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Unilateral uterine ischemia/reperfusion-induced bilateral fetal loss and fetal growth restriction in a murine model require intact complement component 5

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Abstract

The role of complement in ischemia/reperfusion-induced fetal growth restriction and fetal loss is unknown. C5-deficient or wild type timed-pregnant mice were subjected to unilateral uterine ischemia/reperfusion on gestation day 13, either by (1) partial flow restriction by right ovarian artery clamping for 30 min, or (2) total flow restriction by clamping both ovarian and uterine arteries for 5 min. Ischemia/reperfusion-challenged pregnancy outcomes were compared to sham-operated controls five days later. Ischemia/reperfusion-treated wild type mice exhibited significantly increased bilateral fetal loss, which was greater in total flow restriction than in partial flow restriction, and decreased fetal weights, which were the same in total flow restriction and partial flow restriction for the surviving fetuses. Placental weights were unchanged by treatments. Ischemia/reperfusion increased uterine, but not placental, myeloperoxidase activity, which correlated with fetal loss. In contrast, C5-deficient mice were protected from both fetal growth restriction and fetal loss, and exhibited no increase in myeloperoxidase activity. These results demonstrate that unilateral uterine ischemia/reperfusion results in bilateral fetal loss and fetal growth restriction, mediated by a systemic mechanism. In the current model, this pathological process is completely dependent on intact complement component 5.

Keywords

Fetal growth restriction; inflammation; mouse; myeloperoxidase; placenta; uterus

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Xiao-Wu Qu (deceased in January 2012)

Sadly the first author of this paper, Dr. Xiao-Wu Qu, died recently as a result of tragic circumstances. We all cherish the memory of his life and his work.

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1. Introduction

Fetal growth restriction (FGR) is an important cause of fetal mortality and morbidity, underlies postnatal morbidity, and is a risk factor for lifelong adverse outcomes. FGR may be further complicated by preeclampsia, a maternal physiologic reaction to defective placentation, which endangers the lives and long-term outcomes of both the mother and the fetus. Additionally, fetal loss and FGR may share common pathophysiologic mechanisms, since FGR was found to be an independent predictor of stillbirth (Facchinetti et al., 2010; Ogunyemi et al., 1998). Growth-restricted neonates exhibit increased risk of respiratory distress syndrome, intraventricular and intravascular hemorrhage, necrotizing enterocolitis, and adverse neurodevelopmental outcomes. Neonates from FGR-complicated pregnancies are nearly three times more likely to die in the neonatal period than normal weight newborns (Garite et al., 2004; Piper et al., 1996; Sung et al., 1993). Among lifelong consequences of FGR, notable is a propensity to develop metabolic syndrome and mental health abnormalities (Low et al., 1992). Despite intense investigation, the pathogenesis of FGR is not fully understood.

Inadequate uteroplacental perfusion has been commonly observed in human FGR (reviewed by Mayhew et al. (2004)). Histological examination frequently reveals ischemic placental lesions in FGR-complicated pregnancies, and the number/severity of these lesions is correlated with the severity of FGR (Salafia et al., 1995). Thus, it is generally believed that chronic, or intermittent, perfusion defects of the pregnancy compartment play a central role in human FGR. It has been demonstrated that transient, unilateral uterine ischemia/reperfusion (I/R) causes FGR in both uterine horns of treated rats (Thaete and Neerhof, 2006). Using the rat model, mechanistic studies have implicated inflammation in the pathogenesis of FGR. These studies have demonstrated roles for leukocyte infiltration by increased myeloperoxidase (MPO) activity, increased leukocyte numbers detected by immunohistochemistry (CD11), and restoration of uterine blood flow and prevention of FGR using anti-CD11 and anti-CD18 antibodies (Miyakoshi et al., 2001); reactive oxygen species using specific scavengers (Ishimoto et al., 1997); and platelet-activating factor using a specific inhibitor (Thaete and Neerhof, 2006).

Complement (C') activation is a key feature of the innate inflammatory response. Consisting of at least 35 interacting proteins, the C' system mediates vascular activation, chemotaxis, leukocyte activation, and target cell destruction. As summarized in Figure 1, the C' system is activated through four major pathways: the antibody-dependent classical pathway, the antibody-independent alternative pathway, the mannose binding lectin pathway (Zipfel, 2009), and direct activation of the C5 component by thrombin (Huber-Lang et al., 2006). In addition, there may be another C3-independent, so far uncharacterized, activation pathway that is mediated by lipopolysaccharide (Yu et al., 2008). Moreover, it has been demonstrated that C' can be activated by I/R in many organs (Arumugam et al., 2004).

