

† **961** THE MORPHOLOGICAL DIAGNOSIS OF THE WISKOTT-ALDRICH SYNDROME (WAS). Lloyd Cairns, Diane Kenney, Harry Neustein, Eileen Remold-O'Donnell, Fred S. Rosen, Robertson Parkman, Childrens Hospital of Los Angeles, Los Angeles, CA and Childrens Hospital Medical Center and Center for Blood Research, Boston, MA.

We have previously reported abnormalities in the membrane glycoproteins of the T lymphocytes and platelets of WAS patients. Deficiencies in a 115,000 dalton lymphocyte glycoprotein (GPL-115) appear to be diagnostic of WAS. To determine if the membrane glycoprotein abnormalities have morphological consequences, normal and WAS peripheral blood lymphocytes (PBL) and thymocytes were fixed in 1.2% glutaraldehyde and examined by scanning electron microscopy (SEM). Using a 1 to 4 grading scale based upon the character of the lymphocyte surface projections (4 = villus projections on > 75% of lymphocyte surface area; 3 = villus projections on < 75% of surface area; 2 = ridge projections; 1 = no projections), normal PBL had an average score of  $3.60 \pm 1.0$  (SE); thymocytes,  $2.00 \pm 0.2$ ; WAS PBL,  $2.76 \pm 0.7$  (n = 14). The decreased score of WAS lymphocytes was due to a decrease in the percentage of cells with villus projections and an increase in the percentage of cells with ridge or no projections. SEM has been used to confirm the diagnosis of WAS on the cord blood lymphocytes of one patient. WAS represents the first lymphoid immunodeficiency in which morphological abnormalities have been identified that can be used for diagnostic purposes.

● **962** RESTORATION OF IN VITRO FUNCTION OF ADENOSINE DEAMINASE (ADA) DEFICIENT LYMPHOCYTES BY INTERLEUKIN-2 (IL-2)

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We evaluated the effects of recombinant DNA IL-2, a T-cell lymphokine which is essential for normal immune function on the responses to mitogen and alloantigen of peripheral blood mononuclear cells (PBMC) from 3 ADA deficient patients. There was significant enhancement by IL-2 of mitogenic responses to phytohemagglutinin (PHA), pokeweed mitogen (PWM) and alloantigen (MLR) in all 3 patients. Patients with ADA positive severe combined immunodeficiency disease showed no responses to IL-2:

	PHA(+IL-2)	PWM(+IL-2)	MLR(+IL-2)
Control	40255(56566)	4969(4780)	16505(15623)
ADA def #1	606(23449)	3163(8151)	218(1526)
ADA def #2	1190(20354)	2290(6086)	--
ADA def #3	282(23182)	--	--

To determine the responsive cell population we measured T3, T4 and T8 expression in PBMC exposed to PHA + IL-2. Normal PBMC respond with increased T3 cells but no change in T4 or T8 expression. The ADA deficient PBMC response is almost entirely due to T3/T4 cells. Normal PBMC incubated with an ADA inhibitor, deoxyadenosine and PHA cannot be salvaged by IL-2. These results demonstrate a unique feature of the immunodeficiency in ADA deficiency and provide further evidence that purine metabolism is distinct in lymphocyte subsets. Also, the standard in vitro cell model of ADA deficiency may be significantly limited in its use in understanding the pathogenesis of this disease.

**963** COMPLETE TUMOR ABLATION WITH IODINE 131 RADIO-LABELED MONOCLONAL ANTIBODY (Mab) AGAINST HUMAN NEUROBLASTOMA (NB) XENOGRAFTED IN NUDE MICE.

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The antibody 3F8, an IgG3 murine Mab we have developed to human NB, could specifically target iodine 131 to human NB xenograft with tumor to non tumor ratios of 10-100 and a relative radiation dose deposition to normal organs of 1 to 20% of that to the tumor. We therefore studied its efficacy in tumor therapy. Nude mice with actively growing sc human NB (1-2 gm size) were injected with 0.125 to 1 mCi iodine carried on 100 to 200 ug Mab 3F8. Tumor size was followed by direct measurement. Actual tumor weight and measured size showed good correlation ( $r = 0.985$ ). Tumor radioactivity over time was calculated from the gamma images using a known 131-I standard. All the tumors had effective half lives averaging 48 hours. Radiation dose to individual tumors were calculated. Tumor shrinkage only occurred with 131-I 3F8 Mab, but not with nonradioactive 3F8 or radiolabeled irrelevant Mab. While control mice tumor enlarged by 10 fold, treated tumor showed 95% shrinkage by 12 days. Both the rate of shrinkage and duration of response were dose dependent. Only those tumors that received 4700 rads were completely ablated without recurrence. There were no toxicities except reversible weight loss. Thus, human NB xenografts could be effectively eradicated using iodine 131 labeled Mab 3F8 with tolerable toxicities.

† **964** ENHANCED LYMPHOKINE-ACTIVATED CELLULAR CYTOTOXICITY IN CORD MONONUCLEAR CELLS. T. Chin, D. Murakami, B. Ank, S. Strom, and E.R. Stiehm. UCLA Dept. of Pediatrics, Los Angeles, CA.

