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Placental Immunopathology in the FIV-infected Cat: a Role for Inflammation in Compromised Pregnancy?

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Abstract

In utero transmission of feline immunodeficiency virus (FIV) occurs frequently in queens experimentally infected with FIV-B-2542 and other FIV isolates. Fetal infection was detected as early as 3–4 weeks gestation, and the incidence of fetal infection increases with progressing gestation. Reproductive failure occurs commonly, including fetal resorptions and developmentally-arrested fetuses, demonstrating that fetal demise occurs early in gestation. Precise, temporal immunomodulation within the placenta is essential for successful pregnancy. Placental Th1 and Th2 cytokines must be appropriately balanced, typically favoring Th2 cytokines at the maternal-fetal interface. Abnormal inflammatory cytokine expression often accompanies miscarriage. Regulatory T cells (Tregs) play an essential role in maternal tolerance of the semi-allogeneic fetus by suppressing inflammation. We are using the FIV-infected cat to examine the relationship between lentivirus-induced placental immunopathology and reproductive outcome. Using TaqMan real time reverse transcriptase (RT)-PCR, we measured relative expression of key immunomodulators in the placentas of FIV-B-2542-infected and control cats, including placentas from both viable and nonviable pregnancies. Our data associate significantly-increased expression of inflammatory cytokines with failed pregnancies, identify Treg markers in the placentas, and provide preliminary evidence that Tregs or other cells bearing similar activation markers may be involved in pregnancy maintenance. Our data suggest that placental inflammation in the FIV-infected cat may compromise pregnancy.

Keywords

Feline immunodeficiency virus; mother-to-child transmission; placental cytokines; regulatory T cells

Introduction

The FIV-infected cat provides a useful small-animal model for HIV pathogenesis because the two lentiviruses share genetic and biological similarities, and because the clinical manifestations of FIV infection in cats closely parallel those that occur in HIV-infected humans. We are using this system to model HIV mother-to-child transmission (MTCT) as well

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Conflict of Interest

None

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as lentivirus-induced placental immunopathology, which we believe results from aberrant inflammatory changes in the placental microenvironment.

Rate and timing of vertical transmission of lentiviruses

It is generally believed that most perinatal transmission of HIV occurs either very late in gestation or during delivery (Brossard et al., 1995; Chouquet et al., 1999). However, early *in utero* transmission was indicated by the detection of HIV in 8-week fetal tissue (Courgnaud et al., 1991; Lewis et al., 1990) and by the frequent detection of the virus in tissues from electively-aborted 16- to 24- week fetuses (fetuses less than 16 weeks were not examined) from asymptomatic, HIV-infected women (Courgnaud et al., 1991). HIV was detected in four miscarried fetuses that were 18 weeks gestation and younger and in three fetuses that were older than 32 weeks gestation (Langston et al., 1995). Collectively, these data indicate that early *in utero* transmission may be more frequent than recognized.

In queens infected with FIV-B-2542, the *in utero* infection rate increases with progressing gestational age. The virus was detected in 0/16, 1/19 (5%), 6/16 (38%), and 9/15 (60%) kittens delivered at 3, 5, 7, and 9 weeks gestation, respectively. Virus load and hematological parameters in the queens did not vary substantially with gestational age, suggesting that placental or fetal maturation may predispose the timing of *in utero* infection (Rogers and Hoover, 1998). Using the same isolate, we detected proviral FIV in at least one tissue from 21/22 offspring delivered by cesarean section from infected queens during the eighth week of gestation, a 95% *in utero* transmission rate. Fourteen of 15 placentas were virus positive, and provirus was frequently detected in fetal blood, brain, liver, bone marrow, spleen, and thymus (Weaver et al., 2005). We also detected viral RNA, but not provirus, in 12/14 fetuses and 14/14 placentas by week 3–4 gestation in a separate study (Boudreaux et al., 2009). In queens infected with FIV-B-2542 or FIV-AB-2771, 42/83 fetuses (51%) were infected at term delivery and by six months of age in seven foster-nursed kittens, these most likely infected late in gestation or during delivery (O'Neil et al., 1996). At 9 weeks gestation, *in utero* transmission rates of 67% (8/12 fetuses) and 91% (10/11 fetuses) occurred in cats infected with FIV-A-Pet and FIV-C-Pgmr, respectively. Viral load and tissue distribution varied with different isolates, and viral sequestration in fetal tissues frequently occurred in the absence of detectable viremia. Infected fetuses often were detected even when virus could not be detected in the placenta (Rogers and Hoover, 2002).

