Poster 29

SERUM AMYLOID A IN GOOSE

Kovacs BM[1], Szilagyi L[2], Janan J[3], Toussaint MJM[4], Gruys E[4], Rudas P[1]
[1]Department of Physiology and Biochemistry, Szt. Istvan University, Budapest, Hungary. [2]Department of Biochemistry, Lorand Eotvos University, Budapest, Hungary.[3]Department of Applied Ethology, Szt. Istvan University, Godollo, Hungary.[4]Department of Pathobiology, Utrecht University, Utrecht, The Netherlands
Email: Kovacs.Beata.Marianna@aotk.szie.hu

Introduction

Amyloidosis - which is a heterogeneous group of disorders described by the pathologic extracellular deposition in various tissues of some fibrillar protein, amyloid - is a common disease among domestic mammals and birds. Acute phase protein serum amyloid A is the precursor protein of AA amyloidosis, the type of amyloidosis in geese. The amyloid fibril protein deposited in secondary amyloidosis is amyloid protein A, which is derived from serum amyloid A (SAA) and is generally found to be modified by proteolytic removal of C-terminal amino acids. Chronic SAA level increase is an essential but not sufficient precondition in this secondary disease resulting from a variety of infectious and non-infectious inflammatory conditions. Therefore goose SAA might be considered as a significant signal protein of the disease.

Materials and Methods

Cloning and expression of recombinant goose serum amyloid A:

Total RNA was isolated from goose liver and used to synthesise first strand cDNA. The coding region of the goose SAA cDNA was amplified by PCR using primers corresponding to the appropriate conservative regions of duck SAA mRNA. The product was subcloned into pET-15b expression vector to result in a HisTag fusion protein expression. The protein was purified by affinity chromatography. The nucleotide sequence of the construct was verified by automated dideoxy sequencing.

Immunisation and detection of SAA-reactive antibodies:

New Zealand White rabbits were immunised intracutaneously. Subsequent bi-weekly immunisations were followed for 6 weeks. The appearance of the antibody was detected by using standard immunodiffusion techniques. The quality was tested by Western blotting. After testing the sera were purified by ion-exchange chromatography.

ELISA assay:

The antibody was used for developing an ELISA assay measuring SAA concentration in goose sera samples. The method was direct ELISA, coating the sera samples onto a high bonding 96 wells' plate. For the calibration curve wells were coated with the recombinant protein. The purified antisera were used as primary antibody, and then anti-rabbit peroxidase antibody was used for the secondary immuno-reaction. For detection 2,2'azino-bis(3-ethylbenzthiazoline-6-sulfonicacid) was used as substrate.

Results

We defined the nucleotide-sequence of the full-length goose serum amyloid A and compared it to SAA sequences of the duck. The aim of this work was to clone and express recombinant goose SAA and to produce useful antibody against this protein for specific ELISA. The anti-SAA serum was proved to be a highly specific antibody. Using this antibody and the recombinant SAA protein, a sensitive direct ELISA assay was developed.

Conclusion

Our work is the first study on the cloning of goose SAA and on the production of specific antiserum against that protein, thus our ELISA method is the first assay for measuring serum SAA level in geese. This assay will be used to monitor health conditions of goose flocks.