Haptoglobin is present in human endometrium and shows elevated levels in the decidua during pregnancy

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Assuming that haptoglobin, by virtue of its immunomodulatory properties, could be a regulatory factor during reproduction, its presence in the human uterus was determined. Protein extracts from endometrial tissue samples of pregnant and non-pregnant women were analysed by the immunoblot technique and the intensities of specific bands were quantified. Bands corresponding to haptoglobin were identified in tissue samples obtained from both sources. Protein, purified by high-performance liquid chromatography and monitored by Western blot analysis for its haptoglobin identity, was used for amino-terminal sequencing. Sequencing of the 42 kDa protein identified it as the β chain of haptoglobin. Immunohistochemistry was used to corroborate the findings and to visualize the distribution of haptoglobin in the tissue. The intensity of the 42 kDa band derived from decidua graviditatis was significantly higher than the intensity of bands derived from non-pregnant endometrium in the proliferative phase (P < 0.01) and in the secretory phase (P < 0.05). Immunohistochemical staining with anti-human haptoglobin antibody elicited strong signals in the decidua graviditatis and weaker signals in the normal endometrium, with the latter showing menstrual cycle-dependent variation. Moderate staining of stroma and a lack of staining of epithelium in the proliferative phase contrasted with the strong staining of stroma and moderate level of staining of epithelium observed in the secretory phase. Haptoglobin in the uterus may exert several functions such as the known binding of haemoglobin, but could also be involved in the multi-factorial mechanism protecting the fetus from a maternal allograft-like immune response.

Key words: decidua/haptoglobin/human/pregnancy/uterus

Introduction

A number of endocrine and metabolic changes, which together are called the acute phase response (APR), occur in mammals at the onset of an inflammatory process, regardless of whether this is caused by immunological reactions, bacterial or viral infections, or other noxious stimuli. The APR consists of a cellular and a biochemical part, the former is characterized by leukocytosis, whereas the latter is characterized by the synthesis of acute phase proteins (APP) by hepatocytes. In their turn, the APP modulate immune responses (Koj, 1985) and inflammatory processes (Bowman, 1993; Baumann and Gauldie, 1994). Haptoglobin, a member of the APP family, displays a great variety of important biological functions. Besides its well known haemoglobin binding capacity (Giblett, 1968), it also exerts an inhibitory effect on prostaglandin synthesis (Jue et al., 1983), a promotory effect on angiogenesis, the proliferation and differentiation of endothelial cells of blood vessels (Cid et al., 1993), as well as an inhibitory effect on cathepsin B activity (Snellman and Sylven, 1967). Haptoglobin also has immunosuppressive properties: it blocks neutrophil responses to various stimuli (Oh et al., 1990), inhibits lectin-induced lymphocyte transformation (Kudo et al., 1982) and decreases antibody production (Oh et al., 1990).

The possibility that haptoglobin may play a role in human reproduction has recently been discussed. It has been shown that obese patients have a higher level of blood haptoglobin (Hannerz et al., 1995). It is also known that obese patients have a greater incidence of infertility than normal weight
patients (Norman and Clark, 1998). Obesity is associated with infertile conditions such as polycystic ovarian syndrome and recent findings indicate that insulin-signalling pathways play an important role in the regulation of fertility (Burks et al., 2000).

The levels of haptoglobin in serum during pregnancy are elevated, showing a biphasic pattern with peaks in the first and third trimester (Haram et al., 1983). The levels of naturally occurring antibody against haptoglobin in serum have been shown to be lower in infertile women compared with normal fertile controls (Berkova et al., 1997). These clinical findings are complemented by experimental data which show haptoglobin expression in various organs of the reproductive system: the rat ovary (O’Bryan et al., 1997), the mouse uterus and ovary (Friedrichs et al., 1995) and the rabbit uterus (Olson et al., 1997). At present, however, there is no clear picture of how and at what levels the reproductive system can be influenced by haptoglobin. Undoubtedly the pregnant uterus, the ‘interface’ between the maternal and fetal immunosystems, is a place where immunological reactions are likely to happen, and where haptoglobin could be active. The question then would be from what source did the haptoglobin originate. Haptoglobin was originally thought to be synthesized exclusively by hepatocytes (Bowman and Kurosky, 1982). After its release into the bloodstream, a fraction of haptoglobin is taken up by neutrophils (Wagner et al., 1996) from where it can be released again upon stimulation in the periphery (Berkova et al., 1999). However, there is a growing body of evidence which suggests that haptoglobin or haptoglobin-related protein (with similar structures to haptoglobin) originate from other sources including the organs of the reproductive system (Friedrichs et al., 1995; O’Bryan et al., 1997; Olson et al., 1997) including the human uterus (Sharpe-Timms et al., 2000).