An increasing number of studies have linked C' activation to FGR. C3 deposition and increased serum C5a levels were observed in placentae of patients with FGR who had circulating antiphospholipid antibodies (Shamonki et al., 2007). Additionally, various indicators of C' activation were identified in serum samples from patients with preeclampsia and FGR (Derzsy et al., 2010). Increased deposits of the membrane attack complex of the C' system have been associated with fibrin deposits in FGR placentae compared to normal placentae; and treatment of primary trophoblast cultures with serum containing active membrane attack complex altered trophoblast function *in vitro*, especially under hypoxic conditions (Rampersad et al., 2008). Additionally, a function-limiting polymorphism in the C' activator mannose-binding lectin, has been associated with decreased prevalence of preeclampsia and FGR in humans (Derzsy et al., 2010; Rampersad et al., 2008). Finally, C'

mediates anti-phospholipid antibody-induced FGR and fetal loss in mice (Salmon et al., 2003); and CBA/J $\times$ DBA/J mating induces C'-dependent fetal loss and FGR (Girardi et al., 2006; Redecha et al., 2009). However, no definitive experiments showing the involvement of C' in I/R-induced FGR have been reported.

To address specifically the hypothesis that C' activation plays a central role in I/R-induced pathology in pregnancy, we utilized mice that are deficient in C5, a central C' component mediating C' activation through all pathways. We evaluated whether C5-deficient mice react differently to uterine I/R challenge, in comparison to wild type mice, in terms of fetal growth, fetal loss, and changes in MPO activity in uterine and/or placental tissue.

This study is the first to demonstrate that FGR and fetal loss can be differentially induced by different modalities of I/R in mice, that uterine I/R-induced FGR and fetal loss are C5-dependent, that there is a direct positive correlation between uterine I/R-induced MPO activity (suggesting inflammation) and fetal loss, and that the increased MPO activity is also C5-dependent. Furthermore, in the strains of mice we used in this study, we identified the uterus as the main site of C'-dependent pathology, as opposed to the placenta, which has been shown to be the main target organ for C' activation in immunologically induced models of FGR and fetal loss (Cohen et al., 2011).

2. Materials and methods

2.1. Animal experiments

All animal care and experiments were approved by the Animal Care and Use Committee of NorthShore University HealthSystem Research Institute. Virgin male and female mice, C5-deficient (B10.D2-*Hc0 H2d H2-T18c*/oSnJ) and their appropriate wild type controls (C57Bl/10SnJ) (Jackson Laboratories, Bar Harbor, ME), were housed in the animal facility in 12-hour light/dark cycles with free access to a standard laboratory rodent diet and water. Timed pregnancy was produced through overnight mating of 11 to 20-week-old females in estrus. When a vaginal plug was identified in the morning, this was designated gestation day 0.5. Pregnancy was confirmed by palpation on day 12.5 and then pregnant females of each strain were randomly divided among 3 subgroups. On gestation day 13.5 they were anesthetized with a single dose of xylazine and ketamine at 10 and 100 mg/kg ip, respectively. The right uterine horn was exposed through a midline abdominal incision to perform either I/R or sham treatment as follows. I/R challenge was performed either by total blood flow restriction for 5 minutes or by partial blood flow restriction for 30 minutes. Total blood flow restriction was achieved by clamping both the uterine and ovarian arteries of the right horn. The uterine horn was then returned to the abdominal cavity, with the occlusion clips in place until their removal 5 min later for the initiation of reperfusion. The abdominal incision was closed 30 min after exposure of the uterine horn. Partial blood flow restriction was done by clamping only the right ovarian artery, returning the horn to the abdominal cavity with occlusion clip in place, and clip removal and abdominal closure 30 min later. Sham surgery was performed as for I/R but without artery clamping. The animals were euthanized five days later (gestation day 18.5) and the total number of conceptuses and the weights of live fetuses and their placentae in each uterine horn, were documented. Number of conceptuses was defined as: live fetuses + dead fetuses + resorptions detectable at the time of hysterotomy. Resorptions were identified as button-like tissue at the position of placental attachments and IUFDs were near-term fetuses that were no longer alive at the time of hysterotomy. IUFDs were pale, motionless and/or macerated, when compared to pink fetuses that responded with movement when gently prodded shortly after hysterotomy. Uterine and placental samples were collected, frozen in liquid nitrogen and stored at -80° C for further analysis. In the mouse, the decidua remains with the placenta; the uterine samples

included uterine glandular endometrium, myometrium, and serosa for an entire implantation site and adjacent tissue.

