Glycosylation of $\alpha_1$-acid glycoprotein in septic shock: changes in degree of branching and in expression of sialyl Lewis* groups

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The occurrence of differences in acute-phase response, with respect to concentration and glycosylation of $\alpha_1$-acid glycoprotein (AGP) was studied in the sera of patients surviving or not from septic shock. Crossed affinity-immunoelectrophoresis was used with concanavalin A and Aleuria aurantia lectin for the detection of the degree of branching and fucosylation, respectively, and the monoclonal CSLEX-1 for the detection of sialyl Lewis* (SLex) groups on AGP. Septic shock apparently induced an acute-phase response as indicated by the increased serum levels and changed glycosylation of AGP. In the survivor group a transient increase in diantennary glycan content was accompanied by a gradually increasing fucosylation and SLex expression, comparable to those observed in the early phase of an acute-inflammatory response. Remarkably, in the non-survivor group a modest increase in diantennary glycan content was accompanied by a strong elevation of the fucosylation of AGP and the expression of SLex groups on AGP, typical for the late phase of an acute-phase response. Our results suggest that these changes in glycosylation of AGP can have a prognostic value for the outcome of septic shock.

Keywords: $\alpha_1$-acid glycoprotein, orosomucoid, septic shock, sepsis, sialyl Lewis*, fucosylation, concanavalin A, Aleuria aurantia lectin

Abbreviations: AAL, Aleuria aurantia lectin; AGP, $\alpha_1$-acid glycoprotein; CAIE, crossed affinity-immunoelectrophoresis; ConA, Concanavalin A; HSPC, human serum protein calibrator; IL-1, interleukin 1; IL-6, interleukin 6; LIF, leukaemia inhibitory factor; LPS, lipopolysaccharide; SLex, sialyl Lewis*; TNF, tumour necrosis factor.

Introduction

Septic syndrome is the systemic response to bacterial infection and is characterized by fever, tachycardia, tachypnea and multiple organ failure [1]. In about 40% of the cases this syndrome results in septic shock leading to an increased lethality. The disease is caused by the presence of bacteria or products of these bacteria, like lipopolysaccharide (LPS) or endotoxin, in the circulation. LPS induces several cytokines, such as tumour necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-1$\beta$ (IL-1$\beta$), which play a crucial role in the disease [2]. Interleukin-6 (IL-6) and leukaemia inhibitory factor (LIF) are other cytokines induced during septic shock; they are considered as markers of severity and can be used as prognostic indicators [3-5].

It has been reported that $\alpha_1$-acid glycoprotein (AGP, orosomucoid) is able to protect mice from lethal shock induced by TNF or LPS [6]. This positive acute-phase protein, which is a glycoprotein of 43 kDa containing five N-linked glycans of the di-,tri- and/or tetraantennary type [7], is subject to a cytokine-induced increase in diantennary glycan content during acute inflammation (see [8] for review) and in intercurrent infections, e.g. in systemic lupus erythematosus [9] and rheumatoid arthritis [10]. A decrease in diantennary glycan content has been described during chronic inflammation such as rheuma-
Aleuria aurantia

Materials and methods
Materials
Aleuria aurantia mushrooms were collected locally and AAL was isolated as detailed earlier [14]. Con A (Type V), Coomassie Brilliant Blue R250, methyl-\(\alpha\)-D-glucopyranoside and methyl-\(\alpha\)-D-mannopyranoside were purchased from Sigma (St Louis, MO, USA). Human serum protein calibrator (HSPC) and rabbit anti-human AGP IgG (RAH-AGP-IgG) were obtained from Dakopatts (Glostrup, Denmark), polyacrylamide and agarose M from BioRad (Richmond, CA, USA), mouse anti SLeX IgM CSLEX-1 from ATCC (HB 8580), alkaline phosphatase-conjugated goat anti-mouse IgM from Zymed (San Francisco, CA, USA), and Vibrio cholerae neuraminidase from Boehringer (Mannheim, Germany). All other materials used were of analytical grade and obtained from commercial sources.

Source of sera
Ten patients suffering from septic shock (according to international definitions [1]) were studied. Five patients survived (three women and two men) and five patients did not (one woman and four men). Sources of sepsis in the survivors: two pulmonary, two skin and one urinary tract and in the non-survivors: two pulmonary, two abdominal and one mediastinal. The mean age in the survivor group was 51 ± 20 and in the non-survivor group: 62 ± 10 years. The mean APACHE score in the survivor group was 29 ± 6 and in the non-survivor group 30 ± 6. The day of death of the five non-survivors varied between 3 and 14 days after admission into the intensive care unit. Four of the five survivors were discharged from the intensive care unit within the first week of admission. Sera were stored at -20°C until analysis. HSPC consisting of pooled sera from healthy blood donors was used as a standard for determination of control values.

AGP-concentration
Concentrations of AGP were determined by single radial immunodiffusion, according to Mancini [17], using monospecific RAH-AGP-IgG for precipitation; HSPC was used as a standard.

Crossed affino-immunoelectrophoresis (CAIE)
CAIE was performed according to a modification of the Bøg-Hansen method [18], using, instead of 1% agarose in the first dimension gel, 8% polyacrylamide in a 24.3 mM diethylphenobarbituric acid/Tris buffer (pH 8.6) containing 0.4 mM calcium lactate and 0.02% NaN₃. Two mg ml⁻¹ Con A was included in the first dimension gel as the diantennary-specific affinocomponent. Con A binds the unsubstituted groups of \(\alpha\)-linked, 2-O-substituted mannose residues at carbons 3, 4, and 6 with at least two interacting mannose molecules being required for the binding. As a result, Con A binds with di- but not with tri- or tetraantennary glycans. 2.5 mg ml⁻¹ of an AAL preparation (with a haemagglutination titre of 1024) was included as the fucose-specific affinocomponent [14]. Although the binding specificity of AAL is not restricted to the \(\alpha\)1→3 linked fucose residues, in the case of reactivity of AAL with AGP only this type of linkage is detected, because this is the only one present on AGP, as discussed earlier [14]. Separation of the different glycoforms of AGP was obtained by electrophoresis of sera (0.3–1.3 μl) through a lectin containing polyacrylamide slab gel using a MiniProtein II dual slab gel apparatus (BioRad). Detection of the separated glycoforms was achieved by immunoelectrophoresis in the second perpendicular dimension using the precipitating monospecific antiserum (RAH-AGP-IgG) in a 1% agarose gel [14]. The resulting precipitation curves were visualized by staining with Coomassie Brilliant Blue R250. The areas under the curves indicate the relative amounts of glycoprotein, which were determined in triplicate using a Summagraph (ACECAD D-9000) coupled to a 486 DX PC equipped with an area measurement programme [16].

Partial purification of AGP and detection of expression of SLeX on AGP
AGP was isolated from 20–100 μl of the indicated sera by immunoprecipitation in a micromethod [19] using 300 μl anti-AGP:Sepharose beads (1:1, v/v) in phosphate buffered saline. The eluting buffer was 0.05 M sodium citrate (pH 3.0). SDS-PAGE was performed and the AGP preparations were subsequently blotted onto nitrocellulose by electrophoretic transfer [20]. SLeX determinants were detected by incubating nitrocellulose blots of AGP preparations with the mouse monoclonal anti SLeX IgM CSLEX-1, as previously described [20]. AGP isolated from Cohn fraction V according to Hao and Wickerhauser [21] from pooled normal human serum (a kind gift from Dr D.H.