125 ALTERED PROTEIN KINASE C IN A PATIENT WITH COMMON VARIABLE IMMUNODEFICIENCY: A novel type of defect leading to immunodeficiency?

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The aim of our studies is to identify genes involved in the pathogenesis of common variable immunodeficiency (CVI). One of our CVI patients with severely depressed mitogen response and defective B cell differentiation in response to T cell dependent and T cell independent stimulators, shows in addition a reduced CD 25 (IL 2 receptor) expression after T cell stimulation with mitogen. CD 25 expression is lacking on T cells and an EBV-immortalized B cell line (BCL) in response to stimulation with phorbol ester PMA, an activator of protein kinase C (PKC). Binding studies with phorbol ester on patient's BCL show that PKC is expressed at slightly increased numbers and affinity. Inhibition studies of proliferative responses to T cell mitogens with protein kinase inhibitors (H7, H8, HA1004) indicate that PKC is altered in patient's T cells. Our hypothesis is that immunodeficiency in this patient is caused by an alteration in a subspecies of PKC affecting activation of lymphocytes.

126 OUTCOME OF CHRONIC TYPE B HEPATITIS ACQUIRED IN CHILDHOOD.

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This report synthesizes the updated results of a longitudinal study of chronic type B hepatitis including 87 untreated,anti-delta negative children(46 with active,37 with persistent hepatitis and 4 with cirrhosis) followed for 2-13 years(mean 6±3 y.). Of the 76 cases who were initially HBeAg and HBV DNA positive,59 (77%)terminated HBV replication and achieved sustained biochemical remission.Of 11 cases who were anti-HBe positive on entry, all 3 HBV DNA positive terminated replication,9 normalized ALT, while both cases with persistent liver damage had antibodies to hepatitis C virus(HCV).Overall 5 children eventually cleared HBs Ag,while liver histology was improved in all cases with previous active hepatitis or cirrhosis.During follow-up,however,routine alphafetoprotein testing made possible the diagnosis of hepatocellular carcinoma in an asymptomatic boy with cirrhosis.
These data show that most children with chronic hepatitis B terminate HBV replication before reaching adulthood and become asymptomatic HBsAg carriers with normal ALT.Superinfection with HCV has to be considered in patients with increased ALT after loss of HBV DNA.Periodical alphafetoprotein testing may help the early diagnosis of carcinoma.

127 TREATMENT OF CHRONIC TYPE B HEPATITIS WITH RECOMBINANT ALPHA 2a INTERFERON IN HBeAg POSITIVE CHILDREN.

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To evaluate the effect of recombinant alpha 2a interferon(IFN) in Italian children with HBeAg and HBV DNA positive chronic type B hepatitis,two studies have been conducted:1)a multicentre randomized,controlled trial using 3MU IFN thrice weekly for 6 months, 2) an observational study using 9MU thrice weekly for 6 months.
The controlled study included 36 children,aged 3-14 years,with active (12 cases),persistent (21 cases)or minimal hepatitis,that were randomly assigned to either treatment or control group (18 cases each).All patients completed the treatment schedule and a 6-months follow-up.At 12 months 5(27%) treated patients and 3(16%) controls were HBV DNA negative and 3(16%) and one patient,respectively,had lost HBeAg.Only one patient had a significant ALT flare 4 months after therapy withdrawal.
The observational study included 6 patients with chronic persistent hepatitis.Therapy was well tolerated.At 12 months HBV DNA clearance had occurred in 5(75%) cases and HBeAg clearance in 3 (50%).An ALT flare was observed in 3 cases.
These results indicate that low dose IFN therapy does not significantly modify the course of chronic type B hepatitis.High IFN doses,using the same schedule,seem to be more efficient.

128 BINDING OF CLONED S-FIMBRIATED E.COLI TO HUMAN BUCCAL EPITHELIAL CELLS - INCREASED ABILITY OF NEONATAL SALIVA TO INTERFERE WITH BACTERIAL ADHESION COMPARED TO ADULT SALIVA

