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SERUM OPSONIC ACTIVITY AND OPSONIC RESPONSE TO PNEUMOCOCCAL VACCINATION IN SPLENECTOMIZED CHILDREN. G. Scott Giebink, John E. Foker, Priscilla J. Thomas and Paul G. Quie. Depts. of Pediatrics and Surgery, University of Minnesota, Minneapolis, Minnesota.

The risk of life-threatening pneumococcal bacteremia is increased following splenectomy, and there have been conflicting reports regarding serum opsonic activity in these patients. We measured the opsonic activity in serum from 20 children splenectomized because of trauma. Serum was obtained before and one month after vaccination with polyvalent pneumococcal polysaccharide vaccine. Opsonic activity was also measured in a non-vaccinated control group of 78 non-splenectomized children. Pneumococcal opsonic activity was determined by incubating ^{3}H-thymidine labeled S. pneumoniae types 6, 7 and 23 in patient serum and measuring kinetics of uptake by normal human leukocytes. Alternative pathway (AP) opsonic activity was determined by incubating labeled S. typhimurium in patient serum chelated with MgEGTA. While 2 splenectomized children showed mildly depressed AP opsonic activity for salmonella, all 20 showed normal opsonic activity for the 3 pneumococcal types. Thus, the predilection of splenectomized patients for pneumococcal bacteremia is not due to a specific pneumococcal opsonic defect. Following vaccination, 18 children showed increased opsonic activity for at least one of the pneumococcal types; 10 children responded to 2 types, and 4 responded to all 3 types. Thus, serum pneumococcal opsonic activity can be enhanced in splenectomized patients by vaccination with pneumococcal polysaccharide.

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ABSENCE OF SIXTH COMPONENT OF COMPLEMENT (C6) IN A CHILD WITH CHRONIC MENINGOCOCCEMIA. Ronald Gold and Robert H. McLean, University of Connecticut Hlth Ctr., Dept. of Pediatrics, Farmington, Ct 06032.

We report the fifth patient with Neisseria sepsis and absence of functional C6. A six year old Black male developed chronic meningococcemia consisting of 3 episodes of fever, arthritis and rash over a 5 week period. Each acute illness resolved after 3-5 days. During the 3rd attack, Neisseria meningitidis, Group B was recovered from blood and nasopharyngeal cultures. Symptoms disappeared and have not recurred following treatment with penicillin G and sulfasoxazole. Total hemolytic complement (CH50) was undetectable. C6 function was absent by specific hemolytic assay and was partially restored following the addition of functionally pure human C6 to the patient's serum. All of the classic complement components were normal. Mother's CH50 was normal and C6 hemolytic titer was 88% of a normal pool. Granulocyte function as determined by the NBT test was normal (20% unstimulated, 60% stimulated). Immunoglobulin concentrations were normal. Antibody activity in serum obtained 5 days following the positive blood culture was assessed against the patient's strain of meningococcus. Fluorescent antibody titers were: IgG 1:512, IgA 1:64, IgM 1:256. Bactericidal titer of the patient's serum was <1:4. When normal rabbit serum was added, the bactericidal titer increased to 1:8. Recurrent Neisseria sepsis requires evaluation for the congenital absence of one of the terminal C components.

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QUANTITATIVE MICROIMMUNOASSAY FOR IMMUNOGLOBULIN CLASS SPECIFIC HUMAN ANTIBODIES. R.M. Goldblum, J.T. Howell and L.T. May. Dept. of Pediatrics, The University of Texas Medical Branch, Galveston, Texas.

In order to evaluate antibody responses in immunodeficient patients, we have developed a modification of the enzyme linked immunosorbent assay (ELISA) which is 1) sensitive, 2) specific for immunoglobulin class, 3) applicable to numerous antigens, and 4) quantifiable. The method requires sequential addition to microtiter wells of 1) antigen, 2) serially diluted serum, 3) anti immunoglobulin-alkaline phosphatase conjugate, and 4) synthetic enzyme substrate.

In order to express results in absolute amounts of each immunoglobulin bound to antigen, the activities of the conjugates were determined by absorption experiments. These indicated that the assays were sensitive to 1-10 ng of antibody. The immunoglobulin class specificity was demonstrated using sera from patients with selective immunoglobulin class deficiencies.

Twelve patients with either T or B lymphocyte dysfunction or severe combined immunodeficiency were evaluated before and after diphtheria and tetanus toxoid injection. Patients with x-linked B lymphocyte deficiencies did not produce detectable antibodies of any class. No specific IgM antibodies were formed by patients with dysgammaglobulinemia and hyper-IgM. A patient with T lymphocyte deficiency produced only IgM antibody despite normal quantities of all serum immunoglobulins. This ELISA assay has proven to be an effective tool for evaluating in vivo B lymphocyte function and T cell cooperation in primary immunodeficiencies.

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EFFECT OF PRENATAL STEROID ON LYMPHOCYTE FUNCTION IN THE NEONATAL RABBIT: M.Gupta, D.Vidyasagar, M.Zarif, M.M. Yokoyama, ALSM, Uni. Ill., Peds and Immuno., Chgo, Ill.

