5 LOCAL IMMUNE RESPONSE MEASURED BY BLOOD LYMPHOCYTES REFLECTS THE CLINICAL REACTIVITY OF CHILDREN WITH COW'S MILK ALLERGY

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To investigate the intestinal immune responses during a diagnostic milk provocation in patients (mean age 13.5 months) with cow's milk allergy (CMA), total immunoglobulin-secreting cells (ISCs) and specific antibody-secreting cells (ASCs) from peripheral blood samples were assessed by ELISA plaque assay (ELISPOT). Fifteen patients had acute urticarial skin eruptions, 8 patients had slow onset of eczema, and 15 patients showed symptoms from the gastrointestinal tract. A significant increase in IgM secreting cells (geometric means with [95 % confidence intervals]) from 382.2 [265, 552] to 621.4 [381, 1013]/10^6 cells; $t = 2.87$, $p < 0.01$ but not IgA- and IgG-secreting cells was associated with acute urticaria. In patients with eczematous skin eruptions and gastrointestinal symptoms the response involved all these immunoglobulin isotypes. The magnitude of the post-challenge ISC responses in patients with gastrointestinal symptoms in IgM class (from 657.9 [428, 1012] to 3544.0 [1696, 7406]/10^6 cells; $t = 4.44$, $p < 0.001$) and IgA class (from 974.6 [590, 1610] to 2482.4 [1528, 4028]/10^6 cells, $t = 3.99$, $p = 0.001$) significantly exceeded that of the patients with cutaneous symptoms. Notwithstanding the distinct increase in the total number of ISCs, the ASC response specifically directed against beta-lactoglobulin and alfa-casein was small and inconsistent. In patients with acute urticaria no antigen-specific response was found, and in patients with eczema and gastrointestinal symptoms it was restricted to IgM isotype. These findings indicate that immune exclusion of antigens is defective in CMA. The quality and extent of the local immune response varied in the three reaction types, suggesting that different immunopathogenic mechanisms are operative in CMA.

6 HUMORAL IMMUNITY IN CHILDREN WITH CYSTIC FIBROSIS (CF) AND CHRONIC LIVER DISEASE (CLD)

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Altered immune status as evidenced by inappropriate immune reactions to liver membrane antigens occurs in children with CF and CLD, who also have an increased frequency of the "autoimmune" haplotype HLA B8-DR3. To determine whether immune alterations typical of other autoimmune liver disease were present we investigated 143 children with CF, including 25 with CLD. Immunoglobulin levels were measured by nephelometry and autoantibodies (AA) to nuclear (ANA), nucleolar (ANoA), mitochrondrial (AMA), smooth muscle (SMA), gastric parietal cell (GPC) and liver, kidney microsomal (LKM) antigens by indirect immunofluorescence using rat kidney, liver and gut and HEp-2 cells as substrate.

IgG and IgA were significantly higher in children with LD than in those without ($p<0.005$ and <0.007 respectively) whereas IgM levels were similar. AA were found in 96 (67%) children (titres 1:10 to 1:40, median 1:10) : ANA in 56 (39%); ANoA in 55 (38%); SMA in 21 (15%); GPC in 13 (9%); AMA in 2 (1.3%); LKM in 1 (1%). The frequency of AA was similar in children with and without LD but occurred in <2% of normal age matched controls.

Humoral immune abnormalities typical of auto immune diseases are frequent in CF. Their pathophysiological role in CF is unclear. AA including a high and previously unreported incidence of ANoA may arise from recurrent stimulation of the immune system by infection and/or tissue disruption but are not related to LD. High IgG and IgA in CF with CLD may derive from defective clearance by the liver.

7 FREQUENCY OF LIVER MEMBRANE SPECIFIC T LYMPHOCYTES IN AUTOIMMUNE CHRONIC ACTIVE HEPATITIS (aCAH)

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We have previously shown that children with aCAH have circulating activated T lymphocytes specifically sensitised to liver cell membrane, which are involved in mediating liver damage. To determine the frequency of liver membrane specific T lymphocytes (LMSTL) amongst circulating T cells we have established limiting dilution cultures of peripheral blood mononuclear cells from 6 children with aCAH and 6 age matched normal controls. Proliferation of purified T cells, cultured with irradiated autologous B cells as antigen presenting cells in the presence of a human liver cell membrane preparation (HLM), was determined by ^{3}H-thymidine incorporation. Frequency of LMSTL, calculated by Poisson distribution, ranged from 1/3375 to 1/65000 (median 1/20000) in aCAH, being 10 to 20 times higher than in controls (median 1/292961, range 1/30100-1/1286053, $p<0.01$). Children with aCAH have up to a twenty fold increase in the number of circulating T lymphocytes specifically directed to liver cell membrane antigens emphasising their possible involvement in immune mediated liver damage.