2.2. Measurement of tissue myeloperoxidase activity

Myeloperoxidase (a marker enzyme for neutrophils (Malle et al., 2007; Remick et al., 1990)) was assayed as published previously (Qu et al., 1996). Briefly, tissues were homogenized in 0.05 M potassium phosphate buffer containing 0.5% hexadecyltrimethyl-ammonium bromide (Sigma) and EDTA (5 mM), and sonicated. After centrifugation at 14,000g, an aliquot of the supernatant was mixed with the substrate O-dianisidine HCl (Sigma) + H₂O₂ in potassium phosphate buffer, and its optical density read at 460 nm. A standard curve was constructed with serial dilutions of human MPO (Sigma).

2.3. Statistical analysis

Statistical tests were conducted using GraphPad Prism software. One-way analysis of variance (ANOVA, with Bonferroni's Multiple Comparison Test) was employed for the comparison of fetal and placental weights and litter sizes as well as for MPO activities among multiple groups. Linear regression analysis tested for a correlation between MPO activity and fetal loss or FGR. All tests were two-tailed with $p < 0.05$ considered statistically significant.

3. Results

3.1. Transient, Unilateral Uterine I/R Causes Bilateral FGR and Fetal Loss in Wild Type Mice

The two modalities of I/R challenge (total flow restriction and partial flow restriction) were developed because the outcomes of the first are weighted more toward fetal loss, while those of the second are weighted toward FGR. Both total flow restriction and partial flow restriction induced FGR, compared to sham controls, in wild type (C57Bl/10SnJ) mice (Fig. 2a). FGR manifested to the same extent in both the ipsilateral and contralateral horns. Additionally, both modalities of I/R produced FGR to the same extent in surviving fetuses.

Uterine I/R caused fetal loss, defined as (number of resorptions + IUFDs) / (total number of conceptuses) $\times$ 100%, in wild type mice, and the two modalities of I/R resulted in different frequencies of fetal loss. The incidence of fetal death was highest in total flow restriction: nearly 85% of the fetuses in the ipsilateral horn, and 70% in the contralateral horn (Fig 2c). Partial flow restriction has a milder effect with 50% fetal loss in the ipsilateral horn, compared with sham-operated mice (Fig. 2c).

Placental weights associated with live fetuses were not different between sham and I/R treated mice (either by total flow restriction or partial flow restriction), in either wild type (Fig. 2e) or C5-deficient mice (Fig. 2f).

3.2. An Intact Complement System is Required for I/R-Induced FGR and Fetal Loss

In order to test the role of C' in I/R-induced FGR, we performed both modalities of I/R challenge in C5-deficient mice (B10). As shown in Fig. 2b, C5-deficient animals were protected from I/R-induced FGR. C5-deficient mice were similarly protected from I/R-induced fetal loss (Fig. 2d).

3.3. The Number of Conceptuses is not Different in Wild Type and C5-Deficient Mice

We confirmed that the observed FGR and fetal loss differences across groups were not produced by any heterogeneity in the number of conceptuses. Number of conceptuses was not different among the experimental groups or between ipsilateral and contralateral uterine

horns (Table 1). These data indicate that the absence of C5 does not cause deficient embryo implantation or pre-implantation fetal loss. No difference in the rate of occurrence of pregnancy was noted between the C57Bl/10SnJ and the B10.D2-*Hc0 H2d H2-T18c*/oSnJ strains of mice.

3.4. I/R Causes a Pronounced Increase in the Expression of Myeloperoxidase in the Uterine Tissue of Wild Type Mice, but not C5-Deficient Mice

Uterine MPO activity is markedly elevated in I/R-challenged wild type mice. MPO levels increased nearly 10-fold after total flow restriction, and approximately 5-fold following partial flow restriction, compared to the sham group (Fig. 3a). The increase in uterine tissue MPO was completely abolished in I/R-treated, C5-deficient mice, using both total flow restriction and partial flow restriction (Fig. 3b). These differences in MPO activity in the uterine tissue did not include the decidua, which remains with the placenta in mice as noted above.

