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ALPHA-1 ANTITRYPSIN AND MONOCYTES

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Key words: alpha-1 antitrypsin, regulation, monocytes.

Summary
Alpha-1 antitrypsin (AAT) is the main circulating serine proteinase inhibitor. A number of studies suggest that AAT can also exhibit biological activity independent of inhibition of serine proteases. The aim of the study was to make experimental investigation of AAT influence on monocytes stimulated by bacterial endotoxyn . AAT affects monocyte responses to LPS by regulating soluble CD14 release. Here we show that a short-term monocyte exposure to AAT leads to an increase of CD14 levels ($p<0.05$). In parallel, a short-term cell exposure to AAT significantly enhances TNFα release. However, AAT was found to have a dual effect on LPS-induced TNFα release. Probably a rapid increase in AAT concentrations during various inflammatory and infectious conditions may enhance the magnitude of monocyte responses to endotoxin and subsequently accelerate resolution of the inflammatory reaction.

Introduction

Alpha-1 antitrypsin (AAT) is a circulating serine proteinase inhibitor secreted by the liver, which permeates most body tissues where it acts as an inhibitor of a range of proteolytic enzymes [1]. A number of studies suggest that AAT can also exhibit biological activity independent of inhibition of serine proteases [2]. Thus, AAT has been reported to play an immunoregulatory role [3, 4], to reduce development of cancer [5]. *In vivo,* AAT has been shown to protect against TNFα or endotoxin-induced animal lethality and in a mouse model of lung inflammation AAT was highly effective in suppressing inflammation and connective tissue breakdown [6]. Immunoregulatory and antimicrobial effect of AAT, resulting monocytes activation is very important for development of chronic inflammatory diseases like chronic obstructive pulmonary disease (COPD).

Peripheral blood monocytes are a population of circulating mononuclear phagocytes that harbor potential to differentiate into macrophages and dendritic cells [7]. These cells of the monocyte lineage are important elements of immune defence because these cells can phagocytize foreign material, present antigen to T cells, and produce a host of cytokines, including TNFα, IL-1 and IL-6 [8]. Monocytes activation is mainly regulated by expression of membrane CD14 receptors and secretion of soluble serum form (sCD14) [7]. Bacterial lipopolysacharide binds monocyte surface CD14 receptors and triggers cytokine expression [9]. It has been suggested that activated peripheral blood monocytes more easily enter the lung and/or stimulate immune activation when present in the lung. Macrophages are the predominant defence cells in the normal lung and are increased during conditions associated with chronic inflammation [10]. A direct role of AAT on monocytes CD14 expression and secretion is unknown.

Therefore, up till now there are no data about exact mechanisms and cellular receptors for new AAT activities. Thus hereditary deficiency of AAT is a well established genetic risk factor for COPD [11-13]. However AAT deficiency in COPD patients is an under-diagnosed condition worldwide. The same can be said about Lithuania [14].

The aim of the study: to evaluate effect of Alpha-1 antitrypsin on monocyte activity. To determine whether AAT alone or in combination with LPS has any effect on soluble monocytes differentiation factor CD14 (sCD14) levels.

Materials and methods

Monocyte isolation and culture. Human blood monocytes were isolated from buffy coats (in total, blood was obtained from 79 healthy donors) using Ficoll-Paque PLUS (Pharmacia, Sweden). Briefly, buffy coats were diluted 1:2 in PBS with addition of 10 mM EDTA and layered on Ficoll. After centrifugation at 400 *g* for 35 min at room temperature, the cells in the interface were collected and washed 3 times in PBS-EDTA. Cells were seeded into Petri dishes or 12-well cell culture plates (Nunc, Denmark) at a concentration of 4 x 106 cells/ml in RPMI 1640 medium. After 75 min, non-adherent cells were removed by washing

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3 times with PBS supplemented with calcium and magnesium. Fresh medium was added and cells were stimulated with lipopolysaccharide (LPS, 10 ng/ml, Sigma, USA) in the presence or absence of AAT (0.5 mg/ml) at 37 °C, 5% CO_2 for various time points up till 24 h. Cell culture supernatants from monocytes stimulated with AAT or LPS alone or in combination were analyzed to determine soluble CD14 and TNFα levels by using Quantikine ELISA kit (R&D Systems, MN, USA; minimum detection levels less than 125 and 15.6 pg/ml). In some experiments monocytes were stimulated with LPS, AAT or their combination in the presence of 4μg/ml monoclonal anti-human CD14 antibody (R&D Systems, MN, USA).

Statistical analysis was performed with the SPSS 15.0 program (serial code 9880215). Quantitative variables were expressed as means with standard deviations (SD) or median and quartiles. Some values were compared using the Student's t-test and one-way ANOVA. Differences of quantitative data that had not improved the normal distribution were assessed by Mann-Whitney *U* test and Kruskal-Wallis *H* test. Correlation between continuous parameters was determined by Spearman's rank correlation coefficient (*r*). A *p* value of less than 0.05 was considered significant.

Results

AAT effect on monocytes stimulated by bacterial endotoxyn. The first task was to determine whether AAT alone or in combination with LPS has any effect on soluble monocytes differentiation factor CD14 (sCD14) levels. AAT induced a fast sCD14 release from monocytes compared to non-treated controls. Nearly identical induction of sCD14 release was detected in cells exposed to AAT/LPS combination, whereas LPS alone had no significant effect on sCD14 levels.

