Blocking of the placental immune-modulatory ferritin activates Th1 type cytokines and affects placenta development, fetal growth and the pregnancy outcome

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BACKGROUND: Placenta immunomodulatory ferritin (PLIF) cDNA was recently cloned from the human placenta, where it is expressed in syncytiotrophoblast and decidual mononuclear cells. PLIF and its subcloned bioactive domain (C48), expressed in Escherichia coli, are immunosuppressive proteins and induce pronounced IL-10 production in vitro and in vivo. METHODS AND RESULTS: PLIF serum level, measured by enzyme-linked immunosorbent assay, was elevated in pregnant mice throughout gestation and declined towards delivery. Blocking of PLIF activity by vaccination of mice with C48 prior to mating inhibited pregnancy development. Passive transfer of anti-C48 immunoglobulin (Ig) starting at 3.5±12.5 days post coitum (dpc) resulted in high rate of embryo resorption. Furthermore, treatment with anti-C48 Ig resulted in placental and embryonal growth restriction. At gestation day 13.5, growth retardation was especially notable in the placentae, while at 16.5 dpc it was pronounced in the embryos. Histopathological examination revealed that experimental placentae were globally hypoplastic and the labyrinth was strikingly pale and contained less maternal blood compared with control. Immune-activated spleen cells harvested at 13.5 dpc from anti-C48 Ig-treated pregnant mice secreted in vitro increased level of Th1 cytokines (IL-2, TNF-α, IL-12) and decreased level of Th2 cytokines (IL-10, IL-4, IL-5, IL-6) as compared with the level of the respective cytokines secreted by spleen cells from control pregnant mice. CONCLUSION: This study provides the first in vivo evidence that PLIF plays a major role in placentation and embryonic growth.

Key words: abortion/cytokines placenta/PLIF/pregnancy/resorption

Introduction

A most intriguing phenomenon in immunology is for the semiallogeneic embryo (or allogeneic embryo in surrogate mothers) to be exempt from immunological rejection. Understanding this mechanism will have great implications for many immune-related disorders such as allogeneic transplantation and autoimmune diseases (Clark et al., 1999a).

The presence of immunosuppressive regulatory molecules in the placenta is believed to prevent immunological rejection of the embryo. Among them are progesterone (Piccini et al., 1995), progesterone-induced blocking factor (Szekeres-Bartho and Wegman, 1996), prostaglandin E2 (Abe et al., 1997) and early pregnancy factor (Morton, 1998).

It is now accepted that changes in the balance of Th1/Th2 type cytokines occur during pregnancy in the feto-placental unit. These changes contribute to the implantation of the embryo, development of the placenta and survival of the fetus to term (Dealtry et al., 2000).

p43 placenta isoferitin (p43-PLF) is a unique placental protein that has immunoregulatory activity and is composed of a 43 kDa protein (p43) and a ferritin light chain (Moroz et al., 1985). This protein is highly expressed in human syncytiotrophoblasts, Hofbauer cells and in decidual macrophages (Maymon et al., 2000). Additional studies from our group showed that during normal pregnancy p43-PLF level is elevated in maternal circulation as early as day 11, which continues throughout gestation, and declines at term (Maymon et al., 1996a). In contrast, the level of p43-PLF was low or below detection in syncytiotrophoblast of missed abortions (Moroz et al., 1989). This finding correlated well with the low serum level of p43-PLF measured in early pregnancy failures and in intrauterine growth restriction (IUGR) (Maymon et al., 1989; Rosen et al., 1996). It is noteworthy that no correlation was found between PLF and β-hCG, E2 or PP levels (Maymon et al., 1995; Maymon and Moroz, 1996). Furthermore, in IVF patients, even when the level of the β-hCG was rising, low PLF level resulted eventually in missed abortion (Fisch et al., 1996). These correlations have led to the speculation that p43-PLF might play an important role in achieving normal pregnancy.

In an attempt to identify the gene responsible for regulation of normal pregnancy development, our group recently reported the cloning and preliminary characterization of a novel human
pregnancy-related immunomodulator we named ‘placenta immunomodulatory ferritin’ (PLIF) (Moroz et al., 2002). PLIF cDNA was isolated from human placenta cDNA library. The encoded protein has a unique molecular structure composed of an incomplete ferritin heavy chain sequence (amino acids 1–117) linked to a novel non-ferritin 48 amino acid domain named C48 (amino acids 118–165). In immuno-chemical analysis, PLIF was found to cross-react with p43-PLIF and likewise to co-localize in syncytiotrophoblasts of placenta at the fetal–maternal interface (Moroz et al., 2002). Thus PLIF is the gene coding for the previously described and measured p43-PLIF (Maymon et al., 2000).

