Effects of recombinant ovine interferon-τ on ovine lentivirus replication and progression of disease

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The antiviral effects of recombinant ovine interferon-τ (roIFN-τ) were studied in 26 lambs inoculated with ovine lentivirus (OvLV) or mock-infected. Six of the OvLV-infected lambs and three of the mock-infected lambs were treated with 10⁶ antiviral units (AVU) per kg roIFN-τ daily for 30 days starting at day 0 post-inoculation (p.i.) and twice a week thereafter (early treatment). Six of the OvLV-infected lambs and three of the mock-infected lambs were treated with 10⁶ AVU/kg roIFN-τ daily for 30 days starting at day 150 p.i. and twice a week thereafter (late treatment). Six OvLV-infected and two mock-infected lambs were treated either early or late with placebo. Cell-associated viraemia was quantified by an end-point dilution method. The weekly antibody response against OvLV proteins was studied by ELISA. All experimental animals were killed at 27 weeks p.i. and histological sections of lung were scored for the degree of lymphoid interstitial pneumonia (LIP). A 90% reduction in OvLV titres was detected at 4 weeks post-treatment in lambs that received early roIFN-τ treatment (P < 0.01). Differences in virus titres were also found at weeks 2 and 6 (P < 0.05). Scores for LIP degree were higher in infected lambs treated with placebo or late roIFN-τ than in the mock-infected lambs or in the infected lambs that received early roIFN-τ (P < 0.05). LIP scores were not different between mock-infected lambs and infected lambs that received early roIFN-τ. These results indicate that roIFN-τ curtails OvLV replication in vivo and reduces the likelihood of development of lentivirus-induced LIP when infected lambs are treated during the initial phases of OvLV infection.

Introduction

Ovine lentivirus (OvLV), the prototype strain of which is visna/maedi virus, constitutes a subgenus of the genus Lentivirus in the family Retroviridae that shares genotypic, phenotypic and pathogenic features with human immunodeficiency virus (HIV) (Coffin et al., 1995; de la Concha-Bermejillo, 1997). Similairities and differences between these two viruses have provided important information about the basic biology and pathogenesis of lentiviruses (Levy, 1993; de la Concha-Bermejillo et al., 1995a). OvLV infection in sheep results in a slowly progressive, multi-organ disease characterized by cachexia and chronic active inflammation in the lungs, lymph nodes, joints, mammary gland and central nervous system (Cutlip et al., 1988). Pulmonary lesions in OvLV-infected sheep consist of lymphoid interstitial pneumonia (LIP) and lymphocytic alveolitis (Cutlip et al., 1979; DeMartini et al., 1993). Similarly, these pulmonary lesions occur in up to 40% of HIV-infected children and in some adults with AIDS (Joshi et al., 1985, 1990; Scott, 1991).

Chemotherapy during the early phase of HIV infection offers the opportunity to reduce lentivirus load in macrophages and lymphoid organs, therefore delaying the outcome of HIV-
induced disease (Koup & Ho, 1994; Ho, 1996). However, due to the emergence of drug-resistant HIV strains and the toxic side effects associated with continuous treatment of immunocompromised patients (Carpenter et al., 1998), alternative antiretroviral drugs need to be developed.

Interferon-τ (IFN-τ) is a multi-functional cytokine secreted by trophoectoderm of ruminant conceptuses that has antiviral activity in vitro against HIV, feline immunodeficiency virus and OvLV (Pontzer et al., 1988, 1997; Dereuddre-Bosquet et al., 1996; Juste et al., 1996). In addition, IFN-τ has antiproliferative and immuno-modulatory properties similar to those of classical type I IFNs (α, β and ω), but has low cytoxicity even at the high levels found within the uterus during early pregnancy (Dereuddre-Bosquet et al., 1996; Bazer & Johnson, 1991; Pontzer et al., 1991). The unique antiviral and ‘cell-friendly’ properties of IFN-τ suggest that it may be useful as a therapeutic agent for treatment of lentivirus infections. The objective of the present study was to characterize the effects of recombinant ovine (ro) IFN-τ on OvLV replication and the progression of disease.

