ABSTRACT

Regulatory T cells (Treg) support pregnancy maintenance by suppressing placental inflammation, while diminished placental Treg function may accompany reproductive failure. Experimental FIV infection frequently results in vertical transmission and increased pregnancy failure in the cat. The mechanisms of reproductive compromise are unknown. We hypothesized that FIV infection alters placental Treg population dynamics and function, potentiating vertical transmission and reproductive failure. RNA collected from early and late gestation placentas and fetuses from FIV infected and control cats was probed for expression of FIV gag and Treg markers: CD4, FOXP3, and CTLA4, using real time reverse-transcriptase (RT)-PCR. Frequent placental and fetal infection and reproductive failure were detected at early and late pregnancy. Expression of FOXP3 and CTLA4 was higher in early gestation placentas from control cats. FIV infection significantly reduced expression of FOXP3 at early, but not late pregnancy. At late pregnancy, CTLA4 was expressed to higher levels in infected placentas. The number of placentas with decreased co-expression of FOXP3 and CTLA4 was significant in infected cats at early pregnancy. No significant changes in CD4 expression occurred between FIV infected and control placentas at early or late pregnancy. Differences in Treg marker expression were not significant between viable and non-viable pregnancies in infected cats. The detection of Treg markers in feline placental tissues provides the first evidence of feline placental Tregs and suggests that such cells diminish as pregnancy progresses. These cells may be depleting or rendered less functional by viral infection, but understanding their role in pregnancy requires further study.

HYPOTHESIS

FIV infection in pregnant cats causes alteration in placental CD4+CD25+ regulatory T cell populations, allowing transplacental transmission of the virus and frequent damage to the fetus.

OBJECTIVES

1. Quantify the expression of viral RNA in placental and fetal specimens.
2. Quantify the expression of placental cytokines IL-10, FoxP3, and CTLA4 in placental samples from FIV infected and control queens by real time reverse-transcriptase PCR.

METHODS

Tissues:
- Placentas and fetuses were harvested from FIV-2542-infected and control cats at early (week 1-4) and late (week 8) gestation following cesarean delivery.
- RNA was isolated from placenta and fetuses using TRIzol Reagent.

Gene Expression:
- RNA was used in Taqman real time reverse-transcriptor PCR to quantify relative expression of Treg markers CD25, FoxP3, and CTLA4, along with the FIV gag gene.
- Ct values were normalized to 8-actin.
- Mean Ct values for Treg markers from infected versus control cats or viable versus non-viable pregnancies were compared.
- Comparisons were analyzed using ANOVA followed by Wilcoxon Rank Sum analyses.

Immunohistochemistry (IHC):
- 4 micron tissue sections were fixed in 4% paraformaldehyde for 30 min at 4°C, then washed.
- Fixed tissues were treated with 0.1% Triton X-100 in PBS for 5 min.
- Sections were blocked with fetal goat IgG 2 h at 37°C.
- Sections were incubated 1 h with rabbit polyclonal anti-FoxP3 antibody (AbCam), washed.
- Sections were incubated 1 h with goat anti-rabbit IgG (H+L) F(ab)2, washed.
- Cells were counterstained with DAPI, washed, then overlaid with Vectashield.

RESULTS

Frequent placental infection and reproductive failure were detected at early and late pregnancy. Expression of FOXP3 and CTLA4 was higher in early gestation placentas from control cats. FIV infection significantly reduced expression of FOXP3 at early, but not late pregnancy. At late pregnancy, CTLA4 was expressed to higher levels in infected placentas. The number of placentas with decreased co-expression of FOXP3 and CTLA4 was significant in infected cats at early pregnancy. No significant changes in CD4 expression occurred between FIV infected and control placentas at early or late pregnancy. Differences in Treg marker expression were not significant between viable and non-viable pregnancies in infected cats. The detection of Treg markers in feline placental tissues provides the first evidence of feline placental Tregs and suggests that such cells diminish as pregnancy progresses. These cells may be depleting or rendered less functional by viral infection, but understanding their role in pregnancy requires further study.

SUMMARY OF RESULTS

- Vertical transmission of FIV occurred in 12 of 14 fetuses by 3-4 weeks of gestation.
- In normal animals, Tregs may decrease with advancing pregnancy.
- FIV infection decreased expression of FOXP3 at early, but not late pregnancy.
- FIV infection caused decreased expression of CTLA4 at early pregnancy, but infection caused increased expression of CTLA4 at late pregnancy.
- There were no differences in expression of Treg markers in viable versus non-viable pregnancies. Thus, the importance of cells expressing these markers on reproductive outcome is unclear.

CONCLUSIONS

- Tregs are present at the feline maternal-fetal interface.
- In early pregnancy, FIV infection may diminish the Treg population, possibly allowing inflammation and predisposing fetal infection and/or compromising pregnancy.
- Further research is ongoing to determine how FIV infection may alter Treg dynamics and function at the maternal-fetal interface.

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