Role of Alarmins – human-defenisns and HMGB1- in gastrointestinal diseases

Summary of Ph.D. Thesis
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ABBREVIATIONS

α-defensin: alpha-defensin

AGS: human adenocarcinoma cell

β-defensin: beta-defensin

CagA+: cytotoxin associated gene A

CI: confidence interval

EIMs: extraintestinal manifestations

ELISA: enzyme-linked immunosorbent assay

ERK: extracellular signal-regulated kinase

FAM: Carboxyfluorescein

FITC: fluorescein isothiocyanate

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

hBD: human beta-defensin

HNP: human neutrophil protein

LDH: lactate dehydrogenase

LPS: lipopolysaccharide

NF κB: nuclear factor kappa-light-chain-enhancer of activated B cells

NOD2: nucleotide-binding oligomerization domain containing 2

OR: odds ratio

PAI: cag pathogenicity island

PBS: phosphate-buffered saline

PCR: polymerase chain reaction

RAGE: Receptor for Advanced Glycation Endproducts

RNA: ribonucleic acid

ROC: receiver-operating characteristic

SDS: Sodium Dodecyl Sulphate

SNP: single-nucleotide polymorphism

sRAGE: soluble Receptor for Advanced Glycation Endproducts

TNF α: tumor necrosis factor-alpha

VacA+: vacuolating cytotoxin A

I. Introduction

Alarmins are endogenous mediators of innate immunity. They are rapidly released in response to infection or tissue injury. In addition, they they are rapidly released from non programmed cell death but are not released from apoptotic cells. Alarmins recruit immune system and directly or indirectly promote adaptive immune responses. On the basis of their unique activities they have been called „alarmins" molecule. Innate immune mediators possessing alarmin activity include defensins, cathelicidin, eosinophil-derived neurotoxin (EDN) and High Mobility Group Box Protein 1 (HMGB1).

Defensins are antimicrobial peptide components of the innate host defense system. The human defensins are a family of six cysteine 3–4-kD cationic peptides with a characteristic fold that is common to the two subfamilies, α- and β-defensins, despite a difference in the connectivity of their disulfide bonds.

In humans, six human α-defensins have been identified. Four of them, human neutrophil proteins – HNP 1-4 -, are mainly produced by granulocytes. Human α-defensin 5 (HD5) and human α-defensin 6 (HD6) are present in intestinal Paneth cells. Human β-defensins are expressed predominantly in epithelial tissues. Although more than 90 human defensins have been identified by gene-based searches, the function of six human β–defensin (hBD1-6) are to known, they are expressed mainly by epithelial cells. The most prevalent expression of β-defensins is observed in the gastrointestinal and respiratory tracts; however, they are present throughout the entire epithelia.

High mobility group box 1 protein (HMGB-1) is a member of the nonhistone chromatin-associated proteins. Intracellular HMGB-1 has been studied previously for its roles in binding DNA; stabilizing nucleosome formation; as a general transcription factor for nucleolar and mitochondrial RNA polymerases; and as a gene- and tissue-specific transcriptional regulator that can enhance transcription and/or replication. Extracellular HMGB-1 was recently implicated as a "late" mediator of delayed endotoxin lethality. HMGB-1 levels were increased significantly in critically ill patients with sepsis. Extracellular HMGB-1 might play a mediator role in the setting of inadequate tissue perfusion. Other pro-inflammatory mediators of endotoxemia that mediate similar pathological effects (e.g., TNF and IL-1) also function as potent stimulators of monocyte cytokine release in order to amplify and extend the "cytokine cascade". Accordingly, it plausible that HMGB-1 might also function to stimulate monocytes to release TNF and other pro-inflammatory cytokines.