Moreover, PLIF and its novel peptide C48 were independently to inhibit T cell proliferation of human peripheral blood mononuclear cells (PBMC) and mouse splenocytes following allogeneic stimulation in mixed lymphocyte cultures (MLC) or mitogenic stimulation of human PBMC with anti-CD3 monoclonal antibodies (mAb) (Moroz et al., 2002; Weinberger et al., 2003). Accumulated data revealed that C48 represents the bioactive domain of PLIF and exerts in vitro and in vivo immunoregulatory activity independent of any ferritin sequenc (Moroz et al., 2002; Weinberger et al., 2003). The immunoregulatory functions of PLIF/C48 were partly attributed to the induction of pronounced and rapid interleukin (IL)-10 production. This was demonstrated in human monocytes in vitro (Zahalka et al., 2003) as well as in vivo following C48 treatment of mice and rats with Zymosan and adjuvant-induced rheumatoid arthritis respectively (Weinberger et al., 2003).

In the present study we investigated whether PLIF plays a key role in allogeneic pregnancy development in mice by blocking its activity with anti-C48 (PLIF) antibodies. The experiments included female immunization with C48 prior to mating or passive transfer of anti-C48 antibodies starting at 3.5 days post coitum (dpc).

Materials and methods

Prokaryotic protein expression and purification of C48
The cDNA fragment coding for the C-terminal 48 amino acids (C48) of PLIF was subcloned into pGEX 5X-1 prokaryotic expression vector (Amersham Biosciences) resulting in glutathione-S-transferase (GST)–C48 fusion vector as previously described (Moroz et al., 2002). This vector was used to transform Escherichia coli BL 21 strain. Bacterial cultures of transformants were harvested after induction with IPTG (isopropyl thiogalactoside) and lysed in Triton X-100-based lysis buffer. Fusion protein was then absorbed from lysates using glutathione Sepharose 4B beads and subsequently eluted with excess of free glutathione. After dialysis, the fusion protein was cleaved by factor Xa and the purified C48 was obtained by removal of the cleaved GST part using glutathione Sepharose beads.

Preparation of rabbit anti-C48 immunoglobulin (Ig)
Rabbits were immunized with purified recombinant C48 or with GST. Each rabbit was immunized with 50 µg purified protein mixed v/v with complete Freund’s adjuvant, on days 1, 7 and 21. On day 28, rabbits were bled and immunoglobulins were isolated by salt precipitation. Control immunoglobulins from preimmunized rabbits were also purified. Endotoxin levels in the purified anti-C48 Ig and anti-GST Ig preparations used for treatment were <0.1 IU per µg protein. This was determined by the Limulus amoebocyte lysate assay (Biological Industries, Israel).

Anti-C48 titration by enzyme-linked immunosorbent assay (ELISA)
Blood samples were obtained from the immunized mice at the end of the follow-up period for pregnancy outcome. ELISA determined the presence of anti-C48 antibodies as follows: Dynatek microtitre plates (129B) were coated with 100 µl/well of pure C48 (5 µg/ml) or with GST (5 µg/ml) and incubated overnight at 4°C. Plates were then washed with phosphate-buffered saline (PBS) containing 0.01% Tween (PBS-T) and blocked for 30 min with PBS-T plus 1% bovine serum albumin (BSA). Samples containing sera pre-absorbed with GST were diluted, added to C48-coated plates and incubated for 1 h at room temperature. The wells were washed with PBS-T and the anti-C48 antibodies were detected with horse-radish peroxidase (HRP)-labelled anti-mouse IgG (Dako). GST-coated plates were used as above for the measurement of anti-GST antibodies.

PLIF titration in mouse serum by ELISA
Blood samples were obtained consecutively from pregnant mice (n = 10), at days 5.5, 8.5, 13.5 and 16.5 dpc as well as 14 days post delivery (at 35 dpc).

A standard PLIF titration curve was obtained by dissolving increasing amounts of recombinant C48 in pooled non-pregnant normal mouse serum (NMS).

