

974 COLOSTRAL CELLS: IgA RELEASE STIMULATED DURING PHAGOCYTOSIS. Elizabeth A. Weaver, Armond S. Goldman, Randall M. Goldblum, Charles P. Davis. University of

Texas Medical Branch, The Departments of Microbiology and Pediatrics, Galveston.

Human milk leukocytes contain large quantities of IgA and other proteins. During prolonged *in vitro* incubation, the IgA is released. To further examine the potential role of this process in delivering these proteins to the recipient infant, the effect of phagocytosis upon the release of IgA from colostrum leukocytes was investigated. Washed leukocytes were incubated with live *Escherichia coli* or heat-killed *Candida albicans* in the presence or absence of serum opsonins. The degree of phagocytosis was determined microscopically. Controls were incubated without particles. After 15, 30, and 60 minutes, cells and supernatant fluids were recovered and assayed for total IgA and SIgA by a quantitative immunofluorescence assay. The degree of IgA release was enhanced (approximately 40%) with cells exposed to opsonized organisms, as compared to preparations containing no particles. The degree of decrease in the IgA level in the cell lysates was paralleled by an increase in IgA in the supernatant fluids from the cell preparations. In contrast, little release occurred (approximately 4%) with unopsonized organisms. The enhanced release was evident within 15 minutes. Cells incubated with opsonized organisms at 4°C did not release IgA until warmed. The enhanced release of IgA by colostrum leukocytes during phagocytosis may be part of a mechanism to deliver IgA antibodies to the site of microbial colonization or infection in the infant.

975 NORMAL VALUES FOR AGE OF LYMPHOCYTE SUBPOPULATIONS. Kristen A. Weigle, M. J. Hick, Dept. of Pediatrics, Univ. of Tex. Hlth. Sci. Ctr., San Antonio, Tex. and

Dept. of Clin. Pathol., Univ. of Ariz. Hlth. Sci. Ctr., Tucson, Ariz. (Spon. by Philip A. Brunell).

Lymphocyte subsets are frequently measured in children to evaluate immunodeficiency states. Normal values for age are needed for correct interpretation of these measurements. Total leukocytes, total lymphocytes, and lymphocyte subpopulations were measured on 85 healthy children and 53 healthy adults. T-cells were measured by E-rosetting. B-cells were measured by surface immunoglobulin (SIg) on lymphocytes by direct fluorescent staining using anti-μ, anti-γ and polyvalent rabbit anti-human serum. The effect of age on these values was determined by multiple regression analysis. Total leukocytes, total lymphocytes, and percentage SIgM bearing lymphocytes decreased with age ($p < .01$). T-cell percentages increased with age, while SIgG bearing lymphocytes appeared to be independent of age. Normal values for percentage SIgM follow:

| Age(yrs) | Newborn (n=12) | 1/12-6/12 (n=10) | 7/12-2 (n=18) | 2-4 (n=16) | 4-11 (n=19) | 11-21 (n=10) | >21 (n=53) |
|----------|----------------|------------------|---------------|------------|-------------|--------------|------------|
| Mean | 10.9 | 13.6 | 12.0 | 8.9 | 8.4 | 8.3 | 5.5 |
| ±S.D. | 5.6 | 4.2 | 4.6 | 3.8 | 5.7 | 7.0 | 6.9 |

In conclusion, lymphocyte subsets vary with age and should be compared to normal values for age or to age matched controls.

976 AUTOSTIMULATORY ACTIVITY OF ANTIGEN ACTIVATED HUMAN MONONUCLEAR CELLS. Michael J. Welch, Bonnie J. Ank, Dean A. Kujubu and E. Richard Stiehm. UCLA School of Medicine, Department of Pediatrics, Los Angeles, CA.

It has been previously shown that peripheral blood mononuclear cells (PBMC) when activated *in vitro* with antigen are capable of suppressing the proliferative response of autologous fresh cells to the same antigen. We were unable to confirm this using *Candida* antigen (Can) but did find that PBMC are capable of inducing proliferation in fresh autologous cells. PBMC in the absence (control cells) or presence of Can were preincubated for 5 days, irradiated or mitomycin-treated, washed thoroughly, and added to equal numbers of fresh autologous responder cells; these were incubated for 5 days and proliferation assayed by ³H thymidine incorporation. In 12 of 14 experiments with positive *Candida* responders, the presence of Can-preincubated cells caused significant proliferation of fresh autologous cells ($\bar{x}=26,601 \pm 2,171$ SEM) whereas presence of control cells did not ($1,419 \pm 150$). This autostimulatory activity (ASA) was not seen when an individual's *Candida* response was negative or low (N=8). Suppression of the *Candida* stimulation assay by these activated cells was not consistently seen. Similar and even more impressive ASA was noted when phytohemagglutinin was used as the prestimulatory agent. ASA may represent a normal immune amplification mechanism or somehow be related to the now well-described autologous mixed lymphocyte reaction.