Helicobacter pylori infection in the stomach can be found in more than half of the world's population. The bacterium induces gastric inflammation, and the diseases that can follow this infection include chronic gastritis, peptic ulcers and gastric cancer. In the stomach, gastric epithelial cells constitutively express hBD-1, whereas hBD-2 is induced in response to pro-inflammatory cytokines or microbial infection. Recent reports have demonstrated that *H. pylori* infection induces hBD-2, whereas hBD-1 is thought to be expressed constitutively.

Crohn's disease, a chronic, inflammatory disease of the intestinal mucosa, is a complex multifactorial disease, the pathogenesis of which is still not fully understood. A major advance towards a better understanding of the disease was the discovery of mutations in the NOD2 gene in approximately one-third of Crohn's disease patients. NOD may be involved in signaling cascade-mediated defensin expression, and therefore an impaired defensin synthesis has been linked to the occurrence of Crohn's disease. Thus, decreased defensin levels lead to a weakened intestinal barrier function to intestinal microbes and might be crucial in the pathophysiology of Crohn's disease.

II. Aims

The aim of our study was to investigate the role of defensin and HMGB1 as alarmins in multifactorial diseases, where the natural defense system and genetic factors may influence the consequences of infection and/or injury.

Therefore we investigated

The effect of *H. pylori* on defensin-α (HNP1-3) production by human granulocytes

The role of hBD1 SNP in *H. pylori* induced gastritis

The role of hBD1 SNPs in the pathomechanism of Crohn's disease

The relevance of HMGB1 in the severity of acute pancreatitis

III. Patients and methods

Patients with *H. pylori* induced Gastritis and controls

150 *H. pylori*-positive patients with chronic active gastritis included the study. Biopsy specimens were taken during upper gastrointestinal endoscopy from adjacent sites of the gastric antrum and corpus for histology. The 100 members of the control population for mutation analysis were age- and gender-matched serologically *H. pylori*-positive healthy blood donors without gastric or duodenal symptoms.

Patients with Crohn's disease and controls

190 unrelated patients with Crohn's disease were investigated. The diagnosis was based on the Lennard-Jones criteria. The disease phenotype (age at onset, duration, location and behavior) was determined according to the Vienna Classification. The control group for mutation analysis consisted of 95 age- and gender-matched healthy blood donors.

Patients with Acute pancreatitis and controls

Blood samples were obtained from 62 patients with acute pancreatitis. All patients were classified as having mild or severe pancreatitis according to the original criteria of Ranson . Patients with fewer than three positive prognostic signs (n=32) were considered to have mild pancreatitis; while those with three or more positive prognostic signs (n=30) were classified into the severe pancreatitis group. For comparison 20 patients with sepsis of different origin, in the surgical intensive care unit were enrolled in the study. The control cohort considered of a random, unrelated population of 20 healthy blood donors.

Genotyping procedures

For the examination of human beta-defensin polymorphisms leukocyte DNA purified from peripheral blood was isolated using the High Pure PCR Template Preparation Kit in accordance with the manufacturers' instructions (Roche Diagnostic GmbH, Mannheim, Germany). Genotyping was done using Custom TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, Calif., USA). In order to verify the given data, restriction fragment length polymorphism analysis (RFPL) was performed.

Bacterial strains and culture

The *H. pylori* 26695 CagA+ Vac A+ strain was used, which was generously donated by D.E. Berg (Department of Molecular Biology and Genetics, Washington University Medical School, St. Louis, USA). Bacteria were maintained on Brain Heart agar containing 10% sheep blood, and incubated in a microaerophilic atmosphere. Inocula for co-culturing were diluted from suspensions that had been prepared from 72-h subcultures and adjusted by comparison of the absorbance with standards. The cag pathogenicity island (PAI)-negative Tx30a strain of ATCC 51932 was cultivated similarly. For co-culture experiments, both strains were used at a multiplicity of infection (MOI) of 100.

Cells

The human gastric cell line AGS was maintained in RPMI 1640 culture medium supplemented with 10% heat inactivated foetal calf serum (FCS), (GIBCO), 2mM glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin. Cells (106) were seeded into 6-well tissue culture plates (COSTAR) and maintained at 37oC in 5% CO2. Confluent cells were left

uninfected, or were infected with *H. pylori* at a MOI of 100.