ELISA was performed by coating microtitre plates with pregnant mouse serum or C48 diluted in NMS, overnight at 4°C. Plates were washed and blocked with 1% BSA as above. Rabbit anti-C48 Ig (10 µg/ml) was added and incubated for 2 h at room temperature, followed by HRP-labelled goat anti-rabbit IgG (Dako).

C48 vaccination and mating of mice
All mice were purchased from Harlan Laboratories Ltd. Female BALB/c mice were immunized s.c. with C48 or with GST (10 µg purified protein per mouse mixed v/v with complete Freund’s adjuvant). Each mouse was immunized on days 1, 7 and 21. On day 28, the immunized, or control non-immunized, mice were mated with allogeneic C57Bl male mice. Pregnancy development and outcome were followed for each mouse. Mating was repeated with alternating C57Bl male mice, for up to three estrus cycles for non-pregnant mice in all groups.

Treatment of pregnant mice with anti-C48 Ig
In each experiment, pregnant ICR mice were purchased from Harlan Laboratories Ltd and divided into groups of six mice. Anti-recombinant C48 immunoglobulin (anti-C48 Ig) (0.5–2.0 mg) were injected daily i.p. As a control for non-specific cytotoxic and cytopathic effects of rabbit Ig to the embryos, mice received daily injections of rabbit anti-GST Ig. The mice in both groups were treated at the following gestational days: 3.5–6.5, 3.5–12.5 and 3.5–15.5 dpc and killed at 7.5, 13.5 and 16.5 dpc respectively.

In addition, experimental and control mice treated at gestational days 3.5–15.5 were allowed to proceed until term. The date and time (to nearest 0.5 days) of delivery and the number of live pups and their weights were recorded. They were re-examined for viability at 7 days after birth.

Gross and microscopic pathology
Pregnant mice were killed by CO2 overdose on 13.5 or 16.5 dpc. The uterus was removed and cut transversely to separate the swellings containing either viable or resorbed embryos. Each placenta site
was opened and examined. The number of viable and resorbed embryos was compared between the groups. The placentae and embryos were examined with the aid of a macroscope to evaluate any abnormalities and determine the age of the embryos. For measurements, embryos and placentae were separated. Embryos were weighed and their crown–rump length was measured. Placentae were weighed and their largest diameter was determined. Embryos and placentae were fixed in 10% neutral buffered formalin, bisected through their middle and processed routinely for paraffin embedding. Sections were cut at 4 μm and stained with haematoxylin and eosin. Where appropriate, serial sections were obtained. Image-Pro 4.1 was used for morphometric analysis.

Cytokine assay

Mouse spleen cells were harvested from anti-C48 and anti-GST-treated pregnant mice at 7.5 and 13.5 dpc. Splenocytes from three mice in each group were cultured (2×10^6/ml) in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal calf serum (Gibco-BRL, USA) 100 IU/ml penicillin, 100 μg/ml streptomycin (Sigma, USA) without and with Concanavalin A (Con A) 1 μg/ml. The cultures were incubated at 37°C in a 5% humidified incubator and supernatants were collected after 24, 48 and 72 h. Cytokines in supernatants were detected by ELISA using Pharmingen paired Ab (PharMingen, USA), according to the PharMingen cytokine ELISA protocol. PharMingen recombinant mouse cytokines were used as standards for calibration curves. The concentrations of cytokines are shown as the mean ng/ml derived from calibration curves using recombinant cytokines as standards.

Statistical analysis

Results were presented as mean ± SEM. Comparisons were made by Student’s t-test. P < 0.05 was considered statistically significant.

Results

The serum level of PLIF in pregnant ICR mice

The specific detection of PLIF in the serum was carried out using rabbit anti-C48 Ig by ELISA (Figure 1). A quantitative standard curve of increasing concentration of recombinant C48 diluted in NMS is presented in Figure 1A. Further, we measured the serum PLIF level at different time-points during gestation and compared it to the level at 14 days post delivery (35 dpc). As seen in Figure 1B, compared with post delivery, elevated PLIF level was measured as early as day 5.5 of gestation, increasing gradually to the highest PLIF level at day 13.5 and declining towards delivery (Figure 1B). The mean level of PLIF measured during pregnancy was significantly higher in all samples tested compared with the mean serum level at 14 days post delivery (P < 0.001).

