Oral application of cytokines

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Introduction

Oral or nasal administration of interferons has been shown in a number of studies to have a protective effect against viral infections. The majority of the studies suggested that orally or nasally administered interferons exerted their antiviral activity locally [Cummins and Hutcheson, 1983; Cummins and Rosenquist, 1980; Greenberg *et al.*, 1978; Hayden *et al.*, 1986; Higgins *et al.*, 1983; Merigan *et al.*, 1973; Schafer *et al.*, 1972; Smith *et al.*, 1987; Turner *et al.*, 1986]. More recently, a number of studies have suggested that the orally administered interferons might be exerting their antiviral activity through a systemic effect [Cummins *et al.*, 1988; Hutchinson and Cummins, 1987; Koech *et al.*, 1990].

Further studies have extended the original observations with the antiviral activity of orally administered interferons to include demonstrations of immunoregulatory and antiparasitic activities of orally administered interferons [Cummins and Hutcheson, 1986; Fleischmann *et al.*, 1991; Young *et al.*, 1990]. Additional studies have further extended the observations with oral administration of interferons to include immunoregulatory and antibacterial activities mediated by oral administration of other cytokines [Baqar *et al.*, 1993; Koren and Fleischmann, 1994].

The concept that oral administration of interferons could have a systemic effect has been a difficult one to evaluate. It is difficult to understand how orally administered interferons, particularly pH sensitive IFN-γ could survive passage through the acidic and/or peptidase rich environment of the stomach and intestinal tract to trigger a systemic effect.

One concern involves the lack of parallel controls in many of the studies. A further concern involves

questions about the biological relevance of the often relatively modest effects obtained with orally administered interferons. Other concerns relate to the lack of universality in the observation of biological effects of orally administered interferons [Sperber *et al.*, 1993; Witt *et al.*, 1992]. The efficacy of the interferon therapy may depend upon the form of disease (acute or persistent), the daily dosage of interferon [Cummins *et al.*, 1993], and the type of interferon preparation (whether it is made from natural or recombinant sources) [Georgiades *et al.*, 1994]. Furthermore, the type of disease and the duration of therapy may influence the final response to treatment [Georgiades *et al.*, 1994]. These concerns are real and appropriate. It will be attendant upon the researchers investigating the oral administration of cytokines to address all of them effectively.

The oral administration of cytokines was the topic of a number of presentations at a recent workshop presented in conjunction with the 1994 Annual Meeting of the International Society for Interferon and Cytokine Research in Budapest, Hungary.

Local effects of orally administered cytokines

Effects of IFNα on HLA-DR expression in human buccal epithelial cells in culture

The mechanism by which orally administered IFN-α might exert an antiviral effect was probed *in vitro* in studies with cultured primary human buccal epithelial cells [Smith *et al.*, 1994]. David Chi and his colleagues used florescence studies to show that IFN-α treatment significantly increased the number of HLA-DR positive cells. A trend toward greater expression of HLA-DR by HLA-DR positive cells was also observed. The

indication that IFN-α causes an upregulation of HLA-DR expression by human buccal epithelial cells and peripheral white blood cells suggests that orally administered IFN-α may exert an antiviral activity against local viral infections. This antiviral activity may be medicated at least in part by a greater degree of recognition of viral infected cells in the context of HLA-DR by the host immune system.

Effects of IFN-α on established human epithelial cell lines in culture

The mechanism by which orally administered IFN-α might exert antimicrobial action was examined in studies reported by Kunihiro Ohashi and his colleagues [Ohashi *et al.*, 1994]. It was found that the saliva of healthy volunteers contained IL-1α and IL-8. Established epithelial cell lines were also found to produce IL-1α and IL-8 spontaneously. The possible immunological roles of IFN-α in combination with these lymphokines were evaluated *in vitro*.

IL-1α, used at a level equivalent to that in the saliva, and IFN-α were examined for cooperative effects on activation of neutrophil mediated anticandidal action. They were found to have an additive effect. The production of IL-8 was found to be enhanced by treatment of established epithelial cell lines by IFN-α treatment. The IFN-α was also found to partially restore IL-8 production in a monocyte cell line that had been latently infected with human immunodeficiency virus-1 (HIV-1) [Ohashi *et al.*, 1994].

