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Low-dose interferon- α treatment for feline immunodeficiency virus infection

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Abstract

Feline immunodeficiency virus sustains an AIDS-like syndrome in cats, which is considered a relevant model for human AIDS. Under precise enrolment requirements, 30 naturally infected cats showing overt disease were included in a trial of low-dose, oral human interferon- α treatment. Twenty-four of them received 10 IU/Kg of human interferon- α and 6 placebo only on a daily basis under veterinary supervision. The low-dose human interferon- α treatment significantly prolonged the survival of virus-infected cats ($p < 0.01$) and brought to a rapid improvement of disease conditions in the infected hosts. Amelioration of clinical conditions was neither correlated with plasma viremia, nor with proviral load in leukocytes. A good survival of CD4+ T cells and a slow increase of CD8+ T cells were also observed in human interferon- α -treated cats. Interestingly, the improvement of the total leukocyte counts showed a much stronger correlation with the recovery from serious opportunistic infections. As shown in other models of low-dose interferon- α treatment, there was a rapid regression of overt immunopathological conditions in virus-infected cats. This hints at a major role of interferon- α in the control circuits of inflammatory cytokines, which was probably the very foundation of the improved clinical score and survival despite the unabated persistence of virus and virus-infected cells.

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1. Introduction

In vivo experiments of low-dose, oral interferon- α treatment have been described in many animal species

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(e.g. humans, cattle, pigs, horses) (Cummins et al., 2005). Such a treatment was shown to induce dramatic clinical amelioration in models of both infectious and chronic inflammatory and/or autoimmune diseases (Tompkins, 1999). The majority of these findings refer to the use of different preparations of human interferon- α (hIFN- α), which shows wide cross-reactivity with cells of other animal species (Viscomi, 1997). Human IFN- α at the applied low oral doses (1–10 IU/kg body weight) cannot exert direct antiviral activity in vivo of any importance, because of the proteolytic digestion in the gastrointestinal tract (Cummins, 2005). Human IFN- α strengthens instead the *immune response* to viral infections, with a significant modulation of important cytokines like IL-1, IL-5, IL-6, IL-8, GM-CSF (Naylor et al., 1999; Tompkins, 1999).

The favourable effects of low-dose, oral, *natural* hIFN- α treatment were also confirmed in feline leukemia virus (FeLV)-infected cats, which showed longer survival than placebo-treated animals (Cummins et al., 1988; Weiss et al., 1991). Oral treatment with *recombinant* hIFN- α was of some clinical benefit to both FeLV and feline immunodeficiency virus (FIV)-infected cats, although clearly ineffective with regard to lymphocyte depletion (Riondato et al., 2003). FIV infection of cats is a valid model for human AIDS (Willet et al., 1997) because of the similarities between FIV and the human immunodeficiency virus (HIV) (Yamamoto et al., 1988). Both FIV and HIV replicate in lymphoid and monocytoid cell subsets and cause profound immunosuppression that eventually leads to opportunistic infections in the infected host (Kanzaki and Looney, 2004). After entry into the susceptible host, FIV replicates in target cells, i.e. T lymphocytes, macrophages, astrocytes and microglia cells (Brunner and Pedersen, 1989; Dow et al., 1990; Rogers et al., 2002;), and infection proceeds in five distinct clinical phases extensively described in the literature (Ishida et al., 1990, 1992):

1. An acute phase.
2. An asymptomatic phase (AP).
3. A persistent, generalized lymphadenopathy (PGL).
4. An AIDS-related complex (ARC).
5. Overt feline AIDS.

A critical feature of the ARC phase is a plethora of immunopathological conditions (glossitis, uveitis,

follicular hyperplasia of tonsils and so on) (Gaffney, 1992; Kanzaki and Looney, 2004), which are reminiscent of the clinical pictures of autoimmune diseases. Thus, on the basis of previously reported *natural* hIFN- α trials in models of autoimmune disease (Tompkins, 1999), we investigated if such a treatment could be also effective in FIV-infected cats. We set out to study the factors (control of the inflammatory response or containment of FIV replication) underlying the possible clinical improvement in the host. To this purpose, we widened the scope of previous studies and dissected the response to hIFN- α therapy: the observed clinical findings were offset against the virological, immunological, haematological and biochemical data sets accumulated in this study.

