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The effect of recombinant human interferon α B/D compared to interferon α 2b on SIV infection in rhesus macaques.

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Abstract

The model of simian immunodeficiency virus (SIV) infection in rhesus macaques was used to evaluate the effects of recombinant human interferon α, Hu IFN-α 2b and Hu IFN-α B/D, at two doses. Administration began 1 day prior to infection and was continued for 90 days postinfection. Both interferons suppressed SIV antigenemia during the treatment period. Following treatment animals were monitored for 4 years for rate of disease progression. Neither IFN prolonged the asymptomatic period or survival.

Keywords: SIV; HIV; AIDS; Rhesus monkey; Interferon

1. Introduction

Interferon α (IFN-α) has been shown to have antiviral, antiproliferative and immuno-modulating properties in vitro and in vivo. It is used clinically to treat several forms of human cancer and viral infections (Spiegel, 1986; Krown, 1986; Schneider and Goldman, 1989; Nakano et al., 1990). The possible efficacy of IFN-α in human immunodeficiency virus (HIV) infection has been the subject of several clinical trials since 1981 (Krown et al., 1983; Volberding et al., 1987). Because of its anti-tumour effect IFN-α has been studied in AIDS patients with Kaposi's Sarcoma. These studies found evidence for an anti-HIV effect, as shown by decreases of p24 antigen levels (De Wit et al., 1988). Subsequently, IFN-α has

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been tested in clinical trials as single therapy or in combination with other antiretroviral medication (Lane et al., 1990; Fischl et al., 1991; Caliendo and Hirsch, 1994) (for reviews see Lane, 1994; Poli et al., 1994). In asymptomatic HIV-infected individuals treatment with IFN-α may help to stabilize the CD4 count and delay the development of opportunistic infections (Lane et al., 1990; Lane, 1994). The side effects of IFN such as flu-like syndrome, granulopenia and anemia, were reason to refuse further treatment for many of the participants in the study.

A variety of ways to improve the therapeutic index of IFN-α in the treatment of AIDS is still being investigated. These include different doses, treatment schemes and routes of administration or combination with other antiretroviral agents. A very interesting approach is the use of hybrid IFNs consisting of parts of different IFN subtypes. Such molecules may combine the high antiviral efficacy of one subtype with the low toxicity of the other. Hu IFN-α B/D is such an artificial IFN which was constructed with an N-terminal part and C-terminal part of Hu IFN-α 8 (B) and the middle part of Hu IFN-α 1 (D). In two phase I studies in tumor patients Hu IFN-α was better tolerated than other types of IFN-α, while the antiviral activity in vitro proved to be better (Ciba-Geigy, data on file).

Rhesus macaques infected with simian immunodeficiency virus (SIV) develop a disease which closely resembles AIDS. The SIV-macaque model, which is considered the best animal model for AIDS in humans, is widely used to test vaccine and treatment strategies (Schellekens and Horzinek, 1990).

In the SIV-rhesus macaque model of AIDS we compared the antiviral activity of two different types of IFN-α, rHu IFN-α 2b and rHu IFN-α B/D, at both high (5 MU/kg per day) and low (1 MU/kg per day) doses. We tested the efficacy of IFN treatment started 1 day before infection to determine if we could prevent or alter the course of SIV infection and limit SIV antigenemia during the immediate postinfection period. With the aim of limiting virus spread and burden (Fauci, 1993) during the postinfection period, we examined the effect of this treatment on survival in the course of long-term follow-up. This is the first study of SIV-infected monkeys in which the animals have been followed for 4 years.

2. Materials and methods

2.1. Animals

Ten healthy outbred juvenile *Macaca mulatta* free of SIV or related retroviral infections were selected for this study. Animals were all of the same approximate age and weight at the start of the experiment. The animals were all bred at the Primate Center. They were divided into three groups: group A, placebo controls ($n = 2$); group B, rHu IFN-α 2b (Schering Corporation, NJ, USA) ($n = 4$); and group C, rHu IFN-α B/D (Ciba-Geigy Ltd., Basel, Switzerland) ($n = 4$).