The response of lymphocytes to PHA stimulation was studied in prenatally treated (Gr.1) and postnatally treated (Gr.2) rabbit neonates. In the prenatal study, 6 pregnant rabbits with timed mating were given hydrocortisone (10 mg/kg, 0.5 ml) and 7 were given saline (0.5 ml) at 28th, 29th and 30th day of gestation. After delivery, blood was collected from the newborns within 48 hrs. Gr.2 was subdivided into two groups, one was given hydrocortisone (20 mg/kg, 0.5 ml) and the other saline (0.5 ml) on 1st, 2nd, and 3rd day of age. Neonates were sacrificed at 72-120 hrs. of age. The results showed that the stimulation index in Gr.1 when cultured with PHA of 1:100 dilution was 11.15±2.14 saline treated vs. 2.43±1.0 in steroid treated group. This was statistically significant ($p<0.005$). Similar difference in stimulation index was found with PHA 1:1000 dilution, 9.56±2.84 in saline vs. 1.54±0.18 in steroid group, $p<.02$. In Gr.2, the stimulation index both with PHA(1:100) and PHA(1:1000) was significantly depressed in the steroid group $p<.05$. Overall results are shown below. Besides PHA, Pokeweed mitogen was unable to show any evidence of stimulation in both prenatal and postnatal samples. This study demonstrates that steroid when administered prenatally or postnatally suppresses lymphocyte function in the rabbit neonate.

		Saline Mean±SEM	Steroid Mean±SEM	P
PRENATAL	PHA(1:100)	11.15 ± 2.14	2.43 ± 1.06	<0.005
	PHA(1:1000)	9.56 ± 2.84	1.54 ± 0.13	<0.02
POSTNATAL	PHA(1:100)	5.45 ± 2.19	1.20 ± 0.22	<0.05
	PHA(1:1000)	6.52 ± 2.15	1.29 ± 0.43	<0.05

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IN VITRO TESTS FOR CELL-MEDIATED IMMUNITY WITH PROTEIN EXTRACTS OF TISSUE ANTIGENS. Walter L. Henley, Zvi Aviner, and Colette Severin. Mount Sinai School of Medicine of The City University of New York, Departments of Pediatrics and Ophthalmology, New York, New York 10029.

Good correlation of leucocyte migration inhibition (LMI) by human and animal corneal protein was reported in patients with keratopathies. Human and bovine tissue extracts were compared in 288 experiments for migration inhibitory factor (MIF) production and LMI in patients with various eye diseases. LMI and MIF were correlated with the in vivo skin test in patients suspected of immunodeficiencies and tuberculosis. Similar results were obtained in over 90% of experiments comparing MIF production to LMI using human and bovine ocular tissue antigens. Similar results were also obtained in over 90% of experiments comparing homologous to bovine ocular tissue antigens for MIF production and LMI. The in vitro tests were correlated with the in vivo skin test in patients tested for chronic mucocutaneous candidiasis and tuberculosis. The ocular tissue antigen concentration required in the LMI test is generally twice that required for MIF production. The in vivo skin test requires 48 hours for interpretation and remains the yardstick by which the in vitro tests are measured. It can be used with microbial antigens like candida and PPD but cannot be used with tissue protein antigens.

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EVIDENCE FOR EXCESSIVE T SUPPRESSOR CELL ACTIVITY IN X-LINKED IMMUNODEFICIENCY WITH HYPER-IgM. Henry G. Herrod, Donald B. Perlman and Rebecca H. Buckley. Duke Medical School, Department of Pediatrics, Durham, N.C.

Increased T suppressor cell activity has been detected in patients with common variable hypogammaglobulinemia. In the present study, we used both a plaque-forming cell (PFC) assay that detects cells producing IgM antibody to sheep red blood cells (SRBC) and a double antibody radioimmunoassay to measure IgM synthesized by mononuclear blood cells in evaluating two patients with x-linked immunodeficiency with hyper IgM (HypM). Cultures from 18 normal individuals gave a range of 180-2,254 PFC/10^6 viable cells, and co-cultures of cells from pairs of normals averaged 83% of the PFC's predicted from individual culture data. The two HypM patients failed to produce PFC to SRBC, and co-cultures of HypM and normal mononuclear cells showed complete suppression of PFC activity by the normal cells. The HypM patients cells were capable of synthesizing IgM following pokeweed mitogen stimulation but suppressed IgM synthesis by normal cells in co-culture. In patient DM, the nature of the suppressor cell was investigated by co-culturing lymphocyte subpopulations and by adding hydrocortisone (HC). The following data were obtained:

PFC/10^6 Cells	Cont.	DM	Cont. + DM	Cont. + DM + HC	Cont. + DM T cells	Cont. + DM B cells
	2200	0	0	2607	0	1947

The findings indicate that patients with this defect have excessive numbers of hydrocortisone-sensitive T cells that can suppress IgM synthesis by normal B cells and suggest that these cells develop secondary to the patients' excessive IgM production.