SERUM AMYLOID A PROTEIN (SAA) FROM MINK, HORSE, AND MAN:

A COMPARATIVE STUDY

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ABSTRACT

Serum amyloid A protein (SAA) was isolated from mink, horse, and human serum by ultracentrifugation and gel filtration and characterized by two-dimensional gel electrophoresis, Western blotting followed by autoradiography and N-terminal amino acid analysis. SAA was found in similar quantities in the high density lipoprotein (HDL) fraction of serum from a patient suffering from systemic juvenile rheumatoid arthritis (JRA) and mink stimulated with lipopolysaccharide (LPS), and in somewhat smaller quantities in serum from horses stimulated with Escherichia coli cultures. Only very small quantities were present in normal human controls and not detectable in normal mink and horse. Striking similarities were found between human and mink SAA with respect to molecular weight, isoelectric point and degree of heterogeneity, while the molecular weight of horse SAA seemed to be somewhat lower, and no obvious heterogeneity could be demonstrated in this protein using two-dimensional gel electrophoresis. Immunologic cross-reactivity between SAA from the three species was not found. In contrast to human and horse HDL, mink HDL was found not to contain apoA-II and only minute amounts of apoC proteins. Normal horse HDL also contained additional apoproteins not present in HDL from the other species. N-terminal amino acids analysis of SAA from mink and horse demonstrated the same similarity with the corresponding AA protein as previously reported for human SAA/AA.
INTRODUCTION

Amyloid-related serum protein (SAA) is a newly described protein which behaves like an acute phase reactant [1, 2], and is the putative precursor of the amyloid fibril protein AA present in some forms of amyloid disease [3]. SAA was detected in serum of both man [3, 4], mouse [5, 6], rabbit [7], and mink [8, 9], because of its antigenic cross-reactivity with protein AA from the same species. SAA is larger (mol. wt. 11,500), but otherwise structurally identical to protein AA (mol. wt. 8600) [10, 11]. Its physiological role is not characterized, but it may act as a regulator of certain immune functions [12], and may have some importance in the handling of endotoxin [13]. SAA is produced by hepatocytes [14], after stimuli from activated macrophages [15], probably mediated by a monokine, SAA-stimulating factor. The major portion of SAA is present in the high density lipoprotein (HDL) fraction of serum (apoSAA) in man, mouse, and rabbit [7, 16, 17]. The covalent structure of mink AA has shown marked homology with AA from other species [18].

The purpose of this study was to compare apoSAA from the three phylogenetically distant species man, horse, and mink with respect to acute phase response, binding proteins in serum, molecular weight, isoelectric point, possible molecular polymorphism and N-terminal amino acid sequence.

MATERIALS AND METHODS

Reagents

Acrylamide, bisacrylamide, biolyte (ampholines pH 3-10) and glycine (all electrophoresis purity reagents) was purchased from Bio-Rad, California, USA, urea and SDS from BDH Chemicals Ltd., England; Tris and Coomassie brillinat blue R 250 from Sigma Chemicals Co., USA and N,N,N^4,N^1-tetramethylene-diamine (TEMED) from Eastman Kodak Company, USA; Non-idet-NP-40 from Shell Company, USA. Nitrocellulose was from Schleicer and Shull, West-Germany, ^1^25I-protein A from Amersham, England, and Sephadex G-100 from Pharmacia Fine Chemicals, Sweden.

The Sources of apoSAA

Plasma rich in SAA was obtained from a patient with systemic juvenile rheumatoid arthritis (JRA) without amyloidosis. Pooled sera from 20 healthy normal blood donors were used as control. Mink (Mustela vision) were injected subcutaneously with lipopolysaccharide (4 mg LPS from Escherichia coli 026:B6 purchased from Difco, Detroit, Mich.) and exanguinated 24 h later. Pooled sera from healthy, non-stimulated mink were used as control. Two horses (Equus caballus) were injected with Escherichia coli cultures (5 x 10^16 bacterias/horse) and blood samples drawn after 24 h. Serum from a normal horse was used as control. These three sera were investigated separately.

Preparative Ultracentrifugation

Human, horse, and mink serum were subjected to ultracentrifugation for isolation of HDL (d = 1.09-1.21 g/ml) following the method of Havel et al. [19]. Centrifugation was done in a Beckman, Model L3-50 ultracentrifuge (Beckman Instruments, Inc., USA) using a Beckman 60Ti rotor for 21 h at 4°C at 40,000 rpm. Supernatants were aspirated, and the HDL fraction was