

The Oxidative Burst Reaction of Neutrophils Is Reduced by the Acute Phase Serum Amyloid-A Protein

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Introduction

The acute-phase reaction is considered to represent an adaptation of the organism toward meeting such life-threatening incidents as injury, necrosis, inflammation, sepsis, and tumor spread. The acute-phase reaction is induced by cytokines mainly from activated monocytes and macrophages and is characterized by changes in the concentration of definite plasma proteins, called acute-phase proteins. The concentration of such *positive* acute-phase proteins as α_1 -proteinase inhibitor, complement factors, fibrinogen, haptoglobin, C-reactive protein, and serum amyloid-A protein (SAA) is increased, in contrast to a decrease seen in such *negative* acute-phase proteins as albumin or transthyretin [1].

SAA is the most sensitive acute-phase protein in man with a more than 1000-fold increase in concentration due to an increase in the hepatic level of SAA mRNA [2, 3]. SAA is a protein of 12 kDa, appearing in plasma at approximately 200 kDa [4] due to its association with high density [5] and other lipoproteins. It is composed of 104 amino acids [6] and is chemically heterogeneous [7]. SAA has been discovered to be the precursor of the protein thesaurosis amyloid-A (AA) amyloidosis, which is a sequela of recurrent acute-phase reactions in such prolonged inflammations as rheumatoid arthritis and familial Mediterranean fever. The AA protein, the hallmark of AA amyloidosis, is stored extracellularly in amyloid conformation and may result in severe disorders with a fatal outcome [8]. AA protein represents an N-terminal fragment of SAA (typically SAA 1-76). AA-fibrillar deposits can easily be diagnosed at the light and electron microscopic level using monoclonal antibodies [9, 10].

The function of SAA during the acute phase, however, is still unknown although B-cell suppression [11] and autocrine collagenase induction [12] has been described. Following our hypothesis that the humoral changes of the plasma proteins including SAA may influence the concomitantly changed cellular functions during the acute-phase reaction, we set out to examine (a) whether SAA binds to blood cells and (b) whether this binding alters cellular functions, in particular the oxidative burst response responsible for oxidative tissue destruction.

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Experimental Design and Results

Binding of SAA to Neutrophils. Highly purified SAA labeled with fluorescein isothiocyanate (FITC) was incubated with leukocytes isolated from heparinized blood of normal individuals by 1 g sedimentation on ficoll (Histopaque-1077, Sigma). After 1 h of exposure to SAA-FITC, the cells were washed in PBS, fixed in 1% paraformaldehyde, and examined by flow cytometry [13]. The cellular FITC fluorescence was measured simultaneously with side scatter (SSC). SSC permits the distinction of lymphocytes, monocytes, and neutrophils within a mixture of unseparated cells. Strong fluorescence was detected on neutrophils whereas lymphocytes revealed only low fluorescence, similar to the autofluorescence of cells not exposed to SAA-FITC [13].

The Inhibition of the Oxidative Burst of Neutrophils with SAA. To examine whether the binding of SAA was functionally effective, the oxidative burst reaction induced by the bacterial chemotactic peptide *N*-formyl-Meth-Leu-Phe (FMLP) was examined [13]. Indicator of the oxidative burst was the intracellular oxidation of dihydrorhodamine (DHR; Molecular Probes, Eugene, OR, USA) to the fluorescent product rhodamine 123 [14]. White blood cells were preincubated at 37 °C for 15 min with 10 g/ml DHR, followed by cytochalasin B at 1 g/ml for another 15 min, before adding FMLP at 10^{-7} M for a further 15 min. The cells were then fixed (see above) and analyzed by flow cytometry.

The oxidative burst reaction of FMLP-stimulated neutrophils is characterized by a heterogeneous response with a strongly reactive subpopulation (Fig. 1b), in contrast to the basal homogeneous burst activity of unstimulated neutrophils (Fig. 1a). In the presence of SAA, however, the size of the FMLP-reactive neutrophil population is clearly reduced (Fig. 1c). Monocytes have a higher spontaneous burst activity than neutrophils. Yet, after stimulation with FMLP, the oxidative burst is not increased in monocytes, in contrast to that in neutrophils.

The inhibitory action of SAA on the oxidative burst of neutrophils was further confirmed in full acute-phase serum. To examine whether the inhibitory property of acute-phase serum was due to the presence of SAA, the monoclonal anti-AA/SAA antibody mc29 [9] was added to acute-phase serum before being used in the oxidative burst assay. The results revealed blocking of the inhibitory acute-phase serum function on the oxidative burst and proved SAA to be the principal inhibitory component of acute-phase serum.

This functional interference of a specific antibody could be used to localize the ligand by epitope analysis. Using synthetic peptides of the SAA molecule, the antigenic epitope of mc29 has been localized close to the conserved region of SAA [15]. This experiment therefore gives the first clue to the functional importance of the invariant SAA segment.

