Intracellular trypsinogen activation in phagocyting macrophages acts as DAMP fueling severe acute pancreatitis

M. Sendler[1], F.U. Weiss[1], T. Wartmann[2], W. Halangk[2], M.M. Lerch[1], J. Mayerle[1]

[1]Department of Internal Medicine A, University Medicine Greifswald, Germany

[2]Division of Experimental Surgery, Otto-von-Guericke University Magdeburg, Germany

Introduction

Premature, intraacinar cell trypsinogen activation is dependent on cathepsin B and regarded as an initial event in acute pancreatitis. A second peak of trypsinogen activation is thought to be mediated by infiltrating leukocytes. We have studied the role of protease mediated macrophage (MΦ) activation in experimental pancreatitis.

Macrophages during pancreatitis

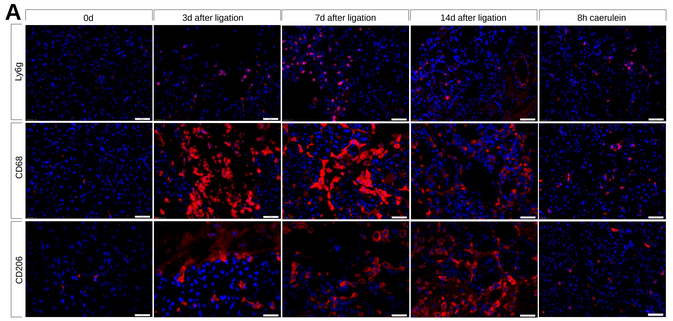

Fig.1: Acute pancreatitis in C57BL/6 mice was induced by partial duct ligation as a model of severe necrotizing pancreatitis or supramaximal caerulein stimulation (50mg/kg/bodyweight). Immunofluorescence stainings of Ly6g (neutrophiles), CD68 (M1 macrophages) and CD206 (M2 macrophages) were used to characterize infiltrating immune cells [**A**]. Quantification of fluorescence staining showed a positive correlation of infiltrating CD68 positive macrophages with disease severity [**B, C**]. Macrophages are able to remove dying cells and cellular debris by phagocytosis. Co-localisation of CD68 and trypsinogen gave evidence for engulfed zymogens by macrophages in necrotic areas of the murine pancreas 3d after partial duct ligation [**D**].

Macrophages activate trypsinogen via cathepsin B

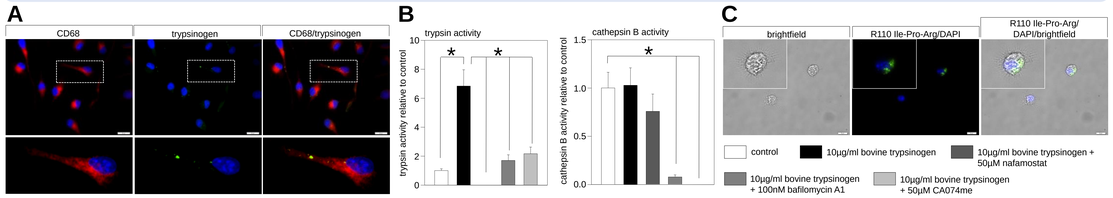

Fig.2: Bone marrow derived macrophages were isolated from C57BL/6 mice and maintained for 5 to 7d under MCSF (macrophage colony stimulating factor). Co-incubation of macrophages with 10µg/ml bovine trypsinogen were performed for 6h. Immunofluorescence staining showed a vesicle confined intracellular localization of trypsinogen within CD68 positive macrophages [**A**]. Activity measurements of trypsin and cathepsin B were performed in macrophage cell lysates [**B**]. Macrophages co-incubated with 10µg/ml bovine trypsinogen showed significant trypsin activity in cell homogenate and this activity was blocked by adding nafamostate (a trypsin inhibitor) to the medium or by blockage of CTSB activity by bafilomycin A1 or CA074me [**B**]. Live cell imaging of macrophages visualized intracellular active trypsin in macrophages after adding of the fluorochrome substrate CBZ R110 Ile-Pro-Arg [**C**]. We detected evidence of intracellular activation of cathepsin B dependent trypsinogen in macrophages in a confined subcellular compartment.

