KANANASKIS INFLAMMATION WORKSHOP

JANUARY 30, 2010

PROGRAM

8:00 - 8:50	**Arrival & Refreshments**
8:50 - 9:00	**Opening remarks** (Tamia Lapointe)
Session 1.	**Chaired by Dr. Jennifer O'Hara and David Prescott**
9:00 - 9:15	*Pseudomonas aeruginosa* small colony variants arising from *gacS*⁻ mutants are associated with increased antimicrobial resistance, biofilm formation and inflammation at mucosal surfaces. L.K. Nelson
9:15 - 9:30	*Escherichia coli* cause altered ion channel expression, which is associated with hyposecretion. C.L. Ohland
9:30 - 9:45	Activation of dendritic cells by secreted components of the enteric parasite *Entamoeba histolytica*. L. Mortimer
9:45 - 10:00	Biofilms of gastrointestinal microflora. K.M. Sproule-Willoughby
10:00 - 10:15	Goblet cell mediators in innate host defense against *Entamoeba histolytica*. V. Kissoon-Singh
10:15 - 10:35	**Coffee Break**
Session 2.	**Chaired by Dr. David Smyth and Christina Ohland**
10:35 - 10:50	AQP3 expression is downregulated in colonic epithelial cells exposed to TNFα and IFNγ. M.A. Peplowski
10:50 - 11:05	Changes in aquaporin-5 expression in the colonic epithelium in long-standing ulcerative colitis. S. Nishimoto
11:05 - 11:20	Molecular pathway responsible for trypsin-induced increase of transepithelial resistance in intestinal epithelial cells. A. Wadhwani
11:20 - 11:35	Colonic epithelial expression of prostaglandin transporter and its role in PGE_2 induced barrier alterations. M. Lejeune
11:35 - 12:15	**Guest Speaker: Dr. Elena Verdu (McMaster University)** Nod 1 and Nod 2 receptors and intestinal barrier
12:15 - 13:30	**Lunch**

Session 3.	**Chaired by Drs. Christina Hirota and Mohammad Imtiaz**
13:30 - 13:45	Lymphatic drainage during intestinal inflammation: a role for inflammatory mediators. S. Rehal
13:45 - 14:00	Proteinase-activated receptor 2 reduces TNFα-induced apoptosis in colonic epithelial cells. V. Iablokov
14:00 - 14:15	Proteinase-activated receptor 1 & 2 regulate porcine coronary artery contractility via activation of distinct tyrosine kinase pathways. M. El-Daly
14:15 - 14:30	Proteinase-activated receptors (PARs) and human proximal renal tubular cell (HPTC) function: Implications for renal fibrosis. H. Chung
14:30 - 14:45	PAR_4 expression in sensory neurons projecting from the knee joint. V. Veldhoen
14:45 - 15:05	**Coffee Break**
Session 4.	**Chaired by Dr. Rithwik Ramachandran and Michael Peplowski**
15:05 - 15:20	Tulathromycin confers anti-inflammatory benefits in the bovine inflamed lung via modulation of neutrophil function. C.D. Fischer
15:20 - 15:35	Modulation of the macrophage response to bacterial infection by Lipoxin A4. D. Prescott
15:35 - 15:50	*Giardia lamblia* attenuates IL-8 secretion in response to proinflammatory stimuli and cleaves the NF-κB p65 independent of caspase-3 activity in intestinal epithelial cells. J.A. Cotton
15:50 - 16:05	Tulathromycin promotes phagocytosis and modulates IL-8, nitric oxide, and PGE_2 secretion in bovine monocyte-derived macrophages to promote anti-inflammatory benefits. J.K. Beatty
16:05 - 16:20	Peripheral activation of the cannabinoid orphan receptor GPR55 reduces nociception in a rat model of acute knee inflammation. N. Schuelert
16:20 - 17:00	**Guest speaker: Dr. Tony Yaksh** The spinal cord and peripheral injury: An inflammatory dialogue
17:00 - 17:05	**Closing remarks** (Dr. Fiona Russell)
17:00 - 18:45	**Free time**
18:45 - 19:30	**Reception**
19:30 -	**Dinner**
7:00 - 9:00	**Sunday Morning Breakfast**

ABSTRACTS

***Pseudomonas aeruginosa* small colony variants arising from *gacS*$^-$ mutants are associated with increased antimicrobial resistance, biofilm formation and inflammation at mucosal surfaces**