Various forms of cellular cytotoxicity have been noted by us and others to be decreased in newborn whole mononuclear cells (WMC) as compared with adult WMC. Diminished cord spontaneous natural killer (NK) cell activity against K562 targets has been observed. However, after incubation for 5-7 days with purified recombinant interleukin-2 (IL-2) cord WMC cytotoxicity against K562 cells increased from  $16 \pm 2\%$  (SEM) to  $74 \pm 5\%$  (n=9). Killing of Raji cells increased from  $7 \pm 1\%$  to  $38 \pm 4\%$  (n=9). Similarly activated adult WMC also increased their killing against K562 from  $31 \pm 4\%$  to  $68 \pm 7\%$  and against Raji cells from  $7 \pm 1\%$  to  $40 \pm 7\%$  (n=10). Dose-dependent responses were observed with the optimal IL-2 concentration at 50-100 units/cc. Increased cytotoxicity was also observed against other cell targets (Molt 4F and EL-4). Cold target inhibition of Molt and Raji killing was equally inhibited in adult and cord lymphokine-activated killer cells (LAK) by K562, Molt or Raji cells. However, cold target inhibition of cord LAK activity against K562 was less than adult LAK, suggesting less specificity and more potent cytotoxic potential. Unlabelled K562 cells decreased cytotoxicity by  $44 \pm 5\%$  for adult LAK (n=7) and  $27 \pm 9\%$  for cord LAK (n=4). Unlabelled Raji cells decreased K562 cytotoxicity by  $34\%$  for adult cells and  $12 \pm 3\%$  for cord cells (p<0.01).

This substantial lymphokine-activated cytotoxicity suggests the possibility of therapeutic intervention in neonatal viral infections and neoplastic disorders with purified lymphokines.

● **965** REGULATION OF IgA SUBCLASS PRODUCTION BY EPSTEIN BARR VIRUS. M.E. Conley, M. Chan, N.H. Sigal, Children's Hospital of Philadelphia; Hospital for Sick Children, Toronto; and Merck Sharp and Dohme, Rahway, N.J.

We have demonstrated by limiting dilution analysis that a high percentage of clones derived from Epstein-Barr Virus (EBV) stimulated peripheral blood lymphocytes (PBL) secrete IgA. To further characterize the IgA produced by these clones the IgA subclass of supernatants from clones stimulated 6-8 weeks earlier with EBV was determined by RIA. 17/17 clones were positive for IgA<sub>1</sub>; none were positive for IgA<sub>2</sub>. Because we have shown an enrichment for IgA<sub>2</sub> precursors in surface IgM<sup>+</sup> B cells, panning techniques were used to separate sIgM<sup>+</sup> B cells from tonsils. 32/32 clones from these sIgM<sup>+</sup> B cells secreted IgA<sub>1</sub>; none secreted IgA<sub>2</sub>. Past experiments have demonstrated a discordance between plasma cell production and immunoglobulin secretion. Therefore cytoplasmic staining for IgA<sub>2</sub> was done on EBV stimulated PBL harvested 7, 10, 14 and 21 days after culture. In all 5 experiments, the percentage of IgA plasma cells positive for IgA<sub>2</sub> decreased with increasing duration of culture. A mean of 25.5% of the IgA plasma cells were positive for IgA<sub>2</sub> at Day 7 and 7.2% at Day 21. These results are unlikely to be due to isotype switching from IgA<sub>2</sub> to IgA<sub>1</sub> as the gene for  $\alpha_2$  is more distal to the  $\mu$  gene than the gene for  $\alpha_1$ . Instead, there may be a difference between limited proliferation and differentiation induced by EBV, and immortalization. Although IgA<sub>2</sub> plasma cell precursors may undergo some proliferation and differentiation after EBV stimulation they are not immortalized. There is selective immortalization of IgA<sub>1</sub> producing cells.

**966** A SEVERE CHROMOSOMAL BREAK SYNDROME WITH PROFOUND IMMUNODEFICIENCY. M.E. Conley, B. Emmanuel, P.C. Nowell. University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, PA

The Chromosomal Break Syndromes: Ataxia Telangiectasia, Fanconi's Anemia, and Bloom's Syndrome are associated with growth failure, microcephaly, neurologic abnormalities, immunodeficiency, failure of secondary sexual characteristics and an increased incidence of malignancy. The relationship between these features is unknown. We recently evaluated a 21 year old female with more severe chromosomal breakage, immunodeficiency and growth failure than in any of the above disorders. It is of note that she has not yet developed a malignancy. Growth failure was apparent in the first year of life and lymphopenia and hypogammaglobulinemia at age 6. At 18 years of age, her weight was 22.6kg (50th% for 7 years) height was 129 cm (50th% for 8 years) OFC was 42 cm (50th% for 6 months). The peripheral blood contained 400-900 lymphocytes/mm<sup>3</sup> with 32% T<sub>11</sub> cells, 17% T<sub>4</sub> and 21% T<sub>8</sub> cells. The proliferative response to the mitogens PHA, PWM and ConA was less than 5% of control. There were 0.2% surface IgM bearing cells (n1 4-15%), and serum IgG was 185 mg/dl, IgM 7 mg/dl, IgA <5 mg/dl. In lymphocyte cultures stimulated with mitogens for T cells (PHA/TPA) or B cells (EBV), nearly half the metaphases examined had one or more chromosome breaks or rearrangements, but there was no evidence of a cytogenetically-abnormal clone. These findings suggest that factors other than the severity of the immunodeficiency or the high incidence of chromosomal damage contribute to the occurrence of malignancy in the Chromosomal Break Syndromes.