Effect of mouse pre-vaccination with C48 on pregnancy development

To investigate whether the expression of PLIF is necessary for placentation and embryonic development, we vaccinated the female mice with the recombinant bioactive domain-C48 prior to mating. Mating of non-immunized female mice or female mice immunized with GST were used as controls. The results of a representative experiment are shown in Figure 2. The mice were grouped according to the type of antibodies detected in their circulation, i.e. none, anti-GST (titre 1:64 000) or anti-C48 (titre 1:8000). Normal pregnancy was observed in 5/5 (100%) of the non-immunized mice, 5/6 (83%) of those with circulating anti-GST antibodies and none (0/4) of the mice with...
circulating anti-C48 antibodies. Importantly, all the pregnancies resulted in normal offspring. These results proved that vaccination with C48 inhibited pregnancy development completely, and suggest that this is due to blocking of PLIF by circulating antibodies.

**Anti-C48 Ig treatment of pregnant mice affects feto-placental growth and viability**

In order to analyse the functional role of PLIF in embryonic development, we passively transferred anti-C48 antibodies to pregnant mice by daily i.p. injections, starting at 3.5 dpc. Injections of escalating doses of anti-C48 Ig (0.5–2.0 mg) revealed a dose-dependent effect on pregnancy outcome (not shown). As a result, a daily dose of 1 mg was chosen for further study. To investigate the effect of this treatment on embryo survival and development in utero, the pregnant mice were killed on 13.5 dpc and 16.5 dpc. The results presented in Table I revealed a significant decrease in the mean embryo number (9 ± 1.3) in the anti-C48 Ig-treated mice (n = 12) compared with the mean embryo number (12 ± 0.37) in the control anti-GST Ig-treated mice (n = 12) (P = 0.05). This finding was consistent with the significantly higher proportion of resorbed embryos per mouse (18 ± 1.7%) in anti-C48 Ig-treated mice compared with the extremely low proportion (0.02 ± 0.01%) observed in anti-GST Ig-treated mice (P = 0.0003).

**Gross pathology**

Gross measurements of placentae and embryos revealed that treatment with anti-C48 Ig resulted in placental and embryonal growth restriction. At gestation day 13.5 (E13.5) growth retardation was especially notable in the placentae, whereas at E16.5 it was more pronounced in the embryos (Table I). At E13.5 many of the experimental placentae were smaller, thinner and paler than in the experimental group (Figure 3A and B). On day 13.5, mean placental weight and diameter was significantly lower in the anti-C48 Ig-treated group (100 ± 2.8 mg and 7.2 ± 0.1 mm respectively, P = 0.0006). This difference in placental weight was narrowed at 16.5 dpc (Table I). Examination of external features of the embryos showed that at either time-point (E13.5 and E16.5) embryos in the control and experimental groups were in equivalent developmental stages, as evaluated by the relevant criteria for each stage. The prominent difference between

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### Table I. The gross effect of placental immune-modulatory ferritin (C48) antibody blockade on pregnancy development

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th>Antibody injected to pregnant ICR micea</th>
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<tr>
<td></td>
<td>Anti-GST Ig</td>
</tr>
<tr>
<td></td>
<td>12 ± 0.37</td>
</tr>
<tr>
<td>Embryo no./mouse E13.5</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>% resorbed embryos/mouse E13.5</td>
<td>135 ± 5.7</td>
</tr>
<tr>
<td>Placenta weight</td>
<td>141 ± 5.7</td>
</tr>
<tr>
<td>E13.5 (mg)</td>
<td>8 ± 0.03</td>
</tr>
<tr>
<td>Placenta diameter E13.5 (mm)</td>
<td>200 ± 2.8</td>
</tr>
<tr>
<td>Embryo weight</td>
<td>1017 ± 21.6</td>
</tr>
<tr>
<td>E13.5 (mg)</td>
<td>200 ± 2.8</td>
</tr>
<tr>
<td>E16.5 (mg)</td>
<td>1017 ± 21.6</td>
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aIgG (1 mg) injected i.p. at days 3.5–12.5 [day of gestation (E) = 13.5] and days 3.5–15.5 (E = 16.5).
bStudent’s t-test. 
cMean ± SEM (n = 12).
GST = glutathione-S-transferase.
embryos in the control and experimental groups was their size and weight. This difference was pronounced at E16.5 (mean embryonal weight 767 ± 54 mg in the anti-C48 Ig-treated group versus 1017 ± 21.6 in the anti-GST Ig-treated group; P = 0.004) (Table I, Figure 4). At E13.5, embryonal size difference was less consistent. The above data demonstrate that rabbit Ig (anti-GST) had no cytotoxic±cytopathic effect on embryos.