Inhibitor studies

AGS cells were pretreated with ERK activation inhibitor peptide (Calbiochem 328005) at 50 mg/ml for 30 min prior to and during bacterial stimulation.

RNA isolation and PCR amplification

Total RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, Calif., USA.) according to the manufacturer's instructions. RNA concentration was determined by the A260 value of the sample. Complementary DNA (cDNA) was generated from 1 µg total RNA using high-capacity cDNA reverse transcription kits (Applied Biosystems) in a final volume of 20 µl. Real-time reverse transcription PCR (RT-PCR) analyses were performed in a fluorescence temperature cycler (LightCycler; Roche Diagnostics GmbH) in accordance with the manufacturer's instructions. After reverse transcription, amplification was carried out using LightCycler FastStart DNA Master PLUS SYBR Green I mix (Roche Diagnostics). All quantifications were normalized to the housekeeping GAPDH gene. Relative expression is given as a ratio between target gene and GAPDH gene expression.

Isolation of granulocytes and mononuclear cells

Peripheral white blood cells were prepared from EDTA-anticoagulated venous blood samples from healthy volunteers by density gradient purification. Peripheral blood mononuclear cells were separated by Ficoll Hypaque (Sigma Chemicals) density gradient centrifugation. Human polymorphonuclear granulocytes were isolated by the same density gradient centrifugation. Purified neutrophils were resuspended in RPMI 1640 medium supplemented with 10 % FCS (fetal calf serum) to achieve final concentration of 1×10^6 cells/ml. Experimental cultures (1×10^6/ml) were incubated without or with $10^{8/}$ ml *Helicobacter pylori* 26695 for 1–4 h, following which the cells were centrifuged and processed for flow cytometry. Cell supernatants were tested for HNP1-3 content by ELISA.

Whole blood incubation method

Venous blood samples from healthy blood donors were incubated in the presence or absence of *H. pylori* for 4 h. Following the incubation period the cells were centrifuged and the supernatants were tested for HNP1-3 content by ELISA.

Flow cytometric analysis

Granulocytes and mononuclear cells were labeled with monoclonal mouse anti human defensin (clone DEF3; Serotec Ltd. UK). As a secondary antibody FITC conjugated goat anti – mouse IgG (SIGMA) was applied. Flow cytometric analysis was performed thereafter with a FACStar plus fluorescence-activated cell sorter (Becton-Dickinson) at 488 nm excitation to estimate intracellular α-defensin in peripheral granulocytes and mononuclear cells.

HNP1-3 ELISA

The HNP 1–3 ELISA kit was obtained from HyCult Biotechnology, The Netherlands. Plasma samples of whole blood cultures and supernatants of granulocytes and mononuclear cells were analysed with standard measurements, according to the manufacturer's instructions. ELISA plates were measured on an Anthos 2010 Reader at 450 nm. Concentration of HNP1–3 in plasma and supernatants were calculated according to a standard curve.

LDH assay

To determine whether the tested granulocytes has been damaged during prolonged cultivation with or without *H. pylori,* which could lead to α-defensin release, 200 µl samples of the supernatants were assayed for lactate dehydrogenase activity using LDH kit Cyto Tax PROMEGA.

HMGB ELISA

Plasma HMGB1 concentration were determined with an established ELISA kit (Shino-Test-Corp., Japan)

according to the instructions of the manufacturer.

sRAGE ELISA

The sRAGE antigen in the plasma was determined by ELISA (R&D Systems, Wiesbaden, Germany) according to the instructions of the manufacturer.

Measurement of Plasma DNA

Plasma DNA levels were estimated by the quantitative PCR method using primers for the β-globin gene, a housekeeping gene.

