●872 A NOVEL IN VIVO HUMAN CYTOTOXIC T CELL. John L. Sullivan, Blake E. Tomkinson. U. Mass. Med. School, Dept. of Pediatrics, Worcester, MA.

Non-MHC restricted cytotoxic lymphocytes appear in vivo during acute EBV induced infectious mononucleosis (IM). Complement depletion with T-cell (OKT3) and NK-cell (Leu11b and NKH-1a) monoclonal antibodies demonstrated this non-MHC restricted activity to be mediated primarily by cytotoxic T-lymphocytes (CTL). Depletion of NK-cells, with a 76% (Leu11b) and 54% (NKH-1a) reduction in the lysis of K562, resulted in a slight (<30% for Leu11b and NKH-1a) reduction in the nonrestricted lysis of two allogeneic EBV infected lymphoblastoid cell lines (LCL). Depletion of T-cells by OKT3 and complement abolished (96% reduction) the nonspecific lysis of these two LCL. Complement depletion with antibodies to CD8 (OKT8) and HLA-DR decreased the nonspecific response by 89% and 62%, respectively. Monoclonal antibody inhibition studies with antibodies to CD3 (OKT3), CD8 (OKT8) demonstrated this nonspecific response to be mediated through the CD3/T-cell receptor and to use the CD8 antigen as an accessory molecule; OKT3 and OKT8 monoclonal antibodies inhibited the nonspecific lysis of three LCL by an average of 79% and 74%, respectively. The specificity of this inhibition was demonstrated by the lack (<10%) of inhibition by the anti-CD5 pan T-cell monoclonal antibody OKT1. These results clearly demonstrate that IM non-MHC restricted CTLs are of T-cell origin. The lack of NKH-1a expression and sensitivity of cytolytic activity to anti-CD8 antibodies distinguishes acute IM CTLs from previously described non-MHC restricted human cytotoxic lymphocytes. (Lanier et al, Immuno. Today vol 7).

●873 MHC-RESTRICTED, ANTIGEN SPECIFIC, GRANULE EXOCYTOSIS FROM HELPER T CELLS: ANOTHER INDICATOR OF RECEPTOR MEDIATED T CELL ACTIVATION Michael Taplits, Pierre Henkart, Richard Hodes Immunology Branch, National Cancer Institute, NIH, Bethesda, MD.(Sp. David Nelson)

Recently, it has been observed that some T cells contain trypsin-like serine esterases. Helper T cell clones derived in our laboratory contain high levels of these enzymes (BLT esterase), which are associated with cytoplasmic granules. Using supernatant release of these enzymes as a marker, we examined granule exocytosis as a consequence of T cell activation via the T cell receptor. Clones 8-5(A^b,KLH), 10-2(A^b,KLH), and A13(A^k,KLH) all released enzymatic activity over background (constitutive release) when cultured with antigen plus syngeneic antigen presenting cells (APC)(37%, 50%, 27% of total activity, respectively). APC alone contained negligible enzyme activity. Incubation of clones with syngeneic APC or with antigen plus non-syngeneic APC gave only background release. Other stimuli which induced strong granule exocytosis were conconavalin A and an antibody against the mouse T3 complex. Using antigen pulsed APC, exocytosis was detected as early as one hour after the initiation of T cell stimulation. In contrast, when activation was induced by incubation of cloned T cells with interleukin 2 (IL-2), no secretion above background was observed despite the fact that substantial DNA synthesis occurred. Thus, it appears that granule exocytosis may be another means of analyzing T cell activation pathways initiated by T cell receptor-ligand interactions. Furthermore, these activation sequences may be distinquished from those occurring after IL-2 interaction with its receptor, since the latter event does not induce granule exocytosis.

●874 CYCLIC AMP INHIBITS NEUTROPHIL ADHERENCE BY BLOCKING SURFACE EXPRESSION OF THE CELL ADHESION MOLECULE, CR3. M. Tosi and M. Berger, Case Western Reserve University, Department of Pediatrics, Cleveland, Ohio.

Elevation of cyclic (c) AMP levels in neutrophils (PMN) has been shown to inhibit cell adherence, but the mechanism of this inhibition has not been established. Since CR3 (Mac-1, Mo-1) is known to be essential for normal PMN adherence, and its expression is rapidly upregulated when PMN are activated, we tested the hypothesis that cAMP might act by blocking CR3 expression. Peripheral blood PMNs were incubated at 37° with the phosphodiesterase inhibitor, isobutyl methylxanthine (IBMX; $3x10^{-4}$M), followed by dibutyryl (db-) or 8-bromo (8Br-) cAMP (10^{-3}M). Cells were then stimulated with n-formyl-methionyl-leucyl-phenylalanine (fMLP; 10^{-8}M) or zymosan-activated serum (ZAS; 1:100). Upregulation of surface CR3 was quantitated using monoclonal antibodies and flow cytometry, and adherence was measured as % PMNs retained by nylon wool. IBMX and dbcAMP together, but not individually, inhibited the increase in CR3 expression (stim./resting ratio) induced by fMLP from 3.98±0.32 to 2.15±0.2 ($p < 0.01$) and by ZAS from 2.56±0.09 to 1.94±0.07 ($p < 0.01$). IBMX + dbcAMP, but neither alone, similarly inhibited PMN adherence induced by fMLP from 33.8±2.1% to 8.2±1.9% ($p < 0.001$), and by ZAS from 25.3±2.5% to 15.6±0.7% ($p < 0.01$). 8BrcAMP gave results similar to dbcAMP. Isobutanol, isobutyric acid, and diluent controls had no effect. Results for CR1 expression paralleled those for CR3. These studies suggest that elevating cAMP inhibits upregulation of surface CR1 and CR3 in PMNs. The decrease in surface CR3 may be responsible for the diminished PMN adherence caused by cAMP.

