Sandwich immunoassays for detecting human adiponectin without cross-reactivity with complement component C1q

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INTRODUCTION

Adiponectin (Adn) is a homo-multimeric protein secreted by adipocytes. It's subunits are composed of a collagen-like fibrous domain and a C1q-like globular domain. Due to the collagen domain adiponectin molecules can bind each other to form trimer (low molecular weight form), hexamer middle molecular weight form) and 12-18-mer (high molecular weight form). Adiponectin is secreted as a mixture of these three oligomeric forms whose ratio is changed upon pathological states. In humans, decreased plasma concentrations of adiponectin have been shown to associate with insulin resistance, type 2 diabetes and cardiovascular disease.

Since Adn is structurally homologous to complement component C1q which is highly abundant in human serum, the development of monoclonal antibodies specific to adiponectin and having no cross-reactivity to C1q is important for the development of precise adiponectin assay. The aim of the present work was to develop anti-Adn monoclonal antibodies (MAbs) without cross-reactivity to C1q suitable for immunodetection of different adiponectin forms in human blood.

METHODS

MAbs raising. BALB/c mice were immunized with native human adiponectin isolated from serum, and monoclonal antibodies were raised using standard hybridoma techniques. Selection of clones was carried out using native adiponectin (positive) and human C1q (negative); only MAbs with no cross-reactivity to C1q were accepted for further studies.

Anti-C1q MAb. MAb against C1q component of complement was from HyTest (Finland).

Sandwich immunofluorescent assay (IFA). Adiponectin in plasma and other samples was tested by immunofluorescent assay using standard sandwich protocol. Coating MAbs were preadsorbed onto plate surface (0.5 μg/well). Then addition of antigen-containing sample and detector MAbs labeled with stable Eu^{3+} chelate (0.2μg/well) were followed and after washing fluorescence was detected.

Plasma proteins separation. Gel filtration was performed on Superdex 200 26/60 column using AKTA-Purifier system (GE Healthcare).

RESULTS

Antibody preparation

All monoclonal antibodies produced were tested in direct ELISA on Adn- and C1q-coated plates (0.1μg/well). About 70% of MAbs showed cross-reactivity. MAbs Adn27, Adn36, Adn94, Adn97, Adn130 recognising And only and having no cross-reactivity with C1q (Fig. 1) were selected for further studies.

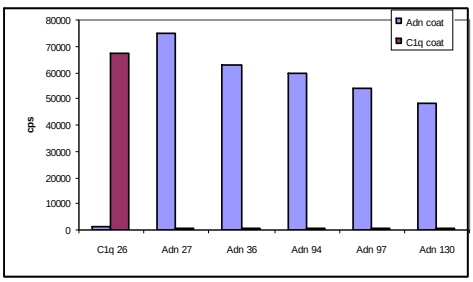

Fig.1. Interaction of selected MAbs with adiponectin and C1q.

Sandwich immunoassay for Adn determination

All antibodies were tested as capture and detection in sandwich ELISA. Several tested two-site MAb combinations demonstrated high sensitivity to Adn and were selected for further work (detector MAbs are marked by asterisk): Adn36 - Adn27*, Adn97 - Adn94*, Adn63 - Adn94*, Adn130 - Adn63*. Typical plasma titration curve for Adn36-Adn27* assay is shown in Fig. 2. It can be seen that adiponectin could be reliably determined if plasma was diluted up to 1000-fold.

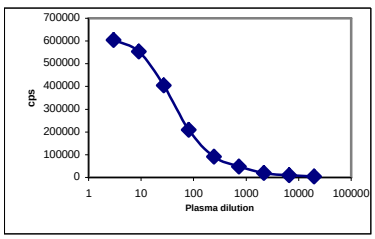

Fig. 2. Human plasma titration in Adn36-Adn27* assay.

Specificity of anti-adiponectin assays for different oligomeric forms

Human plasma proteins were separated by gel-filtration on Superdex 200 column, and Adn immunoreactivity in fractions was measured by several sandwich assays (Adn36-Adn27*, Adn97-Adn94*, Adn63-Adn94* and Adn130-Adn63*). It was found that all assays detected total adiponectin - all three oligomeric forms of plasma Adn (high, medium and low molecular weight forms of Adn) but were different in recognizing forms with different molecular masses (compare Fig. 3, 4, 5). Elution volumes corresponding to three peaks of immunoreactivity are the same, whereas relative intensity of immunoreactivity peaks is different.

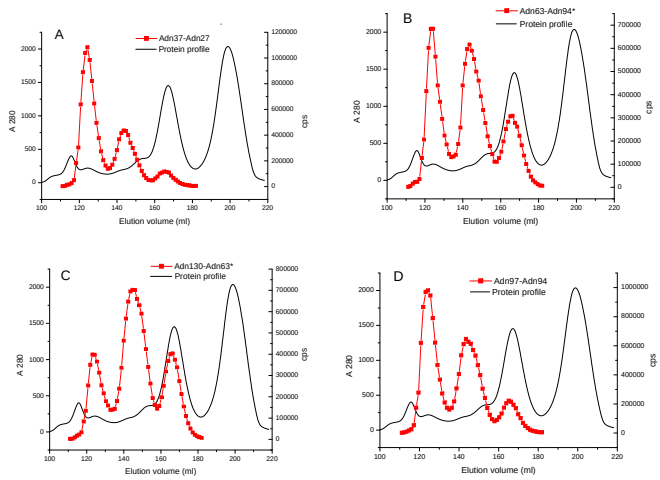

Fig. 3. Adiponectin immunoreactivity in plasma fractions after gel filtration measured by Adn36-Adn27 (A), Adn63-Adn94 (B), Adn130-Adn63 (C), and Adn97-Adn94 (D) assays.

Co-purification of C1q-protein with adiponectin

It was found that C1q is co-purified with Adn during purification step on affinity matrix utilizing Adn36 MAb immobilised on Sepharose CL-4B. Human plasma was applied onto the matrix and eluted fractions contained both adiponectin and C1q. In control experiments, plasma was passed through Sepharose without antibodies or through Sepharose with immobilized mouse immunoglobulins (non-reactive to And or C1q) of the same isotype as Adn36 MAb. In control experiments C1q was not found in eluates. These experiments made us to suggest that complex And-C1q may exist in human plasma. (Fig. 4).

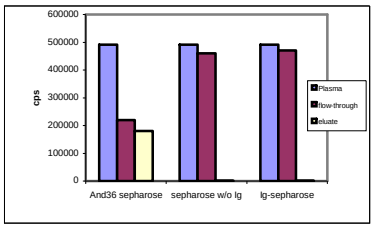

Fig. 4. Detection of C1q immunoreactivity by anti-C1q assay in fraction of the proteins not interacting with affinity matrix and eluates after passing of plasma through Adn-36-sepharose, sepharose without immobilized MAbs and sepharose with immobilized mouse Ig.

CONCLUSIONS

Anti-adiponectin MAbs Adn27, Adn36, Adn63, Adn94, Adn97, Adn130 were raised that recognise only adiponectin and don't show cross-reactivity with C1q component of complement.

Newly developed four adiponectin assays Adn36-Adn27*, Adn97-Adn94*, Adn63-Adn94*, Adn130-Adn63* recognise all three oligomeric forms of adiponectin (total adiponectin) but with different form specificity.

C1q is co-purified with adiponectin on affinity matrix. We assume that C1q forms complex with adiponectin in human blood.

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