Finally, the IFN-α treatment of Detroit 562 (a pharyngeal cancer cell line) and KB cells (oral cancer cell line) caused enhanced binding to a human T cell line, suggesting that IFN-α caused an enhanced recognition of the tumors by T cells. In this regard, the investigators showed an enhanced expression of ICAM-1, CD29, and CD49wb on some oral-mucosal epithelial cells treated with IFN-α *in vitro*.

Taken together, the work provides some *in vitro* evidence to support the concepts that *in vivo* oral administration of IFN-α may (a) locally activate neutrophils in the buccal cavity to exhibit a greater antimicrobial action, (b) affect local lymphokine production by epithelial cells in contact with the IFN-α, and (c) increase cell surface antigens in mucosal epithelial cells making them more sensitive to tumor surveillance mechanisms.

Effects of orally administered IFN-α on lymphoid cell function in mice

Mary Tompkins reported on the effects of intranasally administered IFN-α on lymphoid cell phenotype and function in lymph nodes and the spleen [Tonkonogy *et al.*, 1994]. She and her colleagues showed that lymphocytes isolated from periglandular lymph nodes responded to *in vitro* stimulation with immobilized anti-CD3 activated T cells to produce 4-fold more IFN-γ than control mice. Lymphocytes from superficial cervical lymph nodes of oral IFN-α-treated mice produced 2-fold more IFN-γ than those from control mice. No differences in IFN-γ production were detected for lymphocytes from more distal lymphoid organs such as axillary lymph nodes, mesenteric lymph nodes, Peyer's patches, or spleens from IFN-α and control mice.

Effects of orally administered IFN-α on the expression of cell surface markers of lymphocytes in the lymphoid tissue were also measured. No differences in proportion of expressing cells or degree of expression of cell surface CD4, CD8, MHC Class II or immunoglobulin were seen in the lymphocytes from any of the lymphoid tissues from IFN-α treated and control mice.

The results indicate that intranasal administration of IFN-α establishes a greater responsiveness of lymphocytes in periglandular and superficial lymph nodes to IFN-γ induction by exposure to anti-CD3. This greater responsiveness occurs in the absence of a change in phenotype of the lymphocytes. Further, the results suggested that the effect of intranasal administration of IFN-α and IFN-γ production may be primarily a local effect, since it diminishes with increasing distance from the site of IFN-α administration.

Systemic effects of orally administered cytokines in animal models

Myelosuppressive effects of orally administered interferons in mice

A paper presented by Robert Fleischmann summarized his published work on the myelosuppressive effects of orally administered interferons in mice [Fleischmann *et al.*, 1991, 1992; Koren and Fleischmann, 1993]. The presentation reviewed work showing that oral administration of each of three interferons (IFN-α, IFN-β and IFN-γ) exerted a dose dependent suppressive effect on peripheral white blood cell counts [Fleischmann *et*

al., 1991]. Moreover, the peripheral white blood cell suppression was shown to be reflective of a suppression of bone marrow function by the orally administered interferons [Koren and Fleischmann, 1993]. Of importance in addressing the concerns about the biological relevance of orally administered interferons, the magnitudes of the peripheral white blood cell and bone marrow suppressions induced by oral interferons were equivalent to those induced by subcutaneous or intraperitoneal administration of the interferons.

Studies on the kinetics of establishment of peripheral white blood cell and bone marrow suppression indicated that the suppressive effects developed more slowly for orally administered interferons (2 days for peripheral white blood cell suppression and 3 days for bone marrow suppression) than for injected interferons (1 day and 2 days, respectively).

Finally, the mechanisms of establishment of peripheral white blood cell and bone marrow suppression by orally administered interferons and injected interferons were examined [Fleischmann *et al.*, 1992]. In contrast to observations with injected interferons, the systemic activities of orally administered interferons were not blocked by the presence of circulating antibody. Further, the peripheral white blood cell suppressive effects of orally administered interferons were shown to be able to be adoptively transferred by inoculation of white blood cells to otherwise untreated mice.

These studies show in a mouse model that orally administered interferons are as potent as injected interferons in suppressing both the peripheral white blood cell counts and the bone marrow. Moreover, they indicate that the mechanism by which orally administered interferons exert these systemic effect, is different from that of injected interferons.

