USE OF THE *IN VITRO* BACTERICIDAL ASSAY TO MEASURE FUNCTIONAL OPSONINS IN CONTROL (C) AND WISKOTT-ALDRICH PATIENTS (WA). Rochelle G. Neiburger and Robert L. Baehner, Indiana Univ., Dept. of Ped., Indianapolis.

Functional opsonins against 3 microorganisms which vary in dependence on heat stable specific antibody (HSO) and heat labile complement (HLO) were measured in WA and age matched C by an adaptation of the *in vitro* bactericidal assay. Normal phagocytes were incubated with bacteria and fresh or heat inactivated serum at various dilutions and viable colonies determined over 2 hours. Sera were collected for 4 weeks following administration to WA and C of acellular vaccine containing streptococcal and staphylococcal protein and pneumococcal polysaccharide. Opsonization and killing of *S. pyogenes* was primarily dependent upon HSO since heated sera of WA and C supported killing of this organism. Both sera showed increased opsonization of *S. pyogenes* following vaccination. Opsonization of *D. pneumoniae* depends mainly on HLO; heated sera from WA and C did not support its killing. Unheated C sera showed a small increase of killing following vaccination but there was no change with WA sera. HSO influenced killing of *S. aureus* to a moderate degree; the older WA was equal to C whereas the younger WA developed normal levels with time. This test confirms that WA can make functional opsonins in response to protein antigens as shown by successful killing of *S. aureus* and *S. pyogenes* but fail to respond to polysaccharide antigen following immunization. The assay can be used to demonstrate the relative contribution of HSO and HLO to bacterial killing by phagocytes.

CELL-MEDIATED IMMUNITY IN CHRONIC CANDIDIASIS. Robert D. Nelson and David M. Brown, Departments of Laboratory Medicine and Pathology and Pediatrics, University of Minnesota School of Medicine, Minneapolis, Minnesota.

Cell-mediated immunity in 15 patients with chronic mucocutaneous or systemic candidiasis has been studied by measuring the proliferative response of patients' peripheral mononuclear cells to mitogen and specific antigen *in vitro*. Cells from all patients responded within normal limits to mitogen, but consistently failed to proliferate in response to antigen, including candida antigen. Positive responses to candida antigen were obtained, however, by increasing the density at which the cells were cultured and by addition of monocytes from normal donors. Patient's monocytes failed to reconstitute the response of monocyte-free lymphocytes from normal, candida-sensitive donors to antigen. These results demonstrate that candidiasis patients have circulating candida-sensitive lymphocytes and suggest that failure of these cells to proliferate in response to antigen is due to defective monocytes. Further experiments suggest that this defect may be acquired due to factors present in patients' sera. Work supported by NIH, Minnesota Heart Association, Minnesota Medical Foundation and the University of Minnesota Graduate School.

THE WISKOTT-ALDRICH SYNDROME: A GENETIC DEFECT FUNCTIONALLY EXPRESSED IN MARROW STEM CELL DERIVATIVES. Hans D. Ochs, Sherrill J. Slichter, Lawrence A. Harker, Starkey D. Davis, Wieland von Behrens, Ralph J. Wedgwood. Univ. of Washington Sch. of Med., Depts. of Ped. and Med., Seattle.

Three of four non-related boys presented with thrombocytopenia, eczema, bloody diarrhea and frequent infections. The fourth initially had thrombocytopenia only, was splenectomized, and subsequently developed repeated infections and eczema. Immunoglobulins were variably abnormal. Antibody production to bacteriophage ØX 174 (a protein antigen) was abnormal with low primary and severely impaired secondary response. No IgG antibody was formed. Lymphocyte transformation with non-specific mitogens and allogeneic cells was significantly impaired and the number of E-rosettes diminished.

Volumetric size of platelets was reduced to 50%. Except in the splenectomized patient, autologous platelet survival time was normal. In all patients, *in vivo* and *in vitro* platelet function when corrected for platelet size was normal. Since megakaryocyte mass was adequate, thrombocytopenia must result from ineffective thrombocytopoiesis. Red blood cell (RBC) size was also reduced.

The abnormal B and T-cell function, the ineffective platelet production and decreased size of platelets and RBC in this X-linked disease suggests that a single genetic defect is expressed functionally in all stem cell derivatives.

IMMUNOLOGIC ASPECTS OF VIRAL CROUP IN CHILDHOOD. Pearay L. Ogra, Dept. of Ped., State Univ. of N.Y. at Buffalo.

