ST-908

EXPRESSION OF REGULATORY T CELL ACTIVATION MARKERS IN PLACENTAL TISSUES FROM EARLY AND LATE PREGNANCY IN THE FIV-INFECTED CAT

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ABSTRACT

Regulatory T cells (Tregs) 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 mechanism of reproductive compromise is 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 CD25, 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 CD25 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 depleted 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 (Treg) 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 Treg markers CD25, FoxP3, and CTLA4 in placental samples from in FIV-infected and control queens by real time reverse-transcriptase PCR.

METHODS

Tissues:

•Placentas and fetuses were harvested from FIV-B-2542-infected and control cats at early (week 3-4) and late (week 8) gestation following cesarean delivery.
•RNA was isolated from placentas and fetuses using TRIzol Reagent

Gene expression:

•RNA was used in Taqman real time reverse-transcriptase PCR to quantify relative expression of Treg markers CD25, FoxP3, and CTLA4, along with the FIV gag gene.
•Ct values were normalized to β-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 feline IgG 2 h at 37°C.
•Sections were incubated 1h with rabbit polyclonal anti-FoxP3 antibody (AbCam), washed.
•Sections were incubated ~ 1h with goat anti-rabbit IgG (H+L) FITC, washed.
•Cells were counterstained with DAPI, washed, then coverslipped with Vectashield.

RESULTS

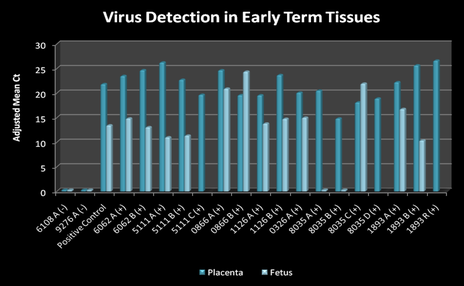

Figure 1. TaqMan real time RT-PCR analysis of FIV gag gene expression in placentas and corresponding fetuses from early (week 3-4) pregnancy in FIV-infected cats. (a) Viral mRNA was amplified from all placental samples (14 of 14 samples). FIV gag was detected in 12 of 14 fetal samples. Negative placental samples (6108 A, 9276 A) were obtained from control queens. Bars represent mean Ct values subtracted from a negative endpoint (adjusted mean Ct).

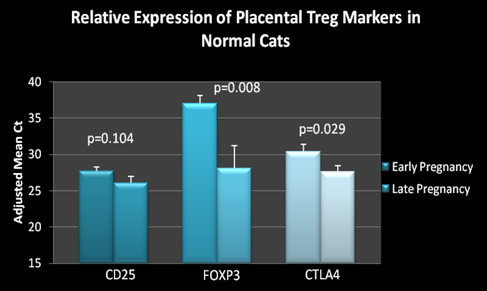

Figure 2. TaqMan real time RT-PCR analysis of placental expression of Treg markers CD25, FOXP3, and CTLA4 in early gestation control (n=18) versus late gestation control (n=13) samples. Bars represent mean Ct values subtracted from a negative endpoint (adjusted mean Ct), and error bars represent standard errors of the mean. P values < 0.05 were considered significant.