Haptoglobin is a multimer consisting of two α (α1 and α2) and two β chains composed in various combinations, giving rise to the different phenotypes of haptoglobin (Bowman and Kurosky, 1982). The three major phenotypes contain two β chains, but can have either two α1 (H-1-1), two α2 (H-2-2), or one α1 and one α2 (H-2-1). In an attempt to correlate the prevalence of the haptoglobin phenotypes to the disposition of diseases, no links were found with respect to the reproductive system (Langlois and Delanghe, 1996). More recently, however, it has been shown that women with the H-1-1 phenotype have a higher natural fertility potential than women with other haptoglobin phenotypes (Bottini et al., 1999).

The major goal of the present study was to see whether the human uterus contains haptoglobin and if so whether there are differences in the uterine levels of haptoglobin between pregnant and non-pregnant uteri. The results are discussed in the context of the regulatory role played by the immunosystem during pregnancy and the possible involvement of haptoglobin.

Materials and methods

Patient material

Biopsy material was obtained from the endometrium of patients admitted for tubal ligation or from the decidua of patients undergoing a Caesarean section at term (38–40 weeks gestation). After removal of the placenta, the decidual sample was obtained from the pregnant uterus outside the area of the attachment of placenta. In order to avoid the results being influenced by conditions which could alter the expression of proteins, certain exclusion criteria for the study were defined as follows: the presence of pelvic inflammatory diseases, overt infection or the intake of anti-inflammatory drugs, steroid derivatives or hormones during the previous 12 months. The phases of the menstrual cycles of the patients were determined according to endometrial morphology. Endometrium of 20 patients admitted for tubal ligation (nine in proliferative phase and 11 in secretory phase) and decidua of 12 patients undergoing a Caesarean at term (38–40 weeks gestation) were subjected to immunoblotting. Endometrium of 20 patients admitted for tubal ligation (10 in proliferative phase and 10 in secretory phase) and decidua of 12 patients undergoing a Caesarean section at term were analysed by immunohistochemical methods.

Prior to participating in the study, all patients signed a consent form, which was approved by the Institutional Ethics Committee, Centre de recherche de St-Francoise d’Assise, CHUQ, Canada, Québec.

Immunohistochemistry

The biopsy samples were fixed in a solution of 10% formaldehyde in 0.1 mol/l phosphate buffer (pH 7.4), then embedded in paraffin wax. Sections, 5 µm thick, were mounted on polylysine-coated glass slides. From each sample, some sections were selected for histological staining and morphological assessment and the rest were kept for immunostaining (Sternberger, 1979). The immunohistochemical study was carried out using tissue showing normal morphology. Sections were incubated sequentially with 2% normal goat serum in phosphate-buffered saline (PBS), with primary rabbit anti-human haptoglobin antibody (Sigma) (10 µg/ml), with a secondary biotinylated goat anti-rabbit antibody (Sigma) (1:1000); and with streptavidin–peroxidase complex, then developed using 3,3’-diaminobenzidine tetrahydrochloride (DAB) (Sigma) as chromogen. As negative controls, normal rabbit antibodies (Sigma) or irrelevant antibodies at the same concentration as the primary antibody were used. After immunostaining, the slides were evaluated by two independent observers. The results were expressed in a semi-quantitative manner as described (Ruck et al., 1994): no staining (−), weak staining (+), moderate staining (++), and strong staining (+++); number of cells stained: a < 10%, b = 10–50%, c > 50%.

Extraction of proteins

Biopsy samples of human endometrium or decidua of ~3 mg wet tissue were used. In order to avoid the consequence of immunoblotting being influenced by the presence of serum haptoglobin, endometrial blood vessels were removed mechanically, and biopsy samples were extensively washed with PBS. Afterwards the samples were chopped and further washed extensively with PBS and homogenized. Tissue proteins were extracted in a buffer solution containing 0.5% Triton X-100, 10 mmol/l HEPES (pH 7.4), 150 mmol/l NaCl, 2 mmol/l EGTA, and 2 mmol/l EDTA. After centrifugation for 20 min at 15 000 g, the concentration of protein in soluble fractions was measured according to a published method (Bradford, 1976). The concentration of total proteins for each sample was adjusted to 0.5 mg/ml.

