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USING SMALL INTERFERING RNA FOR TREATMENT OF FIP

PROJECT STUDY: Immune modulation using small interfering RNA for treatment of feline infectious peritonitis

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Feline infectious peritonitis (FIP) is a highly fatal disease caused by virulent feline coronavirus (FCoV) that has the ability to infect monocytes/macrophages, and the lack of an effective host immune response against the virus. Efficient systemic virus proliferation and progression of the disease has been reported to be associated with T-lymphocyte apoptosis, but to date, the exact mechanisms behind the immune system dysfunction have not been elucidated.

Programmed death-1 (PD-1) is an immune-regulatory receptor whose interaction with its ligand, PD-L1, decreases T-lymphocyte immune responses. This pathway has been implicated as having a role in immune evasion of various chronic viral infections. Consequently, PD-1 molecules may have a role in the immune suppression and immune dysregulation observed in feline chronic viral infections, including FIP.

This current study investigated whether the PD-1/PD-L1 pathway has a role in immune dysfunction associated with FIP. Blood samples were collected from 9 FIP-diagnosed cats, 7 FCoV-infected but otherwise health cats and 12 healthy cats. The Principal Investigators then assessed the expression of PD-1 and PDL-1 mRNA in the collected blood using real-time RT-PCR. The expression of PDL-1 mRNA was significantly increased in blood obtained from 9 FIP-diagnosed cats, 7 wet form and 2 dry form, compared with that collected from 12 healthy uninfected cats and 7 cats with mild enteritis infected with enteric FCoV. The number of each form of FIP was limited and therefore, all the statistical analysis done in this study combine the two forms as one group. The PD-1 mRNA expression was significantly increased in blood obtained from 5 FIP-diagnosed cats (blood samples were no longer available from 4 cats to assess the expression of PD-1), compared with that collected from 10 healthy uninfected cats and 7 cats with mild enteritis infected with enteric FCoV.

To assess the effect of blocking of PD-1/PDL-1 pathway on the immune cells in vitro in the lab, initially, we assessed the ability of short-interfering RNA (siRNA) to inhibit the expression of PDL-1. The PDL-1 mRNA expression was assessed in siRNA transfected cells and non-transfected negative control cells via real-time RT-PCR. The designed siRNAs resulted in 90% reduction in the PDL-1 expression as compared with the negative control. Next, we assessed the effect of the PD1/PDL-1 pathway blockage on T cells apoptosis (death). There was not a significant difference in the percentage of apoptosis in the treated cells as compared with the negative control.

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In conclusion, these results show that there is a **significant increase in PDL-1 and PD-1 mRNA expression in blood collected from FIP-diagnosed cats, as compared** with that collected from healthy uninfected cats and cats with mild enteritis infected with enteric FCoV. Therefore, PD-1 molecules may have a role in the T cell apoptosis (death) associated with FIP, as has also been suggested for FIV.

Currently, there is no definitive anti-mortem test to diagnose FIP and the diagnosis is usually based on the clinical signs as well as the findings of multiple tests. Thus, the increased expression of PD molecules can potentially be used as a biomarker to confirm FIP diagnosis in suspected cases, but further testing with more samples from both wet and dry forms of FIP is necessary to validate this. Blockage of the PD-1 pathway alone was not sufficient to significantly reduce T cells apoptosis (death) in FCoV infected cells. These findings suggest that there are multiple factors and/or pathways that contribute to the immune dysfunction associated with FIP.

Summary prepared by Vicki L. Thayer DVM, DABVP (Feline) © 2017