Methods

Experimental design. Twenty-six newborn Rambouillet or Rambouillet x Suffolk lambs, seronegative to OvLV by the agar gel immunodiffusion (AGID) test (Veterinary Diagnostic Technology Inc., Wheat Ridge, CO, USA) and born from OvLV-seronegative ewes, were allocated randomly into one of six groups. Experimental lambs were infected intratracheally with $5 \times 10^6$ TCID₅₀ OvLV or mock-infected within 24 h of birth. Six of the OvLV-infected lambs and three of the mock-infected lambs were treated with roIFN-τ (Ott et al., 1991) once a day for 30 days starting at day 0 post-inoculation (p.i.) and twice a week thereafter (early treatment). Six of the OvLV-infected lambs and three of the mock-infected lambs were treated with roIFN-τ (Ott et al., 1991) once a day for 30 days starting at day 150 p.i. and twice a week thereafter (late treatment). Six OvLV-infected lambs and two mock-infected lambs that were treated either early or late with placebo (non-adherent cells) were removed by washing with Hank’s balanced salt solution (HBSS) and adherent cells were incubated for 12 more days. At the end of this period, cells were washed with HBSS, fixed in methanol and stained with Giemsa. Flasks and wells were scored as positive or negative for syncytium formation, with a positive score being at least one cell containing five nuclei. The minimum number of syncytium-inducing PBMC per million (Ficoll-separated PBMC) was calculated as described previously (Juste et al., 1998). At the time of necropsy, caudal mediastinal lymph node (CMLN) cells and bronchoalveolar lavage (BAL) cells were collected and OvLV was titrated in these cells as for PBMC.

ELISA. An ELISA was used to determine the antibody responses to the transmembrane (TM) and p25 OvLV structural proteins as described previously (Kwang et al., 1993). Briefly, microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993). Microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993). Microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993). Microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993). Microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993). Microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993). Microtitre plates were coated with 120 µg per well recombinant TM or p25 OvLV structural proteins as described previously (Kwang et al., 1993).
in 10% buffered neutral formalin solution, sectioned at 5 μm and stained with hematoxylin and eosin. The left lung was excised and insufflated with 10% buffered formalin solution at 30 cm of water pressure for 48 h before sectioning. Five lung sections from each lamb taken at the same places (ventral cranial lobe, ventral intermediate lobe, dorsal intermediate lobe, ventral caudal lobe and dorsal caudal lobe) were examined at 200 × magnification and scored for four criteria in a logarithmic scale as follows. Presence of areas of interstitial pneumonia and of lymphoid perivascular or peribronchial infiltrate resulted in a score ranging from 0 to 32 in twofold steps. Presence of lymphoid follicles not associated with airways or blood vessels was considered to be the most specific lesion and was assigned the heaviest weight in the scoring (0, no lymphoid follicles; 24, 1–10 follicles per section; 48, more than 10 follicles per section). The final score for each section was determined by calculating a geometric mean for all four criteria. As a result, histopathological lesions were represented by a single score based on five replicate measurements per lamb.

- **Analysis of results.** Number of infected cells per million cells, ELISA $A_{490}$ readings and the histopathological score from each lamb were transformed into logarithms and data were subjected to analysis of variance to determine differences in virus titrations from PBMC, BAL and CMLN, antibody responses and severity of lesions between treated and untreated lambs (SAS Institute Inc., version 6). Pearson correlations between pairs of variables were also calculated to evaluate the sign and significance of their relationship.

In most analyses, the control lambs for evaluating the effects of roIFN-τ treatment on OvLV infection were the infected, placebo-treated lambs. However, for analyses of effects on virus isolation during the first 16 weeks, results from both placebo- and late roIFN-τ-treated groups were pooled into a single control group of 12 lambs, since the late-treated lambs did not receive roIFN-τ during the first 16 weeks. This provided a larger sample size to evaluate the natural course of infection and reduced the variance. From the week the late-treatment lambs began receiving roIFN-τ up to and including the end of the experiment, the control group was reduced to the six lambs that had only received non-transformed yeast supernatant.