Pregnancy outcome in HIV or FIV infections

HIV infection may contribute to compromised pregnancy and reproductive failure. Evaluating miscarriage rates in a cohort of HIV-infected women from the United States, spontaneous fetal loss occurred in 14/124 pregnancies (11.3%) ranging from 8 to 32 weeks gestation, approximately three times the rate of loss in the general population at comparable gestational stages. HIV was detected in 7/12 (58%) of these aborted fetal tissues (Langston et al., 1995). Adverse pregnancy outcome, including increased levels of miscarriage, stillbirth, pre-term delivery, and low birth weight were significantly increased in HIV-infected women from the U.S., Europe, Asia, and Africa (Anderson, 2001; D'Ubaldo et al., 1998; Goldstein, 2000; Kumar et al., 1995; Rollins et al., 2007). On the other hand, some studies revealed no differences in reproductive outcome between HIV seropositive and negative women (Massad et al., 2004). Pre-term delivery appears to correlate with immune suppression (less than 14% CD4+ T cells) in HIV-infected women (Cohan, 2003).

FIV infection in the pregnant queen may result in pregnancy perturbation. In our study of vertical transmission using FIV-B-2542-infected queens, average litter sizes delivered during the eighth week of gestation were 2.7 kittens/litter and 3.8 kittens/litter in FIV-infected and control queens, respectively (Weaver et al., 2005). Fetal nonviability was 60% (15/25

offspring) in the infected group and 3.2% (1/31 offspring) in the control group. Fetal resorptions and fetuses developmentally arrested early in pregnancy comprised the nonviable offspring, indicating that perturbation occurred early in pregnancy. Arrested fetal development, stillbirth, and abortion associated with *in utero* FIV-CSU-2771 infections were reported (O'Neil et al., 1995). Nine of 17 of the kittens (53%) were either stillborn or dead within 48 hours of birth in that study. More recently, fetuses were delivered from FIV-B-2542-infected and control cats at week 3–4 gestation (Boudreaux et al., 2009). Reproductive failure (resorption) was three-fold higher in the FIV-infected group. Fetal nonviability was 22.2% (6/27 fetuses) in the infected group and 4.7% (2/43 fetuses) in the control group. Average litter sizes for infected and control queens were 3.9 fetuses/litter and 5.4 fetuses/litter, respectively. Collectively, the data demonstrate the deleterious effect of FIV infection on pregnancy and indicate that compromised pregnancy in the FIV-infected cat may parallel pregnancy loss that occurs in HIV-infected women.

The immunology of pregnancy

The immunology of pregnancy remains a curiosity. The fetus can be considered a semi-allograft, and the reasons why this allogeneic tissue is not rejected are not fully understood. Precise, temporal regulation of cytokine expression is necessary for pregnancy to be successful (Raghupathy, 1997). Numerous cytokines are produced in placental tissues and play a role in the maintenance of pregnancy. Th2 cytokines predominate at the maternal-fetal interface and Th1 cytokines are suppressed (Lim, 1998; Lim et al., 2000; Raghupathy, 1997, 2001; Raghupathy et al., 1999; Raghupathy et al., 2000). Th1 cytokine suppression is necessary during early stages of pregnancy because these cytokines induce placental inflammation, leading to pre-eclampsia, pre-term delivery, and/or spontaneous abortion (Lim et al., 2000; Makhseed et al., 2000; Makhseed et al., 2001). These reports and numerous others offer support to the theory that dysregulation of the Th1/Th2 cytokine ratio in placental tissues causes reproductive failure.

