709

ABNORMALITIES IN B CELL DIFFERENTIATION AND T CELL SUPPRESSION IN IMMUNODEFICIENCY WITH HYPER-IgM. H.J. Krantman, E.R. Stiehm, R.H. Stevens, A. Saxon, R.C. Seeger. UCLA School of Medicine Depts. of Pediatrics and Medicine and Microbiology and Immunology, Los Angeles.

We studied B and T cell function in 3 boys (20, 9, and 10 yrs) with Dysgammaglobulinemia I (Dys. I). Patients 1 and 2 are first cousins. Patient 3 is adopted. Peripheral blood mononuclear cells were separated using centrifugation on Ficoll-Hypaque gradients. T and B cells were quantitated using sheep erythrocytes and fluoresceinated anti-human immunoglobulin. Patient and normal T and B cells were separated and cultured in various combinations. Immunoglobulin (Ig) production, which requires cooperation between T and B cells, was assessed by culturing cells for 5 days with pokeweed mitogen and then determining the amount of ^{35}S-methionine incorporated into secreted total Ig. Ig classes were determined by polyacrylamide gel electrophoresis. T and B cell percentages and numbers were normal or nearly so in all patients. Total Ig synthesis was deficient in all 3 boys. Normal B cells with normal T cells produced IgG, M and A whereas each patient's B cells with autologous or normal T cells produced only small amounts of IgM. Addition of T cells from patient 1 or 2 to normal B cells suppressed Ig synthesis; irradiation of these T cells reversed the suppression and allowed them to provide T-helper function. Patient 3 had normal T-suppressor and T-helper activity. We conclude that defective differentiation of B cells into Ig producing cells is a constant feature of Dys. I and excessive T-suppressor cell activity is a variable accompanying abnormality.

710

IMMUNOLOGIC FUNCTION IN CHILDREN WITH IRON DEFICIENCY Herbert J. Krantman, Susan R. Young, Bonnie J. Ank, E. Richard Stiehm, Gary S. Rachelefsky. UCLA School of Medicine, Department of Pediatrics, Los Angeles.

To determine the effect of iron deficiency on immune function, cell mediated immunity and immunoglobulins were evaluated in iron deficient children. Nine children 8-30 months old appearing well nourished with heights and weights 3rd to 97th percentile and arm circumferences 5.25-7", clinically in good health but with Hgb <8.8gm/dl were studied. CBC, immunoglobulins, E-rosettes, and candida, tetanus and PHA stimulation as well as skin tests were performed before and after oral iron replacement. Hemoglobin increased to 10.2-13.3gm/dl with treatment. Evaluating percentage change in E-rosettes $[(E_2-E_1)/E_1 \times 100]$; three children increased rosetting cells; 24.5, 38.6 and 45%. Three children increased E-rosette cells 11.7 to 15.2%. In two children there was no appreciable change and one child had a decrease in E-rosettes. There were no low values of IgG but several children had increases in IgG after iron replacement (200-400mg/dl greater). Changes in delayed hypersensitivity were noted with iron therapy, but pretreatment skin tests may have contributed to these changes. Six of nine iron deficient children showed increases in E-rosettes with oral iron replacement. The presence of subtle T-cell defects in these children may help explain the increased susceptibility to severe infections in anemic children.

711

DIAGNOSING THE CARRIER STATE IN CHRONIC GRANULOMATOUS DISEASE (CGD). Renata Lubens and Robert I. Lehrer, (Spon. by Stephen A. Feig). UCLA Center for Health Sciences, Dept. of Pediatrics and Medicine, Los Angeles, CA 90024

Although laboratory diagnosis of CGD presents no difficulty, present techniques are insufficiently sensitive to detect those CGD heterozygotes in whom the majority of cells are functionally normal. We modified the test proposed by Preisig and Hitzig (Eur. J. Clin. Invest. 1:409, 1971) to improve its sensitivity and accuracy. Peripheral blood leukocytes are exposed to heat killed C. albicans in the presence of 0.05% nitroblue tetrazolium (NBT) and 8% Group AB serum for 15 min. at 37C. Next, an equal volume of 0.2% eosin Y in phosphate buffered saline is added for 15 additional min. The cells are centrifuged, resuspended in a saline solution and examined microscopically. Noningested yeasts whether extracellular or adherent to the cell's surface, are stained red. All intracellular yeasts within normal neutrophils or monocytes are stained blue, by virtue of their content of reduced NBT. Yeast cells within neutrophils or monocytes from patients with CGD lack reduced NBT and appear white in color. Heterozygotes have two populations of leukocytes, one (normal) that contains blue yeast cells and the other (CGD) that contains white ones. In two heterozygous females we studied, only 11.5% and 26% of the neutrophils displayed the CGD phenotype. With the use of eosin Y to stain noningested Candida cells our method is sufficiently sensitive to detect heterozygotes with as few as 1-2% defective cells. We speculate that some females with clinical CGD may prove to be such heterozygotes.