L.K. Nelson, M. Stanton, R.E.A. Elphinstone, J. Helwerda, H. Ceri

In this work, we sought to understand how *P. aeruginosa* biofilms in mucosal surface infections are regulated by the *gacS* gene. When exposed to stress *in vitro, gacS*$^-$ mutants give rise to stable small colony variants (IV-SCVs) that form denser biofilms and are more resistant to antimicrobials than either *gacS*$^-$ or wild-type (WT) bacteria. Therefore, we investigated the hypothesis that under the stress of a mucosal surface infection, *gacS*$^-$ mutants would give rise to mucosal surface derived small colony variants (MS-SCVs) which, akin to IV-SCVs isolates, would show enhanced biofilm formation and antimicrobial resistance. In a test of this hypothesis, the rat prostate was used as a model of mucosal surface infection, and was infected with one of four *P. aeruginosa* strains: WT, *gacS*$^-$, IV-SCVs or MS-SCVs. Infection and inflammation of the prostate was followed for all strains and, subsequently, *P. aeruginosa* colonies isolated from these infections were subjected to testing of antimicrobial susceptibility and biofilm formation. It was found that infections with *gacS*$^-$ bacteria produced two categories of infection which differed based on SCV formation; in cases where MS-SCVs were isolated significantly greater bacterial colonization and inflammation occurred than cases where no MS-SCVs were detected. Furthermore, infections with both IV-SCVs and MS-SCVs resulted in considerable bacterial colonization and prostate inflammation. It was also found that the MS-SCVs isolated from the prostate formed better biofilms and were more resistant to antimicrobials than their GacS$^-$ and WT counterparts. Thus, similar to *in vitro* stress, *in vivo* stress initiated by the immune response causes GacS$^-$ bacteria to throw-off SCVs, which form denser biofilms, are more resistant to antimicrobials, and are associated with increased inflammation at mucosal surfaces.

***Escherichia coli* cause altered ion channel expression, which is associated with hyposecretion**

C.L. Ohland, R. DeVinney, W.K. MacNaughton

Background: The intestinal barrier consists of a single layer of epithelial cells that can actively secrete ions to drive water flux into the lumen and flush away invading bacteria as a defense mechanism. *Escherichia coli* are common enteric bacteria composed of commensal strains and pathogenic strains such as enteropathogenic and enterohaemorrhagic *E. coli* (EPEC and EHEC). We hypothesized that *E. coli* strains alter epithelial ion secretion via alteration of host stimulus-secretion coupling pathways. **Methods:** Monolayers of the human intestinal cell line T84 were exposed to log phase bacteria and mounted in Ussing chambers to measure ion transport. Levels of ion channel mRNA (NKCC1, Na^+K^+ATPase and CFTR) were measured in infected T84 monolayers by quantitative PCR, while total protein levels were measured by Western blot. **Results:** Exposure of T84 cells to either EPEC or EHEC for 4 hr significantly decreased the cAMP-dependent ion secretion, without altering Ca^{2+}-dependent secretion. EPEC deletion mutants of proteins associated with attachment and virulence, as well human commensal strains caused similar hyposecretion, indicating the non-involvement of bacterial virulence factors. qPCR of T84 lysates revealed no significant change in mRNA levels of NKCC1, Na^+K^+ATPase or CFTR after exposure to EPEC. However, Western blots showed a significant decrease in total CFTR protein levels after EPEC exposure, which may be the mechanism by which hyposecretion occurs. **Conclusion:** Pathogenic and commensal *E. coli* strains decrease cAMP-dependent ion secretion of T84 cells. EPEC infection also decreases levels of CFTR protein, which may be the mechanism by which hyposecretion occurs.

Activation of dendritic cells by secreted components of the enteric parasite *Entamoeba histolytica*

L. Mortimer, K. Chadee

The aims of this study were to characterize the cellular responses of the primary antigen presenting dendritic cell (DC) to molecules secreted by the enteric protozoan parasite, *Entamoeba histolytica*. We hypothesize that in asymptomatic *E. histolytica* infections, molecules secreted by ameba induce DC to take on a regulatory phenotype; these molecules can act on intestinal epithelial cells to "condition" DC responses or can directly act on DC. In this study, the latter scenario was addressed. **Results:** Mouse bone-marrow-derived DC exposed to *E. histolytica* secreted products (SP) weakly induced co-stimulatory molecules (CD80 14%; CD86 12%) and inhibited the induction of MHC II. In contrast, whole soluble intrinsic ameba proteins (SAP) completely abrogated MHC II induction. SP and SAP displayed different capacities to induce DC cytokine genes involved in directing T helper responses. Both rapidly induced *IL12p19* (IL-23 specific). In contrast, SP and SAP elicited markedly different effects on *IL12p40* (shared by IL-12 and IL-23) and *IL12p35* (shared by IL-12 and IL-35) expression. SP induced *IL-12p40* and *IL-12p35* that peaked at 6 hr, while SAP weakly induced *IL12p40* at 3 hr and had no effect on *IL-12p35* expression. SP robustly enhanced *COX-2* expression, which peaked at 6 hr, while SAP mildly induced *COX-2*. **Conclusions:** *E. histolytica* SP and SAP have a markedly reduced capacity to induce DC co-stimulatory molecules and MHC II. We have previously shown ameba Gal-lectin (a component of amebic secretions) robustly induces DC co-stimulatory molecules and pro-inflammatory Th1 cytokines. Compared to Gal-lectin, SP and SAP both weakly and transiently induce *IL-12p40.* Taken together, these data suggest that ameba secretory components include mediators that override the co-stimulatory and pro-inflammatory effects of Gal-lectin and directly suppress DC activation. We postulate that *E. histolytica* diverts DC away from a pro-inflammatory response, which may allow ameba to persist in the colon during an asymptomatic infection.

