

SERONEGATIVE JRA PATIENTS EXPRESS THE MAJOR RHEUMATOID FACTOR CROSS-REACTIVE IDIOTYPE. Josiah Wedgwood, Norman Ilowite, Amy Lewison-Nisen, and Vincent Bonagura. Spon. by K. King, SUNY Stony Brook and Schneider Children's Hospital of LIJMC, Dept. Peds., Div. of Immunology, New Hyde Park, NY.

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The majority of children with Juvenile Rheumatoid Arthritis (JRA), are seronegative for rheumatoid factor (RF) by classical Fc binding assays, and are clinically separable from RF⁺ adults with rheumatoid arthritis (RA). The major RF cross-reactive idiotype (RCRI) defined by monoclonal IgM RFs, is preferentially expressed as a dominant idiotype in most RF⁺ and some RF⁻ RA adults. To determine whether the regulation of the dominant RF idiotype in RA adults is similar to RF⁺ JRA patients, we examined sera from 31 individuals with JRA by ELISA, and in PWM induced plasma cells (PCs) from 8 of these patients by cytoplasmic indirect immunofluorescence.

| | RCRI+ by ELISA/ TOTAL PATIENTS | % RCRI+PC/ TOTAL PATIENTS |
|-------------------|-----------------------------------|------------------------------|
| JRA | | |
| 1. Polyarticular | 3/6 (p <.05) | 1/1 |
| 2. Pauciarticular | 4/19 | 4/6 |
| 3. Systemic | 3/6 (p <.05) | 1/1 |
| Controls | 1/18 | 1/9 |

Sera from some RF⁻ JRA patients contain the major RCRI as previously demonstrated in RA adults. Most of the patients studied also express the major RCRI as a dominant idiotype among PWM PCs. Expression of the dominant RF idiotype is therefore similar in some JRA and adult RA patients.

IMMUNIZATION OF INFANTS WITH Haemophilus influenzae TYPE b (Hib) POLYSACCHARIDE-OUTER MEMBRANE PROTEIN (PS-OMP) CONJUGATE VACCINE PRIMES FOR IgG1 BOOSTER RESPONSES TO CONVENTIONAL Hib PS VACCINE. Geoffrey A. Weinberg, Menachem S. Einhorn, Allen A. Lenoir, Paul D. Granoff, and Dan M. Granoff, Washington Univ. Sch. of Med., Dept. of Peds., St. Louis, MO.

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A conjugate vaccine of Hib PS linked to an OMP of *N. meningitidis* is immunogenic in infants as young as 2-3 mo. (Lancet 1986;2:299). 43 children immunized at ages 2-17 mo. have been followed for 10-15 mo. The geo. mean serum Hib PS antibody (Ab) concentration declined but remained higher than that of unimmunized controls (p<0.01). 30 children were reinjected with conventional PS vaccine at ages 14-30 mo., and showed 20-50 fold average increases in total and IgG Ab. Serum Ab has persisted at high levels for >6 mo. The geo. mean Ab response of the reimmunized children was 10-fold higher than that of control children immunized with PS for the first time, and was not significantly different than that of adults immunized with PS vaccine. However, the IgG Ab responses of the adults were composed of both IgG1 and IgG2 (geo. mean G1/G2=1.7), while those of the infants were restricted to IgG1 (G1/G2=9.7, p=0.01). Thus, exposure to PS-OMP primes infants to an IgG1 memory Ab response to PS vaccine. In contrast, infants with Hib disease at ages 3-17 mo., and immunized with PS vaccine 7-21 mo. later did not show a memory Ab response.

| PRIMED IN | AGE AT PS | NO. | PRE | POST | IgG POST |
|--------------|---------------|-----|-------------------------------|------|----------|
| INFANCY | VACCINE (mo.) | | GEO. MEAN ANTI-Hib PS (ug/ml) | | |
| YES (PS-OMP) | 14-20 | 19 | 0.73 | 31.8 | 16.7 |
| YES (PS-OMP) | 22-30 | 11 | 0.60 | 30.0 | 13.4 |
| NO (HEALTHY) | 24-30 | 13 | 0.26 | 2.7 | 0.6 |
| NO (DISEASE) | 24-27 | 8 | 0.67 | 2.0 | ND |
| NO (HEALTHY) | ADULTS | 12 | 2.83 | 51.0 | 10.4 |

POLYMORPHISM OF HUMAN C3: CLINICAL AND FUNCTIONAL CONSEQUENCES. Thomas R. Welch, Linda S. Beischel and Clark D. West. Children's Hospital Research Foundation, Cincinnati, OH.

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Distortions in frequency distribution of the allotypes of human C3 (C3*S and C3*F) have been linked to disease. Explanations for these associations have invoked the possibility of functional differences between the allotypes. We report the first detailed studies of C3 allotype function and distribution in disease.

Purified C3 of each type was prepared from plasma of homozygous donors. In a standard functional assay with sheep erythrocytes (EA's), the hemolytic efficiency of C3S was 1.3 times that of C3F. Correspondingly, C3S uptake on EA's via the classical C3 convertase (assessed by anti C3c binding) was 1.4 times that of C3F.

Interaction of C3S and C3F with regulators was measured using C3b of each type incubated with purified I and increments of purified H or a source of CRI. No difference in the proteolytic formation of C3bi by each allotype was seen.

Labeled BSA and anti-BSA complexes were solubilized in sera containing an excess of C3S or C3F, and uptake of complexes onto erythrocytes was measured. Uptake at 15 minutes was identical, and no kinetic differences were apparent.