No significant difference was found in placental MPO activity (by either total flow restriction or partial flow restriction) between sham and I/R-treated wild type (Fig. 3c) and C5-deficient (Fig. 3d) mice.

3.5. I/R-Induced Fetal Loss is Significantly Correlated with Uterine MPO Activity

Uterine MPO activity was significantly correlated with the incidence of fetal loss (Fig. 4a) but no statistically significant correlation was observed between weights of live fetuses and uterine MPO activity (Fig. 4b).

4. Discussion

4.1. I/R and Bilateral FGR/Fetal Loss

Transient restriction of blood flow to one uterine horn has been used in timed-pregnant rats and, along with the mouse I/R model reported herein, is relevant to human FGR because the I/R used in both rats and mice resembles I/R events observed in humans; it affects only a portion of the uteroplacental tissue, and only for a limited duration. While our murine model uses a single I/R event, it provides an avenue, in an animal model, for gaining insight into the impact on pregnancy of limited ischemia and reperfusion. Furthermore, the unilateral uterine I/R model is uniquely suited to understand the role of circulating mediators in FGR, since growth restriction occurs to an equal degree in both uterine horns, in both rats and mice, despite the unilateral I/R challenge. Bilateral effects of similar intensity to unilateral uterine I/R strongly indicates a systemic response in our mouse model, as has been shown previously in the rat (Thaete and Neerhof, 2006). In order to address mechanistic details of I/R-induced FGR and fetal loss by using genetically modified animals, we reproduced the rat model in mice. The murine model reported herein produces consistent bilateral FGR and fetal loss without affecting the number of conceptuses. Our data are consistent with an inflammatory response following I/R, and the mouse model opens the door for further characterization of molecular pathways involved in this response by using appropriate genetically modified mouse strains.

Early fetal loss in humans has long been suggested to share a common pathogenic pathway with FGR and preeclampsia, since FGR has been found to be an independent predictor of stillbirth (Facchinetti et al., 2010; Ogunyemi et al., 1998). Fetal loss occurs together with FGR in many models, including administration of antiphospholipid antibodies (Salmon et al., 2003) and CBA/J $\times$ DBA/2 mating (Girardi et al., 2006; Redecha et al., 2009). Both of these studies suggested that FGR and fetal loss are parts of a disease continuum, with fetal

loss being a more severe outcome. In our study we found that I/R causes fetal loss in wild type mice, and that the two modalities of I/R result in different frequencies of fetal loss.

Interestingly, compared to the rat model, the C57Bl-based wild type mouse strain exhibits pronounced fetal loss in response to very short, complete uterine flow restriction, while the more moderate partial flow restriction elicits an outcome similar to that in rats, consisting primarily of FGR.

The placenta is pivotal in the maternal/fetal interface and changes in placental weight can reflect the types of insult occurring during pregnancy. For instance, decreased placental weights were found in FGR models elicited with global maternal hypoxia (Neerhof et al., 1997; Thaete et al., 1997), CBA/J $\times$ DBA/2 mating and antiphospholipid antibodies (Girardi et al., 2006; Salmon et al., 2003), endothelin infusion (Neerhof et al., 1997), nitric oxide synthase inhibition (Thaete et al., 2005), or platelet-activating factor infusion (Thaete et al., 2003), but placental weights did not change in a rat model of I/R-induced FGR (Thaete and Neerhof, 2006). Consistent with the findings in rats, we found no difference in placental weights between sham and I/R-treated mice (either by total flow restriction or partial flow restriction). These results suggest that in the I/R-model of FGR and fetal loss, at least based on this gross parameter, the placenta may not be the primary target of pathology.

4.2. I/R-Induced FGR and fetal loss Require an Intact Complement System

Recently, there has been a surge of interest in the role of C' activation in FGR, preeclampsia, and premature labor. Complement activation in the placenta has been observed in human pregnancies in association with FGR, with or without preeclampsia (Cohen et al., 2011; Rampersad et al., 2008). Additionally, in animal models of FGR and preeclampsia, C' activation has been shown to play a pathogenic role. In the well-established immune-mediated model of FGR and fetal loss that is based on CBA/J $\times$ DBA/2 mouse mating, the C3, C5, C5aR complement activation axis has been shown to play a critical role (Girardi et al., 2006). Similarly, the anti-phospholipid antibody-based FGR/fetal loss model is dependent on an intact C' system (Cohen et al., 2011; Salmon et al., 2003). These studies, using complement-blocking antibodies and mice deficient in C1q or factor D, demonstrate the role of C' in immunological models of FGR and fetal loss. Using mice deficient in C5, a central player in all C' activation pathways, the aim of the present study was to test the role of C' activation in I/R-induced FGR and fetal loss.