Chaouat, et al (Chaouat et al., 2004a, b; Chaouat et al., 2002) challenged the Th1/Th2 paradigm of pregnancy, suggesting this model is likely an “oversimplification”. This group identified temporally-expressed and precisely-localized cytokines at the maternal-fetal interface of mice that did not fit the typical Th1/Th2 dichotomy. For example, IL-18, a cytokine which causes increased IFN- γ production by T cells and NK cells, was expressed to lower levels in abortion-prone mice than in non-abortion-prone mice (Ostojic et al., 2003). Both inflammatory (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines were identified at the maternal-fetal interface in mice at various stages of pregnancy, which seems contrary to the Th1/Th2 model (Zourbas et al., 2001). Lower levels of IL-12 in decidual and peripheral blood leukocytes from spontaneous abortion patients as compared to patients experiencing normally-progressing pregnancies were reported (Zenclussen et al., 2006). In contrast to the Th1/Th2 model, expression of TNF- α and IFN- γ was significantly lower in the peripheral circulation of pregnant women than in non-pregnant women, and IL-10 expression was higher in pregnant women. Surprisingly, TNF- α and IFN- γ expression was even more markedly reduced in women with recurrent pregnancy loss (RPL), and IL-10 and IL-4 were more markedly increased in RPL women. Placental cytokines were not measured in this report (Bates et al., 2002).

Placental inflammation resulting from aberrant cytokine expression in HIV-infected women was postulated to be a contributor to transplacental transfer of the virus (Shearer et al., 1997). Virus-induced dysregulation of cytokine expression in placental trophoblasts, cells which secrete many of the cytokines involved in pregnancy maintenance, may contribute to placentitis. Several cytokines, including IL-1 β and TNF- α stimulated HIV long terminal repeat expression in transfected trophoblasts (Zachar et al., 2002) and enhanced HIV

production in trophoblastic cell lines (Vidricaire et al., 2003). Interferons and β -chemokines did not affect viral expression (Lee et al., 2001; Zachar et al., 2002). Enhanced expression of IL-1 β , IL-6, and TNF- α occurred in trophoblasts isolated from HIV-positive placentas, compared to trophoblasts from uninfected placentas (Lee et al., 1997; Shearer et al., 1997).

Placental cytokine and chemokine expression in the FIV-infected cat model

To model lentivirus-induced placental immunopathology and reproductive outcome, we evaluated the effect of FIV infection on placental immune parameters. Using real time RT-PCR to quantify relative gene expression in cats experimentally infected with FIV-B-2542, we detected no significant differences in the expression of inflammatory cytokines IFN- γ and IL-1 β , or the anti-inflammatory cytokine IL-10 between infected and control cats (Figure 1). However, increased placental expression of IFN- γ and IL-1 β occurred in nonviable pregnancies (Weaver et al., 2005) (Figure 2). The data suggest that placental inflammation accompanies pregnancy failure. Recently, we evaluated expression of the cell surface markers CXCR4 and CD134 in late-term placental tissues from queens producing only viable or mostly nonviable offspring (Scott et al., 2008). Both markers were expressed to highest levels in placentas of infected cats producing only viable offspring (Figure 3), as compared to control and the nonviable pregnancy groups. Although not significant when compared to controls, the two markers were expressed to lowest levels in the cats producing mostly nonviable offspring. These data suggest a role for cells bearing CD134 and CXCR4 in pregnancy maintenance. We propose that these cells may be regulatory T cells.