Macrophages phagocytose dying acinar cells

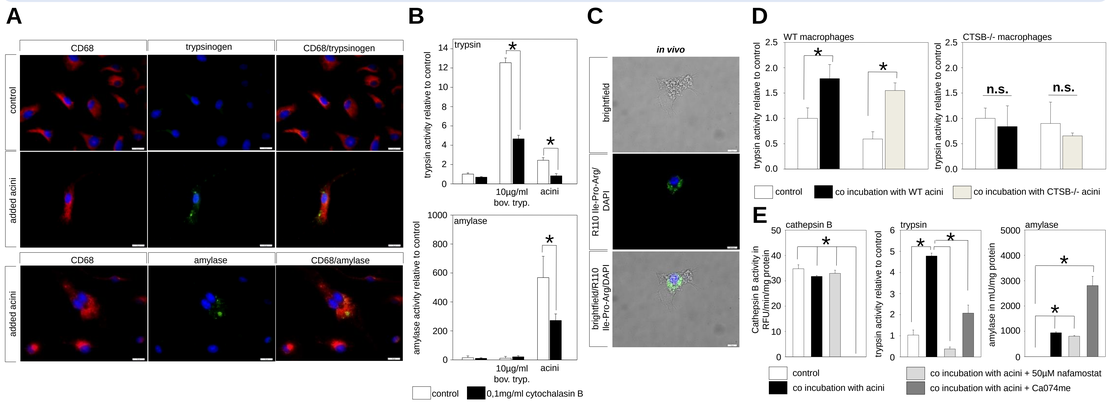

Fig.3: Bone marrow derived macrophages were co-incubated with acinar cells (30min stimulated with 0.001mM CCK). Immunofluorescence revealed a clear staining of pancreatic enzymes (trypsinogen and amylase) within phagocytosing macrophages [**A**]. Inhibition of phagocytosis by cytochalasin B reduced intracelular trypsin activity as well as amylase activity [**B**]. Live cell imaging showed an intracellular localization of active trypsin [**C**]. Increased trypsin activity can also be observed by feeding wild type macrophages with acinar cells from CTSB-/- mice while feeding CTSB-/- macrophages with WT acinar cells did not result in increased trypsin activity [**D**]. Again in this experimental setup inhibition of CTSB by CA074me or nafamostate blocked intracellular protease activation in phagocytosing macrophages [**E**].

Protease activation enhances inflammation

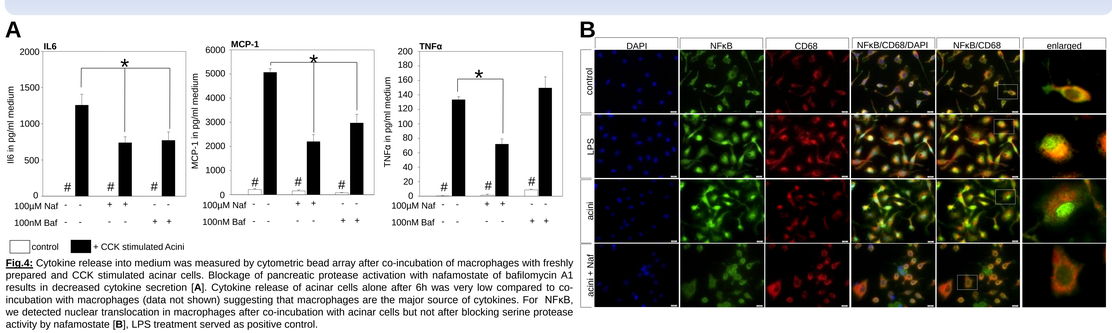

Fig.4: Cytokine release into medium was measured by cytometric bead array after co-incubation of macrophages with freshly prepared and CCK stimulated acinar cells. Blockage of pancreatic protease activation with nafamostate of bafilomycin A1 results in decreased cytokine secretion [**A**]. Cytokine release of acinar cells alone after 6h was very low compared to co-incubation with macrophages (data not shown) suggesting that macrophages are the major source of cytokines. For NFκB, we detected nuclear translocation in macrophages after co-incubation with acinar cells but not after blocking serine protease activity by nafamostate [**B**], LPS treatment served as positive control.

Protease activation in MΦ *in vivo*

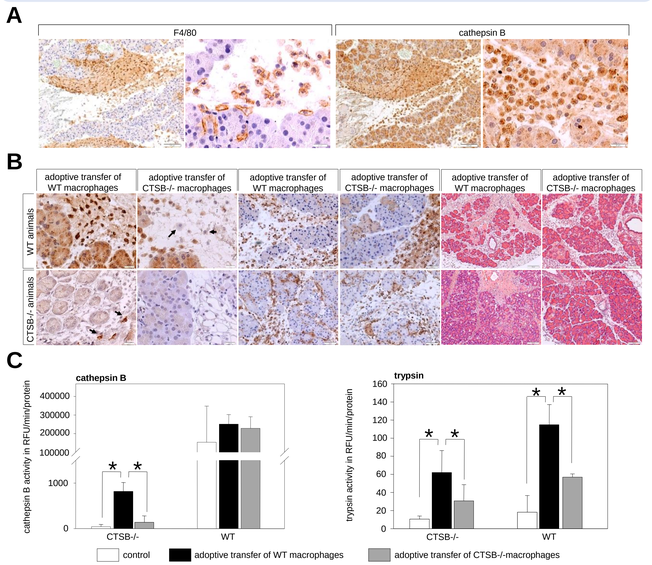

Fig.5: A high number of macrophages infiltrated into the pancreas and they show a high expression of cathepsin B [**A**] We performed an adoptive transfer of wild type and CTSB deficient macrophages after induction of severe necrotizing pancreatitis in C57BL/6 and CTSB-/- mice. Infiltration of wild type macrophages into the damaged pancreas was monitored by CTSB staining [**B**]. Furthermore, enzyme activity measurement showed a significant amount of CTSB in the pancreas of CTSB-/- mice after adoptive transfer of wild type macrophages and this resulted in the activation of trypsinogen and restored a pancreatitis phenotype in CTSB deficient mice [**B, C**].

Conclusion

Intracellular protease activation is not restricted to pancreatic acinar cells. In phagocytosing macrophages trypsinogen is activated in a CTSB-dependent manner. Macrophages activating trypsinogen polarise to M1Φ, release pro-inflammatory cytokines and contribute to disease severity. Intracellular active trypsin in MΦ acts as a danger-associated molecular pattern molecule (DAMP).