2. Materials and methods

2.1. Interferon- α

A single preparation of natural hIFN- α from Sendai virus-stimulated lymphocytes of healthy blood donors (Alfaferone, Alfa Wasserman, Bologna, Italy) was employed in the trial. This is a mixture of at least nine different human IFN- α subtypes, including IFN- α 1, - α 2, - α 8 and - α 21 (Mattana, Scapol, 2002, unpublished data). hIFN- α was diluted to 50 IU mL⁻¹ in phosphate buffered saline (PBS) pH 7.2 supplemented with bovine serum albumin (2 mg mL⁻¹) (PBS-BSA), aseptically filtered through 0.22 μ m absolute cartridges and aliquoted into sterile 50 mL bottles provided with injection stoppers and aluminium seals. The bottles containing the diluted hIFN- α were stored at 4 °C throughout the study. Under these conditions, diluted hIFN- α was shown to be stable for at least 12 months. The biological activity of hIFN- α was determined in the cytopathic effect (cpe) inhibition assay using bovine MDBK cells with vesicular stomatitis virus (Meager, 1987).

2.2. FIV-infected cats

Naturally FIV-infected cats indicated by veterinary practitioners from different Italian Regions and Germany were enrolled in the study. Following preliminary arrangements, cross-bred candidate cats

were put under veterinary inspection in catteries and private homes. Fifty of them were finally approved of for the trial according to the following requirements:

- Serological evidence of FIV infection and FeLV-free status, as established by an immunochromatographic kit for separate detection of antibody to FIV gp 40 and of FeLV p27 protein (DUO Speed FeLV-FIV, Bio Veto Test, Seyne-sur-Mer, France); the test is endowed with high sensitivity and specificity (Hartmann et al., 2001). In some cases, an ELISA for FIV-specific antibody had been performed in advance; no discrepancy was observed between the two sets of results.
- Age between 2 and 10 years (mean age of enrolled cats: 5 years).
- Evidence of clinical symptoms of the ARC or AIDS phases.
- Evidence of immunosuppression and/or immunopathological lesions in form of serious, recurrent, infectious and parasitic diseases and pathologies of the oral cavity. The clinical score of the cats was assessed at the beginning of the trial (see Table 1).

Complete data sets were obtained for 27 of the above cats (22 male and five female; 18 male cats had been castrated). Twenty-two of them were treated with hIFN- α and five with placebo (PBS-BSA only). The placebo group consisted of two castrated male and three female cats. In addition, three FIV/FeLV double-positive cats but fulfilling the remaining criteria were also enrolled. Two of these were allocated to the hIFN- α treatment and one to the placebo group. The large majority of cats did not change their usual life habits during the trial. Only seven of them, coming from catteries, were housed in the Pets' Hospital of the Veterinary Faculty in Parma, Italy, and submitted to the therapy 2 months later. Six cats of the treatment

group and one cat of the placebo group were kept by the owners under free-range conditions; all the other animals were kept in apartments with limited freedom of movement outside. Any difference in gender distribution between test and control groups of *FIV-infected cats* would not be of concern, as opposed to studies about the risk of new infections among seronegative animals (Yamamoto et al., 1989).

2.3. Administration of hIFN- α , clinical examination schedule and blood sampling

After rubbing the surface with ethanol, the stopper of the hIFN- α bottle was pierced with a sterile syringe needle. Aliquots of 1 mL (50 IU hIFN- α) were withdrawn and, after removal of the needle from the syringe, administered over the gums from the lateral rim of the lips. Bottles were discarded in the presence of the earliest cloudiness or precipitate. The same procedure was adopted for placebo (PBS-BSA). Administration of hIFN- α and placebo was performed once a day according to the following regime: 7 days of daily treatment and 7 days of no treatment alternating for 6 months. A second round of therapy was applied 2 months later (months 8–14). No other treatment was performed during oral hIFN α therapy, except the administration of common anti-parasitic drugs. Antibiotics for opportunistic infections were employed only in case of serious clinical worsening.

Clinical examination was carried out every second month until month 16. Blood samples in tubes with/without K₃-EDTA were taken from the jugular vein; sedation with a ketamine/acepromazine mixture or diazepam was carried out if needed.