Regulatory T Cells (Tregs)

Regulatory T cells, comprising 5–10% of peripheral CD4⁺ T cells, are important in suppression of autoimmune diseases, graft rejection, and innate mucosal immunity (Maloy and Powrie, 2001; Maloy et al., 2003). The definitive Treg phenotype, classically identified as CD4⁺CD25⁺ T cells, remains a matter of debate (Askenasy et al., 2008; Yi et al., 2006). CD25, representing the α -chain of the IL-2 receptor, is not reliably expressed on all regulatory T cells. CD25 can be down-regulated following activation in vivo, and potently suppressive CD4⁺CD25⁺ regulatory cells were demonstrated in diabetes and transplant models (Chen et al., 2005; Wood and Sakaguchi, 2003). Forkhead Box P3 (FoxP3) and cytotoxic T lymphocyte antigen 4 (CTLA4), both considered activation markers for Tregs, were expressed to increased levels in the CD25⁺ T cell population in spleens and lymph nodes of highly viremic, SIV-infected macaques (Boasso et al., 2007b). FoxP3 is a transcriptional activator that is fundamentally important in the regulation of Treg function and differentiation (Chang et al., 2006; Fontenot et al., 2003; Ono et al., 2007; Zheng and Rudensky, 2007). Nearly all current reports identify Tregs by their expression of FoxP3.

An additional cell-associated protein used to identify activated Tregs is CTLA4, a constitutively-expressed cell surface marker that serves as negative regulator of T cell activation. B7 receptors on antigen presenting cells initially engage the CD28 receptor on CD4⁺ T cells, transducing a signal that upregulates the production of IL-2. CTLA4 is induced approximately 24–48 hours after activation of the T cell (Krummel and Allison, 1996). CTLA4 inhibits co-stimulation by outcompeting CD28 for B7 binding on antigen presenting cells (Fallarino et al., 2003). The CTLA4/B7 interaction prevents IL-2 production, cell proliferation, and cell cycle progression (Krummel and Allison, 1996).

Treg cells are immunosuppressive either by cell-contact-dependent mechanisms (Askenasy et al., 2008) or by release of cytokines, including IL-4, IL-10 and transforming growth factor (TGF)- β , in part by inhibiting the proliferation of T cells via suppression of IL-2 production by these cells (Thornton and Shevach, 1998). In addition, Tregs suppress the expression of co-stimulatory molecules B7.1 (CD80) and B7.2 (CD86) on dendritic cells (Cederbom et al.,

2000), and selectively destroy antigen presenting B cells (Zhao et al., 2006). TGF- β , in particular, is immunosuppressive to cytotoxic T cells, Th1 cells, antigen presenting cells (Askenasy et al., 2008), and NK cells (Ralainirina et al., 2007). TGF- β is responsible for inhibiting production of cytokines by effector T cells, and it induces expression of IL-10 by Th1 cells (Annacker et al., 2001; Cottrez and Groux, 2001; Thornton and Shevach, 1998; Zheng et al., 2004). TGF- β , most likely in membrane bound form, inhibits NK cytotoxicity by causing down-regulation of the receptor NKG2D (Castriconi et al., 2003). Ligation to this receptor would otherwise lead to destruction of the cells by NK cytotoxic activity. Tregs can indirectly inhibit IL-12-dependent IFN- γ production by NK cells, whereas IL-2 and IL-15-dependent IFN- γ production is not inhibited (Ghiringhelli et al., 2005).

An important means by which Tregs suppress immune activation is via modulation of tryptophan catabolism (Fallarino et al., 2003). Indoleamine 2,3-dioxygenase (IDO), an immunosuppressive, tryptophan-catabolizing enzyme produced by antigen presenting cells and trophoblasts, suppresses T cell proliferation by the rapid depletion of tryptophan (Mellor et al., 2001; Munn et al., 1998). IDO activation by B7-expressing dendritic cells is dependent upon CTLA4 expression by Treg cells and IFN- γ expression by dendritic cells (Fallarino et al., 2003; Grohmann et al., 2002; Miwa et al., 2005). A CTLA4-independent pathway of IDO expression was also reported following in vitro Treg activation by LPS treatment. LPS-stimulated Tregs expressed substantial amounts of IFN- γ and IL-10 which promoted expression of IDO expression from dendritic cells (Fallarino et al., 2003).

