Endometriotic haptoglobin binds to peritoneal macrophages and alters their function in women with endometriosis

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Objective: To evaluate the effects of endometriotic haptoglobin on peritoneal macrophage function.

Design: Prospective laboratory study.

Setting: School of medicine.

Patient(s): Twenty-three women with and without endometriosis.

Intervention(s): Peritoneal macrophages cultured without or with haptoglobin.

Main Outcome Measure(s): Peritoneal macrophage haptoglobin immunoreactivity, adhesion, and interleukin-6 (IL-6) production.

Result(s): In vivo, significantly more peritoneal macrophages from women with endometriosis bound haptoglobin and exhibited reduced adhesion compared to women without endometriosis. In vitro, haptoglobin treatment significantly decreased peritoneal macrophage adherence only in women without endometriosis; this effect was not seen in women with endometriosis, probably owing to in vivo haptoglobin saturation. Conversely, haptoglobin treatment robustly increased IL-6 production only by macrophages from women with endometriosis, suggesting differential immune response in these women.

Conclusion(s): Endometriotic lesions synthesize and secrete a unique form of haptoglobin (endometriosis protein-I) that is up-regulated by IL-6. This study shows that haptoglobin adheres to peritoneal macrophages; decreases adhesion, which may influence phagocytic function; and up-regulates IL-6 production. Hence, a feed-forward loop is proposed whereby endometriotic haptoglobin decreases macrophage phagocytic function while increasing IL-6 production, which in turn increases endometriotic haptoglobin and promotes establishment of endometriosis. (Fertil Steril 2002;78:810–9. ©2002 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, endometrium, peritoneal macrophages, haptoglobin, interleukin-6

Endometriosis is a gynecologic disorder defined as ectopic growth of endometrial glands and stroma (1, 2). Sampson’s theory of retrograde menstruation states that the disease process initiates with endometrial sloughing at menses, with subsequent retrograde flow of endometrial fragments through the fallopian tubes and into the peritoneal cavity (3, 4). Studies have confirmed that retrograde menstruation occurs in most women with patent fallopian tubes (5).

Data in the current literature are conflicting as to whether women with endometriosis have an increased volume of peritoneal fluid that is associated with an increased volume of menstrual debris and the extent of endometriosis (6–9). It is also unclear whether shed endometrium is considered self and has immune tolerance in the peritoneal cavity (10, 11). However, disintegrating menstrual debris contains normal as well as apoptotic and necrotic cells and has increased expression of metalloproteinase enzymes, disordered expression of adhesion molecules, and loss of filamentous action from cell borders (12–15). Phagocytic macrophages recognize these changes in cell surface markers, which are not present on normal cells (16, 17).

Macrophages are the primary line of local host defense (18), and their phagocytic function represents the initiation of the immune response in the peritoneal cavity (16, 19). The phagocytic process involves chemotaxis to the focus site, adherence to substrate, and ingestion...
and destruction of foreign material through such mechanisms as the oxidative burst associated with superoxide anion (O$_2^-$) production (20–22). Macrophage activation also causes synthesis and release of biologically active cytokines and growth factors that may regulate immune response (18). The phagocytic activity of peritoneal macrophages clears extravasated erythrocytes and exfoliated cells and should eliminate disintegrating, retrogradely shed endometrium, which is perceived as foreign material in the peritoneal cavity.

Pioneering work done two decades ago (23–26) showed that maturation and activation of peritoneal macrophages in women with endometriosis were altered compared to women without this disease. Yet, the phagocytic potential of these activated macrophages for shed endometrial tissues and the importance of their secretory products were not known. To date, it remains controversial whether the immune anomalies observed in women with endometriosis are a cause or effect of the disease.