Our data are consistent with the notion that C' plays a critical role in FGR and fetal loss, not only in immunological models, but also in an I/R-based model. These findings from distinctly different animal models, together with the aforementioned data from human studies, strongly infer a role for C' activation in pathological pregnancies.

4.3. Number of Conceptuses is not Affected by C5 Deficiency

Interestingly, C3 deficiency has been linked to impairments in early pregnancy, suggesting that the C' system may play a physiological role in normal pregnancy as well (Chow et al., 2009). In those studies C3 deficiency resulted in reduced numbers of conceptuses due to early fetal loss and limited embryo implantation. In contrast, we used a model of mid- to late-pregnancy fetal loss and FGR in which C5 deficiency proved not to alter the number of conceptuses in a pregnancy. The similarity of numbers of conceptuses across groups in the present study demonstrates that this parameter was not a significant factor in producing the outcomes reported herein.

4.4. I/R Induces Expression of MPO in the Uterine Tissue of Wild Type Mice, but not C5-Deficient Mice

I/R can cause an inflammatory response, which frequently results in increased neutrophil sequestration. Myeloperoxidase, an enzyme abundantly expressed in polymorphonuclear leukocytes (PMN), is frequently used as a marker of PMN sequestration in tissues, and is considered a proxy for the extent of inflammatory response or vascular activation. C5a, a product of C5 activation (see Fig. 1), is a critical soluble factor that mediates PMN sequestration, both as a PMN chemotactic molecule and as an activator of vascular adhesion molecules. In the present study we have found that uterine tissue MPO activity is markedly elevated in I/R-challenged wild type mice and that this increase in uterine tissue MPO is completely abolished in I/R-treated, C5-deficient mice. These results suggest I/R-induced neutrophil sequestration in the uterus, mediated by C5 activation, but it remains to be determined whether the observed signs of potential neutrophil sequestration allude to an actual underlying role for these cells.

Placental MPO activity did not differ among experimental groups. These results, in combination with the placental weight data, suggest that placenta is not the primary site of pathological changes in the murine I/R model of FGR and fetal loss. Given the evidence for the role of placenta in various other rodent models of FGR/fetal loss (Cohen et al., 2011; Girardi et al., 2006), and based on the well-documented role of placenta in human FGR (Redecha et al., 2009; Salafia et al., 1995; Shamonki et al., 2007), other mechanisms not directly influencing placental weight or MPO activity may be involved in this I/R-based murine model. Further investigation will be required to decipher mechanistic explanations, but the dissimilarities in placental development in rodents and in humans (Carter et al., 2006) (e.g., shallow decidual vascular trophoblastic invasion via a perivascular route in the mouse versus deeper endometrial endovascular invasion in humans) may be a possible reason.

4.5. I/R-Induced Fetal Loss Correlates with Uterine MPO Activity

Tissue MPO has been commonly used as a marker of PMN sequestration under inflammatory conditions (Malle et al., 2007; Remick et al., 1990). Our fetal loss data and uterine MPO data are correlated, suggesting that uterine PMN sequestration contributes to the development of IUFD. Interestingly, and in contrast with the fetal loss data, there was not a statistically significant correlation observed between fetal weights and uterine MPO activity. These data suggest that PMN sequestration is more predominant in fetal loss than in FGR. However, an alternative explanation may be the presence of a threshold effect, below which grade of PMN sequestration FGR develops, and above which fetal loss is manifest. It remains to be determined whether the increased MPO activity observed in our model indicates an underlying pathophysiologic role for PMN sequestration, and whether it is a sign of increased vascular activation, PMN chemoattractant inflammatory mediator production in the extravascular tissue, or both. The possibility that the high uterine MPO activity following I/R may be a maternal reaction to IUFD has not been ruled out by our experiments.