Biofilms of gastrointestinal microflora

K.M. Sproule-Willoughby, M. Stanton, K.P. Rioux, D.M. McKay, A.G. Buret, H. Ceri

Background: An imbalance of intestinal microflora or association of specific pathogens with the intestinal mucosa may contribute to the development of gastrointestinal diseases such as Inflammatory Bowel Disease. Recently, studies have demonstrated that microbes grow as biofilms on the gastrointestinal mucosa. The study of biofilms is therefore an important step in understanding the complex nature of the intestinal microflora. *This study aimed to characterize the community structure of biofilms formed by mucosal bacteria from the human colon.***Methods:** Mucosal biopsies of the colon were obtained from healthy patients undergoing screening colonoscopy. Homogenates of the biopsies were used to seed anaerobic biofilms in the Calgary Biofilm Device. Biofilm growth was assessed by viable cell counting and confocal scanning laser microscopy (CSLM), and biofilm community structure was assessed using polymerase chain reaction (PCR), quantitative PCR, and terminal restriction fragment length polymorphism (T-RFLP). **Results:** Anaerobic biofilms developed quickly and continued to increase in size over a period of up to five days. CSLM imaging of the biofilms demonstrated dense, three-dimensional structures, with biofilms reaching a thickness of between 20 and 80 μm after five days of growth. The biofilm communities showed great diversity, and community composition shifting during the growth period. **Conclusion:** Complex biofilm communities were formed using mucosal bacteria from the human colon. These biofilm communities serve as a "representative microflora", which will be useful in further characterization of host-microbe interactions in the gastrointestinal tract. **Acknowledgments:** Biopsies were provided by the Intestinal Inflammation Tissue Bank at the University of Calgary. This work was supported by CCFC, NSERC and AHFMR.

Goblet cell mediators in innate host defense against *Entamoeba histolytica*

V. Kissoon-Singh, V. Srivastava, F. Moreau, K. Chadee

The mucus layer, the first line of host defence against enteric pathogens, is colonized by *E. histolytica* trophozoites through high affinity binding to Gal/GalNAc residues on MUC2 mucin. Polymeric MUC2 protects the underlying epithelial mucosa from invasion and destruction, and is secreted by intestinal goblet cells, along with intestinal trefoil peptide (TFF3), and resistin like molecule (RELM-β). The role of these other goblet cell secretions in host defence against pathogens in the absence of a mucin substrate is not known. We hypothesize that goblet cell mediators play a cooperative role in innate host defence against *E. histolytica*. The aim of this study was to investigate the innate immune response elaborated by goblet cell secretions in wild type (WT) and $MUC2^{-/-}$ mice in response *E. histolytica*. Colonic loops were inoculated with $1x10^6$ virulent log-phase trophozoites and goblet cell responses quantified after 1h and 3h post infection. A significant increase in high molecular weight V_o mucin and non-mucus glycoproteins was observed in WT mice challenged with amoeba as compared to $MUC2^{-/-}$ animals. Histological analysis to quantify muco-polysaccharides corroborated these findings. In an *in vitro* model for amoebiasis, colonic mucins derived from WT animals inhibited amoebic adherence to CHO cells in a dose-dependent manner. Interestingly, live virulent amoebae in $MUC2^{-/-}$ colons did not invade the tissues and animals showed no overt signs of distress. Increased MUC2 and RELM-β mRNA expression was observed in colonic loops of WT mice challenged with *E. histolytica.* In $MUC2^{-/-}$ animals however at the same time points, both TFF3 and RELM-β expression were reduced following amoebic challenge. We conclude that TFF3 and RELM-β are associated with MUC2 in distress conditions. RELM-β (but not TFF3) may mediate resistance to amoebic challenge. These peptides, as well as the pro-inflammatory cytokines TNF-α and IFN-γ are involved in the innate host response to *E. histolytica* infections.

AQP3 expression is downregulated in colonic epithelial cells exposed to TNFα and IFNγ

M.A. Peplowski, W.K. MacNaughton

Aims: Inflammatory bowel diseases (IBD) are characterized by altered water absorption and secretion, leading to the development of diarrhea. Although aquaporins (AQP) are expressed on intestinal epithelial cells and their expression is downregulated in IBD, little is known about the physiological regulation of AQP in epithelial cells of the gastrointestinal tract in health and disease. *We hypothesize that altered AQP expression and localization in intestinal epithelial cells are involved in the barrier dysfunction that characterizes IBD.* **Methods:** Colorectal adenocarcinoma cells (HT29) were incubated with either tumour necrosis factor (TNF) α and / or interferon (IFN) γ. The expression profile of AQP3 mRNA and protein was assessed using quantitative real-time PCR and Western blot respectively. In addition, a 24hr cycloheximide (10μg/mL) study was performed to assess AQP3 protein stability. **Results:** Time-course experiments with a single treatment of TNFα (25ng/mL) or IFNγ (500U/mL) indicated that AQP3 mRNA expression was significantly repressed at 6 to 12 hr post-treatment. Dose-response studies revealed EC_{50} values of 3.77ng/mL and 1.79U/mL for TNFα and IFNγ treatments respectively. Protein expression studies for HT29 cells treated with TNFα or IFNγ every 12 hr for a period of 24 and 48 hr and TNFα for 72 and 96 hr indicate that AQP3 expression remains unchanged. However, treatment with IFNγ for 72 and 96 hr appears to slightly decrease AQP3 protein expression. Results from the cycloheximide study suggest that AQP3 protein is characterized by a long half-life in HT29 cells. **Conclusions:** Our data suggest that TNFα and IFNγ may be responsible for decreasing AQP3 mRNA expression in human intestinal epithelial cells. However, long term exposure to these cytokines may be required to downregulate AQP3 protein levels.