More recently, others have demonstrated that endometriotic lesions and endometrial cells from women with endometriosis are inherently resistant to apoptosis and immune-mediated elimination and can utilize the products of an activated immune system to establish ectopic foci of disease (27, 28). Research has also shown a decrease in the capacity of monocyte- and macrophage-mediated cytolysis of misplaced endometrial cells (28). An important hypothesis is that unique characteristics inherent in the endometrium of women with endometriosis may permit subsets of cells to acquire the ability to quantitatively and qualitatively control the nature of the immune response (27). Others, however, have found no difference in apoptosis or expression of Bcl-2, an anti-apoptotic factor, between endometrial tissues from women with and those without endometriosis (29, 30). Therefore, other anomalies in the eutopic endometrium from women with endometriosis (31), whether present due to genetic predisposition (32) or acquired in an altered peritoneal environment (27), may control the immune response.

Our research has shown endometriotic lesions, eutopic endometrium, and shed endometrial tissue fragments in the peritoneal fluid of women with endometriosis differentially express several genes and proteins that may alter immune response (33, 34). Of note, these tissues synthesize and secrete a uniquely glycosylated form of haptoglobin that we originally called endometriosis protein-I (33–38). Although the liver is the main site of haptoglobin production, haptoglobin is also produced at various extrahepatic sites (39–41). The most studied biological function of haptoglobin is the recapture of hemoglobin; however, haptoglobin has other biological activities distinct from this role (42, 43). In association with certain inflammatory states and some cancers, haptoglobin has immunomodulatory activity (44–49).

We therefore hypothesize that haptoglobin production by shed endometrium and endometriotic lesions in women with endometriosis masks receptors of phagocytic cells and permits establishment and persistence of endometriosis. In this study, we evaluated the effects of haptoglobin on peritoneal macrophage adhesion, because adhesion is a critical step in phagocytic function and peritoneal macrophage activation as measured by interleukin-6 (IL-6) production in women with and without endometriosis.

**MATERIALS AND METHODS**

**Human Subjects**

Peritoneal fluid was obtained from patients routinely presenting to physicians in the Department of Obstetrics and Gynecology, School of Medicine, University of Missouri–Columbia. All patients gave informed consent, and the institutional review board for the health sciences section approved the study.

Patients presenting for non-neoplastic gynecologic surgery were enrolled. The control group (n=12) consisted of fertile women without endometriosis presenting for laparoscopic tubal sterilization. The endometriosis group (n=11) contained women with infertility (8 of 11 [72.7%]) and/or pelvic pain (7 of 11 [63.6%]) presenting for diagnosis of endometriosis. Five of the women had stage I or II endometriosis, four had stage III or IV endometriosis, and stage was not available in two women.

The average age of the two groups was similar (mean ± SD, 30.8 ± 6.8 years in controls and 32.0 ± 6.1 years in women with endometriosis; P=.683, t-test). To control for potential effects of the participants’ hormonal status, all surgeries were performed during the proliferative stage of the menstrual cycle (days 3 to 12). The distribution of participants over cycle days was equivalent between the two groups.

Peritoneal fluid samples from additional donors (n=22) were excluded from these studies because these women used exogenous steroid-modulating medications at the time of surgery or had evidence of blood contamination. Only straw colored fluids were included in the study. Other exclusion criteria were history of acute or chronic inflammatory disease, malignant disease, and liver disease.

Fluids were collected immediately on entry into the peritoneal cavity, and no washings were performed. The concentration of haptoglobin in each fluid sample was evaluated by using an enzyme-linked immunosorbent assay (ELISA) developed in our laboratory (50). The Bradford method was used to measure total protein concentration of the peritoneal fluids.

**Peritoneal Fluid Macrophages**

Peritoneal macrophages were isolated by density centrifugation (24, 51) and washed with phosphate-buffered saline. Replicate aliquots of the macrophages were spotted on microscope slides and immunohistochemically stained with a
monoclonal antihuman CD68 antibody (1:100 dilution; DAKO, Carpentry, CA) with biotinylated antimouse IgG as the secondary antibody to evaluate the macrophage purification technique. A rabbit antihuman haptoglobin antibody (1:5000 dilution; DAKO) previously validated to recognize endometriotic haptoglobin (35), along with biotinylated antirabbit IgG as the secondary antibody, was used to detect haptoglobin immunoreactivity in replicate aliquots. The Vectastain ABC kit (avidin–biotin peroxidase complex; Vector Laboratories, Burlingame, CA) was used according to the manufacturer’s instructions. Peroxidase activity was demonstrated by incubation with 3,3’-diaminobenzidine substrate that yielded a brown pigment. Preimmune mouse and rabbit sera were substituted for the monoclonal and polyclonal primary antibodies, respectively, as negative controls. Whole blood smears were simultaneously stained with the CD68 antibody as a positive control for macrophage staining.

