(57) L’invention concerne un procédé permettant de prévenir les fausses couches pendant la grossesse. Ce procédé consiste à administrer une quantité efficace d’un polypeptide de fétuine.

(57) There is disclosed a method for helping to prevent miscarriages during pregnancy, comprising administering an effective amount of a fetauin-polypeptide.
(54) Title: PREVENTION OF PREGNANCY MISCARRIAGES

(57) Abstract

There is disclosed a method for helping to prevent miscarriages during pregnancy, comprising administering an effective amount of a f托运-polypeptide.
PREVENTION OF PREGNANCY MISCARRIAGES

Technical Field of the Invention

The present invention provides a method for prevention of miscarriages during pregnancy.

Background of the Invention


Recently, spermine, a ubiquitous biogenic amine present in large amounts in the amnion, has been shown to counter-regulate the immune response by inhibiting the production of TNF and other pro-inflammatory cytokines by human mononuclear cells (Zhang et al., J. Exp. Med. 185:1759-1768, 1997).

Fetuin is a globular 341-amino acid protein containing 20-25% carbohydrate (by weight) and 6 internal disulfide bonds. The human fetuin sequence (also known as α2-HS glycoprotein) is provided herein as SEQ ID NO. 1 and SEQ ID NO. 2. Fetuin was first identified over 50 years ago as a major protein component of bovine fetal serum but its biological function remains unclear. Bovine fetuin is a globular 341 amino acid polypeptide with six internal disulfide bonds and three N-linked and two O-linked oligosaccharide chains.


Human fetuin has 3 N-linked oligosaccharide chains (attached to the amine nitrogen atom of asparagine), and 2 O-linked oligosaccharide chains (attached to the oxygen atom of serine or threonine). The sugar moiety directly attached to the fetuin polypeptide is usually a N-acetylglucosamine residue. The terminal sugar residue is usually a sialic acid, in particular a
N-acetylenuraminic acid (NANA) residue, which bears a net negative charge. If one removes the terminal sialic acid residue from fetuin by neuraminidase treatment, the resulting glycoprotein is an asialofetuin. Fetuin is also a carrier protein for growth factors. Fetuin is sometimes referred to as α2-HS-glycoprotein. Thus, it is considered that fetuin's biological effects on cultured cells are related to its carrier function for molecules with growth-promoting properties.

The synthesis of human α2-HS-glycoprotein is down-regulated by cytokines (hIL-1β, hIL-6) (Lebreton et al., J. Clin. Invest. 64:1118-1129, 1979). Human fetuin levels are decreased (25-50%) in trauma patients (van Oss et al., J. Trauma 15:451, 1975). Therefore, there is a need in the art to find a utility for fetuin and to understand fetuin's physiological role and the importance of its many negatively charged (at physiologic pH) sialic acid residues.

Summary of the Invention

The present invention provides a method for helping to prevent miscarriages and pre-term labor during pregnancy, comprising administering an effective amount of a fetuin polypeptide. Preferably, the human fetuin polypeptide has a primary sequence according to SEQ ID NO.1 or SEQ ID NO. 2 or a shortened fragment thereof having at least 250 amino acid residues.

Brief Description of the Drawing

Figure 1 shows the suppression of TNF secretion by spermine in the presence of fetuin (Figure 1A) or fetuin-specific polyclonal antibodies (Figure 1B). HuPBMCs or RAW 264.7 cells were stimulated with E. coli endotoxin (LPS, 100 ng/ml) in the presence of spermine, human fetuin (α2-HS-glycoprotein), or polyclonal antibodies against fetuin. TNF levels in supernatants four hours post-LPS stimulation were determined by ELISA as previously described (Zhang et al., J. Exp. Med. 185:1759-1768, 1997). Note that fetuin increases the TNF-suppressing activity of spermine, and anti-fetuin renders normal LPS-stimulated macrophages refractory to this suppression. A Student's t-test was performed and a P < 0.05 was considered significantly different (*).

Detailed Description of the Invention

The present invention is based upon the new discovery that a fetal plasma glycoprotein, fetuin, is required for the inhibition of TNF production by spermine. Although fetuin was first described more than fifty years ago in fetal bovine serum, and subsequently found to share high homology to human fetuin (α2-HS-glycoprotein), its role in pregnancy and fetal development has, until now, been unknown. While investigating the mechanism underlying spermine-mediated suppression of TNF production in the murine macrophage-like cell line, RAW 264.7, we came upon the surprising discovery that macrophages lost their responsivity to spermine when cultured under low serum conditions. That is, despite the addition of cytokine-
suppressing concentrations of spermine to these cells, the production of TNF was uninhibited by spermine after LPS stimulation.

