Short communication

Circulating TNF-α and its soluble receptors during experimental acute pancreatitis

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Abstract

Clinical and experimental studies have shown increased concentrations of TNF-α and its soluble receptors in serum of patients with acute pancreatitis. In this work, we have investigated the time-course of TNF-α and its soluble receptors during taurocholate-induced acute pancreatitis. In addition, since TNF-α itself could mediate the shedding of its receptors, we have assessed the effect of inhibiting TNF-α production on the release of soluble TNF-α receptors in experimental acute pancreatitis. Our results indicate that soluble receptors are released in the early stages of the disease and this increase is concomitant with the release of TNF-α, which is mainly bound to specific proteins. The increased concentrations of its receptors strongly suggest that they could be these binding proteins. Inhibition of TNF-α generation with pentoxifylline abrogated the shedding of sTNF-αR1, but had no effect on sTNF-αR2. This finding suggests that the shedding of sTNF-αR1 is induced by TNF-α itself, but in the case of sTNF-αR2, the shedding appears to be induced by another mechanism.

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1. Introduction

Acute pancreatitis is a systemic disease with a variable prognosis depending not only on the local conditions, but also on systemic manifestations, in particular lung injury [1]. The mechanism responsible for the involvement of distant organs is still unclear and different pathways have been suggested, including activated complement [2], oxygen-derived free radicals [3] and cytokines, in particular TNF-α and IL-1β [4].

TNF-α is a cytokine produced in response to numerous stimuli and induces acute inflammation by enhancing endothelial permeability, inflammatory cell recruitment and release of superoxide anion and additional cytokines by polymorphonuclear leukocytes. Nucleated cells contain two TNF-α receptors with molecular weights of 55–60 (TNF-αR1) and 75–80 (TNF-αR2) kDa, respectively [5]. Under inflammatory stimuli, the extracellular part of both receptors is shed by proteolytic cleavage and circulates as a soluble receptor (sTNF-αR) that binds to free TNF-α in plasma [6].

Clinical and experimental studies have shown increased concentrations of TNF-α and its soluble receptors in serum of patients with acute pancreatitis that appear to correlate with the severity of the disease [7]. In addition, when circulating TNF-α is blocked or the production of TNF-α is prevented, the severity of pancreatitis and its systemic effects are reduced [8,9]. Furthermore, studies using knockout animals have demonstrated that cytokine receptors are required for
the development of systemic inflammation during acute pancreatitis [10]. The aim of this work was to evaluate the time-course of TNF-α and its soluble receptors during an experimental model of acute pancreatitis. In addition, since TNF-α itself could mediate the shedding of its receptors, we have investigated the effect of inhibiting TNF-α production on the release of soluble TNF-α receptors in experimental acute pancreatitis.

2. Methods

2.1. Animals and pancreatitis model

Male Wistar rats (250–300 g bw) were anesthetized with an i.p. administration of ketamine (80 mg/kg bw) and acepromacine (2.5 mg/kg bw). Pancreatitis was induced by retrograde injection of 0.1 ml/100 g bw sodium taurocholate (3.5%) (Sigma, St Louis, Missouri, USA) into the biliopancreatic duct [11].

2.2. Experimental design

In a first series of experiments, the time-course of free and total TNF-α were performed, by measuring samples obtained at 1, 3, 6 and 9 h after induction of pancreatitis (n = 6 for each group). Control samples were considered as those obtained immediately after pancreatitis induction (t = 0).

In a second series of experiments, TNF-α production was avoided by intravenous administration of pentoxifylline (Laboratorios Robert, Barcelona, Spain). In this series, control animals received an intraductal infusion of saline solution (NaCl 0.9%) (n = 6 for each group). Serum and lung tissue samples were obtained 6 h after induction.

2.3. Lipase assay

Serum lipase was determined by using a commercial kit from Roche (Mannheim, Germany), according to the supplier’s specifications.

2.4. Myeloperoxidase assay

Myeloperoxidase was measured employing 3,3′,5,5′-tetramethylbenzidine as a substrate [12]. Enzyme activity was assessed photometrically at 630 nm. An enzyme unit is defined as the amount of enzyme that produces an increase of 1 absorbance unit per minute.

2.5. TNF-α

To determine the levels of TNF-α in serum, two different commercial kits were used: free TNF-α was measured by a commercial solid phase sandwich enzyme linked immuno-sorbent assay (ELISA) from Biosource International (Camarillo, California, USA) and total (bound and free) TNF-α by a competitive enzyme-immunoassay kit from Chemicon International (Temecula, CA, USA). A common TNF-α standard was used to calibrate the response of the different kits at high and low levels of the standard curve and variation was <10%.

2.6. sTNF-αR1 and sTNF-αR2

To measure the levels of circulating TNF-α soluble receptors -1 and -2, commercial solid phase ELISA from R&D Systems (Minneapolis, USA) were employed. To discard possible interferences due to the presence of TNF-α in the measure of its soluble receptors, assay kits were validated by measuring the effect of adding different concentrations of TNF-α (rat TNF-α, PedroTech, Inc. Rocky Hills, NJ; Ref 400-14) to the standard soluble receptor solution.