In each of the rHu IFN-α treated groups two animals received a high dose (5 MU/kg per day) and two received a low dose (1 MU/kg per day). Treatment started on day 1 and was completed after 90 days. On day 0 all animals received an intravenous (i.v.) challenge dose of 5×10^2 MID_{50} of SIV δ B670 (Murphey-Corb et al., 1986, 1989).

2.2. Clinical and immunological parameters

Blood samples were taken twice weekly in the treatment period and once a month in the follow-up period of 24 months, after which time samples were taken less frequently but at regular intervals. Complete blood counts and clinical biochemistry determinations were performed at each sample interval. Immunological parameters included absolute numbers and percentages of CD3+, CD4+ and CD8+ cells as determined by flow cytometry (FACScan, Becton Dickinson). The monoclonal antibodies to identify CD4 (Leu3a) and CD8 (Leu2a) were obtained from Becton Dickenson. The anti-CD3 (FN 18) monoclonal antibody was developed within the Biomedical Primate Research Center. The animals were monitored for disease progression for 4 years. If animals developed advanced AIDS, they were humanly euthanised and a full patho-

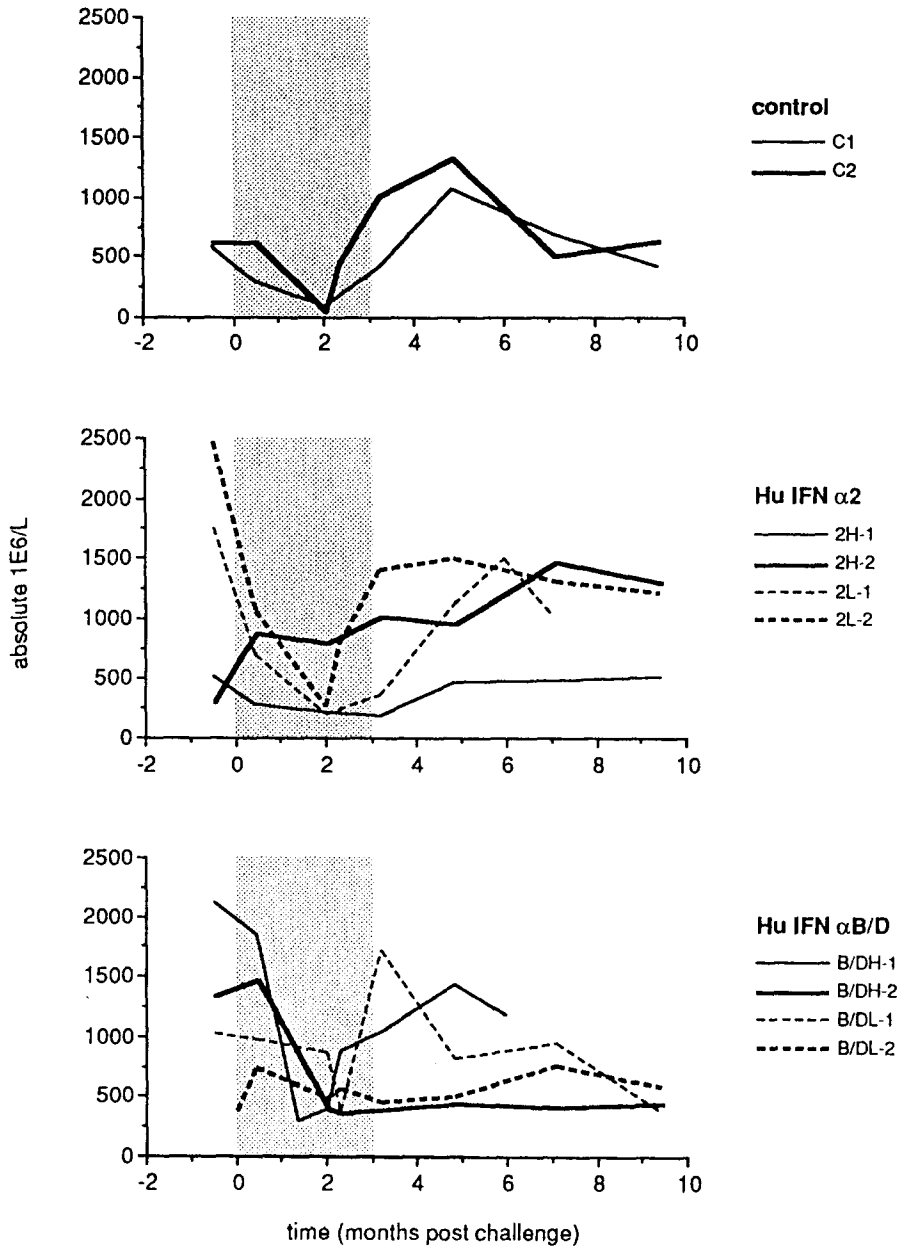

Fig. 1. The effect of Hu IFN-α on the CD4+ cell levels in SIV-infected rhesus monkeys. The absolute CD4+ counts of the individual animals are presented.

logical analysis was performed. Neopterin levels were measured with a commercial radioimmunoassay according to the instructions of the manufacturer (IMMU test Neopterin).

2.3. Serology

Antibodies to SIV antigens were determined by a Western blot (duPont HIV-2 Western blot kit)

and titers determined by enzyme-linked immunosorbent assay (Abbott HIV-1/HIV-2 diagnostic kit). Levels of plasma SIV antigen were measured by a specific SIV antigen capture assay (SIV p27 gag, Coulter, Hialeah, FL, USA). Relative concentrations of SIV p27 were determined by serial 10-fold dilutions and absorption was read according to the directions of the manufacturer.

2.4. IFN and anti-IFN assays