8 COORDINATE EXPRESSION OF LACTASE-PHLORIZIN HYDROLASE (L-PH) mRNA AND ENZYME LEVELS IN RAT INTESTINE DURING DEVELOPMENT

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The development of rat intestinal L-Ph specific activity displays a well-known post weaning decline. In contrast, total lactase activity increases to reach maximal levels around weaning, and remains high subsequently. In order to elucidate the molecular basis for these patterns, a rat lactase cDNA was isolated and characterized, and used in the quantification of lactase mRNA during development. This lactase cDNA uniquely hybridized to a 6.8 kb mRNA in the small intestine. To assess the amount of lactase mRNA encoding for lactase enzyme activity in the small intestine, total intestinal RNA was isolated and analyzed by Northern and dot blot hybridization. The pattern of total lactase mRNA during development followed that of total lactase activity, suggesting that over this time span the level of lactase activity is primarily controlled at the transcriptional level. However, the magnitude of increase of total lactase activity during lactation compared to that of total lactase mRNA suggests that additional mechanisms are involved in regulating lactase levels. Analysis of the regional distribution of lactase mRNA along the small intestine at 14 days revealed that mRNA was high in the proximal three regions, but was dramatically lower in the distal regions. Total lactase activity, in contrast, displayed maximum activity in the mid intestine with decreased levels both proximally and distally. Thus, lactase activity in the intestine appears to be regulated during development predominantly by transcriptional mechanisms, while alterations during lactation, and along the proximal to distal gradient, are the result of other control mechanisms.

9 ADHERENCE PROPERTIES OF ENTEROAGGREGATIVE ESCHERICHIA COLI: A NEWLY DESCRIBED CLASS OF DIARRHOEAGENIC E.COLI

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In a cohort study of children in rural India, E. coli strains that exhibited aggregative adherence to HEp-2 cells, so called enteroaggregative E. coli (EAggEC), were found significantly more often in patients with persistent diarrhoea (29.5%) than in patients with acute diarrhoea (12.8%) or in controls (9.9%). In this study we examined 25 EAggEC strains isolated from cases of persistent diarrhoea for their ability to adhere to human intestinal mucosa which is an important virulence property of diarrhoeagenic E. coli. Human small and large bowel mucosal biopsies maintained in organ culture were infected with EAggEC for 8h. at 37°C and, after thorough washing, assessed for mucosal adhesion of bacteria by scanning electron microscopy. No EAggEC adhered to duodenal mucosa but most EAggEC strains adhered to luminal (but not crypt) colonic mucosa in localised aggregates. 12 strains exhibited good adhesion (>10% surface colonised), 12 showed poorer adhesion (<10% surface colonised) and 1 strain was nonadherent. Transmission electron microscopy showed that EAggEC produce rod-like fimbriae and/or fibrillar surface structures and, in the case of 2 strains examined, fimbriae were shown to promote adhesion of EAggEC both to HEp-2 cells and to the brush border glycocalyx of colonic mucosa; mucosal adhesion of EAggEC was not accompanied by any detectable morphological damage. These observations suggest that EAggEC may cause diarrhoeal disease by colonising the large bowel. The lack of any tissue invasion or mucosal damage suggests a toxin-mediated mechanism of action.

10 INFLUENCE OF THE YERSINIA VIRULENCE PLASMID ON ADHESION TO RABBIT ILEAL BRUSH BORDER MEMBRANE VESICLES, MUCOUS AND INTESTINAL CONTENTS.

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The ability to adhere to the intestinal surface is important for enteric invasive pathogens such as Yersinia enterocolitica. We have examined adhesion of Y. enterocolitica to different constituents from the rabbit ileum. Rabbit ileal brush border membrane vesicles (BBVs) and the water soluble fractions of ileal mucous or ileal intestinal contents were immobilized in polystyrene microtiter plates and adhesion of radiolabeled bacteria was determined. The strains examined were clinical isolates carrying the Yersinia virulence plasmid (pYV$^+$) (serotypes 0:3, 0:8 and 0:9) and their isogenic plasmid cured derivatives (pYV$^-$). pYV$^+$ strains adhered better than pYV$^-$ strains to BBVs ($p<0.01$) as well as mucous ($p<0.01$). No binding to intestinal contents was detected. Incubation of the pYV$^+$ strain Ye0301P+ with mucous or penetration of the same strain through mucous significantly reduced subsequent adhesion to BBVs ($p<0.01$)

The increased ability of pYV$^+$ Y. enterocolitica strains to bind to BBVs may be an important virulence factor. However, this is partly counteracted by the concomitant plasmid facilitated association with the mucous layer. The binding to mucous may reflect a host defence mechanism.