2.4. Clinical score

During the clinical examination cats were weighed and photographed whenever possible. Clinical conditions were evaluated by a score system, whereby points were set during the objective general examination according to the seriousness of the clinical findings: 0 (none), 1 (slight), 2 (moderate), 3 (serious), 4 (very serious). A further point (or proportionally more) was scored for a 500 g change of the initial body weight. Symptoms and lesions of oral cavity (e.g. gum and tongue ulcers, proliferative

Table 1
Clinical score of FIV-infected cats

	Month 1	Month 6	Month 8	Month 14
hIFN- α	6.5 (3.5–10.5)	1.0 (0–3)	1.5 (1–3)	2.0 (1–3)
Placebo	4.0 (1–4)	3.0 (2–4) ^a	ND	ND

Data are expressed in terms of median and interquartile range. ND: not done (all cats but one had died). The data set does not include the three cats with mixed FIV/FeLV infection.

^a Three cats only alive.

stomatitis, tonsil hyperplasia), gastrointestinal tract, lymphatic system, skin, respiratory tract, genital and urinary tracts, heart and circulatory system, locomotor system and nervous system were evaluated. Particular attention was given to signs of immunopathological conditions (rheumatoid-like arthritis, uveitis, retinitis), neoplasias and wasting syndromes (cachexia).

2.5. Plasma viremia measurement

Plasma viremia was evaluated by extracting RNA with the QIAamp Viral RNA kit (Qiagen, Milan, Italy) according to the protocol provided by the manufacturer. Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out as described (Pistello et al., 2003). Briefly, 10 μ L of RNA extracted from plasma were reverse-transcribed in 20 μ L with 900 nM antisense primer M2-AS TTC TGGCTGGTGCAAATCTG, 8 U avian myeloblastosis virus RT (Celbio, Milan, Italy) and 20 U RNase inhibitor (Promega, Milan, Italy). The cDNA was then amplified by TaqMan-PCR on the ABI Prism 7700 Sequence Detection System instrument (Applied Biosystems, Monza, Italy) with the ABI PCR Master Mix (Applied Biosystems), 300 nM sense primer M2-S AGACCGCTGCCCTATTTCACT and 100 nM probe M2-P TGCCTGTTGTTCTTGAGTTAATCC-TATTCCCA. Amplification was carried out by testing samples and controls (negative and positive) in triplicate. Serial 10-fold dilutions (10^{-2} to 10^{-7}) of RNA transcripts were produced by in vitro transcription of gag p24 region cloned pGEM/M2 plasmid (Promega) and used for constructing a standard curve. All the samples positive in only one replica and/or with a coefficient of variation of 50% or greater were re-extracted and tested again in triplicate. Sensitivity of the assay was 100 copies/mL plasma, as evaluated by extracting and amplifying FIV-negative plasma spiked with serial 10-fold dilutions of FIV-M2 gag RNA transcripts.

2.6. Proviral load quantitation

Genomic DNA was extracted from the cells under scrutiny using the QIAamp DNA Blood kit (Qiagen, Milan, Italy). Extracted DNA (0.2–0.6 μ g) was quantified by TaqMan-PCR under the conditions described

above, except that reaction volume was 25 μ L. Serial ten-fold dilutions (10^{-1} to 10^{-7}) of pGEM/M2 in a background of 1 μ g of genomic DNA were used to produce a standard curve and to determine the lowest limit of detection (10 copies of proviral DNA).

2.7. Hematology and clinical chemistry

Blood samples in K₃-EDTA tubes were inserted into an automated analyzer (Medonic CA570, Bromma, Sweden), which provided counts of red blood cells (RBC), total leukocytes, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), platelets, as well as reticulocyte index and total hemoglobin (Hgb). Complete leukocyte formulas were assessed for each sample by visual examination of 200 leukocytes at least in May-Grunwald Giemsa-stained blood smears.

Serum proteins were assessed by electrophoresis on agarose strips and densitometric examination (Super-cello5 apparatus, CGA Strumenti Scientifici, Florence, Italy). A multi-analyzer (Cobas Mira Plus, Roche, Milan, Italy) was employed for determination of total bilirubin, glutamate oxaloacetate transaminase (GOT), gamma-glutamyltransferase (GGT), glutamate pyruvate transaminase (GPT), glucose, urea, creatinine, lactate dehydrogenase (LD), alkaline phosphatase (ALP), amylase, lipase, calcium and total protein.