Morphometric analysis (52) was used to analyze the proportion of macrophages that were immunoreactive with the anti-CD68 and antihaptoglobin antibodies. The proportion of immunoreactive macrophages was calculated by dividing the number of immunoreactive cells by the total number of cells counted and multiplying by 100.

To determine sample size, a preliminary experiment was performed. An ocular grid was randomly placed over three different groups of 100 macrophages from one woman with endometriosis, and then three groups of 100 macrophages from three different women with endometriosis. No significant differences ($P>0.05$) in the proportion of macrophages that stained for CD68 or haptoglobin were found in the three groups of macrophages from one patient or between the groups of macrophages from the three patients with endometriosis. Calculations showed that counting and averaging the results from three groups of 100 macrophages per patient would more than adequately achieve the 95% confidence interval of ±10% of the mean.

Subsequently, three observers who were blinded to the origin of the macrophages counted the three groups of 100 macrophages from the 11 women with endometriosis and the 12 women without endometriosis and averaged the values before statistical evaluation. Interobserver and intraobserver variations were less than 7% and 5%, respectively.

**Peritoneal Macrophage Adherence**

Because adherence is the first step in the cascade of events that lead to the macrophage phagocytic process and activation, differences in peritoneal macrophage adherence capacity were evaluated by quantifying their adherence to a smooth plastic surface. Others have documented that this process resembles adherence to tissue (22, 53). This method is well described and often used by others (22, 53–55) and in our laboratory, with minor modifications.

Macrophages ($1 \times 10^5$ cells) were cultured in 96-well plates in a volume of 0.2 mL of RPMI-1640 (Gibco/BRL) supplemented with 5% fetal bovine serum (no haptoglobin present), penicillin, and streptomycin. At 10, 20, 30, and 60 minutes, the plates were gently agitated and 10-μL aliquots of media containing nonadherent peritoneal macrophages were removed and allowed to dry on glass slides. The nonadherent cells that dried on the slides were stained with anti-CD68 and were quantified by a single observer blinded to treatments. The adhesion index was calculated as $[1-(\text{peritoneal macrophages/mL of supernatant})]/[\text{the number of peritoneal macrophages/mL sample}] \times 100$, as reported elsewhere (22, 53–55). The adhesion index, as opposed to a raw cell count, was used to adjust for removal of cells and sample volume from the total pool at each time point reported (53–55).

First, adherence was evaluated by using freshly isolated, nontreated peritoneal macrophages from women with ($n=6$) and without ($n=5$) endometriosis, as described above. Next, a pilot experiment was performed to determine an effective dose and duration of exogenous haptoglobin treatment. Haptoglobin (0, 0.1, 1.0 or 10 μg/mL; Sigma Chemical Co., St. Louis, MO) was added to replicate cultures in triplicate of peritoneal macrophages from one woman with and one woman without endometriosis. Nonadherent cells were collected from cultures at 10, 20, 30, and 60 minutes in replicates of six.

The results of this pilot dose–response and time course experiment indicated haptoglobin treatment significantly but differentially altered macrophage adherence over time between women with and without endometriosis (see Results for more details). The time course experiment was then repeated in additional women with ($n=5$) and women without endometriosis ($n=5$) in triplicate with 0 or 10 μg/mL of haptoglobin.