Feline Tregs and FIV infection

The feline Treg phenotype is also imperfectly defined. While the cell population expressing FoxP3 is typically the CD4+CD25+ T cell population, small populations of FoxP3-expressing CD4+CD25-, CD8+, and CD21+ lymphocytes were detected in the peripheral circulation. Nevertheless, feline Treg cells have been characterized most definitively by co-expression of CD4, CD25, and FoxP3 (Lankford et al., 2008). Feline Tregs comprise 5 to 10% of the total CD4+ T cells in the blood and 20 to 30% of lymph node CD4+ T cells and express many phenotypic and functional characteristics of human and murine Tregs. They are anergic, do not produce IL-2, are responsive to LPS, and are capable of suppressing Con A-stimulated CD4+CD25- T cells (Vahlenkamp et al., 2004b). While both CD4+CD25+ and CD4+CD25- T cells may be latently infected with FIV, the state of activation of these cells dictates whether or not productive infection occurs. CD4+CD25+ T cells stimulated with low levels of IL-2 support FIV replication. However, stimulation of latently-infected CD4+CD25- T cells with potent mitogens such as Con A or high levels of IL-2 is required to promote productive infection, correlating with up-regulation of CD25 expression in these cells (Joshi et al., 2005; Joshi et al., 2004). CD4+CD25+ T cells express higher levels of CXCR4 and CD134 than CD4+CD25- T cells, and FIV entry is positively correlated with levels of CXCR4 expression. Moreover, CD4+CD25+ T cells constitutively express transcriptional activators which bind the FIV long terminal repeat (LTR) and enhance viral replication. Joshi et al., (2005b) reported that activating transcription factor (ATF) and CAAT enhancer binding protein (CEBP) were expressed at high levels, while activating protein-1 (AP-1) was expressed at low levels. AP-1 expression was enhanced by IL-2 stimulation of the cells. CD4+CD25- T cells constitutively expressed very low levels of the transcriptional activators, all of which were up-regulated by Con A stimulation. FIV infection causes upregulation of expression of co-stimulatory molecules B7.1, B7.2, and their ligand CTLA4 on blood and/or lymph node Tregs (Vahlenkamp et al., 2004b), correlating with disease progression in FIV-infected cats (Tompkins et al., 2002; Tompkins and Tompkins, 2008; Vahlenkamp et al., 2004a). T cells expressing co-stimulatory molecules engage in T cell-T cell interactions that mediate cell death by apoptosis (Bull et al., 2004; Vahlenkamp et al., 2004a).

Tregs and lentiviral infection

This pattern of replication is paralleled in HIV infections. HIV also infects both CD4+CD25⁺ and CD4+CD25⁻ T cells, but in the absence of additional activation, only the CD25⁺ T cells produce viral protein. Elimination of these cells virtually eliminates virus expression (Ramilo et al., 1993). Tregs suppress HIV-specific cytotoxic T cell activity, as well as secretion of IFN- γ , TNF- α , and CC-chemokines from these cells (Kinter et al., 2007a; Kinter et al., 2007b). In an *in vitro* model of HIV infection, CD4⁺ T cell proliferation was suppressed as a result of secretion of IDO by dendritic cells (Boasso et al., 2007a). Likewise, increased expression of IDO, FoxP3, and CTLA4 in gut and lymphoid tissue of SIV-infected macaques correlated well with viral RNA levels in the same tissues (Boasso et al., 2007b). Collectively, these data indicate that these viruses replicate preferentially in Tregs and that the immunosuppressive action of Tregs renders the host incapable of controlling viral infection. These data support the idea that CD4+CD25⁺ Treg cells specific for HIV antigens may contribute to the general immunosuppression that occurs during HIV infection, allowing the establishment of chronic infection (Vahlenkamp et al., 2005).

