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LIVER MEMBRANE-SPECIFIC T CEL CLONES IN AUTOIMMUNE CHRONIC ACTIVE HEPATITIS (aCAH)

L.Wen, A.Lobo-Yeo, M.Peakman, A.P.Mowat, G.Mieli-Vergani, D.Vergani
Dept.of Child Health ' Immunology, King's College Hospital Denmark Hill, London SE5

Patients with a CAH have increased numbers of activated circulating T-lymphocytes expressing interleukin 2 receptors (IL-2R). By sequentially incubating peripheral blood mononuclear cells (PBMC) of a patient with aCAH with recominant IL-2 and PHA at a concentration of 0.3 cells/well, 11 T cell clones were generated, 10 of which were CD4+ and one CD8+. Two CD4+ clones proliferated in response to rabbit liver membrane homogenate (RLM) when co-cultured with irradiated autologous PBMC as antigen presenting cells, but not with allogeneic PBMC. No proliferation was observed when rabbit kidney membrane was used as stimulating antigen. Proliferation of RLM-specific T cell clones was blocked by adding anti-CD4, anti-HLA DR and anti-IL2R, the inhibition being reversed in the latter case with excess IL-2. Six T cel clones (4 CD8+, 2 CD4+) generated from a control under similar conditions were unresponsive to RLM whether presented by autologous or allogeneic PBMC.
These results show that T cell clones can be generated from circulating IL-2R+ T cells of patients with aCAH. Their specificity for liver cell membrane, the likely target of the immune attack on aCAH, indicates their involvement in the pathogenesis of this disease.

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THE RELATION BETWEEN THE EXPRESSION OF ASIALOGLYCOPROTEIN RECEPTOR AND CIRCULATING DESIALYLATED GLYOPROTEINS IN THE DEVELOPING RAT.

Y. Bujanover, E. Lebenthal, J.K. Petall
State University of New York, Dept.of Pediatrics, Buffalo

Previous studies investigating the biological activity of the asialoglycoprotein receptor have demonstrated its importance in the clearance of desialylated glycoproteins from the circulatin. The present study examined the possible physiological role of the receptor during the development in the rat.
Fetuses of individual pregnant Sprague Dawley rats were removed at day 18 to 22 of gestation. Livers and sera were collected. Newborn rates were sacrificed at 1 to 10 days of life and their livers and sera were collected. Adult rats were used as a control group. Total post-nuclei membrane vesicles were prepared from the liver using the sucrose gradient method. Quantitation of the ASGR levels in the membranes was performed using the western method. The quantiation of the serum desialylated glycoproteins was performed using a modified western blot protocol employing radiodinated ricinus communis lectin (RCA 1).
Results: The ASGR levels were very low in the fetus increasing dramatically after birth and reaching the adult levels after the first week of life. In contrast the abundance of the serum desialylated glycoproteins in the serum presented an opposite developmental pattern. High levels were demonstrated in the fetuses decreasing three fold around the time of birth reaching the adult levels in the first days of life. Further the pattern of the desialylated glycoproteins was observed to change during development. These results suggest a direct relation between the level of ASGR and the circulating desialylated glycoproteins during development.

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INCREASED ANTIGEN ABSORPTIN INDUCED BY INFECTION WITH THE ADHERENT ENTEROPATHOGENIC E.COLI STRAIN RDEC-1 IN RABBIT ILEUM AND PEYER'S PATCHES

E.Isolauri, M.Heyman, M.Cotteland, D.Tome, J.F.Desjeux
INSERM U.290, Hopital Saint-Lazare, F 75010 Paris

Intestinal absorption of antigenic (intact) and degraded β-lacto-globulin (β-lg) and horseradish perioxidase (HRP) was studied in rabbits experimentally infected at weaning with the rabbit-specific Escherichia coli strain RDEC-1 (015: NM). Traneptithelial fluxes of both proteins were measured at four stages post infection: early, peak, late and recovery, on segments of ileum and Peyer's patches mounted in Ussing chambers. During this period of observation, absorption of antigenic β-lg and intact HRP decreased significantly in the ileum and Peyer's patches of age-matched controls probably reflecting gut closure to the food antigen. No significant age-related decrease was observed in the controls for the transport of these proteins in degraded form. During the early phase of RDEC-1 infection, absorption of egraded protein fluxes rose across Peyer's patches only. During the peak phase of infection, absorption of antigenic β-lg (74.0 ng/h.cm^2) and intact HRP (21.8 ng/h cm^2) rose fivefold compared to controls (15.8 and 4.4 ng/h.cm^2 respectively). These rises, which were also found in Peyer's patches, delayed the physiologic decrease in protein absorption which occurs with age. These results indicate that increased antigen absorption is observed during RDEC-1 bacterial diarrhea, and that it might interfere with the immune responses of the host.