●875 TRANSFORMING GROWTH FACTOR, TYPE β (TGFβ) INHIBITS THE GROWTH AND DIFFERENTIATION OF NORMAL, BUT NOT TRANSFORMED, B LYMPHOCYTES. L.B. Vogler, S.J. Anderson and M.C. Kinney; Depts. of Pediatrics, Microbiology, and Pathology, Vanderbilt U. School of Med., Nashville, TN

TGFβ, a 25 kD polypeptide, reversibly transforms non-neoplastic murine fibroblasts into cells capable of anchorage-independent growth. In most other cell types TGFβ inhibits cell growth. To assess the possibility that loss of susceptibility to TGFβ suppression may lead to augmented growth in some lymphoid malignancies, we examined its effect on proliferation (^{3}H-thymidine uptake) and differentiation (to plasma cells) of normal human tonsillar B cells and EBV- and malignant-transformed B and pre-B cell lines. Cells were stimulated by anti-μ (15 μg/ml) or TPA (1 ng/ml) with and without B cell growth factor (BCGF 10% v/v) in proliferation assays and by pokeweed mitogen (PWM), EBV or an allogeneic T cell clone (ATC) in differentiation assays. TGFβ (0.1-30 ng/ml) inhibited normal B cell proliferation. At 0.3 ng/ml it suppressed DNA synthesis in cells stimulated by BCGF 23%, by anti-μ + BCGF 42%, by TPA + BCGF 45% and by TPA alone 86%. Differentiation was suppressed 60% in B cells stimulated by EBV, 87% by PWM and 98% by ATC. In contrast, no effects on proliferation or on surface or cytoplasmic immunoglobulin expression were observed in EBV-transformed normal B lymphoblastoid lines or in pre-B or B leukemic cell lines even at TGFβ concentrations of 30 ng/ml.

We conclude that TGFβ profoundly suppresses normal B lymphocyte proliferation and differentiation, but has little effect on EBV-infected or malignant-transformed B or pre-B cells.

876 FAILURE OF ANTI-PNEUMOCOCCAL ANTIBODY FORMATION IN PATIENTS WITH COMPLETE IgA DEFICIENCY AND NORMAL IgG LEVELS. Richard L. Wasserman, (Spon. by Joseph B. Warshaw) University of Texas Health Science Center at Dallas, Children's Medical Center, Department of Pediatrics, Dallas, Texas.

In contrast to common variable hypogammaglobulinemia or IgA deficiency associated with IgG subclass deficiency, selective IgA deficiency has not previously been associated with specific antibody non-responsiveness.

Two patients with recurrent sinopulmonary infection were subjected to an evaluation of humoral immunity. Serum concentrations of IgG, IgM and IgG subclasses 1, 2, 3 and 4 were normal for age. IgA was 0. The patients were immunized with Pneumovax^R and specific antibody reacting with pneumococcal antigen was measured. Antibody concentrations (ng/ml) were:

Patient	Type 3	Type 7	Type 9	Type 14
1	0	148	0	9
2	0	0	54	0
normal	1149	3950	779	2779

The inability of these IgA deficient patients to form IgG anti-pneumococcal antibody may be due a regulatory defect associated with the inhibition of IgA production. This associated defect may explain the observation that only some IgA deficient patients have recurrent infections.

This finding suggests that specific antibody responses of all patients with IgA deficiency should be studied. Patients with a lacunar defect in IgG synthesis may benefit from gamma globulin replacement therapy.

●877 HUMAN ANTI-HAPTEN ANTIBODY DIVERSITY DERIVES FROM COMBINATORIAL MECHANISMS EMPLOYING FEW VH GENES AND SEVERAL JH GENES. Richard L. Wasserman (Spon by Joseph B. Warshaw). University of Texas Health Science Center at Dallas, Children's Medical Center, Department of Pediatrics, Dallas, Texas.

Human B cell hybridomas were produced by in vitro stimulation of normal human B cells with the p-azophenylarsonate (ARS) hapten coupled to keyhole limpet hemocyanin followed by fusion with the HAT sensitive human B cell line LICR-LON-HMy2. ARS specific hybridomas were cloned and analyzed for VH and JH gene usage.

Southern blot analysis of hybridoma DNA using a human JH probe demonstrated limited variability of restriction fragment size suggesting that few germline VH genes are involved in the human anti-ARS response exhibited in these cell lines. Sequential hybridization analysis of DNA from several hybridomas using JH1+2, JH3, JH4, JH1-5 and JH6 probes showed that different JH segments were used. Thus, JH variation appears to be a major contributor to diversity in the human anti-ARS response.

These findings are in direct contrast to the well studied murine anti-ARS response in which a single VH gene and a single JH gene is responsible for the majority of the response and diversity derives from junctional, n-segment and somatic mutational mechanisms. Mechanisms for generating diversity appear to be used to different extents in the human versus the murine anti-ARS response.