Human decidua-associated protein hDP200 appears to be a rheumatoid factor

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The amino acid sequences of the amino-terminal of the two different polypeptide chains of the human decidua-associated protein hDP200, with apparent molecular masses of 55 and 25 kDa, were determined. The amino-terminal sequence of the 55 kDa chain revealed that it is an immunoglobulin heavy chain, of the V H -III subgroup and the amino-terminal sequence of the 25 kDa chain showed that it is a human kappa V-III light chain. The sequence data indicate that hDP200 is an immunoglobulin. The ability of the hDP200 molecules to form high molecular weight complexes with immunoglobulins of other classes suggest that hDP200 is a rheumatoid factor.

Introduction

Endometrial decidualization involves growth and differentiation of a 'new', progestagen-dependent tissue, the decidua, which gradually replaces the functional layer of the endometrium. Pregnancy is not mandatory for growth and differentiation of decidual tissue; it is produced in each normal cycle in the late luteal phase in response to progesterone. The decidua is shed at birth or menstruation. As decidualization involves growth and differentiation of new tissue, the appearance of decidua-associated proteins would be expected.

Halperin et al. (1990a) described the identification, immunopurification by a monoclonal antibody and partial characterization of an apparently novel decidua-associated protein which was named according to its molecular weight in non-reducing SDS-PAGE analysis as hDP200. SDS-PAGE analysis performed under reducing conditions, revealed that hDP200 is composed of two polypeptide chains of apparent molecular masses of 55 kDa and 25 kDa, which were accordingly named hDP55 and hDP25, respectively. We now report that as deduced from amino-terminal sequence information of both polypeptide chains, hDP200 appears to be an immunoglobulin. hDP200 is probably a rheumatoid factor as it has the capacity to interact with other human immunoglobulins.

Materials and Methods

Monoclonal antibody, immunoaffinity purification and western blot analysis

The preparation and characterization of mAb DEC21, immobilization of the antibody on Eupergit C beads (Rohm, Darmstadt), performance of immunoaffinity purification and western blot analysis were performed as described by Halperin et al. (1990a). Briefly, Eupergit C beads were washed with phosphate-buffered saline (PBS) and with 1.0 mol potassium phosphate buffer 1 -1 pH 7.5. Monoclonal antibody dissolved in the above buffer was added to the beads and the mixture was agitated by rotation for 16 h at 4°C. Residual oxirane groups were blocked by reacting them with 1 mol ethanolamine 1 -2 at pH 9.5 for 4 h at 4°C. The mAb–Eupergit C conjugated beads were packed into a column and washed extensively with PBS. The column was loaded with crude decidual extract (2.5 ml ml -1 of column) and then the column was washed extensively with PBS. Bound proteins were eluted with 1 mol ammonium acetate buffer 1 -1 pH 10.0, neutralized, concentrated to 1 ml using a centriflo CF25 ultrafiltration membrane cone (nominal molecular mass cut-off 25 kDa; Amicon, Danvers, MA) and dialysed against PBS. Protein concentration was determined by the method of Bradford (1976).

Double-site enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) plates were coated with 10 µg mAb DEC21 ml -1. Test samples were diluted in PBS containing 5% BSA and incubated in wells for 1 h at 37°C. MAb DEC21 conjugated with horseradish peroxidase (Tijssen, 1986) was added and the plates were incubated for 1 h at 37°C. Activity of the bound horseradish peroxidase was determined with o-phenylenediamine (2 mg ml -1) and H 2 O 2 (0.008%) in 50 mmol citrate buffer 1 -1 pH 5.0. The reaction was stopped by the addition of 50 µl 4 mmol HCl l -1.

Polyacrylamide gel electrophoresis blotting and amino-terminal sequencing

Electrophoresis was performed under reducing conditions as described by Laemmli (1970). Purified hDP200 was separated by SDS-PAGE and blotted onto a PVDF membrane (Millipore, Bedford, MA) treated with polybren (Applied Biosystems, Foster City, CA) (Xu and Shively, 1988). The blotted protein
bands were visualized by Coomassie blue staining. The stained protein bands were cut out and submitted to sequencing, using the Applied Biosystems 470A gas-phase microsequencer. Computer comparisons were carried out by National Biological Research Foundation database.

**Gel filtration on Sepharose 4B**

Samples were separated on Sepharose 4B (Pharmacia, Uppsalas) column (1.2 cm × 40 cm), equilibrated with PBS, at a flow rate of 0.5 ml min⁻¹. Fractions of 1 ml were collected. The fractions obtained were probed by the double-site ELISA for hDP200.