It has previously been proposed that fetuin can function as a carrier of cell-modulating agents. We next showed that fetuin binds spermine by measuring the concentration of spermine after fractionation of a fetuin/spermine mixture (0-20 μM fetuin / 100 μM spermine) via ultrafiltration. These results revealed that one molecule of fetuin is capable of binding 4-6 molecules of spermine. Since spermine and fetuin levels are both extremely high in the fetus and amnion, it now appears that they are ideally poised to counter-regulate TNF production in pregnancy. Accordingly, the present invention provides a method for helping to prevent miscarriages during pregnancy, comprising administering an effective amount of a human fetuin polypeptide, and a method for treating pre-term labor during pregnancy, comprising administering an effective amount of a human fetuin polypeptide.

Example 1

This example illustrates the identification of fetuin as the protein responsible for some of the spermine-based activity observed in macrophage cultures. We added fractionated proteins from normal cells and assayed for their ability to restore the spermine-dependent inhibition of TNF production under serum-free culture conditions, because we hypothesized that these “spermine-non-responsive cells” had become deprived of a protein that was required to inhibit the production of TNF. After anion-exchange chromatography and SDS-PAGE gel elution, we isolated a single protein that mediated the responsiveness of macrophage cultures to spermine. Computer-based protein database analysis of the N-terminal amino acid sequence identified this protein as fetuin.

The role of fetuin as a mediator of spermine inhibition of TNF production was confirmed by adding highly purified fetuin (Sigma, St. Louis, MO), together with spermine, to LPS-stimulated human peripheral blood mononuclear cells (HuPBMCs). As shown in Figure 1A, the level of TNF produced by LPS-stimulated HuPBMCs was significantly reduced by increasing the concentrations of fetuin for a given dose of spermine. Fetuin alone had no effect on TNF production (data not shown), indicating that both spermine and fetuin were required for the suppression of TNF synthesis.

We prepared polyclonal antiserum against purified fetuin, using standard techniques. The anti-fetuin polyclonal antibodies abrogated spermine-mediated suppression of TNF production from LPS-stimulated macrophages, whereas the control (pre-immune) serum did not (Figure 1B). These data show that fetuin is required for spermine to suppress TNF production in normal human monocytes.

SEQUENCE LISTING

(1) GENERAL INFORMATION
(I) APPLICANT: Tracey, Kevin et al.
(ii) TITLE OF INVENTION: Prevention of Pregnancy Miscarriages

(iii) NUMBER OF SEQUENCES: 2

(IV) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Oster, Jeffrey B.
(B) STREET: DAVIS WRIGHT TREMAINE
(C) 1501 Fourth Avenue, 2600 Century Square
(C) CITY: Seattle
(D) STATE: Washington
(E) ZIP: 98101-1688

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
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(vii) ATTORNEY INFORMATION:

(A) NAME: Oster, Jeffrey B.
(B) REGISTRATION NUMBER: 32,585
(C) REFERENCE/DOCKET NUMBER: 0602WO

(viii) TELECOMMUNICATION INFORMATION

(A) TELEPHONE: (206) 628 7711
(B) TELEFAX: (206) 628 7699

(2) INFORMATION FOR SEQ ID NO: 1:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 359 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: N-terminal fragment

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
(2) INFORMATION FOR SEQ ID NO: 2:

(1) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 367 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: N-terminal fragment

(vi) ORIGINAL SOURCE:
   (A) ORGANISM: human

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

1 MKSLVLLLCL AQLWGCHSAP HGPGLYRQP NCCDPETEAA ALVAIDYING 35
51 NLPGWGYKHTL NQIDEVKWVP QPSGELFIEI EIDTLETTCH VLDPTPVARC
35 101 SVRQLEKHAV SGEGDCLQLK LDGKFSVYVA KCDSSPDSAE DVRKVCQDCP
151 LLAPLNDTRV VHAANAKAA VNAQNNNGSNF QLEEISRAQL VPLPRSTYVE
201 FTVSSTDCAV KAATAAACKN LLAEKQYGFCA KATLSEKLLGG AEAVTCTVF
251 QTQPVTSQPQ PEGANAEVPT PVVDPDAPPS PPLGAPGPLPP AGSPPDSHVL
301 LAAPPQGQLH RAYHLRHTF MVVVSLSGPS GEVSHPKTR TVVQPSVGAA
351 AGPVVPPCPG RIRHFKV 367
We claim:

1. A method for helping to prevent miscarriages during pregnancy, comprising administering an effective amount of a human fetuin polypeptide.

2. The method of claim 1 wherein the human fetuin polypeptide has a primary sequence according to SEQ ID NO.1 or SEQ ID NO. 2, or a shortened fragment thereof having at least 250 amino acid residues.

3. A method for treating pre-term labor during pregnancy, comprising administering an effective amount of a human fetuin polypeptide.

4. The method of claim 3 wherein the human fetuin polypeptide has a primary sequence according to SEQ ID NO.1 or SEQ ID NO. 2, or a shortened fragment thereof having at least 250 amino acid residues.