● 956 THE WISKOTT-ALDRICH SYNDROME: A MEMBRANE DEFECT OF THE LYMPHO-HEMATOPOIETIC SYSTEM.

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The Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by eczema, decreased T lymphocyte function, and decreased platelet count with small, poorly functioning platelets. A unitarian hypothesis to explain the various immune and hematological manifestations of WAS has not existed. The size of WAS platelets normalized and their in vitro function improves following splenectomy suggesting the WAS platelet defect is an acquired rather than an intrinsic defect. To determine if the basic defect in WAS patients is a membrane defect present in both platelets and lymphocytes, the size of lymphocytes of a WAS patient was determined before and after splenectomy. The volume of the patient's lymphocytes was markedly reduced before splenectomy ($1264 \pm 20\mu^3$) as compared to normal lymphocytes ($2320 \pm 228\mu^3$, n=6). Seven days after splenectomy two populations of peripheral lymphocytes were identified, one of which was slightly smaller than the patient's original lymphocytes ($1114 \pm 76\mu^3$) while the second was normal size ($2288 \pm 64\mu^3$). Fourteen days after splenectomy only normal sized lymphocytes were detected. No change in lymphocyte size was seen in patients with ITP following splenectomy (n=2). No change in the normal blastogenic response of the patient's T lymphocytes was seen after splenectomy. These results suggest that the basic defect in WAS may be a membrane defect which leads to the production of small platelets and lymphocytes (microplatelets and microlymphocytes) by the spleen.

957 STUDIES OF PRE-B CELLS IN HUMAN BONE MARROW.

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Pre-B cells contain intracytoplasmic IgM but lack surface immunoglobulin (sIg) detectable by immunofluorescence and are postulated to be the precursors of sIgM+ B cells in human bone marrow. Studies of the differentiation capacity of pre-B cells have been hampered by their low frequency and by the presence of B lymphocytes in normal bone marrow. I prepared a marrow cell suspension enriched for lymphoid cells by centrifugation over a 15-35% discontinuous sucrose density gradient. B lymphocytes were depleted by incubation on petri dishes coated with anti-Ig or anti-IgM antibodies. The final cell population was deficient in B lymphocytes and enriched for small pre-B cells.

Unfractionated		Lymphoid Enriched		B Cell Depleted	
%sIgM+	%pre-B	%sIgM+	%pre-B	%sIgM+	%pre-B
3.9(1.2)*	2.5(2.6)	9.2(5.4)	8.6(6.9)	0.6(0.5)	11.5(11.6)

*Mean percent of cells (S.D. of mean) N=5 experiments

Results of preliminary experiments suggest that absolute numbers of sIgM+ cells significantly increase during a brief period of culture under appropriate conditions. CONCLUSIONS: These results provide additional evidence that pre-B cells lack stable sIgM molecules. Human bone marrow enriched for pre-B and depleted of B cells could prove useful in exploring the regulatory events involved in bone marrow B lymphopoiesis in vitro.

958 PRODUCTION OF MUCOSAL ANTIBODY FOLLOWING PARENTERAL VACCINATION WITH *HAEMOPHILUS INFLUENZAE* TYPE B CAPSULAR POLYSACCHARIDE (PRP).

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Anti-PRP antibody (ab) in sera and mucosal secretions from 5 children (>18 mo of age) and 4 adults was quantified by radioantigen binding, before and 3 weeks after immunization with 15 µg of PRP. Total IgA in secretions was quantified by laser-nephelometry. 8 of 9 subjects had detectable serum ab prior to vaccination and all produced an increase (children 4.5-640X, adults 3.3-48X) in serum ab following immunization. Prior to vaccination 3 of 5 children had detectable ab in nasal secretions (519-1828 ng ab/mg IgA). 2 of the 3 children with preimmunization nasal ab had an increase of ab following vaccination (2.3 & 7.7X) and 1 had no response. Both children with nondetectable preimmunization nasal ab levels had detectable levels following vaccination (1026 & 2093 ng ab/mg IgA). All adults had ab in nasal mucus (120-1917 ng ab/mg IgA) and 3 of 4 adults had ab in parotid saliva (127-241 ng ab/mg IgA) prior to vaccination. 3 of 4 adults had an increase (1.6-6.2X) and 1 had a transient decrease of nasal ab and all adults had an increase in salivary ab (3-8.5X in those with detectable preimmunization levels and to 1419 ng ab/mg IgA in the other subject) following vaccination. The subjects who did not have an increase in mucosal ab had the highest preimmunization mucosal ab titers. We conclude that, unlike vaccination with many other non-replicating antigens, PRP immunization often increases mucosal as well as serum ab.