A range of normal values in healthy cats for the above parameters has been described elsewhere (Kraft and Dürr, 1997).

2.8. Staining with monoclonal antibodies and flow cytometry

Three, 100 μ L-aliquots of anticoagulated blood (K₃-EDTA) were used in each test. Tubes were incubated in an ice bath for 20 min and at room temperature for 10 min with 25 μ L of monoclonal antibodies (mAbs) FE1.7B12 (anti-feline CD4), FE1.10E9 (anti-feline CD8) or none (control), respectively; mAbs were a generous gift of Prof. P.F. Moore (Davis University, California, USA).

RBC were lysed with 2 mL/tube of lysing reagent (NH₄Cl 8.26 g/L, NaHCO₃ 1.0 g/L, Na₄EDTA 0.037%, pH 7.2) for 5 min at room temperature and then centrifuged (250 \times g, 5 min). The supernatant was

discarded and RBC lysis repeated once or twice if necessary.

Cells were washed with FACS buffer (Ca and Mg-free PBS, bovine serum albumin 0.2%, sodium azide 0.1%), centrifuged and incubated with a fluorescein isothiocyanate (FITC)-conjugated, goat anti-mouse IgG antiserum (Kierkegaard & Perry Laboratories, Gaithersburg, MD) in FACS buffer for 30 min at 4 °C in the dark. Cells were washed twice with 3 mL/tube of FACS buffer and resuspended in 400 µL of FACS buffer (for immediate evaluation) or in FACS buffer + 1% paraformaldehyde (for later analysis). Data were acquired on a FACScan flow cytometer (BD Biosciences, San Diego, CA) and analyzed using Lysis II software. Lymphocytes were gated by a combination of characteristic forward and side scatter profiles; for each tube data from 8000 viable cells at least were collected and analyzed. The percentages of CD4- and CD8-positive cells were assessed by subtracting background staining with the anti-mouse IgG FITC-conjugate alone.

Absolute counts of CD4+ and CD8+ T cells/µL of peripheral blood were calculated from the total leukocyte counts and the percentages of lymphocytes (leukocyte formula), CD4+ and CD8+ T cells.

2.9. Statistical analyses

Survival curves of hIFN-α and placebo-treated cats were created using the method of Kaplan and Meier. Cats alive at the end of the observation period were scored as censored patients. The two curves were then compared using the logrank test.

A paired *t*-test was applied to different sets of clinical, hematological and virological data (Prism 2.01, GraphPad Software, San Diego, CA).

3. Results

3.1. hIFN-α treatment prolongs the survival of FIV-infected cats

The hIFNα treatment was easily performed by practitioners and the compliance rate was very high (around 95%). Most important, no side effect was observed throughout the study. The treatment was shown to prolong significantly ($p < 0.01$) the survival

of FIV-infected cats (Fig. 1), whose mean clinical score was not significantly different from that of placebo-treated animals at the time of enrolment (Table 1). Data of Fig. 1 include the three cats with mixed FIV/FeLV infection, since there was no significant difference in the clinical response to hIFN-α treatment compared to cats infected by FIV alone. It should be stressed that all but one subject of the hIFN-α group were alive at the end of the observation period; this was a FIV/FeLV double-positive cat enrolled under very serious clinical conditions. On the contrary, all cats but one of the placebo group died during the observation period. Because of the short mean survival time of placebo-treated cats (Fig. 1), the time-course of their clinical, virological, immunological and hematological parameters could not be compared to that of hIFN-α-treated animals over the whole period of this study (see below). The high compliance rate of the treatment was afforded by the drive of both vets and owners on the basis of the rapid clinical improvement of FIV-infected cats.