Finally, the efficiency of the treatments on virus burden was estimated according to the following formula: percentage reduction = $100(V_0 - V_i)/V_0$, where $V_0$ is the mean viraemia in the control group and $V_i$ is the mean viraemia in the treated group. A similar method was used to compare the effects of treatment on the severity of lesions, but in this case the mean score for uninfected lambs was subtracted from the mean of each group of infected lambs before calculating the percentage reduction.

**Results**

No clinical signs of OvLV-induced disease were observed in any of the lambs during the course of the experiment. One of the mock-infected, placebo-treated lambs developed rectal prolapse, unrelated to the experimental treatment, and was euthanized 13 weeks p.i.

OvLV was not isolated from lambs in the mock-infected groups. On the other hand, OvLV was isolated from PBMC on at least one occasion for all OvLV-inoculated lambs. The overall mean cell-associated viraemia titre was lower in the early roIFN-τ treatment group than in the late roIFN-τ and placebo treatment groups ($P < 0.01$). The pool of OvLV-infected lambs not receiving early roIFN-τ treatment showed a peak in mean cell-associated viraemia titre between weeks 2 and 8 p.i., with the mean being greatest at week 2 p.i. (Fig. 1). A 90% reduction in mean cell-associated viraemia was found at week 4 of the experiment in lambs that received early roIFN-τ treatment when compared with the lambs not receiving early roIFN-τ treatment ($P < 0.01$). Lower ($P < 0.05$) mean virus titres were also detected in the early roIFN-τ-treated lambs at weeks 2 and 6. An increase in mean cell-associated viraemia titre was detected in the early roIFN-τ-treated lambs 2 weeks after the roIFN-τ treatment regime was changed from daily to twice a week (week 6). In both roIFN-τ-treated and control lambs, the mean cell-associated viraemia titre declined by week 8 of the experiment. At week 18, 2 weeks after the late roIFN-τ treatment group started to receive daily roIFN-τ, a reduction in cell-associated viraemia was detected in this group when compared with the group receiving no roIFN-τ. However, because viraemia titres were low in all groups at this time, the differences were not significant ($P > 0.05$). An increase in cell-associated viraemia titre was found in the late roIFN-τ-treated group 3 weeks after the roIFN-τ treatment regime was changed from daily to twice a week. The mean number of infected cells per million cells (ICMC) in the early roIFN-τ-treated lambs at week 4 p.i. was 0.096, compared with 3.898 in the placebo-treated lambs. At week 22 p.i., when the maximum difference in cell-associated viraemia titre between late-roIFN-τ- and placebo-treated lambs was observed, the mean number of ICMC was 0.005 for the former and 0.076 for the latter. Throughout the experiment, the highest mean number of ICMC was observed in the placebo-treated lambs during weeks 2–8 (range 2.265–4.358).

Although not significant ($P > 0.05$), the mean virus titre in CMLN cells from lambs receiving early roIFN-τ treatment was lower (titre of 0.059) than for the late roIFN-τ-treated (titre of 0.200) and for the placebo-treated lambs (titre of 0.363). Mean
virus titres in BAL cells were not different (P > 0.05) in any of the three OvLV-infected groups.