Immunoblot analysis

Proteins in the soluble fraction of the tissue extracts were separated under reducing conditions in 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) according to a published method (Laemmli, 1970). The 15 µl samples containing 7.5 µg of proteins in 3-fold-concentrated Laemmli solution (200 mmol/l Tris,
Increased levels of haptoglobin in human decidua

Figure 1. Immunoblot analysis of haptoglobin in normal endometrium and decidua. Constant amounts of protein were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and analysed by Western blot using a rabbit anti-human haptoglobin antibody (1:100). As a positive control, 2 µg of haptoglobin from pooled plasma were run in an adjacent track (haptoglobin lane). Compared to endometrium (lanes 1, 2 and 3), decidua (lanes 4, 5 and 6) showed a much stronger signal. The endometrium samples were obtained from two patients in the proliferative phase (lanes 1 and 3) and from one patient in the secretory phase (lane 2).

Figure 2. Immunochemical characterization of proteins detected with anti-haptoglobin antibody. Immunoblot analysis of haptoglobin standard: 2 µg of haptoglobin from pooled plasma (Sigma) (haptoglobin lane), protein extracted from decidua (lane 1) and protein extracted from endometrium (lane 2), electrophoresed under reducing conditions (12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis), then detected with rabbit anti-human haptoglobin antibody. Preincubation of the primary rabbit anti-human haptoglobin antibody with 4 µg of human haptoglobin (Sigma) resulted in abolition of the specific bands (lanes 3 and 4). As a further negative control, the primary antibody was substituted by normal rabbit IgG, resulting in abolition of the signal (lanes 5, 6).

Figure 3. Levels of haptoglobin in non-pregnant endometrium and decidua graviditatis. Mean ± SEM of optical density (OD) ratios (experimental 42 kDa band/control 42 kDa band) representing relative amounts of β chain of haptoglobin detected in the uteri of pregnant and non-pregnant women. Western blot analysis using a rabbit anti-human haptoglobin antibody was used to visualize the bands. The mean OD ratios of the 42 kDa band in normal endometrium showed cycle-dependent variation, being higher in the secretory phase (0.44 ± 0.07; n = 11) than in the proliferative phase (0.19 ± 0.04; n = 9) (P < 0.05). Much stronger signal was observed in the decidua (0.68 ± 0.11; n = 12). The differences in the mean OD ratios of the 42 kDa bands in decidua and endometrium from either proliferative (P < 0.01) or secretory (P < 0.05) phases were statistically significant.

Figure 4. Purification of the 42 kDa protein extracted from the decidua of pregnant women. Protein extracted from decidua was purified on a Hi Prep 16/60 S-200 Sephacryl column. The fractions containing immunoreactive protein were pooled, concentrated 30-fold and loaded onto a Bio-Scale DEAE Anion Exchange Column. After washing unbound proteins from the column with starting buffer A (20 mmol/l Tris, pH 7.4), the bound proteins were eluted with a gradient of 0–0.5 mol/l NaCl, followed by a gradient of 0.5–1 mol/l NaCl. Eluted protein was concentrated and analysed by immunoblotting with anti-human haptoglobin antibody. One line shows the optical density profile of eluted proteins detected at 280 nm (left axis), and the other line shows the profile of the NaCl gradient (right axis).

In some experiments, controls included primary antibodies preincubated with haptoglobin standard before use in Western blot analysis. Haptoglobin standard was commercially available haptoglobin purified from human pooled plasma (Sigma). In some experiments, tissue proteins were separated on SDS–PAGE without β-mercaptoethanol (non-reducing conditions) before incubation with commercial anti-
N.Berkova et al. Figure 5. Immunohistochemical analysis of haptoglobin in decidua and endometrium. Immunostaining with anti-haptoglobin antibody led to strong reactivity in both the epithelial and stromal cells of the decidua (A) and a weaker signal in endometrium: moderate staining of the stroma and no staining of epithelium in the proliferative phase (C), contrasted with strong staining of the stroma and a moderate staining of epithelium in the secretory phase (E). Substitution of anti-haptoglobin antibody with normal rabbit IgG gave a negative reaction for decidua (B) and endometrium either in proliferative (D) or secretory (F) phases.

human haptoglobin antibodies. The anti-human haptoglobin antibody was developed in the rabbit after immunization with haptoglobin purified from the human pooled plasma (Sigma).