In a study reported earlier [Georgiades *et al.*, 1988], Georgiades and colleagues found that the antiviral state could be transferred from the oral cavity of mice treated with moIFN-α or rat rIFN-γ to peripheral blood lymphocytes and spleen cells. They demonstrated that white blood cells of mice treated with 5–10 IU and 0.005-1 IU IFN-γ could transfer antiviral resistance *in vitro* to fetal fibroblasts. Such a transfer apparently happened by direct cell to cell contact when the spleen cells from mice orally treated with interferon were co-cultured with fetal fibroblasts in the absence of interferon as previously described [Blalock and Baron, 1977]. The phenomenon appeared within 5 hrs after initiation of oral interferon treatment and, with continued oral interferon treatment, persisted through at least 15 days. Neither thymus cells nor plasma of orally interferon-

treated mice had the ability to transfer the antiviral effect.

Taken together, these and other controlled experiments suggest that interferon may be absorbed quickly in the oral cavity by cells of mucosa, somehow activating leukocytes present in mucosal tissue resulting in the activation of leukocytes in the lymphatic system and in the peripheral circulation.

Myelosuppressive effects of orally administered IL-2 in mice

A paper presented by Srecko Koren reported parallel observations for IL-2 [Koren and Fleischmann, 1994]. Oral administration of IL-2 was shown to exert systemic effects by suppressing both the peripheral white blood cell counts and the bone marrow in a dose dependent manner. Moreover, the potency of orally administered IL-2 was equivalent to that of injected IL-2. Taken together with the results of Fleischmann, these observations suggest that the induction of systemic effects by orally administered cytokines may be a general phenomenon.

Taking together all of the above information, it appears that not only IFN-α but also several other interferons and cytokines introduced to the mucosal membrane of the oral cavity can transfer signals through the mucous membrane and induce systemic responses that can be measured by a variety of techniques. This evidence about systemic effects was confirmed by clinical studies carried out on patients with chronic active hepatitis B, and C and on patients with AIDS as discussed below.

Effects of orally administered lymphokines on host response to Campylobacter jejuni in mice

A paper presented by Shahida Baqar reported on the effects of orally administered IL-2, IL-5 and IL-6 on gut mucosal immunity to Campylobacter jejuni [Baqar *et al.*, 1993; Baqar *et al.*, 1994]. She and her colleagues showed that oral administration of IL-6 resulted in a 3 log reduction (relative to control mice) in the amount of *C. jejuni* excreted in the feces within 48 hours after administration of the *C. jejuni*. Oral administration of IL-5 showed a similar reduction though the time course for the reduction was delayed relative to IL-6. No reduction in *C. jejuni* was observed with oral administration of IL-2. These results indicate that oral administration of IL-5 and IL-6 can reduce the initial colonization by *C. jejuni*.

The duration of the effect of the three lymphokines was probed by rechallenging mice that had been treated with oral administration of IL-2, IL-5 or IL-6 to establish a recolonization with *C. jejuni*. The results indicate that, for all three lymphokines, the initial level of recolonization was 2 logs lower than in control animals; however, the recolonization ultimately progresses to the level of the control for IL-5 and IL-6 treated animals. Only IL-2 treated animals showed a durable suppression of *C. jejuni* colonization.

Specific IgA antibody response to *C. jejuni* was also measured for mice treated orally with the three lymphokines. IL-6 treated mice, but not IL-2 or IL-5 treated mice, showed enhanced levels of *C. jejuni*-specific IgA antibodies in the intestine and in the blood.

In further experiments on the effects of orally administered lymphokines on IgA production, IL-2 was evaluated for its potential as an oral mucosal adjuvant for vaccination with formalin-killed *C. jejuni*. A three-fold enhancement in sIgA was observed relative to control mice. This enhancement in sIgA activity was biologically relevant since it led to an enhanced rate of clearance of *C. jejuni* from the intestine and a reduced number of *C. jejuni* that were present in the feces.

These results suggest that oral administration of lymphokines can have important systemic effects on the host response to infection with *C. jejuni*. The results show that IL-5 can result in a faster rate of clearance of *C. jejuni* infection; that oral administration of IL-2 can result in an enhanced humoral immune response to vaccination with *C. jejuni*; and, that oral administration of IL-6 can result in both a faster rate of clearance of *C. jejuni* infection and an enhanced IgA response to *C. jejuni*.

Efficacy of orally administered IFN-α in feline leukemia-positive cats

Oral administration of IFN-α has previously been reported to be efficacious in the treatment of feline leukemia [Cummins *et al.*, 1988]. Thomas Toth reported on a double-blind study evaluating the effects of orally administered IL-2 or IFN-α on various hematological variables in feline leukemia virus-positive cats [Toth *et al.*, 1994]. The hematological parameters measured included total red blood cell counts, total white blood cell counts, differential white blood cell counts, total hemoglobin, mean corpuscular hemoglobin, mean corpuscular-hemoglobin concentration, mean corpuscular volume and hematocrit.

