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TETANUS SKIN TEST: DOES IT ASSESS DELAYED HYPERSENSITIVITY? Thomas C. Borut, Bonnie J. Ank, E. Richard Stiehm, UCLA School of Medicine, Department of Pediatrics, Los Angeles.

To determine if the tetanus skin test (TST) is a valid procedure for the evaluation of delayed hypersensitivity we correlated the results of skin testing with *in vitro* lymphocyte transformation to tetanus (TTx), antibody titers to tetanus by hemagglutination (TAB), and monocyte chemotaxis (MCTX). We tested 6 unimmunized infants less than 10 weeks of age, 6 patients with X-linked agammaglobulinemia (AGG) and 35 immunized controls (8 weeks-35 years) with 0.1 ml of a 1:5 dilution of fluid tetanus toxoid; a positive TST at 48 hours was induration >5 mm in diameter. A transformation index (cpm of 2×10^5 stimulated cells/cpm unstimulated cells) of >2 and a TAB >1:4 were considered positive. MCTX was performed by the agarose gel technique.

All 6 unimmunized infants had negative TST and TTx despite TAB from 1:2 to 1:32. 5/6 AGG had positive TTx and 4/6 had positive TST. In immunized controls 19/35 (54%) had a positive TST, including 14/17 over age 2 and 5/18 under 2. Among the 19 positive reactors all had positive TTx and TAB >1:4. Among the 16 negative reactors 16/16 had positive TTx and 15/16 had TAB >1:4. Of the patients with positive TTx and negative TST 6/6 studied had low MCTX while 11/12 with both positive TTx and TST had normal MCTX. There was a significant correlation between TST reactivity and TTx and MCTX but not between TST and TAB. We conclude that a positive TST is independent of TAB, that TST measures T-cell immunity but is less sensitive than TTx and that a positive TST necessitates both intact TTx and MCTX.

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EFFECTS OF SURFACE ACTIVE AGENTS ON NEUTROPHIL RECEPTORS. Laurence A. Boxer, Susan B. Richardson, Robert L. Baehner. Indiana University School of Medicine, James Whitcomb Riley Hospital for Children, Department of Pediatrics, Indianapolis, IN 46202.

An easily performed assay to identify the C3b and Fc receptor on human polymorphonuclear leukocytes (PMN) was developed. *Salmonella typhi* were directly fluoresceinated and then incubated in non-immune fresh human serum which led to C3b fixation via activation of the alternative pathway. Similarly, Type II pneumococci were fluoresceinated and opsonized with type specific rabbit anti-serum. PMN bearing C3b and Fc receptors formed rosettes with the respective bacteria which were easily readable because of their bright fluorescence. Studies employing various surface agents were employed to provide information on the physical properties of PMN receptors. Incubation of PMN at 37°C with C3-coated bacteria generated 54±5% C3b rosettes whereas PMN incubated with IgG coated bacteria yielded 78±8% rosettes. Heat inactivation of the fresh human serum at 56°C for 30 minutes completely inhibited the formation of the C3b and addition of goat anti-rabbit IgG inhibited the formation of the Fc rosettes. Pre-incubation of PMN with 1 mg/ml Trypsin, 1 mM N-ethyl maleimide, xanthine-xanthine oxidase to generate 20 mM superoxide anion and hydrogen peroxide (H₂O₂), 10 mM H₂O₂, and 8×10^{-4} M hydrocortisone reduced the number of C3b rosettes to values of 25±6%, 7±6%, 22±4%, 36±2%, and 24±5%, respectively, but had no significant effect on the number of Fc rosettes. Thus, this study provides evidence for selective receptors for C3b and Fc on PMN membranes.

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HLA IN A PEDIGREE WITH IMMUNOLOGIC AND ENDOCRINE DISEASE. Stevan J. Cavalier, William Krivit, Kazimiera J. Gajl-Peczalska, Sandra Chartrand, Nancy Reinsmoen, John H. Kersey. Depts. of Pediatrics and Lab Medicine & Pathology, Univ. of Minnesota, Minneapolis, MN 55455.

A pedigree with a variety of rare immunologic and endocrine abnormalities was studied for immunogenetic associations.

Elsewhere in the maternal family hypothyroidism, scleroderma and polycythemia rubra vera occur.

HLA genotyping, plus the presence of an HLA B/D recombinant reveal that all affected share an HLA region of a single sixth chromosome, including the A and B, but not the D region. Suppressor cell assays of the proband indicate that one manifestation of the genetic defect is suppression of B-lymphocyte immunoglobulin production. (Supported by ACS grant IN-13)

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"DISAPPEARING BILE DUCT SYNDROME," RESULT OF A GRAFT VERSUS HOST (GvH) REACTION, AFTER FETAL LIVER AND THYMUS TRANSPLANTATION FOR SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID). Flossie Cohen, Rukmani Raghunath, Chester M. Zmijewski, Eugene V. Perrin & A. Joseph Brough. Wayne State University & Children's Hospital of Michigan, Detroit, MI.