It is yet to be determined which upstream C' activation pathway, i.e., classical, lectin, alternative, thrombin, or possibly lipopolysaccharide, plays the initiating role, but our studies demonstrate that the absence of C5, which is a central player for most known C' activation pathways (see Fig. 1), completely abolishes I/R-induced FGR and fetal loss. The classical pathway, via C1q activation, has been extensively linked to I/R-induced tissue damage in the brain (Ten et al., 2010), kidney (Castellano et al., 2010), and intestine (Lee et al., 2010). Similarly, the lectin pathway has been implicated in I/R damage to the same organ systems (Castellano et al., 2010; Cervera et al., 2010; Lee et al., 2010). Therefore, it is

conceivable that either or both of these pathways participate in I/R-induced FGR and fetal loss, upstream of C5. Downstream of C5, as would be suggested by the correlation between fetal loss and uterine tissue MPO, C5aR-dependent mechanisms are prime candidates for executing the C5-dependent effects, as was observed in I/R-mediated tissue injury in the heart (Zhang et al., 2007), kidney (Zheng et al., 2008), liver (Xu et al., 2009) and intestine (Xu et al., 2009). C5 is indispensable for C' activation and subsequent inflammatory events in at least three ways (reviewed by Arumugam et al. (2004)): 1) C5 is required to sustain the activation cascade; 2) C5b is a component of the membrane attack complex that mediates the destruction of target cells; and 3) C5a is a potent chemotactic factor, which mediates the mobilization of immune cells. In C5-deficient mice, I/R treatment produced no significant evidence of inflammation (i.e., increased MPO activity) in the uterine tissue, despite the fact that the C3 system is still intact in these animals.

Our results have shown that the increased MPO activity, which is closely correlated with the overall pathology, is predominantly altered in the uterus, but not in the placenta, in response to I/R. Salmon et al. (2003) suggested that placenta is the target in anti-phospholipid antibody-induced FGR and fetal loss, which is mediated by C' activation. The same authors also documented monocyte infiltration in the placenta as a result of CBA/J $\times$ DBA/2 mating, and reduced placental weight in association with these growth-restricted fetuses (Girardi et al., 2006). The contrast of these findings suggests that although immunologically-induced and I/R-induced FGR and fetal loss share common mechanisms in terms of participation by various elements of the C' system, there may be significant differences in specific activation mechanisms upstream and downstream of C5 and in the predominant sites of the resultant pathology.

In summary, we have established a murine model of bilateral FGR and fetal loss induced by unilateral transient uterine I/R. This opens the possibility to perform a wide range of studies addressing the mechanistic and genetic bases of I/R-induced FGR and fetal loss. Additionally, we revealed a pivotal role for complement component C5 in this FGR and fetal loss model. Furthermore, we have shown that the major I/R-induced pathophysiologic response is in uterine, not placental, tissue. It remains to be determined whether differences between the relative involvement of placenta versus uterus in distinct murine models of FGR and fetal loss are related to various aspects of human pathologies, especially in view of different placentation mechanisms in mice and men. A better characterization and mechanistic understanding of the role of C' in FGR and fetal loss may have a utility in discovering novel intervention strategies.

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Abbreviations

C'	complement
FGR	fetal growth restriction
I/R	ischemia/reperfusion
IUFD	intrauterine fetal demise
MPO	myeloperoxidase
PMN	polymorphonuclear leukocyte

Fig. 1.

Schematic of the C' activation pathway: Proximal to C5, there are four main activation pathways, and a possible 5th uncharacterized pathway, 1) the C1q-dependent "classic" pathway, typically activated by immune complexes, 2) the mannose-binding lectin (MBL)/mannose-binding lectin-associated serine protease (MASP)-dependent "lectin" pathway, the 3) C3bBp-dependent "alternative" pathway, 4) direct activation of C5 by thrombin, and 5) activation of C5 by lipopolysaccharide (LPS). C5 activation plays a central role, by connecting all proximal activation pathways with the two main distal executing pathways, 1) the C5a/C5aR-dependent vascular activation and inflammatory pathway and 2) the C6-dependent cytotoxic membrane attack complex (MAC) pathways. Solid arrows illustrate the suggested main mechanisms in the I/R-induced model of FGR/fetal loss. MΦ, macrophage.

Fig. 2.