Additional experiments were performed to determine whether the antiadherence effect of haptoglobin could be blocked by an antihuman haptoglobin antibody and were specific to haptoglobin as opposed to other proteins that are found in the sera or peritoneal fluid. Peritoneal macrophages from women without endometriosis (8 women per treatment) were cultured with 0 or 10 μg/mL haptoglobin, haptoglobin plus antihuman haptoglobin antibody (1:500; DAKO), or fetal bovine sera that did not contain haptoglobin (negative control). The antibody-blocking experiment was not performed with macrophages from women with endometriosis, because their macrophages bound haptoglobin on recovery from the peritoneal cavity and because addition of exogenous haptoglobin did not alter their adherence (see Results).

**Interleukin-6 Production by Peritoneal Macrophages**

To test the effects of haptoglobin on IL-6 production by peritoneal macrophages and estimate the effective haptoglobin dose, we performed a haptoglobin dose–response test.
First, peritoneal macrophages from women without endometriosis (n=3) were tested, because endogenous haptoglobin was already bound to the peritoneal macrophages from women with endometriosis on recovery from the peritoneal fluid. These control macrophages (1 × 10^5 cells cultured in 96-well plates in a volume of 0.2 mL of RPMI-1640, as described above) were cultured for 5 hours with increasing concentrations of haptoglobin (0, 0.1, 1.0 or 10 μg/mL; Sigma) in replicates of six.

Culture media were harvested, centrifuged to remove cellular debris, and snap frozen for analysis of IL-6. An ELISA developed in our laboratory was used to measure IL-6 concentrations in the culture media (29). This ELISA detects 0.05 ng of IL-6/200 μL; the intraassay and interassay coefficients of variation are less than 9% and 8%, respectively. Results are reported on a per cell basis because the exogenous haptoglobin treatment altered the total protein content per well.

Subsequently, we tested the effects of haptoglobin on IL-6 production by peritoneal macrophages from women without (n=6) and with (n=6) endometriosis. Macrophages were cultured with 0 or 10 μg/mL of haptoglobin for 5 hours, culture media were collected, and ELISA was used to quantify IL-6 concentration, as described above.

**Statistical Analysis**

Differences in patient age, peritoneal fluid volume, total protein concentration, haptoglobin concentration and haptoglobin concentration/total protein and macrophage density between women with and without endometriosis were analyzed by using the Student t-test. χ^2 analysis was performed to detect the proportion of peritoneal macrophages from women with and without endometriosis that were immunoreactive with the CD68 and haptoglobin antibodies.

To analyze macrophage adherence, arcsin square root transformation was performed to normalize linearly distributed percentage data because by definition, most percentage data are linearly distributed (56). This transformation modifies percentages so that the values more closely follow a normal distribution. The data must be values between 0 and 1, where 0 equals 0% and 1 equals 100%. The arcsin square root transformation supports accurate discrimination between repeated measures (e.g., time course) in smaller sample sizes. Parametric testing is subsequently applicable (56). After transformation, all data sets were normally distributed and passed equal variance testing.

Two-way analysis of variance (ANOVA) was performed to evaluate the adherence index of freshly isolated macrophages from women with (n=6) and without (n=5) endometriosis. The model variables were disease, time, and a disease × time interaction term. Results from the haptoglobin dose response/time course on replicate macrophage cultures from one woman with and one woman without endometriosis were evaluated by repeated-measures ANOVA, in which the model variables were disease, dose, time, and a disease × dose × time interaction term.

Because haptoglobin treatment differentially affected macrophage adherence between women with (n=5) and without (n=5) endometriosis, the effects of subsequent exogenous haptoglobin time course experiments were analyzed separately by two-way ANOVA using the model variables dose, time, and a dose × time interaction term. The effects of exogenous haptoglobin, anti-human haptoglobin-blocking antibody, and fetal bovine sera treatments on the adherence index of macrophages from women without endometriosis were analyzed by using one-way ANOVA. For all ANOVAs, when significant differences were detected, the Tukey multiple comparison procedure was performed.

One-way repeated-measures ANOVA and the Tukey post hoc test were used to evaluate IL-6 production by peritoneal macrophages in response to increasing concentrations of haptoglobin (data were normally distributed). Kruskal–Wallis ANOVA on ranks was used to perform multiple comparisons of IL-6 production by peritoneal macrophages from women with and without endometriosis that had been cultured with and without haptoglobin (data were not normally distributed).

