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**P.5.08.10** IL-2 and IL-4 gene expression in human peripheral blood and intestinal biopsies

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**Introduction:** Cytokines play a fundamental role in the regulation of the immune response. The gastrointestinal tract is one of the largest immune organs in humans, but as yet little is known of the role cytokines play in maintaining normal gut immune regulation or whether the profile of cytokine production changes in intestinal inflammatory conditions. In this preliminary study, using RT-PCR technology, we have examined IL-2 (Th-1) and/or IL-4 (Th-2) production in human peripheral blood mononuclear cells (PBMC), whole or layered intestinal biopsies and epithelial biopsies from healthy and Crohn's disease isolated from PBMC, intestinal epithelium and lamina propria preparations.

**Materials and Methods:** PBMC production of IL-2 and IL-4 was studied in a time course experiment using either mitogenic or antigenic stimuli. Ribonuclease acid (RNA) was isolated from cells cultured after 0, 6, 12, 24, 48 and 72 hours, reverse transcribed into cDNA and the Polymerase Chain Reaction (PCR) carried out with primers specific for either IL-2 or IL-4. Total RNA was also isolated from 6 whole intestinal biopsies and from 2 single cell suspensions of epithelial and lamina propria layers and subsequently analysed for IL-2 and IL-4 mRNA expression. Inflammatory bowel disease (IBD) and Crohn's disease (CD) patients wereCD3+ populations were isolated from PBMC, epithelial and lamina propria cell suspensions from 2 further individuals. RT-PCR for IL-2 and IL-4 was performed on oligo dT coated magnetic beads following extraction of RNA with lyso buffer.

**Results:** In phytohaemagglutinin (PHA) stimulated PBMC IL-2 was detected from 6 to 72 hours and IL-4 was detected at all time points. In gliadin stimulated PBMC IL-2 and IL-4 were detected at all time points. IL-4 was detected in 4 of 6 whole biopsy RNA preparations and in the two separated epithelial and lamina propria layers studied. Finally, in the two patients studied, IL-2 was detected in CD3+ isolated populations from peripheral blood, lamina propria and epithelial layer. In the same individuals IL-4 was detected in both CD3+ isolated populations from peripheral blood, and from the epithelial layer of one individual and the lamina propria of the other.

**Conclusions:** This study demonstrates that it is possible to determine cytokine gene expression in magnetic bead-isolated PBMC, IEL and lamina propria CD3+ populations. It also shows that both IELs and lamina propria T (CD3+) cells can manufacture IL-2 and IL-4 which may play specific roles in immune regulation in the human gastrointestinal tract.
tissue showed a great number of CD4+ and CD8+ T cells that produced IFN-γ and in addition showed an abundant expression of MHC class II molecules on gingival fibroblasts. Therefore, we investigated whether these gingival fibroblasts acquire the capacity to carry out MHC class II restricted functions such as antigen presentation to local T cells.

Materials and Methods: Gingival fibroblasts were cultured from biopsies of chronically inflamed gingiva from patients with chronic adult periodontitis undergoing therapeutic periodontal surgery. First, we studied, by FACS analysis, the effect of IFN-γ on the surface expression of a number of molecules, implicated in antigen presentation, on these fibroblasts. Next, we examined whether the resulting MHC class II-positive gingival fibroblasts are able to function as antigen-presenting cells for relevant antigens, such as bacterial-derived superantigens or conventional antigens from periodontitis-associated bacteria, by measuring T cell proliferation.

Results: In this study, we show that after stimulation with IFN-γ, gingival fibroblasts not only expressed MHC class II molecules but also the costimulatory molecules, CD40, CD80, and CD86. In addition, IFN-γ treated fibroblasts expressed the Th1 cytokines, IL-12 and TNF-α, whereas IFN-γ treated fibroblasts expressed IL-10. These IFN-γ-treated gingival fibroblasts were able to present IFN-γ in the presence of IFN-γ treated fibroblasts. Furthermore, we found that IFN-γ fibroblasts inhibited the presentation of the whole-cell antigens of these bacteria by a reduced availability of IL-2.

Conclusions: These results suggest that gingival fibroblasts play an important role in the local specific immune response in chronically inflamed periodontal lesions by regulating the expression of inflammatory cytokines.

P.5.08.12 Human small intestinal epithelial cells express HLA-DM mRNA

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Human small intestinal epithelial cells (EC) constitutively express MHC class II molecules. It has therefore been proposed that EC may play an antigen presenting role in vivo. The T84 human intestinal epithelial cell line was also included in this study. T84 cells were examined for the expression of HLA-DM mRNA by RT-PCR and flow cytometry was used to confirm the surface expression of MHC class II molecules on IFN-γ treated T84 cells (Table 1).

Table 1

| Intestinal Epithelium | T84 Cells | T84 Cells + IFN-γ |
|-----------------------|-----------|-------------------|
| HLA-DMA (RT-PCR)      | +         | +                 |
| HLA-DMβ (RT-PCR)      | +         | +                 |

These results suggest that MHC class II expressing intestinal epithelial cells also express mRNA for HLA-DM. Moreover IFN-γ treatment upregulates HLA-DMβ, along with hypothesis that human small intestinal epithelial cells may act as classical antigen presenting cells in vivo.