3.2. Rapid improvement of the clinical conditions

FIV-infected cats under hIFN-α treatment showed a dramatic clinical improvement in the first 2 months of therapy and reached stable subclinical conditions by the end of the first cycle of treatment, which persisted thereafter (Table 1). In particular, both fever and lymphadenopathies disappeared on average after seven daily treatments, around day 10 of therapy; these symptoms lasted instead till the exitus in

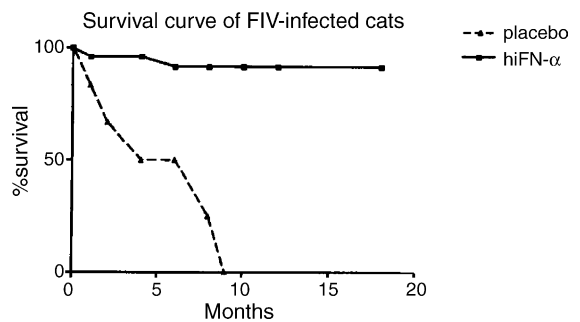


Fig. 1. Per cent survival of FIV-infected cats is depicted in terms of mean. This was assessed on 24 and 6 cats in the hIFN-α and control groups, respectively. The difference between the two curves is highly significant (logrank test, $p < 0.01$).

placebo-treated cats. There was also a rapid regression of typical immunopathological lesions (arthritis, cystitis due to immunocomplexes, uveitis and retinitis); clinically, there was a decline of the inflammatory signs without restitution ad integrum of previous organ lesions (e.g. retinal detachment). Skin ulcers, mycotic infections and hypotrichosis were also on the wane in the first weeks of therapy; no clinical effect was scored instead with regard to neoplasias (basal cell carcinoma of the auditory tract in two cats and squamocellular skin carcinoma in other two cats). Cachexia was the most evident clinical sign in 10 enrolled cats at least. Cachexia was effectively counteracted by the hIFN- α treatment, as shown by the rapid increase of the cats' body weight; this paralleled in turn a strong decrease of the inflammatory response in the oral cavity. The body weight achieved in the first therapy cycle (700 g more on average) lasted till the end of the observation period (see Table 2). Finally, it should be stressed that antiparasitics proved much more effective in FIV-infected cats during hIFN- α treatment than before the trial.

3.3. Clinical conditions are related neither to FIV concentration in plasma, nor to proviral load

There was a sharp decrease of the mean viral load in plasma (from 159,141 to 14,210 copies/mL) during the first 6-month therapy cycle (months 1–6), largely due to a strong reduction of viremia in the two cats with the highest numbers of circulating virus particles. However, there was no significant trend to a reduction of viremia on a group basis ($p = 0.2$). Indeed, the same trend was observed in the placebo group (mean count from 7601 to 1176 copies/mL). Most important, there was clear evidence in this same period of an *inverse relationship* between FIV viremia and clinical score in

five cats under treatment and in one cat of the placebo group. Also, a non-significant reduction of viremia took place during the second 6-month therapy cycle (mean count from 31,469 to 18,176 copies/mL, months 8–14) ($p = 0.2$).

Wide and non-significant fluctuations were also observed with proviral load. There was a non-significant increase of the mean proviral load from 7499 to 13,624 copies/ μ g DNA ($p = 0.44$) during the first therapy cycle. A non-significant reduction (10,769 to 5767 copies/ μ g DNA on average) was observed instead in placebo-treated cats; in this group, the cat mentioned in the previous paragraph also showed an inverse correlation between clinical score (*exitus*) and proviral load. A second, non-significant increase of the proviral load (from 15,400 to 19,200 copies/ μ g DNA on average) was observed during the second therapy cycle ($p = 0.5$). In one cat under treatment, both viremia and proviral load fell to undetectable levels at month 8; the result was always confirmed in the following checks. All the above data do not include the three FIV/FeLV double-positive animals.

3.4. CD4+ and CD8+ T lymphocytes

A better survival of CD4+ T cells (as compared to the placebo group) and a slow, stepwise increase of CD8+ T cells were shown in hIFN α -treated cats. Interestingly, both percentage ($p = 0.07$) and absolute count ($p = 0.017$) of CD4+ T cells were found to decrease after the 1st therapy cycle (difference between months 6 and 8). On closer examination, however, this was due to a “bouncing” effect, i.e. to a sharp decrease at month 8 of all CD4+ T cell counts previously risen beyond 1000/ μ L by month 6. Cats under treatment also showed a progressive decrement of the CD4/CD8 ratio; this parameter eventually levelled off during the 2nd therapy cycle (Table 3).

3.5. Hematology and clinical chemistry

A low number of red blood cells and platelets along with a low hemoglobin content were found in a few enrolled cats, who reacted well to the hIFN- α treatment. On the whole, the group means were always within the range of normal values. A striking difference was observed instead between the leukocyte counts of hIFN- α and placebo groups (Fig. 2).