Sera were tested for the presence of IFN in a bioassay in Hep-2 cells challenged with vesicular stomatitis virus (VSV) as described before (Van der Meide et al., 1985). Antibodies to the two types of IFN-α were determined both in enzyme-linked immunoassay as well as in a neutralization assay (Kawade and Watanabe, 1984; Van der Meide et al., 1985). The IFN neutralizing activity of the sera was determined by incubating serum dilutions with 100 IU IFN for 1 h. After incubation the mixture was tested for the presence of residual IFN activity in the bioassay.

3. Results

During the 90-day treatment period all animals increased in weight. The medication seemed to be well tolerated and did not induce serious toxicity. Two monkeys showed transient leukopenia, one in the control group and one in the rHU IFN-α 2b group. In the posttreatment period individual animals in all groups began to show signs of disease progression as evidenced by diarrhea and weight loss, hypoalbuminemia, anemia, thrombocytopenia or lymphadenopathy.

Table 1
Survival periods of individual animals.

Monkey designation	Treatment	Survival (months)
C1	Control	9.5
C2	Control	32.8
	IFN-α 2b (MU)	
2L-1	1	7.0
2L-2	1	20.8
2H-1	5	13.5
2H-2	5	48.1[a]
	IFN-α B/D (MU)	
B/DL-1	1	16.0
B/DL-2	1	23.0
B/DH-1	5	7.0
B/DH-2	5	32.7

[a] Animal had no evidence of AIDS at time of euthanasia.

In the period after SIV infection, CD4 + cells initially declined and then partially recovered, as has been described in humans with primary HIV infection (Fig. 1). Later all animals showed decreasing CD4 + cell counts. There was no significant difference in decline of CD4 cells between the groups. Except for one IFN-α 2b monkey (2H-2) that remained asymptomatic for more than 48 months, all animals eventually developed AIDS-like disease. Post mortem examination confirmed the clinical diagnosis of AIDS. The most frequently observed opportunistic infections observed histologically included pneumocystis carinii pneumonia, generalized cytomegalovirus (CMV) and other herpes virus infections, candida and cryptosporidium. The survival periods of individual animals are shown in Table 1.