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ESSENTIAL FATTY ACIDS STATUS IN THE THIRD TRIMESTER

K.L. Costeloe, E.A. Lennon, M.A. Crawford, M.J. Leighfield, W. Doyle and N. Meadows.
Dept. Nutritional Biochemistry, Institute of Zoology, London NW1
Academic Dept.Child Health, ST.Barts Hospital MC, London EC1

The aim of this investigations was to study the essential fatty acid (EFA) status of the low birthweight infant.

We have quantified the fatty acids in the EThanolamine and Cholinephosphoglycerides in the umbilical arterial wall of 14 babies over the range of birth weights 820-4310 G. and gestational age 32-39 w.

There was a statistically significant negative correlation with the Mead Acid (20:3 n-9), the triene/tetraene ratio and the pentaene/tetraene ratio and birthweight, ($p<0.01$), and with occipito frontal circumference, ($p<0.05$). The Mead acid was present in amounts varying between 1.74 and 6.4% of total fatty acids. This substance is not usualy found in human tissues in concentrations exceeding 0.1%, its excess production is indicative of EFA deficiency. The ratios described are accepted as indicators of deficiency involving both n-6 and n-2 fatty acids.

These preliminary results indicate tht these babies all show evidence of EFA deficiency by adult standards, but that the deficiency is greatest in the lowest birthweight babies. This fetal EFA deficiency may be related to maternal dietary insufficiency or to defective placental function.

Brain growth and the requirement for long chain EFA derivatives are maximal during the last trimester and it is possible that the EFA deficiency of the lowest birthweight babies is related to the adverse neurologica outcome which is seen in that group. These findings have implications for both maternal and neonatal and nutritional requirements.

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INHIBITION OF ADHESION OF ENTEROTOXIGENIC E.COLI (ETEC) TO HUMAN SMALL INTESTINAL ENTEROCYTES BY HUMAN MILK: EVIDENCE FOR A SPECIFIC ANTIBODY MEDIATED MECHANISM.

K A Nathavitharana, S Knutton, D Catty and A S McNeish
Institute of Child Health and Department of Immunology, University of Birmingham, Birmingham, U.K.

Adhesion of ETEC to the small intestinal mucosa is an essential event in colonisation and the development of diarrhoeal disease. Special classes of protein fimbriae termed Colonisation Factor Antigens (CFA) promote mucosal adhesion of ETEC. We have previously demonstrated secretory IgA (sIgA) antibody to ETEC CFA/I in human milk. In this study we tested the anti-adhesive effect of such antibody using an in vitro isolated human enterocyte adhesion assay. Bacterial adhesion was quantitatively assessed by counting the number of bacteria adhering to the brush border of 100 enterocytes and an adhesion index defined as the number of adherent bacteria/brush border. ETEC strain M109C2 producing CFA/I had an adhesion index of 2.2 (range 2.1-2.4) whereas the same strain lacking CFA/I had an adhesion index of 0.02 (range 0-0.03). At a dilution of 1:10, 6 human milk specimens from Sri Lankan women with demonstrable CFA/I-specific sIgA antibody produced a marked reduction in adhesion index from 2.2 to 0.16 (range 0.05-0.25) whereas 6 milk specimens with no demonstrable CFA/I-specific sIgA showed no reduction in adhesion index (mean 2.2, range 2.1-2.6). These results strongly suggest an anti-adhesive protective role for the CFA/I-specific sIgA in human milk. The presence of anti-CFA/I antibody only in milk from Sri Lankan women and not in any of 75 Caucasian, U.K. women tested presumably reflects prior exposure only of the Sri Lankan mothers to CFA/I-positive ETEC.

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ELEVATED CALCIUM LEVELS AND PROTEIN KINASE C ACTIVATION IN RESPONSE TO INFECTION BY ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC).

T J Baldwin, S Knutton, P H Williams and A S McNeish
Department of Genetics, University of Leicester and Institute of Child Health, University of Birmingham, U.K.

EPEC colonise the small bowel and cause diarrhoea by a mechanism that is unrelated to production of recognised enterotoxins or mucosal invasion. Intestinal ion transport is thought to be regulated by intracellular second messenger molecules which function through activation of their appropriate protein kinases. Since EPEC do not induce elevated levels of cyclic nucleotide second messengers, we considered the possibility that EPEC cause diarrhoea by a mechanism which involves an intracellular calcium second messenger. Cultured HEp-2 cells were used as a model of EPEC infection and levels of intracellular calcium determined fluorimetrically using the calcium indicator dye, Quin-2. HEp-2 cells infected with EPEC for 3 hours showed a 5-6 fold increase in calcium levels (280-320 nanomolar) compared to uninfected cells (55 nanomolar). Alteration in the phosphorylation state of proteins following infection of ^{32}P treated HEp-2 cells with EPEC was analysed by autoradiography following electrophoresis. A protein band of M_r 21-23 kDa showed increased phosphorylation within 2 hours of infection; the same band was phosphorylated a) on treatment of cells with TPA, a potent secretagogue and activator of protein kinase C (PKC), and b) on addition of purified PKC to HEp-2 cell lysates. These results suggest that EPEC pathogenesis involves contact of bacteria with target cells, a calcium intracellular signal and phosphorylation of a critical host cell protein.