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Evaluation of viremia, proviral load and cytokine profile in naturally feline immunodeficiency virus infected cats treated with two different protocols of recombinant feline interferon omega

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ABSTRACT

This study assesses viremia, provirus and blood cytokine profile in naturally FIV-infected cats treated with two distinct protocols of interferon omega (rFeIFN-ω).

Samples from FIV-cats previously submitted to two single-arm studies were used: 7/18 received the licensed/subcutaneous protocol (SC) while 11/18 were treated orally (PO). Viremia, provirus and blood mRNA expression of interleukin (IL)-1, IL-4, IL-6, IL-10, IL-12p40, Interferon-γ and Tumor Necrosis Factor-α were monitored by Real-Time qPCR. Concurrent plasma levels of IL-6, IL-12p40 and IL-4 were assessed by ELISA.

IL-6 plasma levels decreased in the SC group ($p = 0.031$). IL-6 mRNA expression ($p = 0.037$) decreased in the PO group, albeit not sufficiently to change concurrent plasma levels. Neither viremia nor other measured cytokines changed with therapy. Proviral load increased in the SC group ($p = 0.031$), which can be justified by a clinically irrelevant increase of lymphocyte count.

Independently of the protocol, rFeIFN-ω seems to act on innate immunity by reducing pro-inflammatory stimulus.

1. Introduction

Recombinant Feline Interferon Omega (rFeIFN-ω) is an immunomodulator commonly used in feline retroviral infections (de Mari et al., 2004; Doménech et al., 2011; Gil et al., 2013). It is produced as a recombinant protein by means of a baculovirus expression vector which contains the feline interferon omega (IFN-ω) sequence (Ueda et al., 1993). This baculovirus replicates in silkworms, permitting the production of the glycosylated molecule which, after purification, can be used therapeutically (Ueda et al., 1993).

The recommended protocol is based on 3 cycles of 5 daily subcutaneous administrations (1 MU/kg), beginning respectively on days 0, 14 and 60. Following initial *in vitro* studies (Truyen and Schultheiss, 2002), several authors have performed *in vivo* trials in order to assess its clinical and immune properties. The action of rFeIFN-ω in cats naturally infected with feline leukemia virus (FeLV) and co-infected with FeLV and feline immunodeficiency virus (FIV) has been described, showing that this compound induced an important clinical improvement and an increased survival time of treated cats (de Mari et al., 2004). Another group of authors reported that rFeIFN-ω improved the clinical condition of retroviral infected cats, although minor changes were observed on other parameters such as hypergammaglobulinemia, CD4/CD8 ratio, proviral load and viremia (Doménech et al., 2011). Thus, an overall improvement of innate immunity was suspected (Doménech et al., 2011). Recently, our group has reported that, in addition to improving clinical signs, rFeIFN-ω also induces a reduction of concurrent viral shedding (namely herpesvirus, coronavirus, parvovirus and calicivirus) which is particularly relevant in shelter medicine (Gil et al., 2013). In an attempt to understand the immune pathways underlying the therapeutic action of rFeIFN-ω, we also evaluated the effect of this compound on acute phase proteins (APPs) in naturally retrovirally

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infected cats, confirming that it potentiates the innate immune response (Leal et al., 2014).

Despite the clinical benefits of the licensed protocol in naturally retrovirus-infected cats, rFeIFN-ω can be cost-limitative in some cases and alternative protocols have been investigated. After some trials describing the use of lower oral doses of rFeIFN-ω in various conditions such as chronic gingivostomatitis (Hennet et al., 2011; Leal et al., 2013), an oral protocol was recently proposed in naturally FIV-infected cats (Gil et al., 2014). This was based on the daily oral administration of 0.1 MU/cat during 90 consecutive days and, in a similar way to the licensed protocol, revealed a significant clinical improvement of treated cats without relevant changes in hematology, serum biochemistry, serum protein electrophoresis or, in contrast to the licensed protocol, APPs (Gil et al., 2014). This apparent difference in the mechanism of action between the two protocols is in agreement with previous authors who suggested that oromucosal interferon (IFN) therapy seems to act by different mechanisms than parenteral protocols (Tovey, 2002). Therefore, while in the licensed protocol the increased APP seems to denote a potentiated innate immune response (Gil et al., 2014; Leal et al., 2014), in the oral protocol the immune mechanisms underlying the observed clinical improvement remain unclear.

Academically, the immune system can be divided into two general parts: the nonspecific (innate) response and the specific (acquired) immunity (Kennedy, 2010) that interact in order to maintain a competent immune system. This is achieved by the production and release of different cytokines which, being mediators of the immune response, have distinct functions including activation of pro-inflammatory and anti-inflammatory pathways (Day, 2012; Kennedy, 2010; Tizard, 2009a, 2009b).

In spite of the fact that most cytokines are pleiotropic, each part of the immune system can be characterized by different cytokine patterns (Roitt and Delves, 2001). For instance, Interleukin-6 (IL-6), IL-1 and Tumor Necrosis Factor (TNF)-α are pro-inflammatory cytokines strongly involved in the innate immune response, potentiating nonspecific pathways such as acute phase response (APR) or fever (Ceron et al., 2005; Paltrinieri, 2008; Tizard, 2009a). Concerning the acquired immune pathways, IL-2, IL-12 and IFN-γ are strongly related to the cellular immune response (Th1 subset activation) (Locksley and Scott, 1991; Pedersen et al., 1998; Tizard, 2009b; VanCott et al., 1996) while the humoral antibody response (Th2 subset) is associated with IL-4, IL-5 and IL-10 production (Barnard et al., 1996; Osborne et al., 1996; Pedersen et al., 1998; Roitt and Delves, 2001; Romagnani et al., 1994).