The monkeys in the IFN-α B/D group had lower p27 levels compared to the other two groups, both during the treatment period and thereafter (Fig. 2). At day 14 the two animals in the control group and the four animals treated with IFN-α 2b showed more than 1000 pg of SIV p27 gag per ml of plasma. In the IFN-α B/D group only one out of four animals had similar high plasma antigen peaks. However, neither of the IFNs were capable of completely suppressing virus replication, as measured by plasma antigenemia. No correlation was found between immediate postinfection p27 levels and development of opportunistic infections or survival. However, an interesting observation was that the first three animals to die had amongst the highest immediate postinfection plasma antigenemia peaks which either did not return to baseline or increased again within 4 months postinfection.

In Fig. 3 the neopterin levels in the monkeys are shown. These levels were persistently low in those animals which survived the longest and tended to increase in those animals which devel-

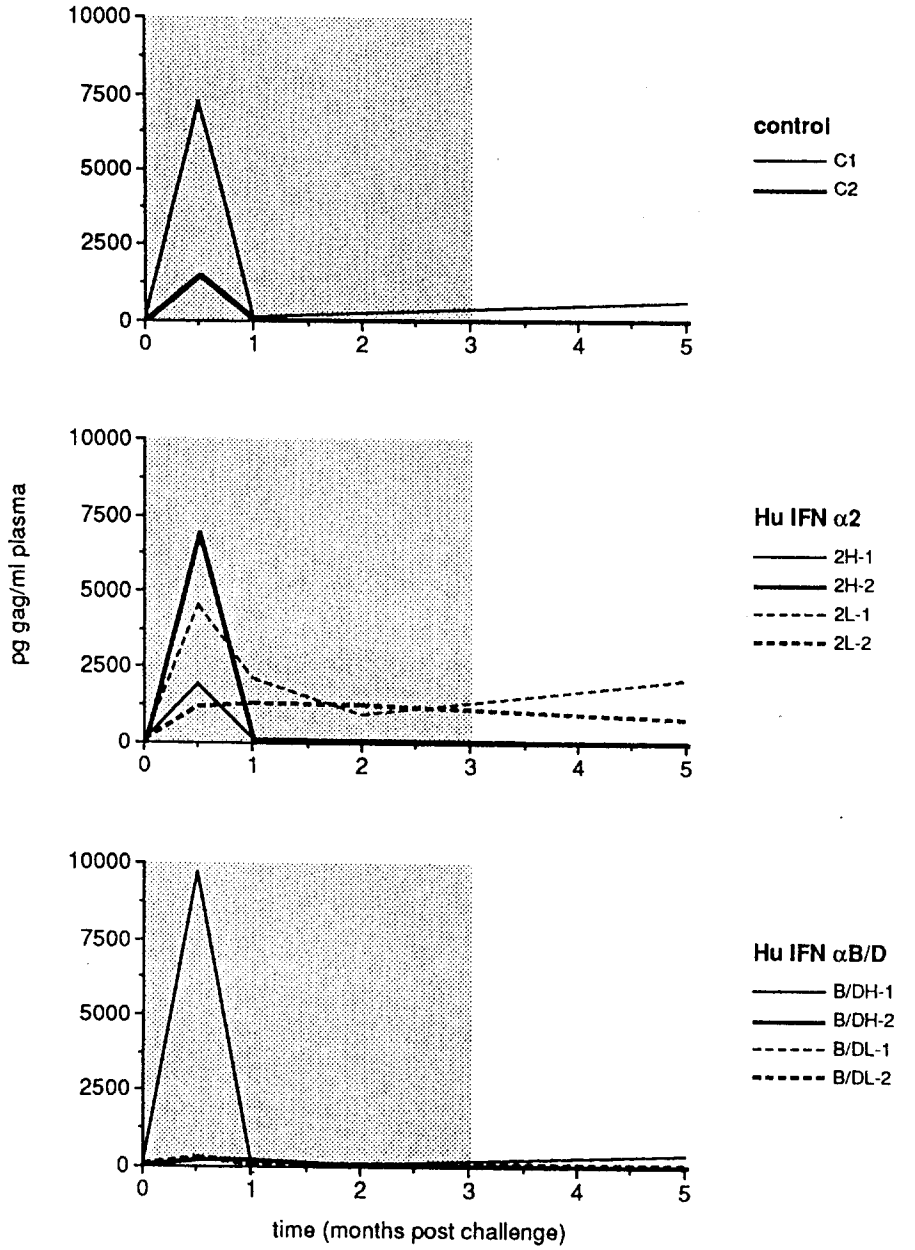

Fig. 2. The effect of Hu IFN-α on the p27 levels in SIV-infected rhesus monkeys. The results of the individual animals are presented.

oped disease. Persistently circulating levels of IFN were found in two animals treated with IFN-α 2b and one control animal. There was no apparent relation with survival (data not shown), although the neopterin levels were higher in these animals. An apparent relationship between the type of IFN-α used and a peak of neopterin levels at 2 months was observed in those treated with IFN-α 2b (Fig. 3). However, this was not related to survival or disease progression.

Antibodies to SIV antigens were detected in all animals. Titers of antibodies against SIV envelope