712

SPLENECTOMY AND PROPHYLACTIC ANTIBIOTICS IN THE MANAGEMENT OF THE WISKOTT-ALDRICH SYNDROME (WAS). L.G. Lum, D.G. Tubergen, and R.M. Blaese. National Institutes of Health, Bethesda, and Denver Children's Hospital, Denver.

The WAS, an x-linked disorder with the triad of recurrent infections, eczema, and thrombocytopenia with hemorrhage, is a difficult management problem. 1/3 of WAS patients die of thrombocytopenia with catastrophic intracranial hemorrhage the principal threat. Because of the experience that splenectomized WAS patients died within a few months of overwhelming sepsis, splenectomy has been considered relatively contraindicated. 18 splenectomized WAS patients were evaluated for their response in terms of hemostatic improvement and subsequent clinical course. 17 of the 18 patients (94%) had elevations of their platelet counts (pc) to normal or near normal levels with control of bleeding. The mean pc rose from 19 to 249 x $10^3/mm^3$. Autologous platelet survival became normal post splenectomy in the one patient studied. 7 of the 18 patients are alive with a mean survival of 8 years post splenectomy. 7 of the 7 survivors are on prophylactic antibiotics and have been free of septic complications while on antibiotics. Of the 9 patients who have died, only one was on long term antibiotic coverage which had been stopped 10 days before the onset of his fatal septic episode. Splenectomy appears to offer a useful therapeutic modality for controlling hemorrhage and increasing survival in WAS when combined with the use of prophylactic antibiotics. The critical requirement for continuous antibiotic coverage, however, cannot be overemphasized when considering this form of therapy.

713

KINETICS OF IMMUNOGLOBULIN RELEASE BY THE BREAST MILK MACROPHAGE. Richard McClead, William Pittard, (Spon. by Avroy Fanaroff). Dept. Ped, CWRU, Cleveland, O.

The breast milk macrophage(Mφ)has been reported to release stored IgA over time. To determine the kinetics of immunoglobulin release, separate aliquots of Mφ from 9 healthy mothers were cultured serially for 7 days. Each culture contained $2x10^6$ Mφ suspended in 1 ml of media. The release of IgA into the media was quantitated using double antibody radioimmunoassays. To determine total cellular IgA at 0 time and 7 days, Mφ cultures were sonicated and ultracentrifuged. The cell pellet obtained was separated from the supernatant and resuspended in a volume of fresh media equal to the supernatant. These two solutions were then assayed for IgA content. Mean ±SEM IgA released into culture media after 0,3 & 7 days was 0.45±0.2, 24.6±15.5, and 29.7±19 µg/ml. The mean ±SEM IgA measured in the sonicate pellet and sonicate supernatant at 0 time was 24.7±16.7 and 24.5±13.2 µg/ml as compared to 12.5±11 and 11.4±0.9µg/ml measured in the sonicate pellet and sonicate supernatant after 7 days incubation. The immunoglobulin released(y)per day(x)can be determined from the equation:

$\log_e y = mx + \log_e b$ $\log_e y = 0.25x + 7.88(\pm 0.5)$

These data indicate that >80% of stored IgA is released by day 3 and the total content of IgA at 7 days can be accounted for at 0 time. Although it appears that the Mφ does not synthesize IgA, because of its reported tolerance for a wide range of pH and osmolality, the cell may play an important role in the transfer of passive immunity from a mother to her newborn.

714

GENERATION OF CHEMILUMINESCENCE (CL) AND HYDROXYL RADICAL (·OH) BY A PARTICULATE FRACTION OF HUMAN NEUTROPHILS. Linda C. McPhail, Daniel R. Ambruso and Richard B. Johnston, Jr. National Jewish Hosp. and Univ. of Colo. Med. Ctr., Dept. of Pediatrics, Denver.

Recent data suggests that phagocyte-generated ·OH and singlet oxygen ($^1O_2$) are the oxygen species essential for bacterial killing. In an attempt to better understand the biochemical basis of conversion of oxygen to microbicidal metabolites, we have studied the generation of ·OH and of CL, hypothesized to be due to $^1O_2$, by a particulate fraction isolated from phagocytizing neutrophils. The CL was NADPH-dependent and peaked in 1-2 min. Very low CL was obtained with fractions from resting cells or from phagocytizing cells of 5 patients with chronic granulomatous disease. The particulate fraction from phagocytizing, but not resting, cells also generated ·OH in the presence of NADPH (mean, 24.9 pmol ethylene/mg). Inhibition of CL was obtained with scavengers of $^1O_2$ (azide, hydroquinone, DABCO) or agents that remove or prevent formation of ·OH (superoxide dismutase, catalase, formate, ascorbate, ethanol, tryptophan). In preliminary experiments with the non-penetrating protein inhibitor, p-diazobenzenesulfonic acid, treatment of intact neutrophils did not prevent subsequent CL by the isolated particulate fraction. This suggests that the oxygen-converting enzyme is located in the cytoplasm or on the inside of the plasma membrane. We conclude that $^1O_2$ is, in fact, generated with the phagocytosis-associated respiratory burst and that ·OH and $^1O_2$ are involved in CL generation. Purification of the respiratory enzyme from the particulate fraction should lead to greater understanding of this critical aspect of host defense.