Monoclonal Antibody Register

New Monoclonal Antibody to Human Apolipoprotein J

J. ČAPKOVÁ, G. GEUSSOVÁ, J. PĚKNICOVÁ

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Background

Apolipoprotein J (apoJ) is a 70 kDa glycoprotein associated with high-density lipoproteins (HDL) in human plasma (de Silva et al., 1990a). HDL transport more than 80% of the plasma cholesterol from extrahepatic tissues to the liver for excretion (Glomset, 1968).

Wide tissue distribution of apoJ was determined by isolation of apoJ mRNA from a variety of human tissues. The presence of apoJ mRNA was mainly detected in the steroidogenic tissues, testis and ovary, and also in the brain (de Silva et al.,1990b).

The apoJ molecule is a dimer which consists of two disulfide-linked subunits designated apoJα (34–36 kDa) and apoJβ (36–39 kDa) (de Silva et al., 1990c). For purification and characterization of the apoJ molecule, monoclonal antibodies were utilized. Six monoclonal antibodies specific for apoJ were generated and described (de Silva et al., 1990a, 1990c).

ApoJ has extensive similarities to the human protein designated as SP-40,40 (or complement lysis inhibitor (CLI)) (Choi et al., 1989) and human TRPM-2/clusterin (Wong et al., 1994). The presence of SP-40,40 was also demonstrated within human seminal plasma at levels comparable to those in serum (Kirszbaum et al., 1989).

Both these proteins, SP-40,40 and TRFM-2/clusterin, have been implicated in a variety of physiological processeses, including sperm maturation, lipid transport, membrane remodelling and inhibition of the complement cascade (Wong et al., 1994).

ApoJ is also secondarily incorporated into the sperm membrane, as sperm travel through the male reproductive tract, and belongs to the sperm-coating proteins. Among monoclonal antibodies against human sperm proteins that we generated, a monoclonal antibody that specifically recognized apoJ was found. The monoclonal antibody was designated Hs-3.

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Corresponding author: Jana Čapková, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. Fax: 420 (2) 444 717 07; e-mail: capkova@biomed.cas.cz.

Abbreviation: apoJ – apolipoprotein J.

Description of the new monoclonal antibody, Hs-3

Production

The monoclonal antibody Hs-3 has been generated by fusion of Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with Triton X-100 human sperm extract. Standard procedures were used for hybridoma production, selection and cloning (Pěknicová et al., 1986; Pěknicová and Moos, 1990).

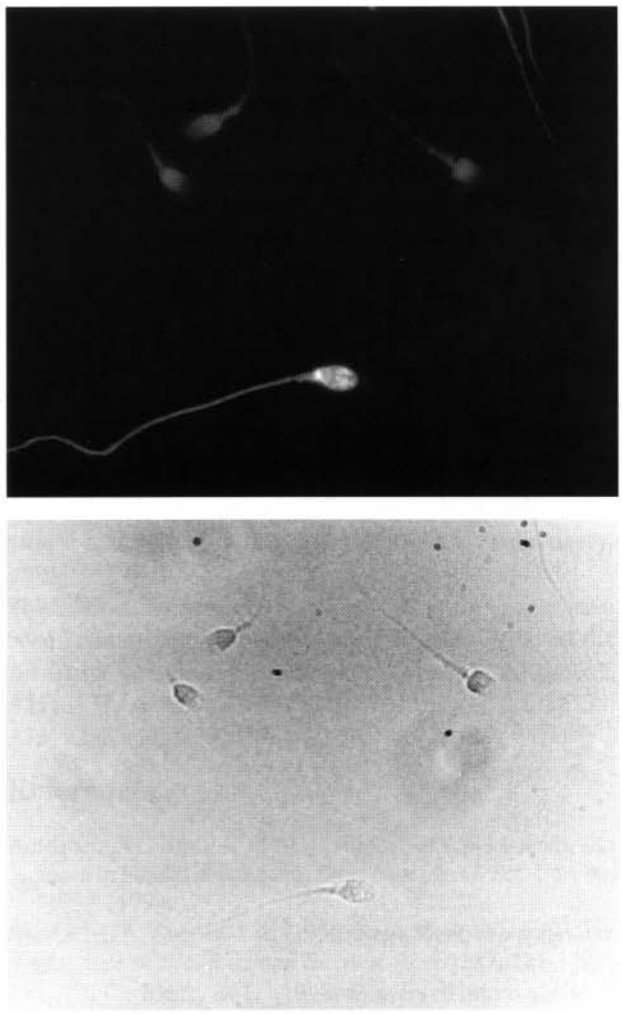

Fig. 1. Immunofluorescence staining of acetone-fixed human spermatozoa with Hs-3 monoclonal antibody. Under UV light whole spermatozoa or their parts were diffusely stained (top). The same field under visible light (bottom). Magnification 400x.

