Extracellular Cleavage of E-Cadherin by Leukocyte Elastase During Acute Experimental Pancreatitis in Rats

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Background & Aims: **Cadherins play an important role in cell-cell contact formation at adherens junctions. During the course of acute pancreatitis, adherens junctions are known to dissociate—a requirement for the interstitial accumulation of fluid and inflammatory cells—but the underlying mechanism is unknown.** ***Methods:*** **Acute pancreatitis was induced in rats by supramaximal cerulein infusion. The pancreas and lungs were either homogenized for protein analysis or fixed for morphology. Protein sequencing was used to identify proteolytic cleavage sites and freshly prepared acini for ex vivo studies with recombinant proteases. Results were confirmed in vivo by treating experimental pancreatitis animals with specific protease inhibitors.** ***Results:*** **A 15-kilodalton smaller variant of E-cadherin was detected in the pancreas within 60 minutes of pancreatitis, was found to be the product of E-cadherin cleavage at amino acid 394 in the extracellular domain that controls cell-contact formation, and was consistent with E-cadherin cleavage by leukocyte elastase. Employing cell culture and ex vivo acini leukocyte elastase was confirmed to cleave E-cadherin at the identified position, followed by dissociation of cell contacts and the internalization of cleaved E-cadherin to the cytosol. Inhibition of leukocyte elastase in vivo prevented E-cadherin cleavage during pancreatitis and reduced leukocyte transmigration into the pancreas.** ***Conclusions:*** **These data provide evidence that polymorphonuclear leukocyte elastase is involved in, and required for, the dissociation of cell-cell contacts at adherens junctions, the extracellular cleavage of E-cadherin, and, ultimately, the transmigration of leukocytes into the epithelial tissue during the initial phase of experimental pancreatitis.**

Cadherins comprise a family of transmembrane proteins that are located at adherens junctions (for review see Troyanovsky[1]) and display calcium-binding motifs in their extracellular domain, which are essential for homophilic cell adhesion. In epithelial organs, E-cadherin is the most abundant regulator of adherens junctions, and its extracellular domain is composed of 5 subunits, EC-1 through EC-5, and the homophilic adhesion activity of this molecule has been mapped to the amino terminal EC-1 domain.[2] X-ray characterization of this domain revealed a 7-stranded (A-G) β-sandwich structure,[3] and adhesive interaction seems to be driven by the β-sandwich topology. This region does not, in itself, participate in Ca^{2+} binding but includes an HAV sequence (in single letter code for amino acids) that mediates adhesive interactions.[4,5] The intracellular domains serve as highly conserved linkers to the cytoskeleton via connecting α- and β-catenins.[6] These intracellularly located proteins are essential for cell-cell adhesion, and mutations in either the E-cadherin-binding site for α- and β-catenin or in the catenins themselves will disrupt cell contacts even in the presence of an intact extracellular E-cadherin domain.[7] Although there is extensive knowledge about the role of E-cadherin mutations, E-cadherin down-regulation, processing, and subcellular relocalization during tumor development or malignant growth,[8] there is only limited information on the role of E-cadherin in inflammation.

Epithelial cell-cell contacts at adherens junctions form a selective barrier and are involved in the active transport of fluids, ions, and small molecules. During inflammatory disorders, cell-cell contacts frequently dissolve, which permits an unregulated movement of fluids and electrolytes into the interstitial space, resulting in tissue edema. In a model system for an inflammatory disorder (experimental pancreatitis), we have shown that this edema formation is associated with a dissociation of adherens junctions between epithelial acinar cells and a

Abbreviations used in this paper: CCK, cholecystokinin; PMN, polymorphonuclear.

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disappearance of E-cadherin from their basolateral membrane.[9]

In the present study, we characterized the early events involved in cell-cell contact dissociation, edema formation, and cell damage during pancreatitis and focussed on the mechanism that causes the disappearance of E-cadherin from the cell surface. In theory, the process involved in the dissociation of E-cadherin-mediated cell contacts should include cytotoxic or proteolytic agents that are released by either inflammatory cells[10–13] or originate from the pancreatic acinar cell itself.[14–16] Prior to the start of our project, the second hypothesis appeared more likely because (1) inflammatory cells were thought to play a role only in the later disease course but not the initial events of experimental pancreatitis, and (2) the early phase of pancreatitis is known to be associated with the generation of large amounts of cytotoxic compounds such as free oxygen radicals and an extensive activation of proteolytic digestive enzymes.[17–20] We were surprised to learn from our experiments that E-cadherin is cleaved very early in the disease course (within the first hour) by polymorphonuclear (PMN) granulocyte elastase, whereas pancreatic proteases are neither required nor involved in this process. Moreover, inhibition of PMN elastase not only prevented E-cadherin cleavage and cell-cell contact dissociation but also pancreatic inflammation and leukocyte infiltration during pancreatitis. These findings provide the first direct evidence for a role of inflammatory cells in the initial disease phase of pancreatitis and designates them a prospective target for future treatment strategies.