Estimation of the relative amounts of antigen
A Bioquant IV system was used to measure the integrated optical density of the 42 kDa bands (representing the \( \beta \) chain of haptoglobin) and the \( \beta \) chain of a haptoglobin standard (4 \( \mu \)g/well) blotted on the same membrane. The normalized optical density of an experimental band was defined as the ratio between the optical density of that band and the haptoglobin reference.

Under the standard set of conditions for immunoblot analysis, with the amount of total protein loaded being constant in each experiment, the normalized optical density of the bands is positively correlated with the amount of haptoglobin in the loaded protein samples.

Gel filtration chromatography
The proteins detected with the rabbit anti-human haptoglobin antibody were purified as described previously (Berkova et al., 1997). A volume of 1.7 ml of decidual protein extract (3 mg/ml) was loaded on a Hi Prep 16/60 S-200 Sephacryl High Resolution gel filtration column (Pharmacia Biotech) at 4°C in 50 mmol/l Na\(_2\)PO\(_4\) and 125 mmol/l NaCl (pH 7.0) using a sample loop. The flow rate was 0.5 ml/min and 2 ml samples were collected. The proteins in eluted fractions were separated electrophoretically and blotted with rabbit anti-human haptoglobin antibody. The fractions containing immuno-
reactive proteins were pooled, then concentrated 30-fold on a Speed Vac concentrator (Savant Instruments Inc., Farmingdale, NY, USA) and dialysed against the buffer containing 20 mmol/l Na2PO4 and 40 mmol/l NaCl (pH 7). Afterwards the samples were purified by high-performance liquid chromatography (HPLC).

**HPLC**

The chromatographic system consisted of two LKB 2150 pumps, an LKB 2152 gradient controller, and an LKB 2221 integrator (LKB Pharmacia). Twenty µg of protein were loaded on a Bio-Scale DEAE Anion Exchange Column 10 mm × 64 mm (Bio-Rad) using the LKB 2154 HPLC injection valve with a 500 µl loop at a flow rate of 1 ml/min. Output was recorded with an LKB 2158 Uvicord SD detector. After sample application, unbound proteins were flushed from the column with 6 ml of starting buffer A (20 mmol/l Tris, pH 7.4). Separation of the proteins by ion-exchange chromatography was accomplished by adding a gradient volume of 10 ml of buffer B (1 mol/l NaCl, Tris pH 7.4) to reach a concentration of 500 mmol/l NaCl (50% B) which was held for 5 min. Afterwards a gradient volume of 2 ml leading to an NaCl concentration of 1.0 mol/l (100% B) was used and held at 1.0 mol/l for 4 h before the column was re-equilibrated with 12 ml of starting buffer A. Eluates were concentrated 100-fold on a Speed Vac Concentrator and the presence of the protein as detected by a rabbit anti-human haptoglobin antibody was assessed by immunoblot analysis. The procedure was repeated five times to obtain a sufficient quantity of protein.

**Determination of NH2-terminal amino acids of the 42 kDa protein extracted from the decidua of pregnant women**

After purification by gel filtration and HPLC DEAE ion-exchange chromatography, proteins (25 µg) were heated at 37°C for 15 min and separated by SDS–PAGE as described above. In order to prevent amino-terminal blocking during electrophoresis, 100 mmol/l sodium triglycolylate (ICN Biomedicals) was added to the upper reservoir. Blotting onto Trans-blot polyvinylidene difluoride (PVDF) membrane (Bio-Rad) was performed as described above. Protein was visualized by staining with Coomassie Blue (0.1% solution in 50% methanol), then the 42 kDa band was isolated and the membrane was destained in 50% methanol. Afterwards the blotted proteins were subjected to NH2-terminal sequencing by the automatic Edman degradation procedure performed on an Applied Biosystems model 474A (Foster City, CA, USA) pulsed liquid protein sequencer.

**Statistical analysis**

The differences in intensity between the 42 kDa bands from decidua and from endometria were assessed by analysis of variance (ANOVA). The values are expressed as mean of the normalized optical density (defined in Materials and methods) and SE. The Tukey’s HSD test was applied for comparison of means between groups. The level of significance was set at P < 0.05.