Distribution of C3S and C3F was examined in groups of patients with diseases in which C3 is thought to be involved; SLE (30), MPGN (33), and IgA nephropathy (31). No significant differences from the distribution of allotypes in 100 healthy controls (C3S = .8; C3F = .2) were evident.

We thus found no association between C3 genetic polymorphism and diseases in which C3 is relevant. More importantly, we identified no important functional differences which could explain such associations, other than trivial differences in affinity for EA's.

INFLUENZA A VIRUS (IAV) MAY INDUCE CYTOSKELETON DYSFUNCTION IN POLYMORPHONUCLEAR LEUKOCYTES (PMNL) BY DEPRESSING PROTEIN PHOSPHORYLATION. J. Gary Wheeler, Lisa F. Cassidy, Susan E. Caldwell, David A. Bass, Jon S. Abramson. Depts. of Ped. and Med., Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC.

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Previous studies have shown that most harvests of IAV depress PMNL end-stage functions (oxidative metabolism, degranulation, and chemotaxis) and are called DV. Some harvests affect none of these functions and are called nonDV. Using both DV and nonDV we studied whether IAV induced alterations in protein phosphorylation or changes in F-actin. Using SDS-PAGE and autoradiography (³²P), PMNL were incubated with buffer, DV and nonDV to determine if differences in phosphorylation exist in cells stimulated with phorbol myristate acetate. DV, but not nonDV, decreased phosphorylation of multiple proteins including some with Mol. Wts. corresponding to cytoskeletal regulatory proteins and to the myosin light chain. Conversion of G- to F-actin was measured by staining fixed cells with NBD-phalloidin and monitoring fluorescence by flow cytometry. Increases in F-actin occurred in DV treated PMNL compared to control. After fMet-Leu-Phe (fMLP) stimulation of PMNL pre-incubated with DV, nonDV or buffer, there was a decrease in peak to prestimulation F-actin fluorescence ratios in both DV and nonDV compared to buffer. Using fluorescence microscopy we compared the regional distribution of labeled virus and the distribution of F-actin in fMLP stimulated PMNL. In polarized cells there was a disassociation between DV (in the uropod) and F-actin (in the lamellipodium). The finding of abnormal phosphorylation may explain the changes in microfilament organization and end-stage functions noted in IAV infected PMNL.

RADIATION EFFECTS ON THYMIC EPITHELIUM ARE PARTIALLY RESPONSIBLE FOR THE T-CELL DEFICITS OBSERVED IN BONE MARROW TRANSPLANT RECIPIENTS. Susan E. Wiedmeier, Wolfram Samlowski, Clark J. Rasmussen, Raymond A. Daynes (spons. by Harry R. Hill). University of Utah Medical Center, Dept. of Pathology, Salt Lake City.

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Experiments were designed to test the hypothesis that irradiation of the thymic epithelium is in part responsible for some of the many deficits in T-cell immunocompetence observed following total body irradiation and successful bone marrow transplantation. C3H/HeN mice were lethally irradiated (7.5 Gy) either immediately before or after the intracranial implantation of fetal thymic epithelial grafts. Syngeneic nucleated fetal liver cells were then provided for hematopoietic stem cell reconstitution. At various times post-irradiation, the animals were analyzed for recovery of T-cell dependent functions. In all instances the lymphoid cells obtained from animals whose thymic grafts were not irradiated showed significantly greater responses to T-cell mitogens and allo-antigen stimulation. It was also determined that the capacity of these animals to elicit contact hypersensitivity responses was significantly greater when compared to animals whose thymic grafts had been irradiated. A reduction in the number of medullary epithelial cells was observed in the thymic tissue exposed to lethal irradiation, while no quantitative differences were found in the number of Thy 1⁺, L3T4⁺/Lyt2⁻ or L3T4⁻/Lyt2⁺ cells in the peripheral lymphoid compartments of the two experimental groups. These data demonstrate that exposure of thymic epithelium to γ -irradiation has a negative influence on its ability to reconstitute T-cell function in lethally irradiated fetal liver reconstituted mice.

LYMPHOCYTE RESPONSES TO HISTAMINE AND CORTISONE ARE ALTERED FOR ONE YEAR FOLLOWING ACUTE EBV INFECTION.

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Peripheral blood lymphocytes (PBL) of 12 normal young adults studied for 1 year after acute Epstein-Barr virus infection (EBV), responded differently from 12 age-matched normals to stimulation with phytohemagglutinin (PHA 3 ug/ml) or pokeweed mitogen (PWM 20 ug/ml) when modified by histamine (HT 10-4 M) or hydrocortisone (HC 10-5 M). Measured monthly by tritiated thymidine incorporation, suppression of PHA stimulation index (SI) by HT in normals (32+/-19% SD of original PHA SI) was less in EBV patients: EBV-PHA + HT/PHA year ave. = 67+/-19% (p<0.001). EBV may have altered PBL H1 and H2 receptors since neither cimetidine (C 10-4M) nor diphenhydramine (D 10-4M) addition could restore the original PHA SI. Further, normal PWM SI suppression by HT (58+/-22% of original PWM SI) was absent: EBV-PWM + HT/PWM year ave = 101+/-21% (p<0.001). While C had no effect on PWM SI, D enhanced the PWM + HT cultures 2-3 times, during most of the year in only post-EBV PBL. Addition of HC to PHA cultures produced normal amounts of suppression (95%) after EBV. In contrast, normal suppression of PWM SI (49+/-18%) by HC addition was significantly lost (p<0.001) at 1 and 4-5 months and at 1 year after EBV. These abnormal responses to HT and HC suggest that profound changes in lymphocyte behavior occur for at least 1 year during the normal recovery from acute EBV, and that they may be related to post-EBV symptoms. Their relationship to serum Immunoglobulin E content is being investigated.