Changes in aquaporin-5 expression in the colonic epithelium in long-standing ulcerative colitis

S. Nishimoto, W.K. MacNaughton

Introduction: Patients with long-standing ulcerative colitis (UC) are at greatly increased risk of developing colorectal cancer (CRC). The molecular events underlying the transition from UC to CRC development are not well known. UC-associated cancer is generally preceded by extended periods of dysplasia. Recent studies have identified ectopic aquaporin-5 (AQP5) expression in early CRC development, during mild dysplasia. We hypothesize that AQP5 is ectopically expressed in the colonic epithelium during long-standing UC, due to hypomethylation of the AQP5 promoter. We will examine changes in AQP5 expression and the methylation status of the AQP5 promoter in the colonic epithelium during UC, using both biopsy samples from UC patients and a mouse model of inflammation-associated colon cancer, the azoxymethane (AOM)/dextran sodium sulfate (DSS) model. **Methods**: C57BL/6 mice will be administered AOM followed by one week of 2% DSS via drinking water, to induce colitis. Mice will be sacrificed at 0, 2, 4, and 6 weeks post-DSS treatment. Human biopsy samples from patients with 10 or more years of UC and normal biopsy samples will be obtained from the Inflammatory Bowel Disease Tissue Bank (University of Calgary). AQP5 expression in the human biopsy and mouse colon samples will be analyzed using reverse transcriptase (RT)-PCR, immunoblot analysis, and immunohistochemistry. The methylation status of the AQP5 promoter in the human biopsy and mouse colon samples will be determined using bisulfite sequencing. **Expected Progress**: By the end of January 2010, we expect to have completed analyses of AQP5 expression in AOM/DSS-treated mice and human biopsy samples. We also expect to have some results from AQP5 promoter methylation analyses of mouse samples.

Molecular pathway responsible for trypsin-induced increase of transepithelial resistance in intestinal epithelial cells

A. Wadhwani, W.K. MacNaughton

Background: We have shown that serine proteases produce a sustained increase in the transepithelial resistance (R_{TE}) of the intestinal epithelial cell lines SCBN, T84 and Caco-2. This response depends upon the enzymatic activities of these proteases, as well as the activation of PKC_{ζ}. However, the role of protease activated receptors (PARs) in the response is unclear and a complete understanding of the pathways that mediate the trypsin-induced increase in R_{TE} is currently unknown. Our aim is to determine if trypsin increases R_{TE} by activating PARs and/or transactivating the epidermal growth factor receptor (EGFR), to cause the intracellular activation of PKC_{ζ}. **Proposed Studies: (1)** Monolayers of the intestinal epithelial cell line SCBN will be cultured on semi-permeable membrane supports for about four days. Confluence will be determined by measuring R_{TE}. Monolayers will be mounted in Ussing chambers to measure the change in R_{TE} upon addition of trypsin to the cells. Cells will be treated with activating peptides of PARs 1,2 and 4, or an inhibitor of EGFR prior to stimulation of R_{TE} with trypsin. **(2)** Cells will be analyzed for phosphorylation of EGFR (post treatment with trypsin) via western blot analysis. **(3)** Cells will also be analyzed for the localization of claudins, zona occludens, and junctional adhesion molecule-1 at the tight junctions via immunocytochemistry, upon activation of PKC_{ζ} by trypsin. **Summary:** These studies will determine the signaling pathways and specifically the role of EGFR in increasing R_{TE} in response to serine proteases.

Colonic epithelial expression of prostaglandin transporter and its role in PGE_2 induced barrier alterations

M. Lejeune, P. Leung, K. Chadee

Prostaglandin E_2 (PGE_2) is an important pro-inflammatory lipid mediator that is produced in excess in inflammatory bowel diseases (IBD). We have previously shown *in vitro* that high output PGE_2 signals through E-Prostanoid (EP) receptor 4 and dose-dependently decreases colonic Trans Epithelial Resistance (TER) indicating a loss of epithelial barrier function. In the present study, we investigated the significance and the role of prostaglandin transporter (PGT) in PGE_2 induced colonic barrier disruption. By immunostaining, EP4 receptors are localized mostly to the epithelial apical plasma membranes. We theorize that PGT is involved in the initiation of colonic inflammation by actively transporting PGE_2 from the lamina propria towards the site of apical EP4 receptors in colonic cells. Thus, to determine if PGE_2 are actively transported across colonic cells towards the apical receptors, we probed T84 colonic cells for the presence of PGT by Q-PCR and Western blot. Not only did PGT transcript and protein were expressed in T84 cells they were also differentially over-expressed in the colonic epithelium of patients with IBD as revealed by immunostaining. Moreover, to establish that PGE_2 is actively transported across T84 epithelial monolayer we traced for the basolateral to apical translocation of ^3H-PGE_2. A gradual increase in the apical translocation was observed with ^3H-PGE_2 with a peak occurring at 24hr. Predictably, knockdown of PGT using shRNA significantly prevented the translocation of ^3H-PGE_2. Thus, it is clear that PGT can play a major role in initiating colonic inflammation by transporting PGE_2 towards their appropriate site of action. Taken together, these studies unravel a complex cellular mechanism whereby PGE_2 plays a role in altering colonic epithelial barrier.

