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Research paper

Systemic and in vitro expression of goat α_1-acid glycoprotein during Caprine Arthritis-Encephalitis Virus infection

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ABSTRACT

The present study was carried out in order to investigate the systemic and local expression of the acute-phase protein α_1-acid glycoprotein (AGP, Orosomucoid) during Caprine Arthritis-Encephalitis Virus (CAEV) natural infection. The aminoacid sequence of goat AGP (gAGP), which was unknown, was determined by cDNA sequencing of the gene. AGP serum concentration was analyzed from 40 healthy and 36 CAEV-induced arthritis-affected goats. The mean concentration of AGP in healthy goats was of 219.8 μg/ml (±178.6 s.d.) and did not statistically differ from that of arthritis-affected goats (157 μg/ml, ±137.8 s.d.). In a second set of experiments, AGP was purified to homogeneity from the serum of healthy and unhealthy goats, and the glycan pattern modifications were analyzed by means of specific binding with lectins. In particular, branching, fucosylation and α(1-6)- and α(1-3)-linked sialylation were analyzed. Goat AGP is not fucosylated in neither physiological nor pathological status. On the contrary, both major (branching) and minor (sialylation) microheterogeneities increase during arthritis.

Finally, the possible local synovial origin of gAGP was determined by means of in vitro expression studies (real-time PCR) which used goat synovial membrane (GSM) as cellular model. It was found that gAGP's mRNA can be constitutively produced by GSM cells, but real-time PCR experiments revealed that the expression of AGP was not influenced by in vitro infection with CAEV.

1. Introduction

Alpha$_1$-acid glycoprotein (Orosomucoid, AGP) is a constitutively expressed acute-phase protein that belongs to the immunocalins, a subfamily of lipocalins (Logdberg and Wester, 2000).

Functions of AGP are still poorly understood: as an immunocalin, AGP combines binding and transport of small hydrophobic molecules with immunomodulatory properties, which include down-regulation of some human (Vasson et al., 1994) and bovine (Lecchi et al., 2008b; Rinaldi et al., 2008) leukocytes defensive properties, as well as the expression of several cytokines during inflammation (Tilg et al., 1993). With some exceptions, most of the activities of AGP identified so far converge toward an overall protection of tissues and cells from collateral damages due to inflammation (Hochepied et al., 2003). This ameliorative function is confirmed by the capability of AGP to reduce the apoptosis rate of mouse hepatocytes (Van Molle et al., 1997) and bovine monocytes

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Abbreviations: CAEV, Caprine Arthritis-Encephalitis Virus; gAGP, goat α_1-acid glycoprotein; SNAI, *Sambucus nigra* agglutinin I; MAA, *Maakia amurensis* agglutinin; ConA, Concanavalin A; AAL, *Aleuria aurantia* lectin.

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(Ceciliani et al., 2007a). As many other acute-phase proteins, AGP is produced mainly by hepatic cells, but local expression has been reported in human breast epithelial cells (Gendler et al., 1982), stimulated alveolar macrophages (Fournier et al., 1999), endothelial cells (Sörensson et al., 1999) and several bovine tissues (Lecchi et al., 2008a). The mature form of AGP is a 183–187 residues long polypeptide, the final MW of 35–37 kDa being reached by adding up to seven, depending on the species, *N*-linked glycan chains (Fournier et al., 2000). Remarkably, inflammatory challenges not only determine an increasing in the net plasma concentration of the protein, but also a modification of its oligosaccharide moiety (Ceciliani and Pocacqua, 2007).

Chronic arthritis is considered a very reliable model for investigating such post-translational modifications during inflammatory reactions. AGP oligosaccharides from human patients with rheumatoid arthritis presented a significant increase in fucosylation and tri-sialylated *N*-glycans expression (Havenaar et al., 1998b). AGP has been found at high concentration in inflamed articulations, but it has been suggested that its increasing in synovial fluid is of hepatic origin (Havenaar et al., 1997). The occurrence of AGP in synovial membrane cells has not been investigated so far and it is still unknown.

