Results: There have been high pronounced blast-cell transformation regarding to autologous saliva in all patients and the highest level in cancer persons. The mitogenetic effect of patients' saliva to heterologous cells in healthy persons has also been reliable more than in a control.

Conclusion: At present mitogenetic factors in saliva resulting in the proliferative response of lymphocytes aren't quite characterized. Perhaps, the highest levels of such activity might correspond to the most serious disorders of oral local immunity.

P.5.08.08 TGEV-specific IgA at different mucosae following infection of pigs with transmissible gastroenteritis virus or the antigenically related porcine respiratory coronavirus

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Introduction: The porcine respiratory coronavirus (PRCV) appeared in 1984 in the European swine population and is antigenically closely related to the enteropathogenic coronavirus, transmissible gastroenteritis virus (TGEV). TGEV infects and destroys enterocytes on the small intestinal villi, whereas PRCV infects epithelial cells in the respiratory tract. Both viruses are antigenically similar for the M, the N protein and for the neutralisation mediating epitopes of the S protein, but show a difference in the antigenic site of the S protein which stimulates non-neutralizing antibodies. Although they have a different cell tropism, they induce neutralizing antibodies to a similar degree. However, PRCV does induce a partial protection against challenge with TGEV, while the reverse could not be demonstrated (1). In order to understand the protection induced by PRCV against TGEV, the presence of TGEV-specific IgA antibodies was determined in blood and different mucosal secretions following infections with PRCV and/or TGEV.

Materials and Methods: Six-week-old TGEV-seronegative piglets were infected intragastrically with 10^7 PID_{50} TGEV strain Miller (n = 4) or by aerosol with 10^7 $TCID_{50}$ TLM83 strain PRCV (n = 2) and challenged 4 weeks later with 10^7 PID_{50} TGEV strain Miller. Nasal, conjunctival, oral and vaginal swabs, faeces and serum were collected at 0, two and four weeks post infection (WPI) and at 2 and 4 weeks post challenge (WPC). All samples were analysed for the presence of TGEV-specific IgA by ELISA.

Results: Both PRCV infected piglets showed anti-TGEV-IgA in nasal, conjunctival and oral swabs at 2 and 4 WPI and in serum at 4 WPI, whereas only one pig was positive in her vaginal swabs at 4 WPI. No antibodies could be demonstrated in the faeces. With respect to the 4 TGEV infected piglets, anti-TGEV-IgA were demonstrated in serum at 2 WPI in 1 and at 4 WPI in all animals. At 4 WPI, 2 and 1 piglets showed IgA in conjunctival and oral swabs, respectively. No anti-TGEV-IgA were detected in faeces, nasal or vaginal swabs. A heterologous challenge with TGEV of both PRCV immunized piglets induced anti-TGEV-IgA in faeces and vaginal swabs at 2 WPC. Four weeks post challenge faecal samples became negative, while the other samples remained positive. Homologous challenge of the TGEV immunized piglets induced only anti-TGEV-IgA in serum.

Conclusions: These data confirm previous results which showed that a PRCV infection of pigs at the age of 6 weeks primes the respiratory and intestinal mucosal immune systems (1, 2). The present results suggest that the PRCV infection also primes mucosa-associated lymphoid tissue at distant mucosae.

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P.5.08.09 Cytokine gene expression in mucosal T lymphocyte populations

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Introduction: The production of cytokines by cell populations within the gastrointestinal tract is believed to play a fundamental role in the mechanisms responsible for oral tolerance to dietary antigens, in the maintenance of homeostasis and in the development of an inflammatory response.

In earlier studies, peripheral blood mononuclear cell (PBMC) responses to a dietary antigen (gliadin) demonstrate that the dominant cytokines produced are IL10 and IL6. The production of T cell cytokines IL2 and IFNγ has also been found but gliadin stimulation does not induce IL4 or IL5 production.

Materials and Methods: To elucidate the importance of local cytokine production, the capacity of mucosal T lymphocytes to express mRNA for IL10, IFNγ and LMIF (leucocyte migration inhibition factor) is examined in this study. RNA was extracted from whole intestinal biopsies and from single cell suspensions of epithelial and lamina propria fractions following treatment with EDTA/DTT and collagenase. RT PCR was performed using cytokine specific primers on the extracted RNA. In addition, CD3+ T cells were isolated from the lamina propria and from the epithelial layers (intraepithelial lymphocytes-IELs) using magnetic beads coated with anti CD3. RT PCR for the above cytokines was performed on oligo dT coated magnetic beads following extraction of mRNA from IELs and from lamina propria T cells.

Results: The results demonstrate the expression of mRNA for LMIF in whole intestinal biopsies, in the fractionated epithelial and lamina propria layers and in isolated IEL and lamina propria T cell populations. The expression of mRNA for IL10 was observed in whole biopsies in 2/5 subjects and was also detected in both the epithelial and lamina propria fractions. mRNA expression for IFNγ was found in 4/5 whole biopsies and in cell suspensions of both the epithelial and lamina propria fractions.

Conclusions: This study demonstrates the use of magnetic beads to extract specific cell populations from small intestinal biopsies. The results illustrate the capacity of both IELs and lamina propria T cells to manufacture cytokines which potentially participate in immune regulation within the gastrointestinal tract

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 biopsy cell suspensions and CD3+ lymphocytes 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 stimulants. Ribonucleic 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-4 message. Finally, using magnetic beads coated with OKT3, CD3+ 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 lysis 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 OKT3 isolated populations from peripheral blood, lamina propria and epithelial layer. In the same individuals IL-4 was detected in both OKT3 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.

P.5.08.11 Antigen-presenting properties of gingival fibroblasts in chronic adult periodontitis

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Introduction: Chronic periodontitis is characterized by dense infiltrations of T lymphocytes in the connective tissue, which mainly consists of gingival fibroblasts. It is becoming increasingly clear that T lymphocytes and gingival fibroblasts are able to influence each other. For example, the T cell cytokine IFN-γ is able to induce MHC class II molecules on the surface of several cells including gingival fibroblasts. Histological sections of chronically inflamed gingival