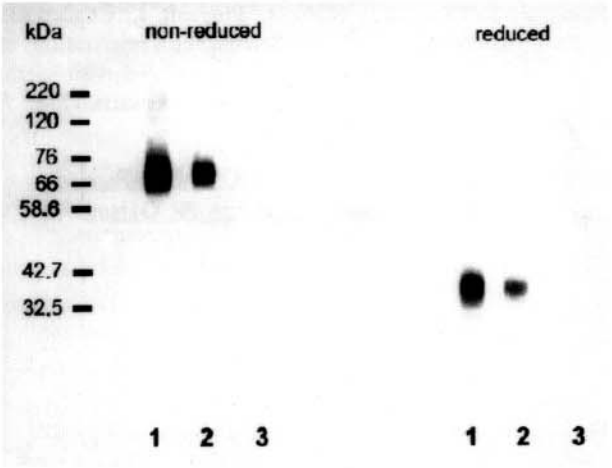

Fig. 2. ECL Western blot of SDS human sperm extract. In lanes 1 and 2, the supernatant of Hs-3 (dilution 100x and 500x) was used as the primary antibody and only one protein band was detected (72 or 35 kDa, respectively). In lane 3, the strip was incubated with the supernatant of Sp2/0 myeloma cells as the negative control. Positions of molecular mass markers are shown on the left side.

Specificity

In indirect immunofluorescence with Hs-3 as the first antibody and with FITC-conjugated swine anti-mouse immunoglobulin (FITC-SwAM, SEVAC, Prague, Czech Republic) as the second antibody, about 30% of acetone-fixed ejaculated human spermatozoa were stained. The Hs-3 mAb diffusely labelled whole spermatozoa or their parts (Fig. 1).

After separation of total human sperm protein extract on 12% acrylamide one-dimensional SDS PAGE, the pattern of immunoreactive sperm proteins observed in Western blot showed that Hs-3 mAb reacted with a sole protein band of approximately 72 kDa (p72). Under reducing conditions Hs-3 mAb recognized a band of an approximately 36 kDa protein (p36) (Fig. 2). The identical picture was found in Western blot when the corresponding protein originated from human seminal plasma extract (not shown). Results of Western blotting indicate a dimeric structure of the protein defined by Hs-3 mAb.

The Hs-3 mAb did not react on immunoblots with separated proteins of SDS boar, bull, dog and cat sperm extracts, respectively. This suggested that Hs-3 mAb was specific for human protein.

The protein that reacted with Hs-3 mAb was further analysed by peptide mass fingerprint (MALDI-MS). SDS human sperm extract was electrophoresed in 12% polyacrylamide gel under non-reducing conditions, and after electrophoresis a small part of the gel was cut off and subjected to the Western blot procedure (Towbin et al., 1979) with the Hs-3 mAb. The immunoblot was fitted in the rest of Coomassie brilliant blue (CBB R 250, Sigma)-stained gel and the Hs-3-corresponding protein band (approximately 70 kDa protein) was cut from the gel. In this gel band, tryptic digestion and sample preparation for MALDI analysis were performed as described by Otto et al. (1996). Extracted peptides were collected from the gel digest and their mass spectra were measured in a MALDI reflectron time-of-flight mass spectrometer BIFLEX II (Bruker-Franzen, Bremen, Germany).

Eight out of nine peptides obtained by tryptic digestion of the Hs-3-corresponding protein matched with apoJ. The sequence coverage of the total apoJ sequence was 28%. One hundred eighteen amino acids have been covered. Peptide mapping was evaluated by using the searching algorithm (see web address in references). On the base of the peptide map, the 70 kDa protein was identified as apoJ.

Properties

The immunoglobulin class and subclass of the Hs-3 mAb was determined with the mouse monoclonal antibody isotyping reagents (ISO-2, Sigma, Prague, Czech Republic) according to the manufacturer's instructions. The antibody is of the IgG_1 isotype.

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