Statistical analyses

The α-defensin levels in the supernatants of *H. pylori* infected and control cell cultures were compared by using the Student t test, and statistical significance was defined as $p < 0.05$. Data are presented as means +- S.E.M. Statistical analyses for comparison of genotype frequencies between groups were performed using the x2 test, and the Fisher exact test if one cell had n=5. The probability level of $p<0.05$ indicated statistical significance. The relationship between genotypes and disease severity is presented as the odds ratio (OR), with a 95% confidence interval (95% CI). The genotype frequencies for each polymorphism were tested for deviation from the Hardy- Weinberg equilibrium by means of the x2 test, with one degree of freedom used. The levels of HMGB1, sRAGE and circulating DNA in the plasma were compared by means of one-way ANOVA. The Bonferroni test was used for post hoc pair-wise multiple comparisons. Statistical calculations were done with the Graph Pad Prism 5 (Graph Pad Software Inc., San Diego, Calif., USA) statistical program.

IV. Results

IV.1. Helicobacter pylori induces the release of α-defensin by human granulocytes

Stimulation of granulocytes with *H. pylori* for 4 h resulted in a decrease in intracellular staining (m. f. i. 250). This decrease was due to the extracellular release of α-defensin; it was proved by measuring the α-defensin levels in the cell supernatants. Following a 4 h incubation with *H. pylori* (108), granulocytes produced 75713 +-8376 pg/ml α-defensin which was significantly higher than the amount released during the 4-h incubation from the non-stimulated cells (5200 +- 2521 pg/ml). The α-defensin levels in the supernatants increased parallel with the decrease in the intracellular α-defensin content determined by flow cytometry. Induction of mononuclear cells (1×10^{6}/ml) with *H. pylori* (10^{8}/ml) did not result in a significant change in the intracellular staining, i.e. α-defensin content of the cells (m. f. i.= 34.5).

IV.2. Potential role of human β-defensin 1 in Helicobacter pylori-induced gastritis

The frequency of AA homozygotes was significantly higher in the group of patients with *H. pylori* induced gastritis (25%) than in the controls (11%) (p=0.005; OR2.745, 95% CI=1.3275.677). When the patients were stratified according to the density of *H. pylori* in the biopsy samples (i.e. mild colonization, or severe colonization) a significant difference in the frequency of GA heterozygotes was observed between the controls and the patients with severe *H. pylori* colonization (p=0.005; OR2.250, 95% CI=1.2863.936). The frequency of AA heterozygotes among patients with severe *H. pylori* colonization was 26%, with an OR of 2.868. The genotypic distribution of the hBD1 C-44G and hBD1 C-20 G polymorphisms was no significant difference in genotype distribution between the patients overall and the healthy controls in any of these two SNPs.

A time-dependent increase in hBD-1 mRNA expression was observed in AGS cells over a 48-h incubation period. Infection by the cag PAI-negative strain showed expression of hBD-1 to a similar extent as observed for the cag A-positive *H. pylori* 26695 strain. There was a significant (p=0.029) reduction in the expression of hBD-1 following pretreatment of AGS cells with the ERK activating inhibitor.

IV.3. Human β-defensin polymorphisms in patient with Crohn's disease

There was no significant difference in genotypic distribution (G-20A) between the Crohn's disease patients overall and the healthy controls. When the patients were stratified according to localization of the disease i.e. ileal, ileocolonic, or colonic a significant difference was observed between the controls and the patients with colonic localization (x2 test, p=0.043) but not between the controls and the patients with ileal or even ileocolonic localization of the disease. At the distribution of hBD1 C-44 G genotypes there was a lower frequency of the GG genotype among the patients with Crohn's disease as compared with the controls. The difference concerning this genotype between patients with ileal localization was not significant, whereas it was marginally significant among the patients with colonic localization (p=0.06), and statistically significant among patients with ileocolonic localization. There were no significant differences in the hBD1 G-52A genotypic distributions between the patients with Crohn's disease and healthy controls.