Guest Speaker

Dr. Elena F. Verdu
Department of Medicine, Division of Gastroenterology
McMaster University

Nod 1 and Nod2 Receptors and Intestinal Barrier

Dr. Verdu received MD training at the Universidad de Buenos Aires (Argentina) and the Université de Lausanne (Switzerland). In 2001, she obtained her PhD in the area of "microbial and hormonal factors in intestinal inflammation" from the Institute of Microbiology, at the Academy of Sciences in Czech Republic. After completing a post-doctoral fellowship at McMaster University in 2006 (ON, Canada), she accepted an Assistant Professor position in the Intestinal Disease Research Program at the same institution. Dr. Verdu's current research interest includes the pathogenesis of chronic inflammatory disorders such as celiac disease. She is also interested in host/microbe interactions, particularly in the context of probiotics and functional gastrointestinal diseases.

Lymphatic drainage during intestinal inflammation: a role for inflammatory mediators

S. Rehal, S. Roizes, P.-Y. von der Weid

Inflammatory bowel disease manifests classical abnormalities, specifically interstitial edema, inflammatory infiltrate and increased bowel thickness. Earlier studies looking at lymphatic vessel morphology in IBD have observed them as being dilated, hypocontractile and often obstructed. These abnormalities in lymphatic drainage could very well be due to the release of inflammatory mediators from the site of intestinal inflammation, specifically prostaglandins. A guinea-pig model of TNBS ileitis was established, along with sham counterparts, and animals were sacrificed at day 1 and 3 (n=5). Lymphatic, arterial, vein and ileum tissue were microdissected out and real time PCR was performed for the following genes: COX-1, COX-2, Prostacyclin synthase, Prostacyclin receptor, Prostaglandin E2 synthase 1/2, EP1, EP2, and EP4 receptors. Furthermore, lymphatic and ileum tissue levels of Prostaglandin E2 were measured in our animals using ELISA (n=4). Our TNBS-ileitis guinea pigs had significant macroscopic damage scores based on erythema, hemorrhage, edema, strictures, ulceration, mucus and adhesion. There was an upregulation of mRNA for COX-2, EP1, EP2 and EP4 in the lymphatic vessels of our inflamed guinea pigs at day 1 with a subsequent downregulation at day 3. Furthermore tissue levels of Prostaglandin E2 were dramatically increased in our lymphatic vessels from inflamed guinea pigs. These results indicate that there is an overabundance of Prostaglandin E2 in our lymphatic tissue, contributing to the dilated, hypocontractile phenotype of the lymphatic vasculature in our animal model of intestinal inflammation. Research project funded by CCFC.

Proteinase-activated receptor 2 reduces TNFα-induced apoptosis in colonic epithelial cells

V. Iablokov, K. Iannuzzi, C.L. Hirota, W.K. MacNaughton

Aims: Patients with chronic inflammatory bowel diseases (IBD) have an increased risk of colorectal cancer and exhibit increased concentrations of fecal serine proteases. Protease activated receptor 2 (PAR_2) is expressed on colonic epithelial cells and is activated upon proteolytic cleavage by serine proteases. In addition, recent studies have shown that activating PAR_2 promotes proliferation in colonic epithelial cell lines. We sought to determine the mechanism responsible for PAR_2 mediated epithelial cell proliferation. **Methods:** HT-29 cells, a human colonic epithelial cell line expressing PAR_2, were treated with the PAR_2 activating peptides for up to 48 hours in serum-free media. Reverse peptides of the agonists and media containing serum were used as negative and positive controls, respectively. DNA synthesis was measured in treated cells by incorporation of 5-bromo-2-deoxyuridine (BrdU). Total HT-29 cell number was measured by cell counting after exposure to serum free media, 2-furoyl-LIGRL or media with serum. Western blots were used to detect cleaved caspase-3 protein levels, as an indicator of apoptosis, after co-treatment with rhTNF-α and PAR_2 activating peptides. **Results:** HT-29 cells showed a significant increase in DNA synthesis as determined by BrdU incorporation and total cell number after treatment with serum media. However, PAR_2 agonists did not significantly alter either parameter compared to controls at any of the time points examined. PAR_2 agonists did not increase epithelial cell apoptosis, as indicated by measurement of cleaved caspase-3, but did significantly decrease the amount of caspase-3 cleavage mediated by rhTNF-α, suggesting anti-apoptotic activity. **Conclusions:** These data indicate that PAR_2 activation does not stimulate DNA synthesis or cellular proliferation in human colonic epithelial cells. Instead, PAR_2 signaling decreases the cell's response to pro-apoptotic signals. Enhanced serine protease levels during IBD may, by activating PAR_2, facilitate the survival of colonic cells harboring cancer-causing mutations.

