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Cytokine 32 (2005) 125–131

Pro- and anti-inflammatory response of acinar cells during acute pancreatitis. Effect of *N*-acetyl cysteine

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Received 19 May 2005; received in revised form 28 July 2005; accepted 29 July 2005

Abstract

We investigate the ability of acinar cells to produce tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) at different stages of acute pancreatitis (AP). Since oxidative stress is involved in the inflammatory response, the effect of *N*-acetyl cysteine (NAC) has also been evaluated. AP was induced in rats by bile-pancreatic duct obstruction (BPDO). NAC (50 mg/kg) was administered 1 h before and 1 h after BPDO. Acinar cells were incubated for 4 h at 37 °C in 5% CO_2 atmosphere in absence and presence of 24-h BPDO-PAAF (20%, v/v) as stimulant agent. Acinar production of TNF-α and IL-10 was analysed by flow cytometry. Plasma amylase activity and histological studies of the pancreas indicated the severity of AP. PAAF significantly stimulated the acinar production of TNF-α and IL-10 in control rats. TNF-α production was also significantly stimulated in acinar cells of rats with AP, although a decrease in the pro-inflammatory response was found from 6 h after BPDO onwards. However, acinar cells failed to produce IL-10 from 3 h after BPDO. The protective effect of NAC treatment against oxidative cell damage reduced the pancreatic injury and maintained and enhanced the ability of acinar cells to produce IL-10 at early AP stages. As long as acinar cells were not severely damaged in the course of AP, greater ability to produce cytokines in response to PAAF was found in those with higher forward scatter (R2 cells). We suggest that the capability of acinar cells to maintain an appropriate balance between the production of pro- and anti-inflammatory mediators could contribute to determine the degree of severity of AP.

Keywords: Acinar cells; Acute pancreatitis; Cytokines; IL-10; NAC; TNF-α

1. Introduction

It is widely accepted that acute pancreatitis (AP) is initiated within acinar cells by premature activation of proteolytic enzymes [1,2]. Pancreatic damage leads to a local inflammatory response mediated by the release of pro-inflammatory factors [3]. Cytokines appear to play a critical role in the pathogenesis of pancreatitis [4]; in fact, whatever the aetiological factor, serum pro-inflammatory cytokine levels are increased in humans [5,6] or experimental models [7–9]. They act not only aggravating the pancreatic injury, but also contributing to the subsequent systemic inflammatory response [3,10], which if marked is the major cause of death. A delicate balance between pro- and anti-inflammatory mediators determines the nature of the inflammatory response [11]. Accordingly, a variety of treatments have been tested in order to palliate the severity of AP. The use of antibodies against TNF-α [12,13] and IL-8 [14] has demonstrated to limit the course of AP. Administration of IL-10, a potent anti-inflammatory agent known to inhibit the production of other pro-inflammatory cytokines [15], attenuated AP experimentally induced in animals by CDE-diet [16] and caerulein [17,18], but not in humans [19].

Although leukocytes infiltrated in the pancreas have been traditionally considered to be the source of the inflammatory mediators in AP [20], recently, there is a body of evidence

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doi:10.1016/j.cyto.2005.07.017

supporting the notion that activated pancreatic acinar cells could provide the first signal that induces the recruitment of inflammatory cells through the initial production of cytokines [21–23] mediated, at least in part, by signal transduction pathways activated by reactive oxygen species (ROS) [24–26]. Increased TNF-α, IL-6 and IL-1β levels have been found in acinar cells after inducing AP by caerulein [22,27], and bile-pancreatic duct obstruction [26]. IL-10 mRNA has been shown to be up-regulated in pancreatic tissue of mice with AP induced by caerulein [28] or pancreatic duct ligation [29]. However, the specific contribution of acinar cells to this anti-inflammatory event is still unknown.