IV.4. Plasma Concentrations of High Mobility Group Box Protein 1 (HMGB1), sRAGE and Circulating DNA in Patients with Acute Pancreatitis

The plasma HMGB1 concentration was significantly higher in the patients with severe acute pancreatitis than in the healthy controls (p<0,001) or in patients with mild pancreatitis (p=<0,001). The highest HMGB1 concentration were detected in the plasma of patients infected necrosis (17,5-42 ng/ml, n=8) The circulating sRAGE levels were compared in the patients with mild and severe acute pancreatitis, in the patients with sepsis, and in the healthy controls. The highest sRAGE level was detected in patients with sepsis. The plasma sRAGE level proved to be inversely associated with plasma HMGB1 level in patients with acute pancreatitis. In contrast, for the septic patients a positive correlation was observed between the plasma sRAGE and HMGB1 concentrations. The highest plasma DNA concentration was measured in the patients with sepsis. The plasma DNA concentration was significantly higher than the controls level also in the case of the patients with severe acute pancreatitis. No significant difference was observed in plasma DNA concentrations between the patients with mild pancreatitis and healthy controls.

V. Discussion

V.1. Helicobacter pylori induces the release of α-defensin by human granulocytes

Our experiments demonstrated the α-defensin inducing capacity of *H. pylori* not only in the case of purified granulocytes, but also when a whole blood incubation method was applied. In the peripheral blood the neutrophil granulocytes are the main source of α-defensin, and we therefore suggest that the elevation of the level of α–defensin following *H. pylori* infection is mainly due to the activity of the granulocytes. Monocytes, immature dendritic cells, lymphocytes also have been reported to contain α-defensin. Our flow cytometric analysis revealed intracellular α-defensin staining in mononuclear cells but with much lower intensity was much less than for the granulocytes, and only 23 % of the mononuclear cells were stained positively intracellularly. A low basal level of α-defensin was measured in the supernatants of these cells, but here was no significant increase in the level following the incubation of mononuclear cells with *H. pylori*. Accordingly, we conclude that the α-defensin in the supernatants of whole blood cultures can be regarded as products of the granulocytes. This is all the more likely, if it is borne in mind, that the neutrophils account for the highest number of leukocytes in the peripheral blood. Whole blood culturing method is a reliable method with which to investigate the effects of activating agents on granulocytes.

V.2. Potential role of human β-defensin 1 in Helicobacter pylori-induced gastritis

We investigated the relevance of three hBD1 SNPs in patients with *H. pylori* induced gastritis. In our study the analysis of hBD1 G-52 A genotypes revealed that the AA and GA genotype comprises a higher risk of chronic active

gastritis among *H. pylori*-infected subjects, with a higher rate of colonization with the bacteria. Although the functional impact is unclear, it is tempting to speculate that this mutation at the -52 untranslated region might cause a lower hBD-1 expression in gastric epithelial cells with deficient function of human defensin. This could lead to increased colonization of *H. pylori* in the stomach, with ineffective clearance, and consequent inflammation. Additional functional studies will be necessary to establish the exact biological role of this SNP. No further significant differences were observed for the SNPs of hBD1 C-44 G and hBD1 C-20 G. Increased hBD-1 expression was likewise observed following infection of AGS cells with the cag PAI strain. In our experiments, however, not only the CagA-positive, but also the cag PAI-negative strain resulted in hBD-1 expression. Therefore it is tempting to speculate that, in contrast with hBD-2, the hBD-1 activation is possible in a NOD1-independent way. In contrast to hBD-2, hBD-3 expression was NOD1-independent, but EGFR and ERK pathway dependent. *H. pylori* infection activates ERK and MEK kinases in AGS gastric epithelial cells, in both a cag PAI-independent and cag PAI-dependent manner. In this way, ERK and MAP kinases are regulated during *H. pylori* infection through the use of secreted factors, and also in an epithelial contact-dependent manner in which cag PAI-positive strains exert the ability to induce higher levels of ERK and MEK phosphorylation. We suggest a potential role for this pathway in hBD-1 expression. In contrast to hBD-2, it seems that hBD-1 expression could also be NOD1 independent, at least independent on cag PAI, but ERK pathway dependent. This might be the reason for a 50% reduction in the expression of hBD-1 following the pretreatment of AGS cells with the ERK activation inhibitor prior to infection with *H. pylori* 26695. As the ERK pathway can be activated by the cag PAI-independent strain, we suggest a potential role of this pathway in hBD1 expression in the presence of the Tx30a strain. Other signaling pathways that might be important in hBD-1 expression cannot be excluded.