Effect of anti-C48 treatment on gestation period, neonatal weight and viability
To determine the effect of anti-C48 treatment on duration of gestation and neonatal viability, the groups of experimental and control mice treated at E3.5–15.5 were allowed to proceed to term. There was no significant difference in duration of gestation between the two groups. The mean number of pups delivered in the anti-C48-treated group (9 ± 2) was significantly lower than in the control anti-GST-treated group (12 ± 1, P = 0.05). The mean weights of the pups were 1690 ± 0.02 and 1780 ± 0.1 mg respectively. The survival of pups during the peri- and post-natal (at 7 days post partum) periods was similar in both experimental and control groups. These results indicate that anti-C48 has no in vivo cytotoxic effect.

Histological pathology
Light microscopic examination of E13.5 placentae in the experimental group revealed that the anatomical micro-architecture is preserved. All embryonal and maternal layers were present (Figure 3C and D). The labyrinth was strikingly pallid and contained fewer maternal erythrocytes. This led to a higher relative concentration of vascular channels due to lumenal collapse of maternal vessels (Figure 3E and F). The glycogen-laden cell population interspersed within the spongiotrophoblast and decidual layers (Georgiades et al., 2002) appeared reduced in the experimental group, but precise evaluation of such an irregularly disposed cellular population was difficult. No other microscopic abnormalities were observed.

In the last trimester of pregnancy in the mouse, the muscular media of the central maternal arterial wall is replaced by acellular eosinophilic material (Georgiades et al., 2002). This physiological degeneration is regarded as one of the mechanisms that ensure adequate blood supply to the developing embryo. Examination of maternal vessels did not disclose, however, any differences between the experimental and control groups (data not shown). At E16.5, a tendency for placentae in the experimental group to be smaller was retained, but the labyrinth was less clearly pale.

Other than the size difference, no microscopic abnormalities were observed in experimental embryos at both time-points.

Th1/Th2 cytokines in Con A-activated splenocyte cultures from antibody-treated pregnant mice
A time-course accumulation (24, 48, 72 h) of cytokines produced by Con A-activated splenocytes was carried out. Spleens were harvested at 7.5 and 13.5 dpc from anti-C48 Ig (n = 3) and anti-GST Ig (n = 3)-treated pregnant mice. The mean levels of Th1 cytokines (IL-2, IFN-γ, IL-12, TNF-α) are presented in Figure 6 and Th2 cytokines (IL-4, IL-5, IL-6, IL-10) are presented in Figure 7. At 7.5 dpc following 4 days of treatments (3.5–6.5) with anti-C48 Ig and anti-GST Ig, the levels and pattern of Th1 (Figure A–D) and Th2 (Figure 7A–D) cytokine secretion were mostly similar. This includes IL-2,
IL-12 and TNF-α (TNF level was below detection), but INF-γ was lower in the C48-treated group compared with the control mice. Among the Th2 cytokines, the level of IL-4 (below detection), IL-5 and IL-10 were comparable in both groups whereas IL-6 was higher in the C48-treated group compared with control.

In comparison, at 13.5 dpc following 10 days of treatment (3.5–12.5), increased mean levels of IL-2, IL-12 and a significantly higher level of TNF-α (Th1) were measured in activated spleen cell cultures from anti-C48 Ig-treated mice compared with control anti-GST Ig-treated mice (Figure 6E–H). This was accompanied by a decrease in the mean levels of IL-4, IL-5, IL-6 and IL-10 (Th2) in the respective cultures (Figure 7E–H). These results indicate a shift from Th2 to Th1 immune response in the spleens of anti-C48 Ig-treated mice.
Discussion

This study demonstrated that in mice, similar to humans, PLIF is elevated in the circulation during pregnancy commencing at the very beginning of gestation, continuing throughout pregnancy and declining towards delivery (Maymon and Moroz, 1996). Further, it was found that blocking PLIF activity by anti-C48 antibodies affected placental and fetal growth in inbred and outbred allogeneic pregnancies. We demonstrated that vaccination with the immunoregulatory domain of PLIF (C48) prior to mating resulted in complete inhibition of pregnancy development. The mechanism(s) has not been completely defined, but could include inhibition of placentaion or fetal rejection. Thus, C48 vaccine acted as an immunocontraceptive and PLIF could possibly be added to the currently investigated vaccines that target strategic points in the reproductive process, fertilization and maternal recognition of pregnancy (Atiken et al., 2002).