A year-old male with SCID was transplanted with fetal liver & thymus from a female fetus removed by hysterotomy from his mother at 16 weeks gestation. The fetus differed from the patient in both HL-A haplotypes. Four days after transplantation, a GvH reaction appeared: fever, diarrhea, maculopapular rash & pos. skin biopsy. By 14 days, liver enzymes & bilirubin were elevated. Max. values: bilirubin 30mg% (tot.), 19mg% (dir.), SGOT 405U & SGPT 350U.

Chimerism was shown by total change in lymphocyte HL-A antigens. T cells rose from 5% to 75%, but did not respond to *in vitro* stimulation by mitogens, antigens & allogeneic cells to date, 7 mos. after transplantation. Immunoglobulins remained essentially absent. Following anti-thymocyte globulin & steroids, bilirubin remains around 10-14mg% (tot.) & 3-8mg% (dir.) & the enzymes have decreased. Liver biopsy 5 mos. after transplantation showed no necrosis, intact lobular architecture, patchy canalicular & hepatocellular bile stasis. Portal bile ducts were totally absent. There were small lymphocytic portal infiltrates. No plasma cells, hematopoietic cells, or fibrosis were seen. No virus was isolated.

The observations show that these fetal cells, capable of effecting a severe GvH reaction *in vivo*, repeatedly did not respond to various stimuli *in vitro* before or after transplantation. The observations also suggest that a GvH type reaction may be one cause of the "disappearing bile duct syndrome."

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BREAST MILK MACROPHAGES. Clarene Cress and Charles L. Paxson, Jr., U. of Nebraska Coll. of Med., Omaha, (Spons. by G. C. Rosenquist).

Animal studies suggest that preterm infants gavage fed human breast milk receive protection against enteric infection by the presence of macrophages (BMM), as well as immunoglobulins. We have been concerned about the effects of collection, storage, route of administration and other factors which may alter the survival of BMM.

Samples of fresh human breast milk from healthy mothers 3 mo. postpartum were collected into plastic containers. BMM were separated by dilution 1:3 with phosphate buffered saline, centrifugation, and resuspension in saline or veronal HCL buffer. Cells were examined by microscopy, counted, and viability tested by phagocytosis of trypan blue dye. Aliquots of BMM were exposed to alterations in temperature to simulate storage procedures (-5 to 56°C), pH changes to simulate gastric vs duodenal feedings (2-8), alterations in osmolality to simulate mixing BMM with infant formulas (200-400 mOsm/kg water), and addition of albumin to simulate variations in formula protein content.

Total BMM counts decreased with increasing postpartum age. Freezing markedly decreased survival, but heating to pasteurization did not. At pH above 7.2 survival and phagocytosis decreased, but the cells survived lowered pH ranges. Osmolality alterations produced no changes, but the addition of albumin diminished phagocytosis. We conclude that methods of procuring and feeding breast milk may markedly affect survival and phagocytic ability of BMM.

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ACQUIRED AGRANULOCYTOSIS SECONDARY TO A SERUM INHIBITOR. William M. Crist, Fabio Pereira, Caroline Feist. The University of Alabama School of Medicine and The Children's Hospital, Birmingham, Alabama. Intro. by Stagno, S.

A 3 year old female presented with perioral and perineal ulcerating skin lesions and 104°F temperature. The peripheral white blood cell count (P.B.) was 3200/mm³ with 0% neutrophils, 11% monocytes, 0% eosinophils, 89% lymphocytes (20% atypical) and 0% basophils. Hb. was 7gm/dl and Hct. 21.9%. Platelet count was 644,500/mm³. Bone marrow (B.M.) smears revealed normal numbers of nucleated red blood cells and megakaryocytes. No myeloid precursor cells were discernible with either Wright - Giemsa or myeloperoxidase staining techniques.

In vitro soft agar culture of P.B. nucleated cells and B.M. cells revealed normal P.B. and slightly dec. numbers of B.M. myeloid colony forming cells (CFC). Patient P.B. nucleated cells supported colony growth from control B.M. cells normally indicating normal colony stimulating activity (CSA).

Serum (0.3cc) mixed with 1cc of normal B.M. cells plated at 10⁵ cells/cc (in quadruplicate) markedly inhibited CFC (mean CFC of 2.5 col/10⁵ cells plated compared to 19.5 col/10⁵ cells plated without serum added). Maturation within the colonies to mature neutrophils and/or monocytes was noted in all situations. The patient clinically improved and had return of normal P.B. white cell count within 2 weeks. When studied 10 months later with normal blood counts and B.M., her serum showed stimulation of normal marrow CFC. These studies suggest that the patient's agranulocytosis was secondary to a serum inhibitor directed against the myeloid stem cell.