V.3. Human β-defensin polymorphisms in patient with Crohn's disease

Ileal and colonic Crohn's disease can be linked with a lowered defensin profile. We analysed the genetic variation in the hBD1 gene in Crohn's disease. The analysis of hBD1 G-20A genotypes among patients with Crohn's disease revealed that the GA genotype comprises a higher risk of colonic Crohn's disease (OR2.393, 95% CI: 1.176 4.869). There was no correlation between this SNP and the ileal or ileocolonic form of the disease. The functional significance of this SNP resides in the formation of a nuclear factor-κB transcription factor-binding sequence, but the functional impact is unclear. As the colonic localization of the disease is connected with impaired hBD1 expression, it is tempting to speculate that this mutation might cause a lower hBD1 expression in colonic epithelial cells. Additional functional studies will be necessary to establish the exact biological role of this SNP. The present study has demonstrated that the distributions of C-44G (according to the initiation codon-based nomenclature), also known as the hBD1 668C/G genotype, were different in patients with Crohn's disease and healthy controls, while the frequency of the GG genotype was significantly higher in the controls. This result indicates that the C-G mutation probably leads to strengthened hBD1 antimicrobial activity, which is less frequent among patients with Crohn's disease. No further significant differences were observed for the SNPs when the patients were stratified according to behaviour of the disease or the extraintestinal manifestations. The only difference in genotypes was observed when patients were stratified as smokers and non-smokers. It is interesting that the correlation between genotype frequencies of SNPs of G-20A and C-44G was striking only among non-smokers. However, a significant difference between the smokers and the controls was not observed for the genotypes. We speculate that defensin SNP might basically influence the susceptibility to Crohn's disease, but regarding smoking habits, other factors might superimpose to worsen the genetically determined situation. This means that smoking increases the susceptibility to and severity of Crohn's disease; moreover, smoking habit and disease location are contributory factors in this process.

V.4. Plasma Concentrations of High Mobility Group Box Protein 1 (HMGB1), sRAGE and Circulating DNA in Patients

with Acute Pancreatitis

We found a significant elevation of the HMGB-1 concentration in the plasma of patients with severe acute pancreatitis. We compared the results with the data on septic patients, as the elevation of HMGB1 level in sepsis is well documented. The HMGB1 level in the patients with severe acute pancreatitis was comparable with the levels in septic patients, and even more higher (however no statistically significantly). In acute pancreatitis HMGB1 secretion can be induced by inflammatory cytokines, which play a definitive role in the pathomechanism of acute pancreatitis. In severe, necrotizing pancreatitis the passive release of HMGB1 from necrotic cells may be an additional source of HMGB1. This may be the reason for a significant difference in HMGB1 levels between patients with mild and severe form of disease. Infected pancreatic necrosis was diagnosed in 8 of the patients with severe acute pancreatitis. It is noteworthy that the highest levels were measured in the plasma of these patients (17.5 – 42 pg/ml) very probably in consequence of the additive effects of inflammation, necrosis and infection.

Extracellular HMGB1 regulates cells through RAGE and Toll like receptors reported an elevation in the level of sRAGE in septic patients. We likewise observed this in our septic patients, but there was an inverse correlation between the levels of sRAGE and HMGB1 in the patients with severe acute pancreatitis. We found, that the level of released sRAGE relates inversely to the HMGB1 level, but only in the case of acute pancreatitis.