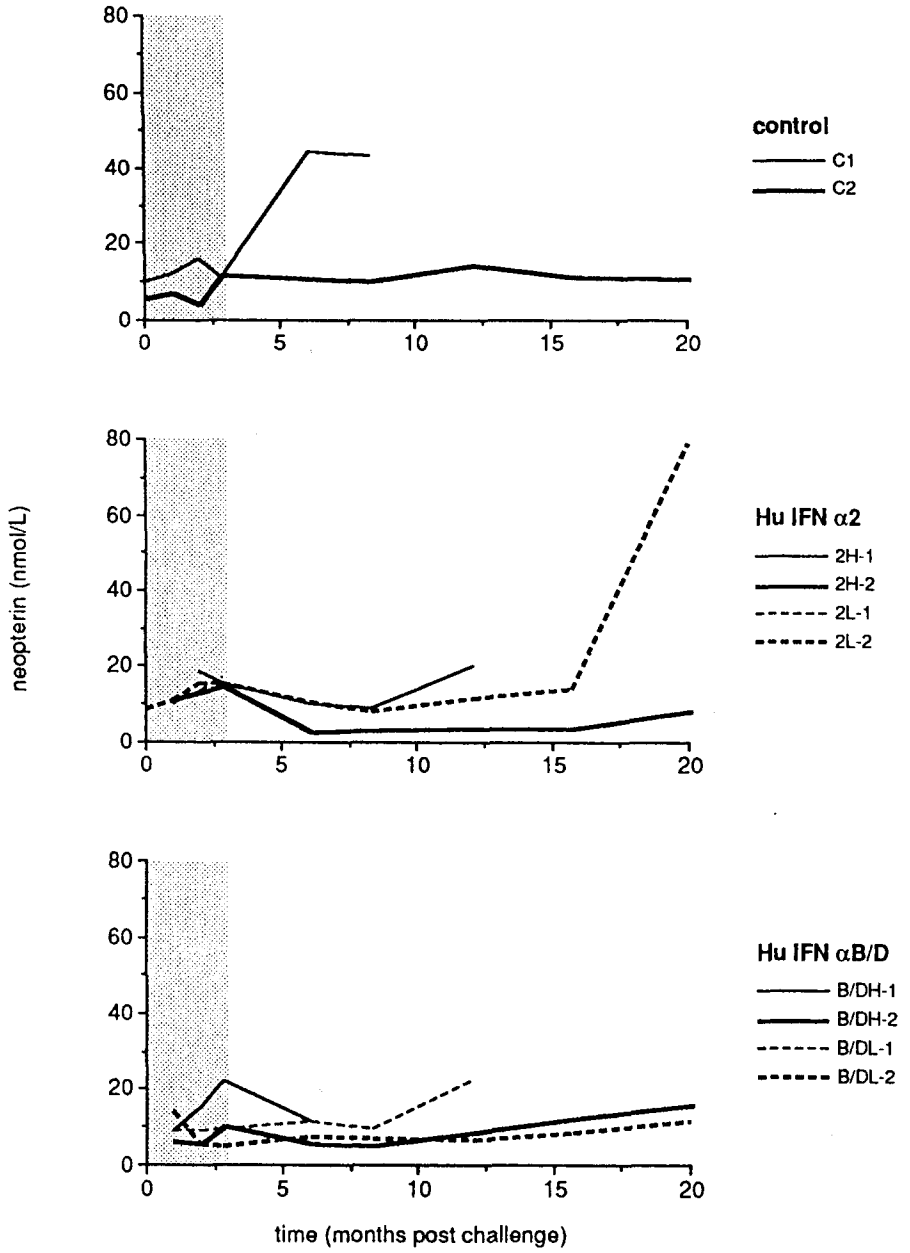

Fig. 3. The effect of Hu IFN-α on the serum neopterin levels in SIV-infected rhesus monkeys. Levels of individual animals are presented.

showed a sharp increase approximately 1 month postinfection. The kinetics of all antibody production showed no significant difference between the groups during the treatment period. The induction of antibodies to the IFNs was also studied. In all animals treated with Hu IFN-α B/D, binding antibodies were found during the treatment period, which disappeared 3 months after the treatment was stopped. In three animals, antibodies neutralizing IFN-α B/D were also found. IFN-

α 2b seemed to induce lower levels of antibodies in rhesus monkeys. Only in two animals were both binding and neutralizing antibodies found which in one animal persisted until 18 months after treatment was stopped (data not shown).

4. Discussion

The effect of IFN-α in the late asymptomatic and symtomatic phase of HIV infection has been the topic of a number of clinical studies. In this study we investigated the effect of IFN-α 2b treatment initiated 1 day prior to infection and continued throughout a postinfection period of 12 weeks, and then monitored the animals for survival for 4 years. We wished to evaluate the possible effect of IFN-α 2b on limiting establishment of infection and subsequently slowing the rate of disease progression. However, neither one of the rHu IFN-α types, at the investigated doses, prevented infection, nor did they significantly alter disease progression or prolong survival.