All OvLV-infected lambs seroconverted by week 6 p.i., as determined by the AGID test, and remained seropositive until the end of the experiment. Precipitating antibodies were not detected in serum from any of the mock-infected lambs during the course of this experiment. Weekly mean ELISA A$_{105}$ in the OvLV TM antibody assay increased slowly over time in all OvLV-infected groups (Fig. 2), and differences from the mock-infected lambs (controls not shown in graph) were significant (P < 0.05) by week 5 p.i. The overall mean TM ELISA A$_{105}$ was lower (P < 0.05) in the early roIFN-r-treated group than in the late roIFN-r- and placebo-treated lambs. Weekly mean ELISA A$_{105}$ in the OvLV p25 antibody assay also increased over time in all OvLV-infected groups, and became significant (P < 0.05) in the mock-infected lambs by week 3 p.i. (data not shown). The overall mean p25 ELISA A$_{105}$ was lower (P < 0.05) in the late roIFN-r-treated lambs than in the early roIFN-r- and placebo-treated lambs. However, no differences (P < 0.05) in weekly mean p25 ELISA A$_{105}$ were detected between roIFN-r- and placebo-treated lambs.

Macroscopic lesions were not observed in the mock-infected lambs at necropsy. Characteristic OvLV-induced lesions, consisting of multiple, discrete, 1–3 mm foci of grey discoloration, were present in the lungs of two OvLV-infected lambs, one belonging to the placebo-treated group and the other to the late roIFN-r-treated group. Histologically, varying degrees of perivascular and peri-bronchiolar lymphoid infiltration or lymphoid follicles were observed in some lung sections from all groups. Lymphoid follicles not associated with airways were specific to OvLV-infected groups. Histopathological scoring of lung sections revealed no differences in the degree of mononuclear cell infiltration between the OvLV-infected, early roIFN-r-treated and mock-infected lambs. However, the degree of lung inflammation was more severe (P < 0.01) in the OvLV-infected, placebo-treated and in the OvLV-infected, late roIFN-r-treated lambs than in the OvLV-infected, early roIFN-r-treated and mock-infected lambs (Fig. 3). There were no differences (P < 0.01) in histopathological scores between OvLV-infected, placebo-treated and OvLV-infected, late roIFN-r-treated lambs. After subtraction of the mean score for the mock-infected group, the OvLV-infected group that received early roIFN-r-treatment showed a reduction of 100% in histopathological score in comparison with the OvLV-infected, placebo-treated group. The late treatment group showed no reduction in histopathological score.

A clear positive correlation was detected between mean individual cell-associated viraemia titres and lung lesion scores (P < 0.01).
level of humoral response defined by about half of the lambs that point on, experimental lambs seem to fall into two groups, viraemia titre and anti-TM transformed values of about 0 of three parts. The first part corresponds to cell-associated degree equation (Fig. 5). In fact, the plot seems to be composed of mean OvLV TM individual mean cell-associated viraemia titres and individual regression equations. In particular, the relationship between individual mean cell-associated viraemia titres and individual mean OvLV TM A105 had a positive linear relationship ($P < 0.006$), but the relationship was best described by a second-degree equation, as discussed in the text. ▽, △; Points fitted using two different second-degree equations, as shown.

### Discussion

In HIV-1 infection, the measurement of virus load in plasma is a useful guide for prognosis and for determining the efficacy of antiretroviral therapy (Mellors et al., 1996; Katzenstein et al., 1996). Recent results show that reducing HIV load below the limit of detection is essential for long-term treatment strategies (O’Brien et al., 1997). Multiple target combination therapy, using combinations of drugs to inhibit several different enzymes or regulatory proteins necessary for HIV infection and replication, has proven to be a highly effective way of reducing HIV load and enhancing T cell counts (Autran et al., 1997). However, even in patients successfully treated with highly active antiretroviral therapy for up to 30 months, replication-competent virus can be recovered routinely from resting CD4+ lymphocytes (Finzi et al., 1997). In addition, drug resistance to and adverse side effects from antiretroviral therapy continue to be a problem (Carpenter et al., 1998).