3. Results

Total TNF-α levels in serum increased significantly 1 h after induction of pancreatitis and remained increased up to 9 h post-induction (Fig. 1). By contrast, free TNF-α levels showed a transient increase within the first hour and low levels when compared to the total TNF-α.

Both sTNF-αR1 and sTNF-αR2 showed increased levels in plasma 1 h after pancreatitis induction and remained increased during the course of the experiment (Fig. 2). Levels of sTNF-αR2 were three-fold higher than those of sTNF-αR1.

The presence of increased concentrations of TNF-α does not affect the performance of the immunoassays since measures obtained after overloading with two different concentrations of TNF-α does not differ from the expected values (Table 1).

The effect of pentoxifylline has been evaluated 6 h after pancreatitis induction. This treatment completely inhibited the plasma total TNF-α increase. It had no significant effect on lipase activity and only a moderate effect reducing the neutrophil infiltration into the lungs (Fig. 3). Concerning the release of soluble receptors, pentoxifylline treatment abolished the increase observed in sTNF-αR1 levels, but had no effect on sTNF-αR2 levels (Fig. 4).

4. Discussion

Cytokines, particularly TNF-α, are suspected to play a central role orchestrating the inflammatory response in the early stages of acute pancreatitis [4], but TNF-α is often undetectable in patients even with severe disease.
This lack of detection could be related to high levels of soluble forms of its receptors present in plasma that could bind circulating TNF-α and interfering with its detection.

Our results indicate that soluble receptors are released in the early stages of the disease and this increase is concomitant with the release of TNF-α. By using two different assays that discriminate between the free form of TNF-α and that bound to proteins we observed that TNF-α is mainly present bound to specific proteins. The increased concentrations of its receptors strongly suggest that they could be these binding proteins. Only

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<th>sTNF-αR2 expected (pg/ml)</th>
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The presence of the cytokine does not interfere with the measurement of its receptors.
a transient increase in the free form of TNF-α in the initial stages of the disease could be observed.

The release of soluble receptors could be a protective mechanism against excessive TNF-α activity. Proteolytic cleavage (shedding) is a well-documented mechanism to down-regulate the cell response to TNF-α. Less clear is the physiological role of circulating soluble receptors. It has been suggested that they act by buffering the effects of this cytokine. Of course, the effect of other non-specific binding-proteins could not be excluded. The fact that we have found similar levels of TNF-α and its receptors could suggest a role stabilizing the trimeric structure of TNF-α more than binding to soluble receptors. Of course, the effect of other non-specific binding-proteins could not be excluded. The fact that we have found similar levels of TNF-α and its receptors could suggest a role stabilizing the trimeric structure of TNF-α more than buffering the effects of this cytokine. Of course, the effect of other non-specific binding-proteins could not be excluded. The fact that we have found similar levels of TNF-α and its receptors could suggest a role stabilizing the trimeric structure of TNF-α more than buffering the effects of this cytokine. Of course, the effect of other non-specific binding-proteins could not be excluded. The fact that we have found similar levels of TNF-α and its receptors could suggest a role stabilizing the trimeric structure of TNF-α more than buffering the effects of this cytokine. Of course, the effect of other non-specific binding-proteins could not be excluded.

Fig. 3. Effect of pentoxifylline treatment in lipase activity (upper), lung myeloperoxidase activity (middle) and total TNF-α (lower) levels, 6 h after induction of pancreatitis. Pentoxifylline inhibits the production of TNF-α, partially reduced the inflammatory process in the lung, and has no effect on pancreatic damage. * = p < 0.05 vs control; + = p < 0.05 vs pancreatitis.

Fig. 4. Effect of pentoxifylline (Ptx) treatment on the release of soluble receptors 6 h after induction of pancreatitis. Inhibition of TNF-α production prevents the release of sTNF-α R1, but has no effect on sTNF-α R2. * = p < 0.05 vs control; + = p < 0.05 vs pancreatitis.
shedding of TNF-α receptors demonstrated differences between sTNF-αR1 and TNF-αR2. It has been reported that down-regulation of its receptors by TNF-α is due to the shedding of sTNF-αR75 and the internalization and shedding of sTNF-αR55. In this regard, our results suggest the involvement of a TNF-α-dependent mechanism for sTNF-αR1 and a different mechanism, non-directly related with TNF-α for TNF-αR2. In fact, pancreatitis is characterized by the simultaneous activation of different pro-inflammatory pathways, including free radical generation, cytokine synthesis and complement system activation [2–4]. In addition, damaged pancreatic cells release proteolytic enzymes. Some of these mechanisms could act inducing the shedding of TNF-α receptors.

In conclusion, our results indicate that during early stages of pancreatitis, TNF-α and its soluble receptors are released to systemic circulation and maintained at a high concentration. The shedding of sTNF-αR1 seems to be induced by TNF-α itself, but in the case of sTNF-αR2, the shedding appears to be induced by another mechanism. In plasma, TNF-α is present bound to its soluble receptors, but the precise role of this binding remains to be elucidated.

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