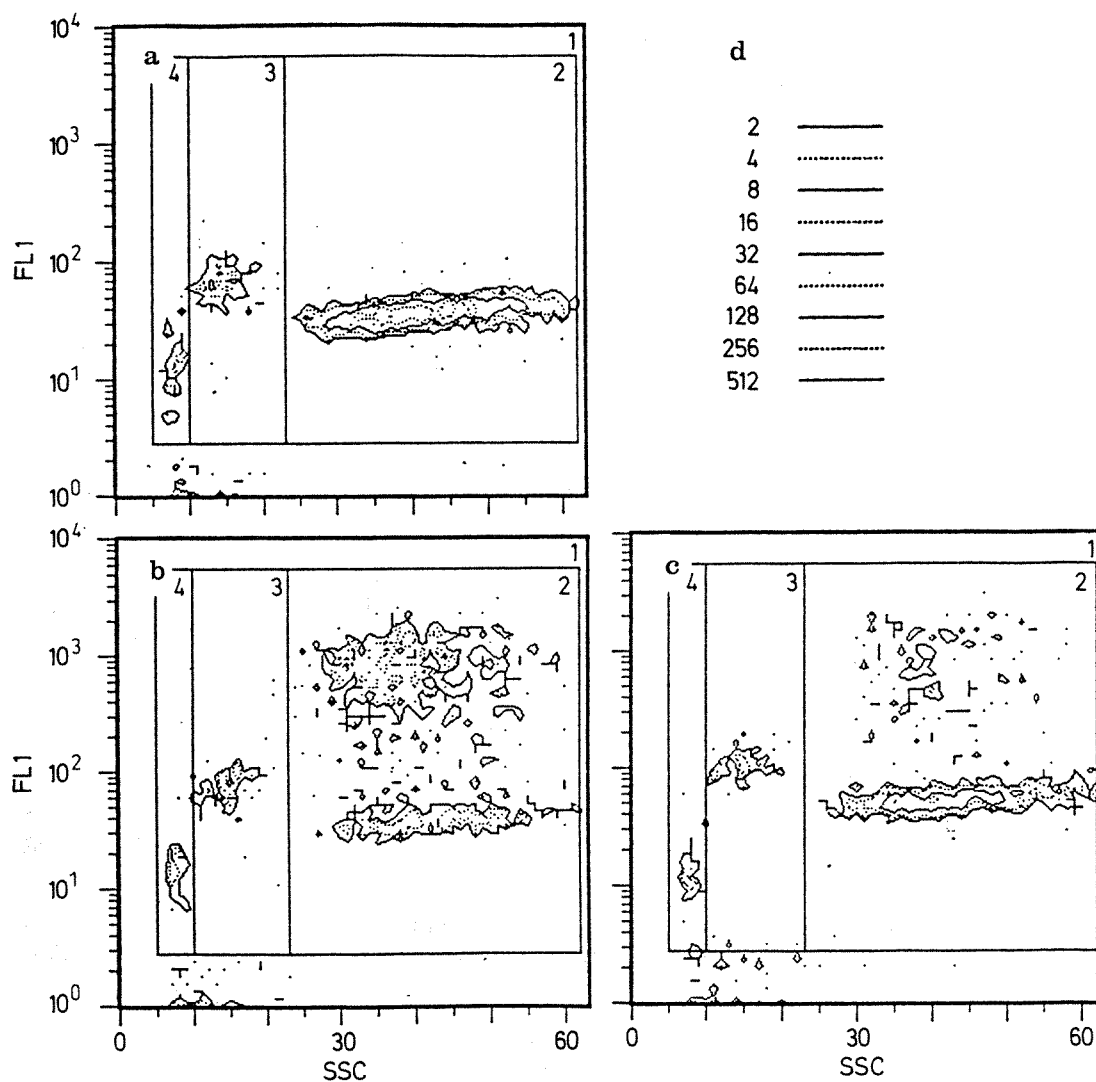


Fig. 1a-d. Reduction of the oxidative burst reaction in the presence of serum amyloid-A protein (SAA), as measured by dihydrorhodamine (DHR) oxidation and shown by flow cytometry. **a** Basal oxidative burst. **b** Oxidative burst reaction after stimulation with *N*-formyl-Meth-Leu-Phe (FMLP). **c** Reduction of the FMLP-induced oxidative burst reaction in the presence of SAA. **d** Conditions of evaluation: 2–512 density of events (contour levels). The position of neutrophils, monocytes and lymphocytes is indicated in *frames 2, 3 and 4*, respectively. FL, fluorescence, SSC, side scatter

Comment and Outlook

This data demonstrates that SAA binding to a specific binding site on neutrophils reduces the action of $\text{TNF-}\alpha$ and possibly that of other cytokines (IL-1 β , IL-6) in a negative feedback regulatory loop. By way of successively increasing plasma levels, this leads to an increased oxidative burst response during sepsis [16]. In reducing the effect of these cytokines, SAA may reduce intravasal neutrophil activation and the ensuing self-destruction of the organism. The ligand domain of SAA for the interaction with a specific binding site on

neutrophils was localized by the monoclonal antibody mc29. If this inhibitory principle can be utilized therapeutically, it could reduce the consequences of the overshooting neutrophil activation in inflammation, tissue injury, and sepsis.

References

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