977 IN VITRO COMPARISON OF HUMAN COLOSTRAL AND MATERNAL PERIPHERAL BLOOD T-LYMPHOCYTE. Leonard E. Weisman, George Brown, Fred Rangel, Gerald B. Merenstein.

(Spon. by Frederick Battaglia). Fitzsimons Army Medical Center, Newborn and Clinical Investigation Services, Aurora, Colorado.

We compared *in-vitro* mitogen induced lymphoproliferative response (IVMILR) between paired samples of colostrum lymphocytes (CL) and blood lymphocytes (BL) using equal numbers of T-cells or a T-cell subpopulation. Standard lymphocyte counting, sheep erythrocyte rosetting (SER), "active" sheep erythrocyte rosetting (ASER) and IVMILR techniques were used. Optimal mitogen concentration was established by dose response curves. Cultures had 95% viability by trypan blue staining.

Morning colostrum and blood was donated at 3 days postpartum by 18-35 year old primiparous Caucasians with normal pregnancy & delivery. Twelve samples were evaluated for % of SER and ASER cells: BL had 60% SER and 40% ASER cells, CL had 30% SER and 20% ASER cells. Six samples of 95% SER cells and 3 of 95% ASER cells were evaluated for IVMILR after isolation over Ficoll-Hypaque. BL SER cells had stimulation index (SI) 2-10 times greater than CL SER cells. BL ASER cells had SI 2-5 times greater than CL ASER cells. Three samples of CL and BL were co-cultured and IVMILR revealed no change in slope of dose response curve.

IVMILR of CL T-cells was hyporeactive when compared to equal numbers of matched BL T-cells. The IVMILR of CL "active" T-cells, a subpopulation of T-cells, was hyporeactive when compared with an equal number of matched BL "active" T-cells. There is no evidence of suppressor cell or substance produced by milk cells. The CL T-cell is different than the BL T-cell.

978 MURAMYL DIPEPTIDE (MDP) ENHANCES SUPEROXIDE (O₂⁻) PRODUCTION BUT NOT ANTIMICROBIAL ACTIVITY OF HUMAN MACROPHAGES (Mφ). Christopher B. Wilson, John Bon-

sack, William M. Weaver. (Spon. by A.L. Smith) Univ. of Wash., Sch. of Med., Dept. of Peds. and Child. Ortho. Hosp. Seattle, WA

MDP, a subunit of bacterial peptidoglycan, enhances resistance of animals to certain infections; MDP treatment enhances animal Mφ phorbolmyristate acetate (PMA) stimulated O₂⁻ production and antimicrobial activity (indices of Mφ activation). We examined *in vitro* effects of MDP on human monocyte-derived Mφ O₂⁻ production and antimicrobial activity against *T. gondii* (T), an intracellular pathogen, and *S. aureus* (S), an extracellular pathogen. PMA stimulated O₂⁻ generation by MDP treated Mφ (MDP-Mφ) was always increased (55% ± 12%) compared to control Mφ (NMφ) (n=7; p < .02); without PMA no O₂⁻ was generated. In contrast, O₂⁻ generation by MDP-Mφ and by NMφ phagocytosing T,S or zymosan was similar. Survival of T within NMφ (53% ± 11%) and within MDP-Mφ (43% ± 12%) did not differ significantly; T replicated equally well in either Mφ. Phagocytosis of [¹⁴C]-labelled S, determined after removal of extracellular S with lysostaphin, by NMφ and by MDP Mφ was equal (log₁₀ 5.4 ± .1); killing of intracellular S by NMφ (63% ± 11%) and by MDP Mφ (61% ± 8%) was comparable. By enhancing Mφ release of inflammatory mediators such as O₂⁻ in response to non-specific stimuli, while failing to enhance Mφ antimicrobial activity, MDP might adversely affect human response to infection. Chemical modification of MDP alters its effects in animals; modifications may yield drugs that enhance human host defenses without enhancing inflammation.

979 IMMUNOLOGICAL ABNORMALITIES IN JUVENILE RHEUMATOID ARTHRITIS (JRA). Carlos M. Arroyave and Silvia Mejia. Clinica Nova, Mont-

rrey N.L. and Unidad de Investigaciones Biomedicas, Guadaluajara Jal. Mexico.

The sera of twenty five patients with JRA and the skin of ten of them were studied. Six patients had typical JRA rash. Their ages ranged from 5 to 15 years. Twelve patients with JRA demonstrated elevated levels of immune complexes (IC). The IC decreased during treatment and control of the disease. Five patients showed the presences of rheumatoid factor and antinuclear antibodies. There was good correlation between these abnormalities and treatment. The sera complement levels were normal in twenty patients. Punch biopsies were obtained from the site of rash or normal skin (control). Immunofluorescence staining revealed deposition of immunoglobulins or complement in 8 patients, six of whom had the rash and had systemic onset of disease. The fluorescence was positive for IgM (8), C3 (4) and IgG (1) mainly at the dermal-epidermal junction and vessels. Controls were negative. These data suggest, 1) positivity of IC in some patients with JRA, 2) Good correlation between IC and disease activity and, 3) The rash probably is immunological mediated.