Materials and Methods

Materials

Cerulein was obtained from Pharmacia, Freiburg, Germany. Collagenase from *Clostridium histolyticum* (EC.3.4.24.3) was from SERVA (lot No. 14007, Heidelberg, Germany; collagenase activity, 1.50 PZ U/mg). Human neutrophil elastase was purchased from Calbiochem (San Diego, CA; catalog No. 324681; protein concentration, >20 U/mg protein specific activity; in 50 mmol/L Na-acetate, pH 5.5, and 200 mmol/L NaCl; purity >95%). Bovine pancreatic trypsin, human myeloperoxidase, porcine pancreatic elastase, bovine pancreatic chymotrypsin, and bovine α-amylase were obtained from Calbiochem (Schwalbach, Germany). The substrates rhodamine 110 (R110)-(CBZ-Ile-Pro-Arg)$_2$ and R110-(CBZ-Ala$_4$)$_2$ were purchased from Molecular Probes (Eugene, OR). The substrate 7-amino-4-methylcoumarin (AMC)-(Suc-Ala$_2$-Pro-Phe) was obtained from Bachem (Heidelberg, Gemany). The amylase quantification kit "Amyl" is commercially available from Roche (Ingelheim, Germany). Elastase inhibitor II was from Calbiochem (catalog No. 324744; San Diego, CA). The biologically active phosphorylated cholecystokinin (CCK) octapeptide [Tyr(SO3H)27]-cholecystokinin fragment was obtained from Sigma (Taufkirchen, Germany, catalog No. 2175). For the detection of E-cadherin, 2 different antibodies were used: monoclonal anti-E-cadherin clone 36 directed against the C-terminus (catalog No. 20820; Transduction Laboratories, San Diego, CA) and polyclonal rabbit anti-E-cadherin H108 directed against the N-terminus (catalog No. SC-7870; Santa Cruz, CA). For the detection of human neutrophil elastase, mouse monoclonal antibody clone AHN-10 (catalog No. MAB1056, lot 258CCD; Chemicon International, Temecula, CA) was used. Fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat CD45 (leukocyte common antigen) monoclonal antibody was used to label and quantitate inflammatory cells in paraffin sections during different intervals of pancreatitis (clone OX-1; BD Pharmingen, Heidelberg, Germany).

All other chemicals were of highest purity and were obtained either from Sigma-Aldrich (Eppelheim, Germany), Merck (Darmstadt, Germany), Amersham Pharmacia Biotech (Buckinghamshire, United Kingdom), or Bio-Rad (Hercules, CA). Animals were bred at Charles River Breeding Laboratories (Sulzbach, Germany). All animal experiments were conducted according to the guidelines of the local animal use and care committee. PaTu-8988-S cells were purchased from DSZM (Braunschweig, Germany).

Induction of acute, cerulein-induced pancreatitis, male Wistar rats (140–250 g) were anesthetized with pentobarbital 30 mg/kg. A cannula was placed into the jugular vein, and the animals were infused with supramaximal concentrations of cerulein (10 μg/kg per hour) for up to 48 hours or treated with neutrophil elastase inhibitor II at a concentration of 50 μmol/L (Calbiochem, San Diego, CA) for 2 hours or a mixture of cerulein (10 μg/kg per hour) and neutrophil elastase inhibitor (50 μmol/L). Saline-infused animals served as controls. After exsanguination under ether anesthesia, the pancreas was rapidly removed and trimmed of fat, and tissue blocks were embedded in OCT (Tissue Tek, Sakura Finetek, Zoeterwoude, The Netherlands) for cryosections or fixed in 5% formaldehyde for electron microscopy (EM) cryolabelling or embedding in paraffin. The main part of the pancreas was frozen in liquid nitrogen and stored at −80°C for later protein analysis and detection of enzymatic activity.[21] Pancreatic tissue was homogenized with a Dounce S glass homogenizer in iced Triton X-100 lysis buffer containing protease inhibitors and subsequently immunoprecipitated and immunoblotted (Braun-Melsungen, Melsungen, Germany). Iced Triton X-100 lysis buffer contained protease inhibitors (1 mL/mg tissue, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 0.01 mol/L sodium pyrophosphate, 0.1 mol/L sodium fluoride, 1 mmol/L hydrogen peroxide, 1 mmol/L L-phenylmethylsulfonylfluoride [PMSF], and 0.02% soybean-trypsin inhibitor). Protein concentration was determined by a modified Bradford-assay (Bio-Rad, Unterhaching, Germany), and equal amounts of protein were used in subsequent experiments. DNA content of homogenates was determined with propidium iodine using a fluorescence reader (excitation 350 nm/emission 630 nm) and used to standardize cell homogenates to comparable cell numbers and corrected for