On the other hand, preferential replication of HIV in the Treg population in chronic infections leads to depletion of those cells (Baker, 2007; Eggena et al., 2005). Using surface expression of CD62L as a definitive marker for Tregs in a cohort of 81 HIV-positive Ugandan volunteers, Tregs were depleted with the significant correlation of T cell activation (Eggena et al., 2005). Likewise, depletion of Tregs occurred in the gut-associated lymphoid tissue of SIV-infected macaques with a concomitant loss of immunosuppressive function in these tissues, possibly leading to persistent inflammation in this location (Chase et al., 2007).

Tregs and pregnancy maintenance

Recently, a role for Tregs in the maintenance of pregnancy was recognized, perhaps largely explaining maternal tolerance of the semi-allogeneic fetus. The median percentage of these cells in the peripheral circulation doubled during the first and second trimesters of normal human pregnancy, and these cells suppressed proliferative responses of CD4+CD25⁻ T cells to allogeneic dendritic cells (Somerset et al., 2004). In the human decidua, CD4+CD25⁺ T cells comprise approximately 10% of CD4⁺ T cells during early pregnancy and 14% at term (Chao et al., 2002; Heikkinen et al., 2004). Higher levels of activated CD4+CD25⁺ T cells in both the periphery and decidua were associated with successful pregnancy (Sasaki et al., 2004; Zhu et al., 2005), while failed pregnancy and pre-eclampsia were accompanied by reduced number and function of these cells (Sasaki et al., 2007; Sasaki et al., 2004). Tregs begin to accumulate in the human uterus as the female approaches estrus, in preparation for possible implantation (Kallikourdis and Betz, 2007). Adoptive transfer of Tregs from normal pregnant mice prevented fetal rejection in abortion prone mice, while implantation failure occurred when an anti-CD25 monoclonal antibody was administered at day 0.5 to 2.5 in a mouse semi-allogeneic model of pregnancy (Zenclussen, 2005; Zenclussen et al., 2005). In mice, highly suppressive Treg cells display CCR5. Using the mice model, the Treg phenotype CD4+CD25⁺CCR5⁺ accumulated in the uterus of allogeneic, but not syngeneic matings (Kallikourdis et al., 2007). In humans, the CD4+CD25^{high}CTLA4 phenotype is apparently most responsible for successful pregnancy. Increased numbers of this Treg phenotype accumulate during normal pregnancy but decrease to pre-pregnancy levels if pregnancy fails (Sasaki et al., 2004). The expression of CTLA4 induces antigen presenting cells to produce IDO.

It is now clear that IDO plays a major role in tolerance of the fetal allograft. Performing matings between allogeneic and syngeneic transgenic mice clearly demonstrated that IDO was required for successful pregnancy (Mellor et al., 2001; Munn et al., 1998). Rejection of fetal allografts increased in a dose-dependent manner in mice treated with increasing concentrations of 1-

methyl tryptophan, an IDO inhibitor. Failed pregnancies were a result of inflammation at the maternal-fetal interface marked by T cell recruitment, deposition of complement components, and hemorrhagic necrosis. Likewise, up-regulated IDO was detected in both peripheral and decidual dendritic cells and monocytes during normal pregnancy. Conversely, IDO expression was reduced in these cells in failed pregnancies (Miwa et al., 2005).