The passive transfer of anti-C48 antibodies to pregnant mice at 3.5 dpc produced a unique model suited to address the role of PLIF in placentation and embryo development. In our mouse model, blocking PLIF by antibodies resulted in a high rate of embryo resorption, diminished placental growth and reduced maternal blood supply, resulting in IUGR without affecting fetal organogenesis.

The results obtained in the current study, resemble our previous observations where a high association between circulating PLF levels and complications of pregnancies was found. It was reported that low level or deficiency of p43-PLF (currently designated PLIF) is accompanied by abnormal pregnancy outcome such as early abortions (Fisch et al., 1996; Moroz et al., 2002), preterm delivery (Maymon et al., 1989), IUGR (Rosen et al., 1996) and pre-eclampsia (Bar et al., 1998).

Normal placental development depends on passage of oxygenated maternal blood and nutrition to the fetus. Poor uterine blood flow or small placental surface area can adversely affect fetal growth. Proper development of the placental vascular system is essential for nutrient and gas exchange between mother and developing embryo. For example, pre-eclampsia, which is an important clinical condition of altered placental growth and fetal IUGR, is caused by impaired placental vascularity (Redline et al., 1995). We demonstrated that blocking of PLIF (C48) impaired placental growth and function, leading to growth restriction of the embryo. The possibility that it is a result of reduced oxygen and nutrient supply throughout the placenta cannot be ruled out.

Recent data support the notion that pregnancy-associated suppression of maternal cell-mediated immune responses contribute to the physiologically compatible environment for the conceptus (Raghupathy et al., 1997; Clark et al., 1999b; Dealtry et al., 2000; Mellor et al., 2000).

Evidence in rodents suggests that successful pregnancy is associated with predominance of anti-inflammatory cytokines such as IL-4 and IL-10 in the uterine microenvironment while prevalence of Th1 cytokines such as IFN-γ and TNF-α may adversely affect pregnancy outcome (Wegmann et al., 1993; Chaoa et al., 1999). Human studies on IVF patients showed significantly increased Th1 cytokine expression (low level of IL-10 and high level of TNF-α and IFN-γ) in women with recurrent spontaneous abortion or implantation failure (Gilman-Sachs et al., 2002). Interestingly, PLIF has been shown to act as a vigorous inducer of IL-10 both in vitro and in vivo, exerting its effect both locally as well as systemically (Weinberger et al., 2003; Zahalka et al., 2003). How blocking of PLIF in pregnant mice induced a high rate of fetal resorption and IUGR is not yet established. However, it may be related to the finding of increased Th1 (TNF, IL-2, IL-12) and decreased Th2 (IL-4, IL-5, IL-6, IL-10) type immune response in anti-C48 Ig-treated mice.

Since PLIF is produced by syncytiotrophoblast cells (Maymon et al., 2000) and is elevated in the maternal circulation, it may exert its immunosuppressive effect both in the placenta as well as systemically, leading to suppression of activated clones at different sites. This mechanism may be involved in the remission observed during pregnancy in patients with rheumatoid arthritis (RA) (Ostensen and Husby, 1983) and multiple sclerosis (MS) (Confavreux et al., 1998).

Indeed, we have shown previously that recombinant C48 administration to mice with RA induced a disease remission accompanied by Th2 type immune response of activated splenocytes (Weinberger et al., 2003). In the current study, the opposite effect occurred when anti-C48 was administered to pregnant mice. This treatment resulted in increased Th1 (IL-2, TNF, IL-12) and decreased Th2 (IL-4, IL-5, IL-10, IL-6) cytokine secretion by activated splenocytes and affected the pregnancy outcome.

This assumption is supported by previous observations that TNF and IL-12 are associated with early pregnancy loss (Hill et al., 2000).

The outbred allogeneic pregnant mouse model we developed by blocking PLIF with antibodies to C48 is compatible with the previously reported association between PLIF deficiency in human pregnant patients and abortion, premature delivery and IUGR (Maymon et al., 1996). PLIF may be viewed as a major regulatory cytokine governing the Th1/Th2 cytokine balance operating during pregnancy.

In summary, our results provide in vivo evidence that PLIF plays a major role in placental and fetal development. Thus, pathological pregnancies associated with deficiency of PLIF may be candidates for treatment with recombinant C48/PLIF as a novel immunological strategy to improve the success of pregnancy.

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