili isolated from K. pneumoniae.

Thus, the secretion of SIgA by human milk leukocytes is initiated not only by phagocytosis of intact microorganisms, but also by important components of certain enteric bacterial pathogens. Furthermore, these findings support the hypothesis that SIgA released from those leukocytes plays a role in protecting the breast-fed infant from bacterial infections of the gastrointestinal tract.