The importance of Tregs in pregnancy maintenance was further supported by examining Tregs from the perspective of the fetal tolerance of maternal alloantigens. Human Tregs are present in fetal cord blood and secondary lymphoid organs at levels higher than those of adults (Michaelsson et al., 2006), and their contribution to immunosuppression is becoming clear. Fetal CD4⁺ and CD8⁺ T cells are highly reactive to stimulation in utero in the absence of CD4⁺CD25⁺ Tregs, a subpopulation of these cells expressing the early activation marker CD69 and secreting INF- γ . Adding back autologous CD4⁺CD25⁺ T cells suppressed INF- γ production by these cells (Michaelsson et al., 2006). Moreover, following depletion of CD25⁺ T cells, fetal T cells were highly reactive to maternal APCs, but only slightly more reactive to unrelated APCs. Evaluating mesenteric lymph node tissue from 18–22 gestational week fetuses in very recent study (Mold et al., 2008), a high rate of maternal microchimerism (15/18 lymph node samples) was found, likely a result of engraftment of maternal cells that cross the placenta with fetal tissue. Fetal spleen and lymph node lymphocytes were highly reactive to alloantigens in vitro, clearly establishing the potential for fetal T cells to target noninherited maternal alloantigen (NIMA). The fetal T cells differentiate into CD4⁺CD25^{high}FoxP3⁺ T cells in response to NIMA in utero, suppressing immune reactivity to maternal antigens (but not paternal antigens). These Tregs persists in children at least into young adulthood. Likewise, maternal Tregs specific for their children's alloantigens persist well past birth as well.

Collectively, our studies (Scott et al., 2008; Weaver et al., 2005) lead us to suspect that feline placental Tregs may play a role in pregnancy maintenance in the FIV-infected cat model. We are currently evaluating expression of the Treg markers CD25 (IL2R α), FoxP3, and others in placental tissues from viable and nonviable pregnancies. RNA extracted from sections of placenta collected during cesarean delivery at week 8 gestation from FIV-infected and control cats (Weaver et al., 2005) was used in TaqMan real time RT-PCR (Scott et al., 2008), targeting CD25, FoxP3 (Lankford et al., 2008), and the housekeeping gene β -actin (Table 1). The relative placental expression of the two markers in FIV-infected versus control cats, as well as expression in viable versus nonviable pregnancies is shown (Figure 4). We did not detect significant differences in expression of either marker in infected versus control or viable versus nonviable placental tissues. CD25 expression was very similar among all comparisons, suggesting its consistent expression. FoxP3 expression varied among the different comparisons, but due to variability among individual specimens, differences between groups did not reach the level of significance ($P < 0.05$). The trend toward the highest level of expression of FoxP3 occurring in the infected, viable pregnancy group suggests the possibility that Treg activation is enhanced in this group. If this is the case, the data support the predicted pattern of activated Treg immunosuppression allowing fetal tolerance.

Conclusion

In conclusion, MTCT is frequent in experimentally-FIV-B-2542-infected cats by week 3–4 gestation (Rogers and Hoover, 1998, 2002; Weaver et al., 2005), and maternal infection often results in reproductive failure (Boudreaux et al., 2009; Weaver et al., 2005). Using the FIV-infected cat model to evaluate lentivirus-induced placental immunopathology, we have accumulated data which suggest that placental inflammation accompanies failed pregnancy. We are continuing to examine the expression of placental Th1 and Th2 cytokines and Treg activation markers in viable and nonviable placental tissues collected during early (week 3–4) and late (week 8) gestation in control and FIV-infected cats. It is our intent to determine how

FIV infection contributes to inflammation in the placental microenvironment fostering MTCT and adverse reproductive outcome.

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Figure 1.

Relative expression of placental cytokines. TaqMan real time RT-PCR was used to quantify relative expression of representative inflammatory (IFN- γ and IL-1 β) and anti-inflammatory (IL10) cytokines in 8 week gestation placentas from FIV-infected (infect) and control (cont) cats. Adjusted mean Ct values were obtained by subtracting mean Ct values from a negative endpoint. Bars represent adjusted mean Ct values for gene expression in control placental samples (n=12) and FIV-infected placental samples (n=13). Standard errors of the mean are bracketed. P values obtained from single-factor ANOVA are annotated. P values < 0.05 were considered significant.

Figure 2.