Oral treatment with IL-2 was given 3 times (Days 1, 3 and 5) at 2 dosage levels (40 and 400 units/treatment). The hematological variables were monitored on Days 7 and 14. Orally administered IL-2 affected only one hematological variable as it reduced the mean corpuscular hemoglobin concentration to a significant level by Day 7 in cats treated with 400 units IL-2. By Day 14 the mean corpuscular hemoglobin concentration returned to the control level.

Oral treatment with IFN-α was given 8 times (Days 0, 3, 7, 10, 14, 17, 21 and 28) at 3 dosage levels (0.05, 0.5 and 5.0 IU/treatment). The hematological variables were monitored on Days 0, 7, 14, 21 and 28. Oral administration of IFN-α caused significant and dose dependent elevations in total red blood cell count, total hemoglobin, and hematocrit on Days 14, 21 and 28. Interestingly, with 0.5 IU/treatment, monocyte counts were also significantly elevated on these days. The other hematological variables were at the control level.

These results indicate that oral administration of IL-2 and IFN-α can affect several hematological variables, suggesting that these biological response modifiers cause systemic effects. The biological importance of these systemic effects and their relevance for the control of the feline leukemia virus infection are as yet unknown.

Effect of orally administered IFN-α on bacterial infections in mice

Finally, Miklos Degre [1994] reported on his studies on the effect of oral administration of two biological response modifiers on the course of an enteric bacterial infection in mice [Degre, 1994]. He found that orally administered tumor necrosis factor did not have an effect on the course of infection. However, orally administered IFN-α reduced the mortality of infected mice relative to controls. The degree of mortality with orally administered IFN-α was the same as that observed for intraperitoneally administered IFN-α. The results suggest that oral administration of IFN-α may exert a protective effect against enteric bacterial pathogens.

Orally administered IFN-α for treatment of inflammatory airway disease in horses

Race horses suffer from inflammatory airway disease that impairs their ability to perform on the race track. Bonnie Moore presented the results of a double-blind,

placebo controlled study on the efficacy of oral IFN-α in the treatment of inflammatory airway disease [Moore et al., 1994]. Horses were randomly assigned (8 horses/group) into a placebo group and three IFN-α dosage groups (50, 150 and 450 IU/day for 5 days). Fifteen days after initiation of therapy, horses were evaluated for the degree of inflammatory airway disease by semi-quantitative endoscopic examination score and by cytologic evaluation of bronchoalveolar lavage fluid. Oral administration of 50 and 150 IU of IFN-α resulted in a reduction in the endoscopic examination score as well as a reduction in the number of white blood cells in the bronchoalveolar lavage fluid. Administration of 450 IU of IFN-α was not as efficacious. The relative percentages of the various lymphocyte subpopulations was unaffected by the IFN-α treatments. The results suggest that orally administered IFN-α may have a beneficial effect on inflammatory airway disease in horses.

Clinical studies in man

Efficacy of orally administered IFN-α in HIV-infected patients

Previously published work suggested that oral administration of IFN-α may be useful as a treatment of patients with AIDS [Hutchinson and Cummins, 1987; Koech et al., 1990]. Two presentations discussed work evaluating the efficacy of orally administered IFN-α against HIV, while one presentation evaluated the efficacy of orally administered IFN-α against feline leukemia virus.

Musabbir Mian reported on a study in Poland of 48 patients with AIDS or ARC (AIDS related complex) who had been given low dose oral treatment with IFN-α [Babiuch and Mian, 1994]. This report represented the results of studies initiated four years earlier. Part of these studies were already published [Babiuch et al., 1993; Georgiades and Babiuch, 1994] and found confirmation by others [Jordan, 1994]. It was reported that most patients with initial CD4+ cell counts above 200 became asymptomatic, gained weight, had improved Karnofsky performance scores and had either increased or stable CD4+ cell counts and total lymphocyte counts. Patients with initial CD4+ cell counts below 200 did not respond to oral IFN-α.