**RESULTS**

**Peritoneal Macrophages**

Peritoneal fluid volume, total protein concentration, haptoglobin concentration, haptoglobin concentration as a proportion of total protein, and macrophage density were similar in women with and without endometriosis (Table 1). The proportion of isolated cells with positive CD68 immunostaining was also similar between women with and without endometriosis and demonstrated that peritoneal macrophage preparations were at least 85% pure (Table 1, Fig. 1B).

CD68-positive cells displayed a mixture of macrophage morphologic characteristics representing different stages of maturation, as described elsewhere (25, 57), whereas non-staining cells appeared morphologically similar to lymphocytes. Non–CD68-staining cells were immunoreactive with anti-CD45, a lymphocyte marker (data not shown). These results parallel those reported by other investigators using this macrophage purification technique (26, 51, 57). Significantly more of the isolated peritoneal macrophages, as identified by CD68 immunostaining and morphologic characteristics, from women with endometriosis showed immunoreactivity with the haptoglobin antibody compared to women without this disease (P<.001; β=.989) (Table 1, Fig. 1).

**Peritoneal Macrophage Adherence**

The adherence index of freshly isolated, nontreated peritoneal macrophages from women with endometriosis was reduced compared to women without endometriosis (P=.043; β=.437) (Fig. 2) and did not change over time.
within each group ($P=.713; \beta=.500$) (Fig. 2). No significant interaction was found between disease and time ($P=.949; \beta=.500$).

The pilot haptoglobin dose–response and time course

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The pilot haptoglobin dose–response and time course

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Women with endometriosis (n=11)</th>
<th>Women without endometriosis (n=12)</th>
<th>P and ( \beta ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>8.9 ± 7.8</td>
<td>2.5 ± 2.2</td>
<td>0.522, 0.398*</td>
</tr>
<tr>
<td>Total protein level (mg/mL)</td>
<td>42.4 ± 3.0</td>
<td>47.2 ± 4.7</td>
<td>0.888, 0.802*</td>
</tr>
<tr>
<td>Haptoglobin level (mg/mL)</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.643, 0.500*</td>
</tr>
<tr>
<td>Haptoglobin/total protein (µg/mg)</td>
<td>8.5 ± 4.9</td>
<td>9.3 ± 4.7</td>
<td>0.793, 0.785*</td>
</tr>
<tr>
<td>Macrophage density</td>
<td>1.0×10^6 ± 1.2×10^6</td>
<td>0.8×10^6 ± 0.4×10^6</td>
<td>0.513, 0.750*</td>
</tr>
<tr>
<td>CD68-positive macrophages (%)</td>
<td>91.1 ± 5.8</td>
<td>85.0 ± 13.5</td>
<td>0.277, 0.719*</td>
</tr>
<tr>
<td>Haptoglobin-positive macrophages (%)</td>
<td>91.3 ± 5.5</td>
<td>62.5 ± 21.8</td>
<td>0.001, 0.989*</td>
</tr>
</tbody>
</table>

Note: Data are the mean (± SD).

\( ^a \) t test.

\( ^b \) \( \chi^2 \) test.

The effect of haptoglobin treatment on the index (mean ± SD) of peritoneal macrophages from women with endometriosis and those without endometriosis. Addition of exogenous haptoglobin adhesion (right bar of each time point pair) had no effect on the peritoneal macrophage adhesion index in women with endometriosis (open bars; n=5 per bar; P=.316; β=.613) at any time point (P=.118; β=.284). However, haptoglobin (10 μg/mL) significantly reduced the peritoneal macrophage adhesion index in women without endometriosis (striped bars; n=5 per bar; P=.032; β=.664) at all times tested (*P<.001; β=.518).


experiment showed that in vitro, haptoglobin treatment significantly decreased adherence over time in macrophages from women without endometriosis (P<.001). The time course experiment was repeated in triplicate in additional women with (n=5) and without (n=5) endometriosis with 0 or 10 μg/mL haptoglobin. Administration of exogenous haptoglobin (10 μg/mL) did not affect adherence of peritoneal macrophages in women with endometriosis (P=.316; β=.613) at any time tested (P=.118; β=.284) (Fig. 3). In contrast, haptoglobin significantly decreased the peritoneal macrophage adhesion index in women without endometriosis (P=.032; β=.664) at all times tested (P<.001; β=.578) (Fig. 3).