● 959 ABNORMAL LYMPHOCYTE ACTIVATION IN CARTILAGE HAIR HYPOPLASIA.

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Cartilage-Hair Hypoplasia (CHH) is an autosomal recessive form of short-limbed dwarfism which occurs in increased frequency in the Amish. In previous studies of Amish CHH patients we found markedly decreased lymphocyte proliferative responses to phytohemagglutinin, concanavalin A (Con A), pokeweed mitogen and allogeneic cells. The present study was undertaken to determine if these abnormalities were due to imbalances in lymphocyte subpopulations or defective lymphocyte activation. Studies on 9 CHH patients with specific monoclonal antibodies revealed a decrease in the absolute number of T- and B-lymphocytes but a normal helper to suppressor cell ratio (OKT4+/ OKT8+). The number of monocytes assayed by non-specific esterase, latex ingestion and anti-OKM1 were normal; CHH monocytes functioned normally as helper cells and did not demonstrate suppressor activity when cocultured with CHH or normal T-lymphocytes.

CHH lymphocyte membranes bound Con A and the mitogenic monoclonal anti-OKT3 normally, however CHH T-lymphocytes showed markedly reduced proliferative responses to Con A, OKT3, phorbol myristate acetate and Ca++ ionophore A23187. CHH B-lymphocytes proliferated poorly after stimulation with S. aureus.

These data suggest that CHH lymphocytes have a defect in activation at a post-membrane, post-calcium influx step. CHH lymphocytes may be a useful model for the study of defects in cell proliferation which may also exist in other cells of CHH patients (e.g. chondrocytes).

960 THE ACCURACY OF IDENTIFYING T CELL LEUKEMIA USING E ROSETTING OF BONE MARROW LYMPHOBLASTS.

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The identification of sheep erythrocyte rosettes (ER) formed by acute lymphocytic leukemia (ALL) cells (blasts), at 4° C has permitted investigators to distinguish patients with presumed T cell ALL from those with non-T ALL (less than 10-20% ER(+) blasts). The identification of T antigen (T-ag) on the blasts, detected by monoclonal or carefully absorbed xenoantisera, now permits an analysis of how accurately ER positivity distinguishes T from non T ALL. Bone marrow blasts from 354 pediatric patients with untreated ALL were examined for ER and T-ag. The following chart summarizes the results:

Patients with % E Rosette Positive ALL:

	<10	10-19	20-40	>40	Total
T-ag (-)	256	27	8	2	293
T-ag (+)	19	7	12	23	61

Using the reference point of > 10% (20%) ER (+) blasts to define T cell disease would have led to the incorrect judgement that 37(10) T-ag (-) patients had T cell ALL, a 60%(16%) excess, while 19 (26) T-ag (+) patients would have been excluded, a 31%(42%) deficit. This degree of error in subclassification could substantially affect the results of descriptive studies on therapeutic trials in T cell leukemia, indicating the need for careful T-ag detection in classifying ALL.

961 MATERNAL-INFANT TRANSFER OF NON-HUMORAL IMMUNITY TO INFLUENZA IN THE MOUSE.

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Evidence was sought for the transfer of influenza specific non-humoral immunity from mother to infant mouse. We studied infant mice born to 3 groups of mothers: (1) influenza immune mothers in whom influenza-specific serum antibody was suppressed by passive antibody received prior to non-lethal influenza infection (I-Ab), (2) influenza immune mothers with antibody (I+Ab) and (3) non-immune controls.

I-Ab and control mothers as well as their infants showed no evidence of influenza specific serum antibody 7 days after infection. In contrast, high levels of serum antibody were found in all I+Ab mothers and infants 7 days after infection ($P<0.001$). After a lethal influenza challenge, no infant mortality was found in either immune group (I-Ab, I+Ab), whereas 11 of 16 infants in the control group died. Three days after lethal challenge, I-Ab infants showed a lower but not statistically different mean lung virus titer when compared to controls ($P=0.11$). At 7 days, lung virus titers of control infants continued to rise, while titers of I-Ab and I+Ab infants declined. Nasal virus titers of I-Ab and control infants did not differ, whereas, nasal virus titers of I+Ab infants showed a decline from day 3 through 7 after infection.

We conclude that influenza specific non-serum-antibody mediated immunity is 1) transferred from mother to infant, 2) protects against death, 3) plays a role in reducing lung virus shedding and 4) has no effect on nasal virus shedding.