A comparative evaluation of serum and secretory antibody responses to influenza A2/HK, para-influenza and other viruses was undertaken in groups of children with acute viral pharyngitis, croup or bronchiolitis. The techniques of tissue culture infectivity, neutralizing antibody assay, and indirect immunofluorescence were employed to recover infectious virus from the respiratory tract and to characterize specific antibody response in the serum and nasopharynx. Serologic evidence of infection or recovery of influenza A2/HK or parainfluenza 1 and 3 viruses was most frequently observed in patients with croup. The sequence of appearance and the levels of serum γ G, γ A and γ M influenza and para-influenza antibodies in patients with pharyngitis, croup or bronchiolitis were generally similar. However, nasopharyngeal antibody levels to influenza or para-influenza viruses were significantly lower in patients with croup when compared to the levels obtained in patients with pharyngitis or bronchiolitis. Furthermore, nasopharyngeal antibody response to influenza virus was conspicuously absent in a few patients with severe influenza A2/HK croup who required tracheostomy on hospitalization. The reduction or absence of nasopharyngeal antibody levels was not related to virus-antibody complexes and no change was observed in nasopharyngeal antibody levels for as long as 3-4 months after recovery from the infection. These observations suggest that alterations of secretory immune response in the respiratory tract may be related to the pathogenesis of viral croup.

USE OF GNOTOBIOTIC METHODS FOR A CHILD WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID) TRANSPLANTED WITH MARROW FROM AN UNRELATED MIXED LYMPHOCYTE CULTURE (MLC) COMPATIBLE DONOR. Immunodeficiency Study Group: Mem. Sloan-Kettering Cancer Ctr., New York City, Tissue Typing Lab., Rigshospitalet and State Serum Inst., Copenhagen. Presented by Richard O'Reilly. (Intr. by E.M. Smithwick). Dept. Ped. MSKCC.

A male infant in a family with the autosomal form of SCID was delivered vaginally. SCID was diagnosed on cord blood and later confirmed. He was kept free of infection by reverse isolation in a laminar flow hood from age 1 week until transplant 5 months later. Before transplant, decontamination was begun with chlorhexidine for the skin and oral cephaloridine, neomycin and mycostatin for the upper respiratory and GI tracts. Within 3 days and thereafter all cultures (including washed stools) revealed no growth.

The infant has received 2 marrow grafts from an unrelated female donor differing by one allele but matched by MLC. Ten days after the 1st, the maculopapular rash of graft vs host (GVH) began. Eosinophilia and hepatosplenomegaly are the only other signs of GVH. The patient shows early reconstitution: palpable nodes, synthesis of IgM and IgA, weak PHA responses, lymphocytes with donor specificity. Previous marrow grafts in SCID using an unrelated donor have led to severe GVH with fatal infectious complications. This infant has had mild GVH without infection.

ENHANCEMENT OF *IN VITRO* CELLULAR IMMUNE RESPONSE BY L-TETRAMISOLE. Henry F. Pabst, Joan A. Crawford (Intr. by E.E. McCoy). Univ. of Alberta, Dept. of Ped., Edmonton, Alberta.

L-tetramisole, a potent antihelminthic, has recently been shown to enhance the ability of anergic cancer patients to both become sensitized and respond to the contact sensitizing agent 2,4-DNCB (New Engl. J. Med. 289: 354, 1973). L-tetramisole is hypothesized to stimulate the T-cell system (*ibid*, 375), and we have tested this hypothesis by examining the effects of L-tetramisole on the response of normal human lymphocytes to T-cell stimulating antigens. Following ficoll-hypaque isolation, 2×10^6 lymphocytes were cultured in triplicate for 5 or 8 days, with or without specific antigens (candida, measles virus, or PPD), in the presence or absence of L-tetramisole (1 mg/culture, optimal dose), in 4 ml RPMI 1640 containing 20% autologous serum. Each culture received 2 μ C³H-thymidine 24 hours prior to harvesting, and *in vitro* cellular immune response was measured as ³H uptake. Cells cultured in the presence of L-tetramisole showed significantly greater response to each of the three antigens than those cultured without the drug ($p < 0.01$). Cells from donors with pre-existing antigen immunity responded within 5 days, but those with mild or absent pre-existing sensitivity responded after 8 days. Our results clearly indicate an *in vitro* immunostimulant activity of L-tetramisole, and suggest the potential importance of this drug both in the treatment of patients with primary cellular immunity defects and in the management of cancer.