P.5.08.13 Oral tolerance in CD8-knockout mice; Cholera toxin abrogates induction but cannot break established tolerance

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Oral feeding of KLH-antigen to CD8-knockout mice (CD8−/−) resulted in the development of oral tolerance (OT) with antigen-specific humoral as well as T cell mediated responses being significantly reduced as compared to non-knockout control mice. The OT was dose-dependent in CD8−/− mice and could not be transferred by CD4+ T cells to naive recipients. Antigen-restimulation in vitro of CD8−/− mice gave markedly depressed IFNγ and IL-4 production. Moreover, consistent with findings in normal mice, cholera toxin (CT) adjuvant co-administered orally with antigen abrogated completely the development of OT, indicating that CT can break induction of OT even in the absence of CD8+ T cells. However, if wild-type and CD8−/− mice were first allowed to develop OT and later challenged with oral antigen plus CT-adjuvant then OT was retained. Thus, CT could not break already established OT in either CD8−/− nor in normal wild-type mice. By contrast, oral immunization with KLH plus CT-adjuvant to tolerant mice promoted strong mucosal anti-KLH IgA responses in the gut lamina propria of CD8−/− mice whereas in wild-type mice local gut anti-KLH IgA immunity was impaired in the presence of CT. Based on the data we propose that gut mucosal immunity in normal mice after feeding of conventional antigens may be locally down-regulated by CD4+ T cells, whereas systemic OT develops independently of CD8+ T cells by rendering CD4+ T cells of both Th1 and Th2 type allergic, and CT-adjuvant appears to protect against induction ofergy.

P.5.08.14 Impaired mucosal IgA responses but intact oral tolerance in IFN-γ receptor deficient mice

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IFN-γ receptor gene knock-out (IFN-γR−/−) mice were used to analyse the role of IFN-γ in local and systemic immune responses following oral immunization. We show here that IFN-γR−/− mice respond with 50% reduced numbers of antibody producing cells in the gut lamina propria and in the spleen after oral immunization with a soluble protein antigen (KLH) + cholera toxin (CT) adjuvant. Serum levels of IgG2a, IgG3 and IgA were 5–10 fold lower in IFN-γR−/− mice compared with wild type mice. The mutants mice exhibited 10–fold reduced total serum KLH-specific antibody levels compared with wild type mice. After intravenous immunization no such difference was seen suggesting that IFN-γR−/− mice have a selective impairment of mucosal immune responses.

Levels of total IgA and IgG responses in gut lavages after oral immunization with CT were similar in IFN-γR−/− and wild type mice and both groups of mice developed gut anti-toxoid protection as assessed by the ligated loop test. In vivo restimulated T cells from immune IFN-γR−/− mice generated lower levels of IFN-γ than T cells from wild type mice. No differences were seen in the production of IL-4. Oral feeding with KLH followed by parenteral immunization resulted in strongly suppressed numbers of antibody forming cells and reduced cell mediated immunity i.e. Ag-specific T cell IFN-γ production in both wild type and IFN-γR−/− mice indicating that induction of oral tolerance is independent of IFN-γ. Moreover, CT adjuvant abrogated the induction of oral tolerance in the IFN-γR−/− mice. In conclusion, IFN-γR−/− mice have impaired mucosal immune responses while induction of oral tolerance is unaffected by the lack of IFN-γ functions.

P.5.08.15 Antileukoprotease; An endogenous protein in the innate mucosal defense against fungi

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Introduction: Previous studies have suggested that endogenous protease inhibitors may participate in the mucosal host defense. Antileukoprotease (ALP) is an important protease inhibitor found on various mucosal surfaces including those of the respiratory and genital tracts. This study reports on the antimicrobial activity of ALP towards the human fungal pathogens; Aspergillus fumigatus and Candida albicans

Materials and Methods: A. fumigatus conidia and C. albicans yeast cells were incubated with various concentrations of recombinant ALP or its NH2- and COOH-terminal domains. Fungal killing by rALP was scored by enumerating colony forming units and compared to the antifungal activity of human defensins and lysozyme. Yeast cell growth inhibition was quantified spectrophotometrically.

Results: ALP expressed pronounced fungicidal activity towards metabolically active A. fumigatus conidia and C. albicans yeast cells, however, metabolically quiescent A. fumigatus conidia were totally resistant. In contrast with the production of lysis activity, the ALP fungicidal activity was found to be localized primarily in the NH2-terminal domain. On a molar base, the fungicidal activity of ALP was comparable with the fungicidal activity of human defensins and lysozyme. In addition, rALP caused inhibition of C. albicans yeast cell growth.

Conclusion: By exhibiting antifungal activity ALP may play an important role in the innate mucosal defense against human pathogenic fungi.

P.5.08.16 Expression of monoclonal d-lgA in mammary gland epithelial cells of transgenic mice during lactation

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Introduction: In the murine mammary gland dimeric IgA (d-lgA) is expressed and secreted by plasma cells, transported across the epithelial cell layer by the