Relative expression of cytokines in placentas from resorbed and non-resorbed fetuses. TaqMan real time RT-PCR was used to quantify relative expression of representative inflammatory (IFN- γ and IL-1 β) and anti-inflammatory (IL10) cytokines in 8 week gestation placentas from FIV-infected resorbed (resorb) fetuses (n=5–6) and all non-resorbed (nonresorb) fetuses (n=17–20). Adjusted mean Ct values were obtained by subtracting mean Ct values from a negative endpoint. Standard errors of the mean are bracketed. P values obtained using Wilcoxon rank sums test are annotated. P values < 0.05 were considered significant.

Figure 3.

Relative expression of FIV receptors in placental tissues. TaqMan real time RT-PCR was used to quantify relative expression of FIV receptors CXCR4 (top) and CD134 (bottom) in 8 week gestation placentas. Placentas from three groups of cats were evaluated: control queens producing only viable offspring (n=3), FIV-infected queens producing only viable offspring (FIV+ viable) (n=3), and FIV-infected queens producing viable offspring among mostly nonviable litters (FIV+ nonviable) (n=3). Adjusted mean Ct values were obtained by subtracting mean Ct values from a negative endpoint. Bars represent adjusted mean Ct values for gene expression. Standard errors of the mean are bracketed. P values obtained from single-factor ANOVA are annotated. P values < 0.05 were considered significant.

Figure 4.

Relative expression of placental Treg markers. TaqMan real time RT-PCR was used to quantify relative expression of Treg markers CD25 (top) and FoxP3 (bottom) in 8 week gestation placentas. Three sets of placentas were evaluated: placentas from FIV-infected cats producing viable offspring (n=9), FIV-infected cats producing nonviable offspring (n=9), and uninfected control cats (n=24). Adjusted mean Ct values (bars) were obtained by subtracting mean Ct values from a negative endpoint. Standard errors of the mean are bracketed. P values obtained from single-factor ANOVA are annotated. Significant differences ($P < 0.05$) between the comparisons were not detected.

Table 1

Sequences of PCR Primers and TaqMan Probes Used in Real Time RT-PCR

Targeted gene and Genbank access. #	Sequence (5'to 3')	Purpose	Reporter
β-actin	GACTACCTCATGAAGATCCTCACG	forward	-
AB051104	TCTCCTTGATGTCACGCACAATT	reverse	-
	ACAGTTTCACCACCACCGCCGAGC	probe	HEX
IFN-γ	CTTCCTCAAGCTGATTCAAATCCCC	forward	-
D30619	GCTCTACGGCCTCGAAACAG	reverse	-
	TGGCTCCTTTTCCGCTTCCTCAGGT	probe	FAM
IL-10	TCTGTGTTTCTGGTCTTCCTG	forward	-
AF060520	GTGGGTGCAGTTGTCCTCAG	reverse	-
	TAGGAGCCAGCCGACACCAGAGCAC	probe	FAM
IL-1β	GGAGATGAGGGCATCCAGTTG	forward	-
M92060	ATAGCCACAATGACCGACACG	reverse	-
	CTCCCACCAGCCGACAACAAGAGT	probe	FAM
CXCR4	AAGGCAGTCCATGTCATCTACAC	forward	-
AJ009816	AGACCACCTTTTCAGCCAACAG	reverse	-
	ACCTCTACAGCAGTGCCTCATCCTGGC	probe	FAM
CD134	CAGGTTATGGGATGGAGAGTCG	forward	-
AY738589	TGCAAGGCTCGTAGTTCACG	reverse	-
	TGACCAGGACACCAAGTGCCTCCAGTG	probe	FAM
FoxP3	GCCTGCCACCTGGAATCAAC	forward	-
EF419427	GTGTGCTGGGGCTTGGGA	reverse	-
	CAGTGCTGGCTCCCTGGACACCCA	probe	FAM
CD25 (IL2R α)	CCACGTGACAGAACTGTGTG	forward	-
NM001009355	GTGCCCCGTCTTGTATGTGAG	reverse	-
	CCGCCAGATATCCAACACGCC	probe	FAM

FAM - 6-carboxyfluorescein; HEX - hexachloro-6-carboxyfluorescein