**Results**

The decidua-associated protein hDP200 was purified by immunoaffinity chromatography on a column prepared from mAb DEC21. hDP200 was copurified with hDP25 (Halperin et al., 1990b). The preparation was separated by SDS-PAGE and blotted onto a PVDF membrane. The hDP55 and hDP25 protein bands were excised and amino-terminal sequencing was performed. The resulting sequence for hDP55 was: Glu-Val-Gly/Gln-Leu-Val-Glu-Ser-Gly-Glu-Leu-Val-Gln-Pro-Gly. This sequence is identical to the sequence of the Human Ig gamma heavy chain of the V₁–II subgroup which is: Glu-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly-Asp-Leu-Val-Gln-Pro-Gly. The resulting sequence for hDP25 was: Glu-Ile-Val/Gly-Leu/Met-Thr-Gln-Ser-Pro-Gly/Ala-Thr-Leu. This sequence is identical to the sequence of the human kappa light chain of the V–III subgroup which is: Glu-Ile-Val-Leu-Thr-Gln-Ser-Pro-Gly-Thr-Leu. The above results indicate that hDP200 is an immunoglobulin. The monoclonal antibody DEC21 recognizes an antigenic determi-

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**Fig. 1.** Analysis of the apparent molecular mass of hDP200 in menstrual fluid by size exclusion chromatography. A crude menstrual fluid specimen was subjected to chromatography on a column of Sepharose 4B, and fractions were collected and probed for the presence of hDP200 by double site ELISA.

**Fig. 2.** A western blotting analysis demonstrating that hDP200 is complexed with non-IgG immunoglobulins. Immunoaffinity purified hDP200 from menstrual fluid was separated by SDS-PAGE, and blotted onto a nitrocellulose membrane. Detection of the bound antibodies was performed using a second antibody conjugated with horseradish peroxidase (HRP) and the HRP chromogenic substrate chloronaphthol. (A) Staining for total proteins with Ponceau S, (B) staining with anti-human γ heavy chain, (C) staining with anti-human α heavy chain, (D) staining with anti-human μ heavy chain, (E) staining with anti-human kappa light chain.

A double site ELISA for hDP200 was established. MAb DEC21 was used as both the capture and detector antibody. When this ELISA was used hDP200 was detected and quantified in decidual extracts (12 samples), uterine washes (over 200 samples), amniotic fluids (17 samples), menstrual fluid samples (over 250 samples) and seminal fluid samples (five samples). The determinants recognized by mAbs usually appear only once in polypeptide chains. The success of the double site ELISA therefore implied that the antigenic species recognized in the ELISA is a multi-molecular complex of hDP200 and perhaps additional molecules. The presence of the putative complexes in crude menstrual fluid was demonstrated by subjecting a crude menstrual fluid sample to chromatography on Sepharose 4B column and the fractions containing hDP200 were identified by the ELISA (Fig. 1). The activity of hDP200 was eluted from the column in a high molecular mass fraction (> 900 kDa) and not at the expected molecular mass of 150 kDa typical for immunoglobulins. This finding supports the idea that the antigenic species identified by the ELISA exists as a complex containing at least two units of hDP200. hDP200 was purified from crude menstrual fluid by
immunoaffinity chromatography using mAb DEC21. Identification of the proteins in the immunoaffinity purified preparation of hDP200 by western blotting analysis (Fig. 2) revealed heavy chains of IgG, IgA and IgM and kappa light chains. Thus, apparently, the putative complexes formed by the hDP200 contains IgG (the hDP200) and also IgA and IgM.

Discussion

Analysis of hDP200 revealed that it is an IgG. The heavy chain is of the V₁–II subgroup and the kappa light chain is of the V–III subgroup. The kappa light chain of the V–III subgroup is found in a high percentage of monoclonal rheumatoid factors (Kunkel et al., 1974; Newkirk et al., 1986). The presence of a light chain of the V–III subgroup in hDP200 therefore indicates that it is a monoclonal rheumatoid factor. MAb DEC21, which was used for identification and immunoaffinity purification of hDP200, recognizes a specific determinant of hDP200 that is not found on other immunoglobulins. Further support for the contention that hDP200 is a rheumatoid factor is the discovery that hDP200 forms complexes with other immunoglobulins of the IgM and IgA classes.

There are no previous reports of the presence of an Ig binding protein or rheumatoid factor in the uterus. Several Ig binding proteins were described in the seminal plasma (Witkin et al., 1983; Thaler et al., 1989; Liang et al., 1991) but none was identified as an immunoglobulin. The observations reported here indicate that the distribution of Ig-binding proteins, rheumatoid factor or other, is common to both the male as well as the female genital systems. Cells bearing the surface antigen Leu-1 (CD5) produce rheumatoid factors in humans (Hardy et al., 1987). This cell type is probably similar to murine lymphocytes, bearing the cell surface antigen Ly-1 (Casali et al., 1987). Lympocytes, bearing the Ly-1 marker, were identified in murine decidual tissues (Lala et al., 1986). It is therefore reasonable to assume that homologous cells, bearing the Leu-1 marker would be found in the human decidua and produce the rheumatoid factor(s) found in the human decidual tissue.

The physiological function of the rheumatoid factor found in the human uterus is currently unknown. However, it is possible that it serves as one of the mechanisms that ensures suppression of the immune response toward sperm cells and the fetus.

References


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