Proteinase-activated receptor 1 & 2 regulate procine coronary artery contractility via activation of distinct tyrosine kinase pathways

M. El-Daly, M. Saifeddine, Z. Yu, C.R. Triggle, M.D. Hollenberg

Proteinases (thrombin, trypsins and kallikreins), signaling via proteinase-activated receptors (PARs: Pharmacol. Rev. 54(2): 203) regulate vascular function and inflammatory processes (Br J Pharmacol. 153 Suppl 1:S263-82). PARs regulate vascular tension via endothelium-dependent and endothelium-independent mechanisms. We previously found that Angiotensin-II-mediated porcine coronary artery (PCA) vasoconstriction was inhibited by tyrosine kinase (PYK) inhibitors (CJPP 72(9): 1075; Pharm. Commun. 1:177). We hypothesised that, like angiotensin-II, PAR-mediated PCA contractions are PYK-pathway-dependent. Contractions of isolated PCA rings +/- a functional endothelium were monitored in the absence and presence of several signal pathway inhibitors, including PP1 (Src kinase), tyrphostin-AG-18 (nonspecific PYKs), AG 1478 (EGF receptor-kinase), AG1296 (PDGF receptor-kinase), L-NAME (NO-synthase) and indomethacin (cyclo-oxygenase). We found that PP1 inhibited endothelium-independent contractions caused by PAR_1 and PAR_2 agonists, but not by angiotensin-II and prostaglandin-F2α (PGF2α), whereas tyrphostin AG18 inhibited contractions caused by all four agonists. The EGF-kinase inhibitor, AG1478, inhibited contractions cause by PAR_2, but not by PAR_1, Angiotensin-II or PGF2α. Indomethacin attenuated PAR-mediated contractions but not those caused by Angiontensin II and PGF2α. We conclude that PCA contractions via PARs 1 and 2 are Src-kinase dependent and mediated by a cyclo-oxygenase product, but employ distinct PYK pathways that involve the EGF receptor kinase for PAR_2-mediated contractions but not for contractions caused by PAR_1 and other GPCR agonists.

Proteinase-activated receptors (PARs) and human proximal renal tubular cell (HPTC) function: Implications for renal fibrosis

H. Chung, D. Muruve, M.D. Hollenberg

Epithelial-to-mesenchymal transition (EMT) is a process characterized by loss of epithelial phenotype and increased proliferation of fibroblasts and excessive accumulation of extracellular matrix. Emerging evidence indicates that tubular epithelial cells can undergo EMT and that this process is fundamentally linked to the pathogenesis of renal fibrosis. Many studies have suggested potential mechanisms underlying the activation of inflammatory and fibrogenic pathways in human proximal tubular epithelial cells (HPTCs). However, the significant role played by proteinases in EMT and the process of renal fibrosis has not been investigated. The kidney is an organ that highly expresses Proteinase-activated receptors (PARs). Serine proteinase such as trypsin or kallkrein, found in the urine, can activate PARs by proteolytic cleavage within the extracellular N-terminus of the receptors (1-4). While a role for PARs in inflammation and cell proliferation has been established, the identities of the PAR-regulating proteinases that are present in inflammatory settings have not yet been determined. These multifunctional hormone-like proteinases may be implicated in renal pathology due to their ability to regulate PARs. Therefore, we hypothesize that HPTCs can secrete proteinases that in turn can induce renal fibrosis via PAR activation. To test this hypothesis, primary cultures of HPTCs were isolated from adult human kidneys and were analyzed for their content of PARs and their ability to secrete proteinases. Confluent cell monolayers demonstrating a normal human karyotype were studied at 7 days after seeding and cells that have been passaged up to 4 times. We found that HPTCs (1) express functional PAR1 and PAR2 (mRNA and calcium signaling responses to PAR activation), and (2) secrete proteolytically active proteinases into the cell medium. These data provide a basis for the further study of the roles of PARs and the HPTC-secreted proteinases in EMT as it relates to the development of renal fibrosis.

PAR_4 expression in sensory neurons projecting from the knee joint

V. Veldhoen, F.A. Russell, J.J. McDougall

Introduction: Proteinase activated receptors (PARs) are G-protein coupled receptors that are activated by serine proteinases that are released into the joint during inflammation. The most recently identified PAR, PAR_4, was discovered in human platelets. We have shown that PAR_4 has a pro-nociceptive effect in the joint but it is unclear whether this is due to a direct effect of PAR_4 on sensory neurons. The aim of this study was to determine whether PAR_4 is expressed in joint sensory neurons. **Methods**: 10 μL of a 2% Fluoro-Gold (FG) solution in saline was injected into the knee joints of three naïve rats. After 4 days the rats were euthanized and DRGS from L3, L4 and L5 were removed and fixed in 4% paraformaldehyde overnight. DRGS were then placed in 30% sucrose solution overnight at 4°C and then stored in OCT at -80°C until sectioning. DRG sections then underwent an immunohistochemical procedure utilizing a primary antibody specific for PAR_4 and a secondary antibody labeled with Cy3. Each DRG section was examined under filters appropriate for FG and Cy3. The diameter of all FG positive cells was measured. **Results**: FG labeling of neuronal cells was present in all L3, L4 and L5DRGs. Overall 2964 FG positive cells were found, with an average of 533 ± 66 in each knee joint. The diameter of the FG neurons was found to be broadly distributed across a range for all DRGs. 60 ± 2% of the FG positive cells were also positive for PAR_4. **Conclusion**: These data show that PAR_4 is expressed in the sensory neurons projecting from the knee joint of male Wistar rats.