Thus it may be speculated, that the more HMGB1 there is in the circulation, the lower will be the level of sRAGE:in severe acute pancreatitis. The question arises whether the low level of sRAGE is simply due to its consumption to bind HMGB1, or whether there are any other components which could decrease the level of sRAGE. The elevation of other (as yet undefined) components in the circulation, that can suppress the amount of soluble RAGE receptor can not be excluded. Whatever the reason, the relatively high HMGB1 levels relative to the low sRAGE levels make the feedback mechanism less effective, and further enhance the inflammatory loop in acute pancreatitis.

Our data revealed, that the circulating DNA significantly elevated in patients with severe pancreatitis, or in sepsis. A significant elevation was not observed in those with mild pancreatitis. Our data are in contrast with those of Bagul et al., who found a decreased rather than an increased level of plasma DNA in severe acute pancreatitis. There may well be some methodological differences between the two studies. First, our study involved the use of a real time quantitative PCR method for the β-globin gene, which is a widely accepted method. We applied the two-step procedure of sample centrifugation, while Bagul et al. separated the plasma only with one centrifugation step at 2000 rpm. This could result in a high variation in DNA concentration, originating not only from cell the free DNA, but also from contaminating leukocytes. From the aspect that both inflammation and necrosis are involved in the pathogenesis in acute pancreatitis, a complex study of the levels of HMGB1, sRAGE, and plasma DNA might be informative in an evaluation of the effects of different levels of severity of acute pancreatitis.

Summary: Conclusions and potential significances

The major new findings of our experiments are as follows:

1. *H. pylori* induce α-defensin release from granulocytes which may well be important in local host response to *H. pylori* infection in gastroduodenal diseases.
2. The association of the higher frequency of the G-52 A SNP of hBD1 with chronic active gastritis, and the elevated level of hBD-1 mRNA following *H. pylori* infection draw attention to the importance of hBD-1 in *H. pylori infection*. This indicates that not only the inducible, but also the constitutive form of hBD-1 plays a role in the development of chronic active gastritis following *H. pylori* infection.

3. The colonic Crohn's disease could be due to defective β-defensin 1 production. This means that not only the inducible, but also the constitutive form of hBD1 plays a definitive role in the colonic localization of Crohn's disease.

4. The circulating HMGB1 level was significantly higher in the patients with severe acute pancreatitis than in the healthy controls or the patients with mild pancreatitis, and the level of HMGB1 correlated inversely with that of sRAGE. The plasma DNA level was increased in severe acute pancreatitis. These observations underline the role of HMGB1 and its receptor in the pathogenesis of acute pancreatitis, and in the severity of the disease. Blockade of HMGB1 should be considered as possible future therapy in sepsis and severe acute pancreatitis.

Publications with results incorporated in the thesis:

I. **Kocsis AK, Ocsovszky I, Tiszlavicz L, Tiszlavicz Z, Mándi Y.:**

Helicobacter pylori induces the release of alpha-defensin by human granulocytes. *Inflamm Res*. 2009 May;58(5):241-7.

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II. **Kocsis AK, Kiss ZF, Tiszlavicz L, Tiszlavicz Z, Mándi Y.:**

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IF:1,98

III. **Kocsis AK, Lakatos PL, Somogyvári F, Fuszek P, Papp J, Fischer S, Szamosi T, Lakatos L, Kovács A, Hofner P, Mándi Y.:**

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Total impact factor: 8,46

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Mannose-binding lectin level and deficiency is not associated with   inflammatory bowel diseases, disease phenotype, serology profile,   and NOD2/CARD15 genotype in   a large Hungarian cohort. *Hum   Immunol.* 2010 Jan 25

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Total impact factor: 7,854

Cumulative impact factor: 16,314