The haptoglobin-induced reduction in the adherence index of peritoneal macrophages in women without endometriosis (n=8) was reversed by more than 50% with the addition of antihuman haptoglobin antibody but was not elicited by treatment with fetal bovine sera (fetal bovine sera), a complex mixture of proteins that does not contain haptoglobin (P<.001; β=.994).

Interleukin-6 Production by Peritoneal Macrophages
Haptoglobin at concentrations of 1.0 and 10 μg/mL significantly increased IL-6 production by peritoneal macrophages from women without endometriosis compared with concentrations of 0 or 0.1 μg/mL (P=.028; β=.569) (Fig. 4). Peritoneal macrophages from women with endometriosis who received haptoglobin produced more IL-6 (P=.023; β=.800) than did peritoneal macrophages from women with endometriosis who did not receive haptoglobin or those from controls who received or did not receive haptoglobin (Fig. 5).

DISCUSSION
Our studies show for the first time that freshly isolated peritoneal macrophages from women with endometriosis bind haptoglobin have a reduced adherence capacity, and more robustly produce IL-6 in response to haptoglobin treatment compared to peritoneal macrophages from women without endometriosis.

It is not known why peritoneal macrophages from women with endometriosis bind more haptoglobin in vivo than do those from women without this disorder, when the concentration of haptoglobin in the peritoneal fluid is similar in both groups. This effect may be due to fundamental differences in macrophage receptors between women with and without endometriosis or to the presence of an altered form of haptoglobin in the peritoneal cavity of women with endometriosis that binds to peritoneal macrophages.

We hypothesize that the copious haptoglobin bound to the peritoneal macrophages in vivo in women with endometri-
osis is the uniquely glycosylated haptoglobin that is secreted by endometriotic tissues from women with endometriosis and up-regulated by IL-6 (50). We previously called this protein endometriosis protein-I (33–38). The antihuman haptoglobin antibody used in this study to detect haptoglobin binding to peritoneal macrophages recognizes de novo synthesized and secreted endometriotic haptoglobin isolated from culture media and peritoneal fluid by Western blot analysis (35, 50). It also recognizes endometriotic haptoglobin localized in the stroma of tissue sections from endometriotic lesions and eutopic endometrium of women with endometriosis by immunohistochemical localization (35, 50).

A key concept is that endometriotic haptoglobin is differentially glycosylated than is serum haptoglobin. Endometriotic haptoglobin has more α-fucose and α(2,3)-linked sialic acid residues than does hepatic haptoglobin (58, 59). To initiate the phagocytic process, receptors on the macrophage must see a ligand found on the cell that is not present on normal, healthy cells. Ligands fitting these criteria include changes in the patterns of protein glycosylation (16). Although the mechanism by which endometriotic haptoglobin binds to macrophages is yet to be defined, it has been shown that haptoglobin binds to other immune cells, including monocytes, granulocytes, a subset of CD8+ T cells, and natural killer cells through specific carbohydrates or glycan chains present on haptoglobin (48, 60). Hence, the unique glycans on endometriotic haptoglobin compared with those on normal hepatic haptoglobin may differentially interact with macrophage receptors to elicit the anomalies in immune response seen in women with endometriosis.