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A common feature of E.Coli strains causing sepsis and meningitis in the newborn period are K1 capsules and S-fimbriae. Because adhesion of pathogenic microorganisms to mucosal surfaces is a fundamental prerequisite for infection we investigated adhesion of S-fimbriated E.Coli to buccal epithelial cells obtained from newborn babies. FITC-labelled E.Coli (strain H8101 pANN 801-4) carrying plasmid pANN 801-4 encoding for S-fimbriae were incubated with mucosal epithelial cells. Analysis by fluorescence mikroskopy showed that bacteria had been bound to 75 - 90% of epithelial cells. This binding was specifically mediated by glycoproteins containing neuraminic acid which could be shown by inhibition experiments using fetuin, α_1-acid glykoprotein, methyl-α-D-mannoside and sialic acid. Using this test system the inhibitory activity of saliva on bacterial adhesion was analyzed. The capacity of newborn saliva to inhibit bacterial adhesion was much higher than that of adult saliva: newborn saliva could be diluted 4 - 5 fold to show inhibitory activity equal to adult saliva. Chemical analysis of newborn saliva specimens revealed a 4-5 fold increased total content of sialic acid (mostly protein bound). Using Western blot analysis of newborn and adult saliva we identified sialoglycoprotein bands with relative molecular masses > 200 KD reactive with wheat germ agglutinin which, accordingly, have to be classified as mucins. These data suggest that saliva mucins could represent a major defense mechanism against bacterial infections at a stage of ontogeny were the secretory IgA system is not yet developed.

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129 GRANULOCYTE/MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) FOR TREATMENT IN GLYCOGEN STORAGE DISEASE TYPE IB

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Type Ib glycogen storage disease is caused by a defect in the glucose-6-phosphatase translocase of the microsomal glucose-6-phosphatase system. In addition to metabolic problems patients exhibit a predisposition for infections which is related to neutropenia. Various degrees of impairment of the neutrophil function have been observed including reduced chemotaxis and superoxide (O_2^-) production. In vitro, GM-CSF stimulates growth of myeloic progenitor cells and improves functions of mature neutrophils such as formation of oxygen radicals and chemotaxis. In clinical trials, GM-CSF has been shown to increase the neutrophil count in a dose-dependent manner.
A 13 year old girl with type Ib glycogen storage disease, inflammatory bowel disease (resembling Crohn's disease), and obstinate neutropenia (neutrophil counts below 500/µl) sufferd from severe oral mucosal lesions. The girl was treated with human recombinant GM-CSF at a daily dose of 250 $\mu g/m^2$ subcutaneously during a 2 week course with the parents' informed consent. From the third day of treatment the neutrophil count increased continuously from 216/µl to 6052/µl at day 14. At the same time, normal superoxide production was measured in the isolated granulocytes activated by PMA or zymosan. Oral ulcers resolved completely. Except for some painful hyperemic areas at the sites of injections no side effects appeared. After GM-CSF administration was discontinued leucocyte counts decreased to pre-treatment levels within 7 days.
From the results of this limited clinical trial we conclude that GM-CSF can increase the neutrophil count and normalize specific granulocyte functions in glycogen storage disease Ib. GM-CSF might be of clinical benefit in managing severe inflammatory complications in neutropenic patients with this disorder.

130 INHIBITION OF ADHESION OF S-FIMBRIATED E.COLI TO BUCCAL EPITHELIAL CELLS BY HUMAN MILK FAT GLOBULE MEMBRANE MUCINS: A NEW ASPECT OF PROTECTIVE FUNCTION OF THE NON-IMMUNOGLOBULIN FRACTION

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In search of factors of human milk that inhibit invasion of pathogenic bacteria we have analyzed the effect of human milk fat globule membrane (HMFGM) components on adhesion of cloned S-fimbriated E.Coli to human buccal epithelial cells. S-fimbriae are a common feature of E.Coli strains causing sepsis and meningitis in the newborn period and are bound to epthelia via sialyl-(α 2-3) or sialyl-(α 2-6) galactoside structures. Whole milk fat globules (MFG) could be agglutinated by the above mentioned bacteria. Agglutination could be inhibited by fetuin, human glycophorin and $\alpha_1$acid glykoprotein. In addition, pretreatment of MFG with Vibrio cholerae neuraminidase markedly reduced bacteria - induced agglutinations indicating the involvement of neuraminic acid containing glykoproteins. Lipid droplets of infant formula or artificial lipid emulsions (Intralipid®), in contrast, could not be agglutinated. MFG were present in stools of breast fed newborn babies as shown by staining with a FITC-labelled monoclonal antibody directed against HMFGM and could be agglutinated by bacteria. To further characterize relevant HMFGM components they were separated by gel chromatography. Of the fractions obtained mucins whose quality was confirmed by Western blot and monosaccharide analysis showed the most pronounced inhibitory effect on adhesion of S-fimbriated E.Coli to human buccal epithelial cells. Our data suggest that MFG besides their nutritive function provide protection against bacterial infections by inhibition of bacterial adhesion over the length of the entire intestine.

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