Tulathromycin confers anti-inflammatory benefits in the bovine inflamed lung via modulation of neutrophil function.

C.D. Fischer, J.K. Beatty, D.W. Morck, M.J. Lucas, E.J. Robb, A.G. Buret

While neutrophils (PMNs) mainly serve as a defensive role, they have been shown to be injurious in several acute and chronic pulmonary diseases. Neutrophil clearance is imperative for the resolution of inflammation at the site of injury. Tulathromycin (TUL), a new antibacterial agent for bovine respiratory disease (BRD), offers superior clinical efficacy for reasons not fully understood. Studies have shown that modulation of immune cell function by macrolide antibiotics confers anti-inflammatory benefits. **Aims**: 1) to determine whether TUL alters PMN function in Holstein calves challenged intra-tracheally with live *Mannhemia haemolytica* or zymosan and 2) to determine the potential anti-inflammatory mechanisms of TUL *in vitro*. **Results**: ELISA and fluorescent microscopy showed that bronchoalveolar lavage (BAL) fluid from TUL-treated infected calves challenged with *M. haemolytica* had significantly higher levels of apoptotic PMNs. ELISA also revealed that in both the *M. haemolytica*- and zymosan-infected calves, TUL-treatment reduced levels of LTB_4, a potent PMN chemoattractant. In addition, TUL induced apoptosis in bovine PMNs *in vitro* in a capase-3 dependent manner, as determined by Western bloting and activity assays. TUL also reduced levels of phosphorylated IkappaB (IκB) in LPS-stimulated PMNs. **Conclusion**: In addition to its antibacterial properties, TUL anti-inflammatory benefits via modulation of PMN function. Indeed, this study revealed that TUL induces PMN apoptosis, inhibits NF-κB signaling and reduces the accumulation of LTB_4 in the bovine lung.

Modulation of the macrophage response to bacterial infection by Lipoxin A4

D. Prescott, D. McKay

Aims: Lipoxins are arachadonic acid-derived eicosanoids that play a major role in the resolution of inflammation. However, it is unclear how this role changes in the presence of bacteria. The aim of this study is to determine the influence of LXA_4 on macrophage phagocytosis and cytokine production in response to live *E. coli*. **Methods**: The human THP-1 monocytic cell line ($2.5x10^5$ cells/well) was differentiated into a macrophage-like phenotype by PMA treatment (10nM, 48h), and exposed to live *E. coli* (10^6 CFU/well) or inert fluorescent beads (10^7/well) ± aspirin-triggered lipoxin (ATL: a stable analog of LXA_4, 100nM). Six hours later, internalization of bacteria was assessed by the gentamicin exclusion assay, and bead internalization was assessed fluorometrically. Culture supernatant levels of IL-1β, IL-8, IL-10, and TNF-α were measured by ELISA. **Results**: Treatment with ATL resulted in increased phagocytosis of inert beads (23.0 ± 5.4% increase over control, n=6, $p<0.05$) and live *E. coli* (0.044 ± 0.00921% vs. control = 0.018 ± 0.00136% internalized, n=6, $p<0.05$). ATL treatment did not affect THP-1 production of IL-8 and TNF-α in response to E. coli, but IL-1β levels were increased (ATL + *E. coli* = 890 ± 210 vs. *E. coli* = 500 ± 160 pg/ml, n=3, $p<0.05$). **Conclusion:** LXA_4 and its analogs are able to promote the uptake of live bacteria and inert polystyrene beads. However, THP-1 macrophages treated with ATL do not down-regulate the release of pro-inflammatory cytokines in response to live bacteria. This suggests that while the macrophage plays a role in the clearance of bacteria from a damaged tissue, it also supports a prolonging of the inflammatory response until the tissue is free of infection.

***Giardia lamblia* attenuates IL-8 secretion in response to proinflammatory stimuli and cleaves the NF-κB p65 independent of caspase-3 activity in intestinal epithelial cells**