Evidence suggests that haptoglobin glycans bind to at least some immune cells through interactions with the αMβ2 integrin receptor (also called CR3 and CD11b/CD18) (48). It is known that macrophages express the αMβ2 integrin receptor (61), but it is not known whether haptoglobin glycans serve as ligands for this receptor on these immune cells. Of note, activation of the macrophage αMβ2 integrin receptor by ligands other than haptoglobin markedly alters macrophage phagocytic function and enhances production of inflammatory mediators, including IL-1, IL-6, and tumor necrosis factor-α (49, 62, 63). In turn, increased production of these cytokines stimulates production of acute phase reactants, including haptoglobin, by hepatocytes (64). This suggests the existence of a positive feed-forward loop between
immune cells and hepatocytes in the control of the inflammatory response.

Despite several decades of investigation that has documented immunologic changes in women with endometriosis, it remains unclear whether women with endometriosis have an immune deficiency that allows endometriotic lesions to develop. We found that the total peritoneal macrophage counts in women with and without endometriosis were equivalent (Table 1), which agrees with the reported peritoneal fluid leukocyte concentrations in healthy patients of 0.5 to 2.0 \( \times 10^6 \) cells/mL, of which 85% are macrophages (65). Other studies have identified elevated peritoneal macrophage counts in women with mild endometriosis compared with fertile controls or other infertile patients (25, 65, 66). Our study did not identify differences in peritoneal macrophage counts when patients with mild or minimal disease (n=5) were compared to patients with moderate or severe disease (n=4). Additional studies are warranted to identify such differences. Cross-study comparisons are difficult: Reports with divergent or poorly described study populations debate whether macrophage concentration varies during the menstrual cycle, by tubal patency, degree of retrograde menstruation, extent of endometriosis, and presence of chronic peritoneal inflammation (66, 67).

Recent studies have identified a decrease in the ability of peritoneal macrophages to mediate cytolysis of misplaced endometrial tissues in the peritoneal cavity, associated with an increased resistance of these cells to apoptosis, in women with endometriosis compared with controls (28, 68). We have found that shed endometrial tissue fragments recovered from the peritoneal fluid of women with endometriosis express the endometriotic haptoglobin gene at levels equivalent to those in established endometriotic lesions (38). Taken together, these observations suggest that misplaced endometrial cells in women with endometriosis are more resistant to apoptosis and produce more endometriotic haptoglobin, offering a plausible but most likely partial explanation for the immune anomalies observed in women with endometriosis.

It is not known why macrophages from women with endometriosis responded more robustly to exogenous haptoglobin treatment with a threefold increase in IL-6 production compared to peritoneal macrophages from women without endometriosis. In vivo exposure of peritoneal macrophages to endometriotic haptoglobin in women with endometriosis may have up-regulated receptors on the macrophages, enhancing their activation status and IL-6 production in response to haptoglobin treatment in vitro. The elevated levels of IL-6 in peritoneal fluid from women with endometriosis (66, 67, 69) and in vitro production of IL-6 by ectopic and eutopic endometrial stromal cells from women with endometriosis (50, 70) may originate from peritoneal macrophages and resident endometrial macrophages, respectively.

In conclusion, we hypothesize that a local, positive feedforward loop exists between haptoglobin-expressing endometriotic lesions and peritoneal macrophages producing IL-6 (Fig. 6), similar to the positive feed-forward loop between macrophages and hepatocytes in control of the inflammatory response. Thus, by expressing haptoglobin, endometriotic cells may both avoid phagocytosis by peritoneal macrophages and stimulate them to produce IL-6, which propagates additional haptoglobin expression by the ectopic endometrial cells. Elimination of endometriotic haptoglobin by passive removal, active therapy, or modulation of the glycan-integrin mechanism may be an important adjunct immunotherapy for endometriosis.

References

Model of the effects of endometriotic haptoglobin on peritoneal macrophage function. Endometriotic haptoglobin is a differentially glycosylated form of haptoglobin, synthesized and secreted by endometriotic lesions, that binds to peritoneal macrophages and reduces their phagocytic capacity by blocking adherence while up-regulating macrophage interleukin-6 (IL-6) production. Hence, endometriotic lesions that express ENDO-I may both avoid phagocytosis by peritoneal macrophages while stimulating them to produce cytokines that propagate ENDO-I expression by the ectopic endometrial cells.

![Figure 6](image-url)