Adiponectin

Adiponectin is an abundant protein hormone that belongs to a family of adipokines. It is expressed mostly by adipocytes and is an important regulator of lipid and glucose metabolism. Adiponectin is an insulin-sensitizing hormone with anti-diabetic, anti-inflammatory and anti-atherogenic properties (1). It has been shown that the amount of adiponectin in blood reduces in cases of patients suffering from Type 2 diabetes mellitus or coronary artery diseases, or who are insulin-resistant (2-6).

Human adiponectin consists of 244 amino acid residues and has a distinct domain structure: it contains both collagen-like and globular C1q-like domains. Collagen-like parts of three adiponectin molecules can form a triple coiled coil structure very similar to that in collagen (7). C1q-like domains form a “head” of adiponectin globula (Figure 1) and share a considerable degree of structural similarity to complement component C1q.

In blood, adiponectin is found as trimers (low-molecular weight form, LMW), hexamers (medium molecular weight form, MMW) and higher order multimers (high molecular weight form, HMW). The exact structure of the HMW form of adiponectin is not yet known. Most likely several combined hexamers and/or trimers form the high-molecular weight form of adiponectin. It has been suggested that different oligomeric forms exist in blood as separate moieties and do not convert into one another (8). Adiponectin oligomers are capable of binding Ca²⁺ ions, which are thought to participate in the maintenance of conformational stability of adiponectin (9).

The concentration of total adiponectin in the blood is approximately 3-30 μg/ml, whereas the concentration of the closest structural homolog of adiponectin, C1q, is approximately 80-200 μg/ml. Therefore, it is critical that anti-adiponectin antibodies do not cross-react with human C1q (10). Some authors describe significant gender differences in adiponectin level in healthy adults. These differences could contribute to discrepancies in adiponectin concentrations reported by various authors. It has been suggested that the concentration of the HMW form of adiponectin or HMW/total adiponectin ratio correlates with insulin resistance and metabolic syndrome better than just the concentration of total adiponectin (11-12).

Reagents for the development of a reliable adiponectin assay

HyTest offers several anti-human adiponectin monoclonal antibodies and a native purified adiponectin that enable the development of an adiponectin specific immunoassays.
Monoclonal antibodies specific to human adiponectin

Hybridoma clones were derived from hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with either human recombinant adiponectin or native human adiponectin.

All antibodies were tested in direct ELISA for cross-reaction with C1q, which is the most abundant adiponectin homolog in blood. None of the selected MAbs showed any cross-reaction with human C1q.

Sandwich immunoassay

All MAbs were tested in two-site combinations as capture or detection antibodies in sandwich ELISA with native adiponectin. Seven two-site combinations were selected for the development of sandwich immunoassays on the basis of sensitivity and specificity to different oligomeric forms of adiponectin:

- Adn20 - Adn23
- Adn36 - Adn27
- Adn94 - Adn63
- Adn279 - Adn94
- Adn214 - Adn27
- Adn222 - Adn94
- Adn305 - Adn279

A representative curve demonstrating detection of purified native adiponectin by the assay Adn279-Adn94 is shown on Fig. 2.

All assays were tested with serial dilutions of normal human serum to evaluate the interaction of MAbs with native adiponectin in a complex environment. All assays demonstrated a steady decrease of signal correlating with the degree of serum dilution. The representative titration curve for the assay Adn94-Adn63 (capture antibody-detection antibody, respectively) is shown in Fig. 3.

Assays Adn36-Adn27 and Adn20-Adn23 react differently with adiponectin in serum and citrate plasma (Fig. 4). Other MAbs two-site combinations (Adn94-Adn63, Adn279-Adn94, Adn214-Adn27, Adn222-Adn94, Adn305-Adn279) react identically with antigen in serum and plasma identically.

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A representative curve demonstrating detection of purified native adiponectin by the assay Adn279-Adn94 is shown on Fig. 2.

All assays were tested with serial dilutions of normal human serum to evaluate the interaction of MAbs with native adiponectin in a complex environment. All assays demonstrated a steady decrease of signal correlating with the degree of serum dilution. The representative titration curve for the assay Adn94-Adn63 (capture antibody-detection antibody, respectively) is shown in Fig. 3.

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Recognition of adiponectin by assays Adn20-Adn23 and Adn36-Adn27 in serum is Ca²⁺-sensitive (Fig. 5). Chelating of Ca²⁺ ions by EGTA leads to the rearrangements in adiponectin structure and changes in the interaction of one of the antibodies with the antigen. Other assays do not demonstrate Ca²⁺-dependence in the antigen recognition and react identically with adiponectin in serum or citrate plasma.

**Assays detecting total, HMW or LMW forms of human adiponectin**

To establish an oligomer specificity of HyTest assays, serum proteins were separated according to their molecular masses by means of size-exclusion chromatography and immunoreactivity in fractions was measured. The assay Adn20-Adn23 detects two oligomeric forms of adiponectin: mostly HMW and to a lesser extent, the MMW form (Fig. 7A). The assay Adn94-Adn63 recognizes all three Adn oligomeric forms - total adiponectin (Fig. 7B) and the assay Adn214-Adn27 reacts primarily with the LMW form of adiponectin (Fig. 7C).

**Western blotting**

All MAbs were tested on their ability to recognize adiponectin in Western blotting. Only five of the tested antibodies - MAbs Adn20, Adn23, Adn63, Adn214 and Adn222 - reacted with adiponectin transferred onto nitrocellulose membrane after SDS-PAGE in reducing conditions (Fig. 6).

**Figure 6. Immunodetection of native adiponectin with anti-Adn MAbs in Western blotting after SDS-electrophoresis in reducing conditions.**

40 ng of native purified adiponectin was loaded onto each track. Nitrocellulose membrane was stained with 5 μg/ml of various anti-adiponectin MAbs in phosphate-buffered saline, containing 5% dry milk and 0.1% Tween-20.

1: Adn20, 2: Adn23, 3: Adn63, 4: Adn214, 5: Adn222, MW markers are marked by arrows.

**Figure 7. Sandwich ELISA in protein fractions after size-exclusion chromatography, measured by three different capture-detection antibody combinations.**

(A) Adn20-Adn23, (B) Adn94-Adn63 and (C) Adn214-Adn27. 1 ml of normal human serum was applied onto the column. Positions of oligomeric forms of adiponectin and molecular weight markers are depicted in the picture. The black line presents the optical density detected at 280 nm.
Native purified adiponectin

Native adiponectin purified from normal human plasma is the best calibrator for immunoassays. Native adiponectin was isolated from normal human plasma using a combination of chromatographic methods. Its purity is approximately 95%.

Native purified adiponectin fully recovers its immunoreactivity after lyophilization and reconstitution by the addition of deionized water (Fig. 8).

Purified native adiponectin contains all three oligomeric forms of Adn (Fig. 9) and can therefore serve as a calibrator for all types of Adn assays: total Adn, HMW- or LMW-specific.

FIGURE 8. Lyophilization does not affect immunological activity of native purified adiponectin measured by assay Adn94-Adn63.

FIGURE 9. Native purified adiponectin contains all oligomeric forms. 3 μg of adiponectin was applied onto a gel-filtration column and immunoreactivity in fractions was measured by the sandwich ELISA using Adn94 and Adn63 as capture and detection antibodies respectively. Molecular weight markers are depicted by arrows on the x-axis. The black curve represents the optical density measured at 280 nm.

Ordering information

MONOCLONAL ANTIBODIES

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References