**Results**

**Detection of haptoglobin in endometrium and decidua by Western blot analysis**

Proteins extracted from endometrium and decidua were separated electrophoretically and subjected to immunoblot analysis. Using rabbit anti-human haptoglobin antibodies three bands with mol. wts of 16, 20 and 42 kDa were obtained (Figure 1, lane 1–6). Among the 32 tissue samples examined all showed the 42 kDa band, but only 11 showed all three bands (Figure 1, lane 2, 6), while the haptoglobin standard revealed all three of the bands in the same molecular weight ranges (Figure 1, haptoglobin lane).

As a positive control haptoglobin standard, commercially available haptoglobin purified from pooled human plasma (Sigma) was run in an adjacent track of the PAGE gel and blotted with anti-human haptoglobin antibody in each Western blot analysis. Electrophoresis under reducing conditions showed three bands with apparent mol. wts of 16, 20 and 16 kDa. As a negative control, the primary rabbit anti-human haptoglobin antibody was substituted with normal rabbit IgG, resulting in abolition of the signal (Figure 2). As a further control, the primary rabbit anti-human haptoglobin antibody was blocked by an excess of haptoglobin standard and this also resulted in abolition of the specific bands (Figure 2).

In a complementary experiment, proteins extracted from endometrium and decidua were separated electrophoretically...
in non-reducing conditions and further analysed by immunoblot analysis using anti-haptoglobin antibody. Rabbit anti-human haptoglobin antibody revealed a high mol. wt band of 110 kDa corresponding to the polymer form of haptoglobin. A faint band was also detected at ~42 kDa. Haptoglobin standard analysed in the same way under non-reducing conditions showed a similar pattern (data not shown).

**Variation of haptoglobin levels in normal endometrium and decidua**

In order to compare the relative amount of haptoglobin in decidua and in normal endometrium at different phases of the menstrual cycle, the normalized optical densities of haptoglobin β chains in the examined samples were determined. The mean of OD ratios of the 42 kDa band in normal endometrium showed menstrual cycle-dependent variation, being higher in the secretory phase (0.44 ± 0.07) than in the proliferative phase (0.19 ± 0.04) \( (P < 0.05) \). Decidua showed a much stronger signal (0.68 ± 0.11), and this was significantly higher than that for endometrium in the proliferative phase \( (P < 0.01) \). The signal from decidua showed a smaller increase compared to that from the endometrium in the secretory phase (0.44 ± 0.07), but the difference was still significant (Figure 3).

**Purification and amino-terminal sequence of the 42 kDa haptoglobin protein**

Gel filtration chromatography was carried out on protein extracts from decidua. The fractions containing immunodetectable bands were pooled, and protein was purified by HPLC. Western blot analysis resolved the protein containing the 42, 20 and 16 kDa bands in the fraction corresponding to 0.26 mol/l NaCl (Figure 4). After separation by electrophoresis, the purified proteins were transferred onto a PVDF membrane for amino-terminal sequence analysis. The following sequence was obtained for the first 12 amino acids of the 42 kDa protein: Ile-Leu-Gly-Gly-His-Leu-Asp-Ala-Lys-Gly-Ser-Phe. The sequence was identical to the first 12 amino acids of the β chain sequence of human haptoglobin in the Swiss protein data bank (NCBI).

**Immunohistochemical staining of haptoglobin in decidua and normal endometrium**

In order to confirm the above findings and to define the histological distribution of haptoglobin, endometrial and decidual tissue were analysed immunohistochemically. Immunostaining of all decidual samples with rabbit anti-human haptoglobin antibodies led to strong reactivity in both the epithelial and stromal cells (Figure 5A).

Immunohistochemical staining with anti-human haptoglobin antibody showed weaker signals in the normal endometrium compared to that in decidua graviditatis. Contrasting variations of signal in normal endometrium were detected between the different phases of the menstrual cycle. Neither surface nor glandular epithelium were stained in the proliferative phase (except in one sample showing moderate staining in glandular epithelium), while moderate epithelial staining was observed in the secretory phase of the menstrual cycle. Stromal cells of normal endometrium were stained with anti-haptoglobin antibody in both phases of the cycle (Figure 5C, E), but the staining for haptoglobin in stroma in the proliferative phase was less than that for the secretory phase (Table I). Substitution of primary antibody either with rabbit normal IgG (Figure 5B, D, F) or irrelevant antibody (data not shown) at an equal concentration gave negative reactions.