I/R-induced FGR and fetal loss require intact C5. Timed pregnant wild type (a,c,e) or C5 gene-targeted (b,d,f) mice were challenged by unilateral uterine I/R, by either 30-min partial flow restriction (PFR, grey bars) or 5-min total flow restriction (TFR, black bars), on day 13.5 of pregnancy. These groups were compared to sham-operated controls (see methods; empty bars). IL and CL refer to ipsilateral and contralateral, respectively, relative to the uterine horn that was subjected to I/R. Data shown (mean ± SEM) are fetal weights among survivors (a,b), fetal loss as a percent of the number of conceptuses (c,d) and placental weights (e,f) on day 18.5 of pregnancy. Bilateral decreased fetal weights and increased fetal loss can be observed in I/R-challenged wild type mice, but not in C5 gene-targeted mice.

Placental weights were the same in all groups. For C5 (+/+) the number of pregnancies were 7 sham, 10 partial flow restriction, 7 total flow restriction, the number of surviving fetuses and their placentae for weight measurements were 31 sham, 27 partial flow restriction and 12 total flow restriction. For C5 (-/-) the number of pregnancies were 6 sham, 6 partial flow restriction, 7 total flow restriction, the number of surviving fetuses and their placentae for weight measurements were 27 sham, 26 partial flow restriction and 26 total flow restriction. * $p < 0.05$, ** $p < 0.01$, compared to sham-operated controls; # $p < 0.05$ between PFR and TFR in corresponding uterine horns.

Fig. 3.

I/R induces an increase of uterine, but not placental, MPO activity in wild type, but not in C5 gene-targeted mice. Timed pregnant wild type (a,c) or C5 gene-targeted (b,d) mice were challenged with unilateral uterine I/R by partial flow restriction (PFR, grey bars) or total flow restriction (TFR, black bars) on day 13.5 of pregnancy. These groups were compared to sham-operated controls (see methods; empty bars). Graphs depict MPO activities in the uteri (a,b) and in placentae (c,d). MPO increased 5 fold in partial flow restriction and 10 fold in total flow restriction in uteri, but not in placentae and only in wild type mice. For C5 (+/+) the number of pregnancies were 7 sham, 10 partial flow restriction, 7 total flow restriction, the number of placentae for MPO activity measurement were 31 sham, 27 partial flow restriction and 12 total flow restriction. For C5 (-/-) the number of pregnancies were 6 sham, 6 partial flow restriction, 7 total flow restriction, the number of placentae for MPO activity measurements were 27 sham, 26 partial flow restriction and 26 total flow restriction.

* $p < 0.05$, ** $p < 0.01$, compared to sham-operated controls; # $p < 0.05$ between PFR and TFR in corresponding uterine horns.

Fig. 4.

Significant positive correlation between uterine tissue MPO activities and the incidence of fetal loss. Timed pregnant wild type mice were challenged by unilateral uterine I/R by partial flow restriction (PFR, grey circles) or total flow restriction (TFR, black circles), or they were sham-operated (clear circles), on day 13.5 of pregnancy. Data shown are linear regression plots of MPO vs fetal loss (a) and MPO vs fetal weights (b). A significant positive correlation was observed between MPO and the incidence of fetal loss, but not between MPO and fetal weight. The number of pregnancies represented in the chart is the same as in figures 2 and 3, however, the analysis was conducted using data from each ipsilateral and contralateral horn of each uterus, therefore the number of observations was twice the number of pregnancies. Because many of the points overlap, the number of dots in a given group may not correspond with the number of observations actually present in the analysis.

Table 1
Numbers of conceptuses in C5-deficient and wild type mice (mean $\pm$ SEM for each uterine horn)

Treatment	B10 (C5-deficient)		C57/10Sn (wild type)	
	Ipsilateral horn	Contralateral horn	Ipsilateral horn	Contralateral horn
Sham	3.3 $\pm$ 0.7	3.0 $\pm$ 0.9	3.9 $\pm$ 0.6	3.1 $\pm$ 0.5
PFR	2.8 $\pm$ 0.3	3.5 $\pm$ 0.8	3.3 $\pm$ 0.4	2.8 $\pm$ 0.4
TFR	2.9 $\pm$ 0.4	3.1 $\pm$ 0.6	2.7 $\pm$ 0.6	3.5 $\pm$ 0.6

PFR = partial flow restriction; TFR = total flow restriction.

Number of conceptuses included the total number of live fetuses + dead fetuses + resorptions in the uterine horns at the time of hysterotomy.

There were no statistically significant differences among the groups; by ANOVA.