Table 2
Weight of FIV-infected cats

	Month 1	Month 6	Month 8	Month 14
hIFN- α	4.3 \pm 1.2	5.0 \pm 1.1	5.3 \pm 1.2	5.0 \pm 0.9
PLACEBO	3.6 \pm 0.8	4.0 \pm 1.0 ^a	ND	ND

Data are expressed in terms of mean kg \pm one standard deviation. ND: not done (all cats but one had died). The weight increase of hIFN α -treated animals between month 1 and 6 is statistically significant ($p = 0.012$, paired *t*-test). The data set includes three cats with mixed, FIV/FeLV infection.

^a Three cats only alive.

Table 3
Time-course of CD4+ and CD8+ T cells in FIV-infected cats

	Month 1	Month 6	Month 8	Month 14
(a) Percentage and count of CD4+ T cells				
hIFN- α	23.3 (19.1–25.0)% 778 (610–1172)/ μ L	23.1 (13.9–29.6)% 757 (322–2617)/ μ L	19.9 (17.1–24.4)% 787 (562–931)/ μ L	20.7 (18.8–23.6)% 857 (682–1167)/ μ L
Placebo	35.0 (17.7–40.0)% 1293 (814–2127)/ μ L	16.8 (16.4–19.4)% ^a 676 (448–1421)/ μ L ^a	ND ND	ND ND
(b) Percentage and count of CD8+ T cells				
hIFN- α	28.4 (18.7–34.0)% 1090 (530–1509)/ μ L	27.2 (21.5–32.2)% 1392 (497–1631)/ μ L	35.2 (25.1–42.7)% 1096 (700–1895)/ μ L	35.2 (27.7–41.3)% 1570 (1087–2242)/ μ L
Placebo	15.7 (13.0–19.9)% 1317 (476–1763)/ μ L	26.1 (21.3–38.3)% ^a 1052 (609–3941)/ μ L ^a	ND ND	ND ND
(c) CD4+/CD8+ T cell ratio				
hIFN- α	0.91 (0.63–1.21)	0.93 (0.58–1.1)	0.57 (0.43–0.93)	0.69 (0.49–0.74)
Placebo	1.36 (1.01–2.55)	0.64 (0.48–0.99) ^a	ND	ND

Data are expressed in terms of median and interquartile range. ND: not done (all cats but one had died). The data set does not include the three cats with mixed FIV/FeLV infection.

^a Three cats only alive.

Also, there was a significant diminution of the total leukocyte count in hIFN- α -treated cats after the first therapy cycle (difference months 6–8, $p = 0.02$). No significant changes of the leukocyte formula were observed instead in both groups. Finally, only cats of the placebo group had dramatic increases of total bilirubin, GGT and lipase, as signs of stepwise liver failure before death (data not shown).

4. Discussion

The antiviral, immunostimulating and anti-proliferative activities of IFN- α , as well as the crucial roles of drug concentration and timing of administration have been known for a long time (Tompkins, 1999).

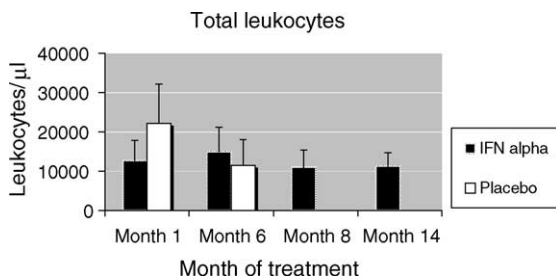


Fig. 2. Total leukocyte counts are shown in terms of mean \pm one standard deviation. Data of the placebo group after month 6 are not reported since one cat only was still alive.

An affective modulation of the immune system takes place at low to moderate IFN- α concentrations (Cummins et al., 2005), as also confirmed by the results obtained with the low-dose, oral IFN- α treatment in several models of infectious and autoimmune diseases. The signal provided by IFN- α and conveyed by a powerful amplification system mediated by oral cavity lymphocytes is induced by very low oral doses (1–10 IU/kg body weight) and it disappears at higher daily doses (Rollwagen and Baqar, 1996). Higher doses induce pro-inflammatory Th1 responses (Belardelli and Gresser, 1996), whereas the parenteral injection of the highest one can directly trigger inflammatory and pyretic responses in both humans and cattle (Straub, 1995).