A corresponding model of systemic multiple joint inflammations can be found in goats affected by CAEV (Caprine Arthritis-Encephalitis Virus). CAEV is an ungulate lentivirus that causes severe, progressive central nervous system disorders, arthritis, mastitis and eventually death of infected animals (Cheevers and McGuire, 1988). CAEV is responsible of a severe, systemic, inflammatory status. It is therefore possible, but still not thoroughly investigated, that CAEV is also accompanied by an acute-phase reaction since, notwithstanding its name, the so-called "acute-phase response" may occur in both acute and chronic systemic inflammation (Gabay and Kushner, 1999). CAEV-induced arthritis has been already studied as a model of inflammatory disease which may alter glycan microheterogeneity of plasma proteins (McCulloch et al., 1995). It was found that the number of the agalactosyl glycoforms were increased during CAEV-induced arthritis. CAEV model allows also a throughout investigation about the local (articular) expression of AGP, since several cell culture systems can be used to study goat synoviocytes including, for example, goat synovial membrane (GSM) cells.

The present study was carried out in order to gain insight into the occurrence, the glycosylation pattern and the local expression of α_1-acid glycoprotein in goats affected with chronic arthritis caused by CAEV infection. Thus, detailed aims of this study were to assess the difference between healthy and pathological condition of the serum concentration of goat AGP (gAGP) and the possible modification of the carbohydrate moiety expressed on its surface. In particular, major (branching) and minor (sialic acid and fucose content) glycan microheterogeneity will be studied by specific lectins interaction. Furthermore, the possible local (articular) expression of gAGP in an in vitro model using GSM cells infected with CAEV was also explored.

Finally, since the aminoacid sequence, as well as number and positions of glycosylation sites, were still unknown, a prerequisite of this investigation was the determination of the primary structure of gAGP, which was carried out by cDNA sequencing of the gene.

2. Materials and methods

2.1. Collection of samples

This study was conducted with animals from a commercial dairy flock of about 300 Saanen lactating goats where no CAEV control measures were implemented. All blood samples were collected by jugular venipuncture. Serum was separated from the blood clot and tested with MVV/CAEV Elisa test (Institut Pourquier, Montpellier, France) for serology determination (courtesy of Prof. G. Bertoni, University of Bern). Molecular characterization of CAEV infection was done with PCR amplification as previously described (Pisoni et al., 2005).

AGP concentration was assayed from 40 clinically healthy (and CAEV-negative) goats (2–4 years) and 36 clinically ill, CAEV-affected, goats with clinical signs of carpal joint disease.

α_1-acid glycoprotein was purified from 12 healthy and 36 CAEV-affected goats. Of the latter group, only 22 animals contained the amount of AGP sufficient for multiple analyses with lectins.

No animals selected for this study showed any alteration of other body functions (body temperature, hematologic values and mental state).

2.2. Purification of gAGP from non-pathologic and pathologic sera

Purified gAGPs were obtained by conventional HPLC anion exchange chromatography, followed by Heparin affinity chromatography and a final RP (reverse-phase)-HPLC step as previously described for bovine AGP (Ceciliani et al., 2007a). The presence of gAGP was detected by SDS-PAGE and Western blotting on nitrocellulose, using as primary antibody an anti-bovine AGP polyclonal antibody raised in rabbit (Ceciliani et al., 2007a) which has been shown to cross-react with goat AGP (Supplemental Fig. 1). Pre-stained protein standards (ProtoMarkers™, National Diagnostic Inc., Atlanta, GA, USA) were used to assess molecular weight. The homogeneity of the purified fractions was assessed by Coomassie blue staining. The protein content was determined by direct spectrophotometric measurement at 280 nm.

2.3. gAGP cDNA sequence determination

Total RNA was extracted from the goat liver using the RNeasy Mini Kit (Qiagen, Milano, Italy). The reverse transcription reaction was carried out with the iSCRIPT cDNA SYNTESIS Kit (Bio-Rad Laboratories, Segrate (MI), Italy). The thermal profile was as suggested by the protocol. Purified cDNA was used as template for PCR (Eppendorf Mastercycler®).