J.A. Cotton, H.L. Sayer, A.G. Buret

Introduction. *Giardia lamblia* is a non-invasive protozoan parasite that causes diarrheal disease in humans. In response to *G. lamblia* trophozoites, intestinal epithelial cells (IECs) do not release proinflammatory mediators, such as the potent neutrophil chemoattractant interleukin (IL)-8, that lead to intestinal inflammation. Expression of these mediators, including IL-8, is regulated by the nuclear factor κB (NF-κB) pathway. This pathway is regulated by several mechanisms, including caspase-3 mediated cleavage of NF-κB proteins. We hypothesized that *G. lamblia* reduces IL-8 secretion in IECs by modulating the NF-κB pathway. **Methods.** Caco-2 monolayers incubated with *G. lamblia* (strains NF, S2, PB or WB) trophozoites for 24 hours at an MOI of 10:1 were challenged with pro-inflammatory IL-1β. Samples were processed for ELISA assays and Western Blotting of whole cell lysates or cytosolic/nuclear fractions. **Results.** IL-1β induced IL-8 secretion in Caco-2 monolayers was significantly reduced when monolayers were incubated with *G. lamblia* for 24 hours; this effect was independent of caspase-3 activity. Physical contact, between trophozoites and monolayers, was not required to reduce IL-8 secretion from Caco-2 monolayers. *G. lamblia* reduced total levels of the NF-κB p65 subunit in Caco-2 monolayers and induced cleavage of p65 into a fragment approximately 37 kDa in size that translocates to the nucleus following IL-1β stimulation. **Conclusion.** The mechanism by which *G. lamblia* attenuates proinflammatory signaling in IECs is partially dependent on a caspase-3 independent modulation of the NF-κB pathway.

Tulathromycin promotes phagocytosis and modulates IL-8, nitric oxide, and PGE_2 secretion in bovine monocyte-derived macrophages to promote anti-inflammatory benefits

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Some macrolide antibiotics have been shown to confer anti-inflammatory properties in addition to antimicrobial capabilities. A new macrolide, tulathromycin (TUL), is used in the treatment of Bovine Respiratory Disease, and has recently been shown to induce apoptosis in bovine neutrophils, however, associated effects of TUL on macrophage phagocytosis of these cells have not yet been investigated. Additionally, direct effects of TUL on macrophage production of inflammatory mediators in the lung: interleukin (IL)-8 and nitric oxide (NO), prostaglandin E_2 (PGE_2), remain unknown. **Aims:** 1) to determine the effects of TUL on neutrophil phagocytosis by bovine monocyte-derived macrophages (BMDM) and 2) to examine the direct effects of TUL on the expression of cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) and secreted levels of IL-8, PGE_2, and NO. **Results:** Diff-Quick staining and light microscopy suggest that TUL-induced neutrophil apoptosis is associated with an increase in neutrophil phagocytosis by BMDM. Western blotting revealed TUL increases expression of COX-2 and decreases expression of iNOS, while ELISA and Greiss reaction illustrated that TUL decreases secreted levels of IL-8, and NO and increases levels of PGE_2 in lipopolysaccharide (LPS)-stimulated BMDM. **Conclusion:** TUL promotes macrophage phagocytosis of apoptotic neutrophils and modulates inflammatory protein levels (COX-2 and iNOS) and secreted levels of inflammatory mediators (IL-8, NO, PGE_2) in LPS-stimulated BMDM. Together, the findings illustrate novel mechanisms through which an antibiotic may deliver potent anti-inflammatory benefits.

Peripheral activation of the cannabinoid orphan receptor GPR55 reduces nociception in a rat model of acute knee inflammation

N. Schuelert, J.J. McDougall

Aim of the study: Aim of this study was to investigate, if activation of the cannabinoid orphan receptor GPR55 by local application of the selective GPR55 receptor agonist O-1602 can reduce nociceptive activity of afferent nerve fibres in an acute inflammatory model of the rat knee joint. **Methods:** An acute synovitis was induced in the right knee joint by an intra-articular injection of 2% kaolin followed by 2% carrageenan. Joint nociception was objectively measured in these animals by recording electrophysiologically from knee joint primary afferents in response to noxious rotation of the joint both before and following close intra-arterial injection of O-1602 (100µg) and co-administartion of the GPR55 antagonist O-1918 (50 µg). **Results:** A single injection of O-1602 caused afferent firing rate to be significantly reduced by up to 50% during noxious rotation of the rat knee joint. This desensitizing effect was found to be maximal 15 min after O-1602 injection. Co-administration of the selective GPR55 receptor antagonist O-1918 (50µg) abolished the antinociceptive effect of O-1602, confirming that the analgesic effect is mediated via this atypical cannabinoid receptor. **Conclusions:** These findings indicate that peripheral activation of the cannabinoid GPR55 receptor alleviated peripheral sensitization of knee joint afferents and reduces pain transmission during movement of the joint under inflammatory conditions. The results provide further evidence that atypical cannabinoid receptors are involved in mediating inflammatory pain. Selective ligands directed towards GPR55 might have the potential to be novel therapeutics in the treatment of joint pain, minimizing centrally mediated side effects.

Guest Speaker

Dr. Tony Yaksh
Department of Anesthesiology
University of California, San Diego

The spinal cord and peripheral injury: An inflammatory dialogue

After completing his MSc degree at the University of Georgia, Dr. Yaksh obtained his PhD degree in the field of Neuropsychology at the Perdue University in 1971. After working as a research scientist at the University of Wisconsin and University College London, he became a Professor of Pharmacology and Neurosurgery at the Mayo Clinic (Rochester, MN) for more than 10 years. Dr. Yaksh joined UCSD in 1988 as Professor and Vice Chairman for Research in the Department of Anesthesiology and Professor of pharmacology. His research focus is primarily in the area of the physiology and pharmacology of pain processing. He has a particular interest in the role of non-neuronal cells and lipid mediators in pain transmission.

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