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SIMILARITIES OF AN AUTOANTIGEN IN ANEURYSMAL DISEASE OF THE HUMAN ABDOMINAL AORTA TO A 36-kDa MICROFIBRIL-ASSOCIATED BOVINE AORTIC GLYCOPROTEIN

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Summary: Immunoglobulin G (IgG) from human aneurysmal aorta was used to partially purify an aortic protein with an apparent MW ~80 kDa. Amino acid sequencing of a tryptic digest revealed two sequences with homology to mouse tenascin-X. The autoimmune IgG was then shown to react with purified human tenascin, and a rabbit polyclonal anti-human tenascin antibody was found to react with the purified autoantigen. These observations suggest that the autoantigen of abdominal aortic aneurysm disease may be homologous to a calcium-binding member of the tenascin superfamily that has been identified by others in pig and cow.

BACKGROUND

Brophy et al reported the presence of immunoglobulin G (IgG) in specimens of human abdominal aortic aneurysms (AAA) in 1991.(1) IgG was suspected after noting Russell bodies in a histological study of AAA pathology,(2) and IgG was shown to be present by immunoblotting experiments with Protein A.(1) B-cells have been shown to be present in AAA tissue by fluoresence-activated cell counting(3) and immunohistochemical(4) (5) techniques. Gregory et al(6) extracted IgG from AAA specimens for use as a probe to determine whether a putative autoantibody could be demonstrated immunohistochemically in the normal human aorta. An immunoreactive matrix protein was found to codistribute with matrix fibers, predominantly in the adventitia. Further experiments using AAA IgG as a probe in Western immunoblots of soluble extracts from human aorta suggested that the putative autoantibody has an apparent molecular weight of ~80 dDa. The present experiments were carried out to purify and partially characterize this protein.

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METHODS

Protein preparation. Homogenized specimens of human aorta were sequentially extracted with salt, Brij, and urea as we have previously reported.(7) Specimens of aneurysmal aorta were similarly extracted, and IgG was purified using Protein A (Sigma, Inc)-Sepharose affinity column chromatography. Repetitive preparations of the salt and Brij extracts were done by electroeluting SDS PAGE gel slices excised at ~80 kDa apparent molecular weight, then pooling and drying the eluted protein. When sufficient protein had been purified to detect by Coomasie Blue staining in a single lane, the band was excised and submitted to the Keck Laboratory for further analysis.

Tryptic digestion of the gel slices, HPLC separation of the resulting peptides, laser desorption mass spectrometry, and peptide sequencing were all carried out at the Keck Foundation Biotechnology Resource Laboratory in the Boyer Center for Molecular Medicine at Yale University School of Medicine. A blank gel control and a transferrin control were digested and eluted along with the purified sample. The protein digest was fractionated by HPLC. Fractions were selected for sequencing with laser desorption mass spectrometry on a VG Tofspec as a tool to check peak purity. Amino acid sequencing was carried out on an Applied Biosystems sequencer equipped with on-line HPLC.

Computer searches for homologous proteins: Sequences were searched against the National Center for Biological Information's non-redundant database (blastp), which includes Protein Identification Resource (PIR), Genpept and Swiss Protein databases.

Western immunoblots were carried out by conventional methods as previously described.(5) Human tenascin standard was obtained from ICN Biomedicals, and rabbit polyclonal anti-human tenascin was obtained from Gibco BRL.

RESULTS

Although the initial amino acid analysis on a fraction of the gel slice containing the protein of interest indicated a total of 147 pmoles of protein at a density of .24 ug/mm^3, the gel slice digested poorly yielding with lysylendopeptidase (Lys-1). Therefore, the gel was redigested with trypsin, but this also yielded a poor digest. Nevertheless, two peaks (one from the Lys-1 digest and one from the tryptic digest) revealed tentative sequences. The first (peak #82) had four residues sequenced (with an ambiguous residue at position 3): lys-phe-(phe)-leu. These residues of the human protein match four residues of mouse tenascin-X (gp|x73959; residues 237-240).

The second peak (# 80) was a mixture of peptides, including one that could be assigned to albumin. After deleting the albumin sequence, another short sequence was noted (asn-ile-ser) that also occurs in mouse tenascin-X.

After finding that the putative human autoantigen had homology to a member of the tenascin superfamily in mouse, we obtained a rabbit polyclonal antibody to human tenascin. When soluble extracts of human aorta were probed with this antibody on Western blots, an immunoreactive protein was observed at ~80 kDa, corresponding to the putative autoantigen observed by Gregory et al.[6] Finally, when authentic human tenascin was probed with IgG purified from AAA, it was found to be immunoreactive.

DISCUSSION

The putative autoantigen in AAA disease may be a cleavage product of one of the large members of the tenascin superfamily, but an especially interesting possibility is that it might be a human homologue to an aorta-specific microfibril-associated glycoprotein (MAGP-36) of pig(8) and cow.(9) MAGP-36 has both calcium-binding and tenascin-like domains. It has a MW of 36 kDa, but it occurs naturally as a dimer. Its tissue localization is limited to the aortic adventitia in the pig, and fine structural immunomicroscopy further specified its localization to elastin-associated microfibrils.[8] About 40% of the amino acid sequence has been reported in cow.[9] Because of these interesting similarities, MAGP-36 is a candidate for further study in relation to the etiology of the abdominal aortic aneurysm. It seems reasonable to postulate the existence of a human homologue and to speculate that this protein may play an important role in aneurysmal disease of the abdominal aorta (AAA).

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