In vitro, hIFN- α at low concentrations exerts a potent control on the response of leukocytes to bacterial endotoxin; this includes the down-regulation of the LPS receptor (CD14) and a strong decrease of both TNF- α secretion and TNF- α gene expression (Begni et al., 2005). Such a control over inflammatory cytokines may underlie the favourable results obtained with low-dose hIFN- α treatments in models of chronic autoimmune diseases (Tompkins, 1999), as well as in the cats with immunopathological conditions described in this study. This conclusion is in line with accumulated evidence in models of autoimmune disease like relapsing allergic encephalomyelitis (EAE) in various animal species, sialoadenitis and

lacrimitis in Sjögren's syndrome in humans and kerato-conjunctivitis sicca in dogs: the progression of clinical disease can be consistently prevented or reduced by oral administration of IFN- α (Cummins et al., 2005). Furthermore, the time-course of the clinical response to hIFN- α in this study suggests that the above control over inflammatory cytokines could start early and greatly contribute to both recovery and survival of FIV-infected cats. In this respect, the clinical benefit of *natural* hIFN- α used in this study was more evident than that of *recombinant* hIFN- α and of high-dose, recombinant, feline interferon- ω (De Mari et al., 2004; Riondato et al., 2003).

The unabated persistence of viremia and virus-infected cells during hIFN- α treatment had little if any prognostic value for the animals' clinical conditions and survival. Our results are in agreement with the virological findings in the FeLV model, in which hIFN α -treated cats became persistently viremic but failed to develop any of the FeLV-related diseases (Cummins et al., 1988). In our study, there were indications of a reduced virus replication during the treatment and of a certain increase after the therapy was interrupted (14,210 copies/mL plasma at months 6 and 31,469 copies/mL at month 8 on average); however, this did not lead to any relapse of disease or malaise in infected cats. Circumstantial evidence showed that such an event could take place in some animals 6–12 months after the study was over, thus inducing the practitioners to resume the hIFN- α treatment (data not shown).

The hIFN- α therapy failed to maintain the balance between CD4+ and CD8+ T lymphocytes; in particular, there was a stepwise increase of CD8+ T cells in agreement with previous results (Riondato et al., 2003). However, as described in another study (Novotney et al., 1990), the stepwise decrease of the CD4/CD8 ratio had no evident correlation with the clinical conditions. A better correlation was found instead with the leukocyte counts: the higher values in hIFN- α -treated cats underlie a strengthened innate immune response and a better control of opportunistic infections.

Only one old female cat of the placebo group (10 years) survived beyond month 8 of this study; retrospectively, this was probably a subject in the AP rather than ARC or FAIDS phase; the low number of CD4+ T lymphocytes and the clinical conditions at the time of enrolment were probably due to the advanced age.

The clinical benefit of the hIFN- α treatment should be also viewed in terms of a better compliance to the therapy protocol, as compared to antiviral drugs. Both AZT (3'-Azido-3'-deoxythymidine) and PMEA (9-2-phosphonyl-methosietethyl-anine) treatments induce serious side effects, as well as a frequent selection of drug-resistant FIV strains (Hartmann et al., 1992; Haschek et al., 1990; Remington et al., 1991). Finally, because of the proteolytic digestion of hIFN- α in the gastrointestinal tract, the low-dose oral treatment is not likely to induce a neutralizing antibody response to hIFN- α after high-dose parenteral injection, which takes place in human patients, too (Antonelli, 1995).

In conclusion, this study confirms the potential efficacy of low-dose, oral IFN- α treatment in models of viral infections, inducing major disorders of the homeostatic control circuits within the immune system. As shown in other animal models, the activity of IFN- α would have a two-fold effect:

- A modulation of the innate immune system for a better control of environmental pathogens.
- An early regulation of different immunopathological conditions, probably due to an effective control over inflammatory cytokines in diseased organs.

The combination of these two components would contribute to the improvement of clinical scores and life expectancy of patients. Future research should investigate the cell populations, the regulatory circuits and the immune effector mechanisms involved in this fascinating, still mysterious model of immunological therapy.

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