**Discussion**

The original understanding of the function of haptoglobin was its ability to bind free haemoglobin (Bowman and Kurosky, 1982). More recently, it has also been identified as an acute phase plasma protein (Koj, 1985). Now it seems that haptoglobin also plays an important further role in the functioning of both the male and female mammalian reproductive system. While the presence of haptoglobin in Sertoli, Leydig and germ cells appears to be essential for keeping iron levels sufficiently high for spermatogenesis (O’Bryan et al., 1997), the presence of haptoglobin in the uterus requires further explanation (Friedrichs et al., 1995; Olson et al., 1997).

Previously we have shown that when compared with normally fertile women, infertile women have a decreased level of anti-haptoglobin antibody in their serum (Berkova et al., 1997). This suggested that haptoglobin might play a role in fertility. In the context of haptoglobin modulating immune responses and the uterus being regarded as the ‘interface’ between the maternal and fetal immunosystems, we looked for the presence of haptoglobin in the uterus. Our first results obtained from the analysis of decidual tissue indicated such a presence (Berkova et al., 1998). Indirect evidence was also derived from a study showing substantial haptoglobin levels in the uterine fluid during the secretory phase (Beier and Beier-Hellwig, 1998). The present study confirms and extends these findings. Using anti-human haptoglobin antibodies, we have isolated three proteins of 16, 20 and 42 kDa from tissue samples of pregnant and non-pregnant uteri. These mol. wts correspond to the two forms of α chains and the β chain of haptoglobin respectively. Although mol. wts have been documented in the literature (Kuhajda et al., 1989; Hoffman et al., 1996) other sizes for haptoglobin subunits have also been reported (Bowman and Kurosky, 1982). The differences, however, are due to the various analysis methods used. SDS–polyacrylamide gel electrophoresis, as used in our experiments, renders significantly higher values than, for instance, sequence analysis: 40–42 versus 33 kDa for the β and 16 versus 9 kDa for the α₁ chain (Bowman and Kurosky, 1982). Although the results from the Western blot analysis were sound, we confirmed the nature of one of the proteins by amino-terminal sequencing. The sequence of the first 12 amino acids of the 42 kDa protein was identical to that known for the human haptoglobin β chain. We therefore concluded that human endometrium contains haptoglobin.

Immunostaining and Western blot analysis of endometrium samples taken at different menstrual cycle phases showed phase-related haptoglobin level variations. Immunohisto-
chemistry showed moderate staining of stroma and negative staining of epithelium in the proliferative phase in contrast to strong staining of stroma and moderate staining of epithelium during the secretory phase. The relative amount of haptoglobin in the Western blot analysis was significantly higher in the secretory phase than in the proliferative phase. Similar results have been obtained (Sharpe-Timm et al., 2000) using a different methodological approach to show a non-significant increase in uterine haptoglobin during the secretory phase. With both methods, immunostaining and Western blot analysis, we further showed that decidual tissue of the pregnant uterus contains significantly higher levels of haptoglobin than does normal endometrium. Since we did not investigate the synthesis of haptoglobin in decidua, the sequestration from the circulation is a possibility, although an unlikely one.

The angiogenic potential of haptoglobin may account for the elevated haptoglobin levels in the decidua. It is evident that placental angiogenesis is an important process for embryonic development. However, the angiogenic property of haptoglobin has been shown in connection with pathological angiogenesis such as systemic vasculitis or endometriosis (Cid et al., 1993; Oosterlynck et al., 1993; Berkova et al., 1998; Sharpe-Timms et al., 2000) and might not be relevant in the physiological environment.

Haptoglobin may also participate in immune mechanisms which take place in the pregnant uterus and which are necessary for the maintenance of the integrity of the feto-placental unit. Haptoglobin has been shown to possess general immunosuppressive properties (Kudo et al., 1982; Oh et al., 1990) some of which can be mediated through the binding of haptoglobin to B-cell lectin CD22 (Hanasaki et al., 1995). Haptoglobin may also interact with natural killer (NK) cells which are present in human decidua (Loke and King, 2000). Since decidual cells express ICAM (Ruck et al., 1994), NK cells express CD11b/CD18 integrin (Timonen et al., 1988) and there is a high binding affinity between ICAM and CD11b/CD18 (Diamond et al., 1993) and there is a low binding affinity between haptoglobin and CD11b/CD18 (El Ghimati et al., 1996), it is possible that haptoglobin may act by modifying NK-decidual cell interaction.

These two mechanisms could contribute to attenuate maternal immune responses provoked by feto-placental antigens when accidentally in contact with the maternal immune system.

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References

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