Our purpose was to stimulate acinar cells to produce cytokines in order to ascertain their ability to produce pro- (TNF-α) and anti-(IL-10) inflammatory cytokines during AP induced in rats by bile-pancreatic duct obstruction (BPDO). Acinar cell activation was carried out with pancreatitis-associated ascitic fluid (PAAF) in accordance with previous results [23], revealing that heat resistant factors, different from trypsin and TNF-α, which are present in ascites obtained from rats with BPDO-induced AP are able to stimulate the TNF-α production in the acinar cells of control rats, through mechanisms partially mediated by NF-κB activation. Since ROS are involved in the production of cytokines, the time course of the acinar pro- and anti-inflammatory response has also been analysed in rats with BPDO-induced AP treated with *N*-acetyl cysteine (NAC) – a thiol compound with potent antioxidant properties [30].

2. Materials and methods

2.1. Reagents

N-acetyl cysteine (NAC), collagenase type XI, soybean trypsin inhibitor (STI), amino acid mixture, bovine serum albumin (BSA), *N*-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), brefeldin A, L-glutamine, buprenorphine were supplied by Sigma Chemical Co. (Madrid, Spain). Pharmingen (San Diego, CA, USA) supplied phycoerythrin (PE)-labelled monoclonal antibodies against TNF-α and IL-10. The Fix & Perm kit for staining of intracellular antigens was purchased from Caltag Laboratories (San Francisco, CA, USA). Medium 199 (Gibco, Paisley, Scotland), RPMI 1640, and calf foetal serum (Biowhittaker, Walkersville, MD, USA) were also used. Other standard analytical grade laboratory reagents were obtained from Merck (Darmstadt, Germany).

2.2. Animals and experimental model

All experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of the University of Salamanca (Spain) in accordance with European Community guidelines on ethical animal research, established by the European Community (86/609/EEC).

Male Wistar rats weighing 250–300 g were used. After 12-h fasting, the rats were anaesthetized with ether and AP was induced by ligation of the common bile-pancreatic duct at the distal part, close to its exit to the duodenum. Afterwards, the abdominal wall was closed in a double layer and the animals were returned to their cages with free access to water and food. Postoperative analgesia was maintained in all animals by subcutaneous injections of buprenorphine (0.2 mg/kg/8 h).

2.3. Animal groups

Rats were randomly divided into four groups, each containing four subgroups.

Group I Rats with AP induced by bile-pancreatic duct obstruction (BPDO) as described above.
Group II Sham-operated rats, surgically treated as group I but without ligation of the bile-pancreatic duct.
Group III Rats receiving 50 mg/kg of NAC by intraperitoneal injection 1 h before and 1 h after the ligation of the bile-pancreatic duct.
Group IV Sham-operated rats receiving NAC as described above.

In all four groups, studies were carried out at the following time periods: 3, 6, 12 and 24 h after surgery. The number of animals was six per group in each experimental period.

2.4. Collection of samples

After 12-h fasting and under anaesthesia with sodium pentobarbital (3 mg/100 g body weight, intraperitoneally), blood samples were taken by cardiac puncture into heparinized tubes. Afterwards, the entire pancreatic gland was removed, in order either to isolate acinar cells or to carry out histological studies.

Acinar cells were isolated by collagenase digestion as previously described [26]. Briefly, pancreata were minced in a previously oxygenated solution composed of (in mM) 25 HEPES (pH 7.4), 110 NaCl, 5 KCl, 1 $CaCl_2$ 14 D-glucose, 2 L-glutamine as well as 2% (w/v) BSA, 0.01% (w/v) STI and 2% (v/v) amino acid mixture. Collagenase (25 U/ml) was then added and the suspension was vigorously shaken in a water bath at 20 °C for 10 min under continuous oxygenation. Afterwards, the medium was replaced by a fresh oxygenated solution without collagenase and then gently pipetted on ice through tips of decreasing diameter (3–1 mm). After filtering through a double layer of muslin gauze acinar cells were washed twice by centrifugation (540 g, 3 min, 4 °C).

2.5. Treatment of acinar cells

Acinar cells were resuspended in Medium 199 supplemented with 10% of heat-inactivated calf foetal serum, streptomycin (0.1 mg/ml) and penicillin (100 U/ml) and plated at a density of 4×10^6/ml in 24-well primary tissue culture plates (Becton Dickinson Biosciences, San José, CA, USA). After adding 10 μg/ml brefeldin A, as a cytokine-secretion blocking agent, acinar cells were incubated for 4 h in a 5% CO_2 humidified sterile atmosphere at 37 °C in the absence