In feline medicine, particularly in FIV, several studies have been performed not only in cell cultures but also in experimentally infected cats in order to characterize the cytokine profile after infection (Dean and Pedersen, 1998; Dean et al., 1998; Kipar et al., 2004; Lawrence et al., 1995; Lerner et al., 1998; Liang et al., 2000; Linenberger and Deng, 1999; Tompkins and Tompkins, 2008; Wood et al., 2012). Despite the fact that there is no clear Th1 to Th2 shift in response to FIV infection, this retrovirus induces a cytokine dysregulation with alterations in cytokine transcription, leading to an inadequate innate and cell-mediated immune response to other pathogens (Kipar et al., 2004; Levy et al., 1998; Tompkins and Tompkins, 2008).

To the authors' knowledge, no studies (*in vivo*) have been performed in order to assess the cytokine-based immunological pathways underlying the clinical improvement and restored control of other pathogens induced by rFeIFN-ω therapy (Gil et al., 2013, 2014). Therefore, this study aims to evaluate the anti-viral and immunomodulatory properties of rFeIFN-ω by monitoring changes in viremia, proviral load and blood cytokine profile in naturally FIV-infected cats receiving oral or subcutaneous rFeIFN-ω therapy.

2. Materials and methods

2.1. Animals and sample collection

The biological samples used in this study were collected from 18 naturally FIV-infected cats that had been previously enrolled in two past works from the group (Gil et al., 2013, 2014; Leal et al., 2014). In detail, 7/18 cats living in an animal shelter had received the licensed protocol (SC group) while 11/18 cats admitted/referred to the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine – University of Lisbon received the oral protocol (PO group), following the protocols previously described (Gil et al., 2013, 2014; Leal et al., 2014).

The animals had been monitored and submitted to blood collections before (D0) and after therapy (D65 and D90, respectively for SC and PO groups). All the procedures were approved by the Committee for Ethics and Animal Welfare of the Faculty of Veterinary Medicine – University of Lisbon (CEBEA – FMV-ULisboa).

Similar to studies previous published (Gil et al., 2013; Leal et al., 2014), a single-arm trial policy was applied in each group meaning that for each parameter, values on D0 were set as baseline and were taken as the individual control for each cat.

2.2. Relative quantification of cytokine expression by real-time qPCR

At each specified time point, whole blood was collected in RNA protected tubes (RNAprotect Animal Blood Tubes, Qiagen) and, according to the manufacturer's instruction, mRNA was extracted using specific kits (RNeasy protect animal blood kit, Qiagen). Thereafter, cDNA was synthesized using Transcriptor High Fidelity (Roche) following the manufacturer's instructions and used as a template for Real-Time quantitative Polymerase Chain Reaction (qPCR).

The primers used for each gene were published in the literature and the respective authors and sequences are presented in Table 1. Despite the DNAse step performed during the RNA extraction, in order to preclude genomic DNA amplification, primers covered putative exon–exon junctions. Optimization experiments and efficiency assessments for each amplification system were previously performed (data not shown). Primers were obtained from a commercial manufacturer (STAB Vida, Portugal). Relative expression of each cytokine was quantified using Miner software (http://www.miner.ewindup.info), following the computed algorithm for Quantitative Real-time PCR system (Zhao et al., 1995). Beta-actin was set as the housekeeping/reference gene (Table 1).

Real-time qPCR was performed using the StepOne Plus real-time analyzer (Applied Biosystems). The PCR assays comprised, in

Table 1
Primers used to evaluate cytokine expression by Real-time qPCR in naturally FIV-infected cats treated with rFeIFN protocols.

Gene	Oligo	Sequence (5′–3′)	Reference
B-Actin	For	GACTACCTCATGAAGATCCTCACG	Scott et al., 2011
	Rev	CCTTGATGTCACGCACAATTTCC	
IL-1β	For	ATTGTGGCTATGGAGAAACTGAAG	Scott et al., 2011
	Rev	TCTTCTTCAAAGATGCAGCAAAAG	
IL-4	For	CCCCTAAGAACACAAGTGACAAG	Taglinger, Van Nguyen, Helps, Day, & Foster, 2008
	Rev	CCTTTGAGGAATTTGGTGGAG	
IL-6	For	GTGTGACAACTATAACAAATGTGAGG	Scott et al., 2011
	Rev	GTCTCCTGATTGAACCCAGATTG	
IL-10	For	ACTTTCTTTCAAACCAAGGACGAG	Scott et al., 2011
	Rev	GGCATCACCTCCTCCAAATAAAAC	
IL12p40	For	TGGCCTTCTGAAGCGTGTTG	Scott et al., 2011
	Rev	GAAGTACACAGTGGAGTGTCAGG	
IFN-γ	For	TGCAAGTAATCCAGATGTAGCAG	Taglinger et al., 2008
	Rev	GTTTTATCACTCTCCTCTTTCCAG	
TNF-α	For	CACATGGCCTGCAACTAATC	Taglinger et al., 2008