Therefore, development of new antiretroviral drugs with low toxicity that target different aspects of the virus replication cycle continues to be an important area of research. rolIFN-τ was shown to have potent antiviral activity in vitro against OvLV, probably by interfering with virus assembly (Juste et al., 1996). In the present experiment, treatment of experimentally OvLV-infected lambs with rolIFN-τ curtailed the overall cell-associated viraemia titres. The reduction in cell-associated viraemia titre was most drastic (over 90%) at week 4 of the experiment ($P < 0.001$), but also at weeks 2 and 6 p.i. ($P < 0.05$). Furthermore, scores for LIP/BALT were higher ($P < 0.01$) in infected lambs treated with placebo or the late rolIFN-τ regime than for control lambs or infected lambs treated early with rolIFN-τ. LIP scores were not different between mock-infected and OvLV-infected lambs treated early with rolIFN-τ.

Long-term follow-up studies of early versus later treatment of HIV-infected patients with zidovudine showed that early zidovudine therapy delayed progression to AIDS (Simberkoff et al., 1996). Similarly, IFN-τ treatment given from the early stages of infection, but not after the appearance of AIDS symptoms, can prolong survival (Rivero et al., 1997). Results of the present study confirm that early treatment of lentivirus infections is highly effective in curtailing viraemia titres and in preventing the development of lentivirus-induced disease.

A mild reduction in cell-associated viraemia titre was observed 2 weeks after daily rolIFN-τ treatment was started in the late rolIFN-τ group (at week 16); however, because viraemia titres had already declined to a minimum in all groups as a result of the normal immune response against OvLV, differences among treatment groups were not significant. Nevertheless, daily treatment with rolIFN-τ starting 4 months after infection did not have significant beneficial effects on virus-induced LIP. The majority of HIV-infected individuals go through an acute phase of viraemia during the first few weeks after primary infection (Koup & Ho, 1994; Pantaleo et al., 1994; Daar et al., 1991), when HIV is widely disseminated throughout the lymphoid system by infected macrophages (Pantaleo et al., 1993). Subsequently, a specific HIV immune response leads to a decline in viraemia (Koup et al., 1994). Therefore, reducing the initial peak of viraemia in the few weeks following infection is critical for preventing or delaying the pathological outcome of lentivirus infections.

In both early and late rolIFN-τ treatments, mean viraemia titres increased after the frequency of treatment was changed from daily to biweekly, indicating that daily treatment is necessary to maintain a low virus load. Lentivirus biological clones, such as OvLV 85/34, are composed of a multitude of genetically diverse virus types or quasispecies (Cichutek et al., 1992; Woodward et al., 1995). The fact that rolIFN-τ did not give 100% inhibition of OvLV may suggest that some virus types within the strain are resistant to rolIFN-τ treatment. It is possible that rolIFN-τ treatment exerts selective pressure for replication of the resistant virus types. Primary isolates of HIV derived from donors at various stages of infection display a
broad range of sensitivity to IFN-α2 (Kunzi et al., 1995), but the prevalence of IFN-α2 resistance was low in the absence of AIDS and increased dramatically once the infection progressed to AIDS. For these reasons, further experiments are necessary to determine the role of rolIFN-τ-resistant virus types in lentivirus-induced disease and immune responses.

In the present study, individual viraemia titres were positively correlated with LIP scores, confirming previous observations that viraemia levels can predict disease progression in lentivirus infections (Katzenstein et al., 1996). The antibody response against OvLV TM protein was significantly lower in lambs that received the early rolIFN-τ treatment, suggesting that initial viraemia influences the magnitude of the antibody response against this protein. The antibody response to OvLV TM protein was also correlated positively with LIP scores. A previous report also suggested that the humoral response in OvLV infection plays a role in disease development (Petursson et al., 1992). Therefore, the beneficial effect of early rolIFN-τ treatment may include reducing virus load, decreasing the TM antibody response and reducing immunopathology. Because the relationship between cell-associated viraemia and antibody response against OvLV p25 protein was negative, the reduction in anti-TM antibody levels was not likely to be the result of a suppressed humoral response caused by rolIFN-τ.