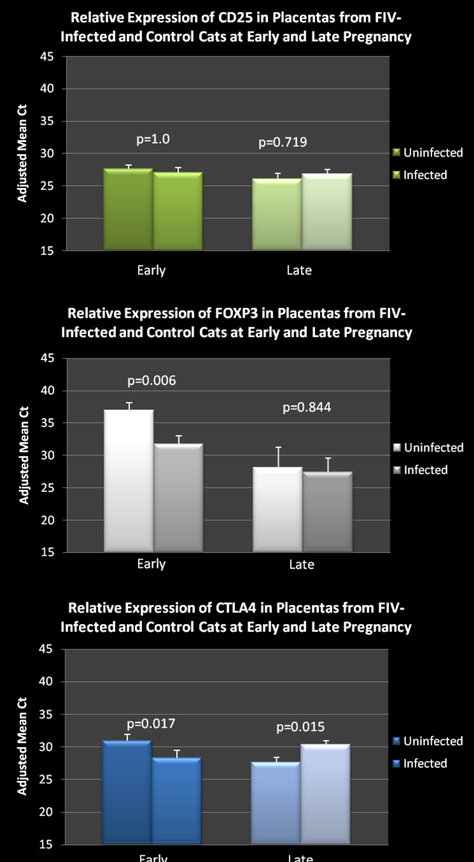

Figure 3. TaqMan real time RT-PCR analysis of placental expression of Treg markers CD25, FOXP3, and CTLA4 in control and infected placentas at early and late pregnancy. Placental samples were evaluated as follows: infected (n=17) versus control (n=18) at early pregnancy; and infected (n=18) versus control (n=13) at late pregnancy. Bars represent mean Ct values subtracted from a negative endpoint (adjusted mean Ct), bracketed by standard errors of the mean. P values < 0.05 were considered significant. (top) CD25 (middle) FOXP3 (bottom) CTLA4.

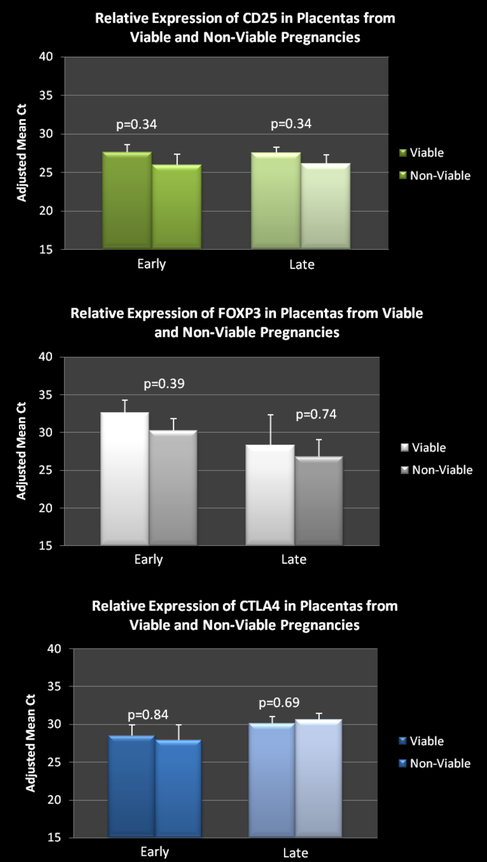

Figure 4. Relative expression of Treg markers in early and late term infected placentas producing viable versus non-viable fetuses. The placental samples were evaluated as follows: infected cats producing viable offspring at early pregnancy (n=11) vs infected cats producing non-viable offspring at early pregnancy (n=5); and infected cats producing viable offspring at late pregnancy (n=9) vs infected cats producing non-viable offspring at late pregnancy (n=9). Bars represent mean Ct values subtracted from a negative endpoint (adjusted mean Ct), bracketed by standard errors of the mean. P values < 0.05 were considered significant. (top) CD25, (middle) FOXP3, (bottom) CTLA4.

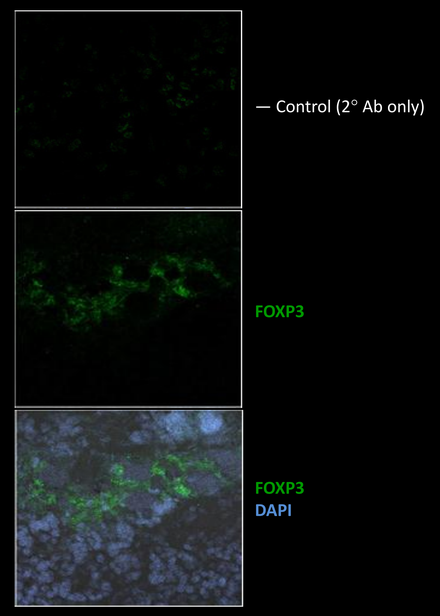

Figure 4. Immunohistochemistry of a representative, late- term, FIV-negative feline placental tissue section. Rabbit polyclonal antibody to FoxP3 labeled with FITC was used to detect FoxP3-expressing cells.

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 caused 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.
•FIV infection did not affect expression of CD25.
•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 are unclear.
•FOXP3-expressing cells were detected at the maternal-fetal interface of tissue specimens using IHC.

CONCLUSIONS

•Tregs are present at the feline maternal-fetal interface.
•At 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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