Although the number of animals studied is small, our results suggest that rHu IFN-α B/D is more effective than rHuIFN-α 2b in suppressing SIV antigenemia during treatment. In addition, p27 levels in the posttreatment period remained relatively low in the Hu IFN-α B/D treated group compared with the other groups. These low p27 levels in the Hu IFN-α B/D treated group were observed despite the development of antibodies against IFN. In clinical studies, the development of neutralizing IFN antibodies has been related to disease remission during IFN treatment (Giannelli et al., 1994).

We have also studied a number of parameters of disease progression. The initial SIV-antigenemia observed in our experiment did not always correlate with the initial drop in CD4 cells. In the rHu IFN-α 2b treated group we observed that the monkey with the highest p27 peak showed no CD4 + decrease in the postinfection period. This monkey was the longest survivor (48.1 months) of the experiment. Circulating neopterin was shown to relate to disease progression. Low neopterin levels were found in the longest survivors. Urine neopterin excretion has been reported as a disease marker in rhesus monkeys infected with SIV and IFN-α has been shown to induce neopterin in vitro (Nathan, 1986; Wachter and Hunsmann, 1989). In this study the effect of a hybrid interferon, r-Hu IFN-α B/D was compred with r-Hu IFN-α 2b. Although the number of animals studied does not allow firm conclusions to be drawn, the data may suggest that the hybrid is slightly more effective than r-Hu IFN-α 2b in transiently suppressing viremia. However, neither IFN prevented infection or altered disease progression. These results confirm the study in which a combination of 3′-azido-3′-deoxythymidine (ZDV) and Hu IFN-α was ineffective in preventing SIV infection in rhesus monkeys (Fazely et al., 1991).

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