The finding that AAT affects sCD14 level prompted us to investigate whether AAT also affects monocyte responses to LPS in a time-dependent manner. Thus, LPS (10 ng/ml) was added to human monocytes with or without AAT (0.5 mg/ml) for 30 min, 1, 2, 4, 6, 8, 12, 18 and 24 h, and cell supernatants were analyzed for TNFα release. Cells stimulated with AAT alone served as a negative control. LPS triggers a release of TNFα by monocytes in a time-dependent manner. However, AAT was found to have a dual effect on LPS-induced TNFα release. Thus, during the first 4 h AAT enhanced, while after 8, 12, 18 and 24 h it inhibited LPS-stimulated TNFα release. The most potent enhancement of LPS-stimulated TNFα release by AAT was observed at 2 h.

Discussion

AAT, one of the major serine proteinase inhibitors, is classified as an acute phase protein and increases in concentration during various inflammatory responses. We analyzed short-term (2 h) and long-tem (18 h) monocyte responses to LPS and AAT separately or in combination. Our results clearly show that within 2 h AAT alone as well as with LPS, strongly up-regulates sCD14 secretion. We also measured sCD14 concentrations in cell culture supernatants even after 18 h, and found that the concentration of sCD14 was much higher in monocytes treated with AAT and AAT/LPS combination compared to controls or LPS-treated cells. This latter observation provides evidence that a direct relationship exists between the accumulation of sCD14 and acute inflammatory phase duration. The biological function of sCD14 is so far not clear. *In vitro*, an excess of sCD14 is shown to inhibit LPS binding to mCD14 and hence block cellular activation [15-18]. Data show that binding of LPS to monocytes and LPS-induced cell activation are abrogated by an exogenously added high dose of sCD14 [19]. sCD14 itself appears to interact with LPS and play a role in the neutralization of LPS [20]. On the other hand, low amounts of sCD14 are suggested to play a role in sensitizing normal human phagocytes to low endotoxin concentrations [18, 20]. Our findings reveal that after 2 h incubation with AAT the monocyte supernatant levels of sCD14 are about 16 ng/ml, which is similar to previously described optimal levels needed for enhancement of LPS-induced cell activation. Accordingly, our data show that monocyte exposure to LPS for 2 h led to an activation of NF-κB (p50/p65) in concert with a large release of pro-inflammatory cytokine. Indeed, simultaneous treatment of monocytes with LPS and AAT amplified LPS-induced pro-inflammatory cytokine TNFα release. However, sCD14 levels in AAT-stimulated monocytes increased to about 30 ng/ml after 18 h, which suggests that the long-term effects of AAT on LPS-induced monocyte activation might be related to the highly elevated sCD14 levels that lead to reduction in monocyte responsiveness to LPS. As predicted, a long-term (18 h) exposure of monocytes to LPS, AAT or their combination shows that AAT significantly inhibits LPS induced pro-inflammatory cytokine TNFα secretion. Here we show that neutralization of sCD14 with anti-CD14 antibody significantly reduced AAT capacity to enhance monocyte response to LPS in the short-term (2 h), whereas it had no effect in the long-term (18 h). The data support the hypothesis that a modulation of LPS-induced monocyte activation by AAT may be related to the AAT-induced modulation of CD14 levels. This may be a physiologically important mechanism by which AAT damps inflammatory

processes. A rapid increase in AAT concentrations during various inflammatory and infectious conditions may enhance the magnitude of monocyte responses to endotoxin and subsequently accelerate resolution of the inflammatory reaction [21].

Conclusions

Short-term stimulation of monocytes with alpha-1 antitrypsin induces expression of differentiation marker CD14 and secretion of soluble CD14. It helps monocytes in neutralization of bacterial endotoxin. Long-term stimulation of monocytes with alpha-1 antitrypsin decreases expression of CD14 and induces secretion of soluble CD14; thus, monocytes are protected from hyperstimulation of bacterial endotoxin.

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ALFA-1 ANTITRIPSINAS IR MONOCITAI

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Raktažodžiai: alfa-1 antitripsinas, reguliacija, monocitai.

Santrauka

Alfa-1 antitripsinas yra vienas svarbiausių serino proteazių inhibitorių. Pirminė alfa-1 antitripsino funkcija yra blokuoti neutrofilų elastazę bei kitas proteazes, tačiau naujausi moksliniai tyrimai rodo, kad alfa-1 antitripsinas turi ir kitų savybių. Tyrimo tikslas nustatyti alfa-1 antitripsino poveikį monocitų aktyvumui. Buvo tirta, ar alfa-1 antitripsinas derinyje su bakteriniu lipopolisacharidu turi poveikį tirpaus monocitų diferenciacijos žymens (sCD14) sekrecijai. Nustatyta, kad, palyginus su kontrole, alfa-1 antitripsinas sukelia staigų monocitų sCD14 sekrecijos padidėjimą. Panašų poveikį sCD14 sekrecijai sukėlė AAT ir LPS derinys, tačiau vienas LPS neturėjo žymesnio poveikio sCD14 sekrecijai. Ilgalaikės stimuliacijos (18 val.) alfa-1 antitripsinu poveikis sCD14 sekrecijai buvo ypač ryškus. Tyrimo rezultatai įrodė, kad, dalyvaujant monocitų diferenciacijos žymeniui CD14, staigus alfa-1 antitripsino koncentracijos padidėjimas įvairių uždegiminių ir infekcinių būklių metu pradžioje gali sustiprinti monocitų atsaką į bakterinę infekciją, o vėliau pagreitinti uždegiminių reakcijų nuslopinimą apsaugant monocitus nuo hiperstimuliacijos.

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