The levels of TM antibody were correlated positively with viraemia titres and lung lesions, suggesting that this antibody response may be used as a surrogate marker to estimate virus load and the outcome of OvLV-induced lung pathology. However, the relationship between TM antibody levels and viraemia titres is best described by a second-degree equation, because it is positive up to a transformed viraemia titre of approximately 0·35 and a transformed TM A₁₀₀ of approximately 0·6, but thereafter the relationship becomes negative. Although OvLV does not cause clear immunodeficiency, as seen in humans with AIDS, opportunistic infections often occur during the terminal stages of this infection (de la Concha-Bermejillo, 1997). Therefore, the relationship between viraemia and TM antibody response suggests that OvLV may overwhelm the humoral response in sheep with extremely high virus load. In fact, other immune disregulations such as reduced delayed hypersensitivity reactions (Myer et al., 1988; Pyrah & Watt, 1996), failure of IgG₂ response to the virus (Bird et al., 1995), depressed levels of lymphocyte-generated interleukin-2 (Ellis & DeMartini, 1985 a), impaired pulmonary lymphocyte activation (Begara et al., 1995) and decreased concanavalin A-induced activity of suppressor cells (Ellis & DeMartini, 1985 b) have been reported for OvLV-infected sheep. It is possible that opportunistic infections associated with OvLV-infection may be the result of a compromised immune function.

Virus load in BAL cells may correlate with the degree of LIP (de la Concha-Bermejillo et al., 1995 b; Brodie et al., 1992), but the virus load in BAL cells was not decreased by rolIFN-τ treatment in the present study. However, virus load was highest in BAL cells in late rolIFN-τ-treated lambs, followed by early rolIFN-τ-treated and placebo lambs. Because treatment was administered subcutaneously, the concentration of rolIFN-τ in the alveolar space may have been limited. In addition, treatment with rolIFN-τ alters the integrity of the surface coat of pulmonary intravascular macrophages (PIMs), causing their disappearance from the lungs (Singh et al., 1998). The same report showed that PIMs, cells that are not obtained regularly in lung lavages, play a role in lentivirus-induced lung pathology. Thus, the lack of LIP in lambs treated with rolIFN-τ early after OvLV infection may have been due in part to the effects of this cytokine on PIMs rather than on alveolar macrophages. In the case of CMLN, the early rolIFN-τ treatment group had the lowest virus load, followed by the late rolIFN-τ treatment group and then the placebo treatment group. While these differences were not significant (P > 0·05), the virus load in CMLN cells correlated positively with viraemia levels. Although the lung is the main target organ for OvLV-induced pathology, the impact of treatment in lentivirus infections can be better assessed in lymphoid tissue reservoirs, where most of the virus is produced in macrophages and stored in immune complexes on the surface of follicular dendritic cells (Cavert et al., 1997). In HIV infection, the pool of virus on follicular dendritic cells is at least an order of magnitude greater than that in mononuclear cells (Haase et al., 1996).

In summary, the results of this experiment demonstrated clearly that early rolIFN-τ treatment decreased OvLV replication in vivo and prevented development of lentivirus-induced LIP. Although human recombinant IFN-α is active in vitro against HIV (Kornbluth et al., 1990; Gendelman et al., 1990) and has beneficial effects in the treatment of HIV-related Kaposi’s sarcoma and early HIV infection (Francis et al., 1992; Lane, 1991; Stuart-Harris et al., 1992), treatment with rIFN-α also results in a high incidence of toxic side effects (Lane et al., 1990). Because of its low toxicity and potent antiviral activity, rolIFN-τ may prove to be a promising new IFN therapy to delay the pathological outcome of lentivirus infections. However, because rolIFN-τ treatment did not reduce OvLV viraemia below the limit of detection, its effectiveness in combination with other antiretroviral agents needs to be investigated.

The authors express appreciation to Mrs Phyllis Benge for editing the manuscript.

References


Begara, I., Lujan, L., Hopkins, J., Collie, D. D., Miller, H. R. & Watt, R. A. Juste and others

The virus load in BAL cells was not decreased by rolIFN-τ treatment in the present study. However, virus load was


Received 1 July 1999; Accepted 28 October 1999