Martin Cummins reported on a double-blind, placebo controlled study in Zambia involving 147 patients

divided randomly and equally into three treatment groups: Group 1, placebo; Group 2, 150 IU/day IFN-α administered on an alternate weekly basis with placebo for 24 weeks; and, Group 3, 150 IU/day IFN-α administered continuously for 24 weeks [Mukunyandela et al., 1994]. The study showed that oral IFN-α treatment had a significant effect in reducing HIV-related signs and infections. Both Groups 2 and 3 had a significantly greater resolution of pre-existing HIV-related skin infections than the placebo treated Group 1. Group 3 had a greatly reduced incidence of new, mucocutaneous HSV infections (2%) compared to the placebo treated Group 1 (25%), with Group 2 having an intermediate value (13%). Interestingly, patients in the alternate IFN-α/placebo Group 2 (but not the continuous IFN-α Group 3) showed a reduction in global symptom score and small, but significant, increases in absolute and mean CD4+ counts compared with placebo treated Group 1.

Taken together, these studies provide significant support for the concept that orally administered IFN-α may have beneficial effects for AIDS patients, provided they are treated in the early stage of disease. As a caution, however, several negative studies have been reported showing a lack of efficacy of orally administered IFN-α in the treatment of HIV infected patients [Hulton et al., 1992; Sperber et al., 1993]. These negative observations may relate to the limited period of oral IFN-α treatment employed in these studies or to the use of recombinant as opposed to natural IFN-α. While it is not now possible to be certain of the anti HIV effects of oral IFN-α in humans, several studies provide additional support.

The observation that AIDS patients with baseline CD4+ cell counts below 200 cells/mm^3 responded poorly to oral IFN-α therapy was in agreement with reports of Kaiser et al. [1992] in Germany, Hutton et al. [1992] in Canada, and Sperber et al. [1993] in the USA. All of these studies reported similar findings: patients having < 200 CD4+ cells/mm^3 did not respond very well to IFN-α therapy. However, patients with baseline CD4+ cell counts > 200 cells/mm^3 survived longer than control patients and required much less medical attention than patients in the control group [Babiuch and Mian, 1994; Georgiades and Babiuch, 1994]. These observations were confirmed by a report from California where physicians had similar experiences with HIV-1-infected patients [Jordan, 1994].

Interestingly, the beneficial effects of IFN-α therapy may not be constant through the course of IFN-α therapy. Babiuch reported that, from time to time,

patients may apparently lose their sensitivity to oral IFN-α therapy [Babiuch and Mian, 1994; Georgiades and Babiuch, 1994]. This apparent loss of sensitivity to IFN-α therapy (or deterioration of clinical status) can be reversed by slightly increasing the dose of IFN-α [Babiuch and Mian, 1994]. These observations may represent clinical manifestations of a phenomenon described in vitro by Yamamoto and colleagues [Yamamoto et al., 1993]. These investigators examined the effects of IFN-α using long-term culture of HIV-1-infected white blood cells. They observed a transient loss of sensitivity of the white blood cells to IFN-α. The transient loss was observed twice during a 60 day culture period. Further studies will be needed to determine if HIV-1 resistance observed in vivo and in vitro occur by the same mechanism.

Clinical studies with orally administered IFN-α in patients with chronic active hepatitis (CAH)

Small scale trials with 14 hepatitis B patients with chronic active hepatitis have previously suggested that oral administration of IFN-α might be of therapeutic benefit [Zielinska et al., 1993]. Similar beneficial results were seen in a small scale trial with six hepatitis C patients with chronic active hepatitis who were treated with oral IFN-α [Zielinska et al., 1993]. Zielinska reported on the long-term follow-up of 30 hepatitis B patients with chronic active hepatitis who were treated with oral IFN-α [Zielinska et al., 1994]. The diagnosis of chronic active hepatitis was based on clinical and biochemical signs of active disease. Oral IFN-α was given daily at a dose of 25–150 IU/day, depending on the body weight of the patients, for an average of 7 months. Seroconversion from HBeAg to antiHBe was observed in 18 of 30 patients. The median time to seroconversion was 36.2 weeks. In addition, two patients seroconverted from HBsAg to antiHBs.

In all 18 patients who seroconverted to antiHBe, HBV DNA was not found to be present in serum. Also, biochemical markers associated with liver function returned to the normal range. Further, 7 of the 12 patients who did not seroconvert to antiHBe were negative for the presence of HBV DNA in the serum. The remaining 5 patients had serum HBV DNA levels that were significantly lower than at the initiation of oral IFN-α treatment. Taken together, these observations suggest that oral IFN-α may be an effective therapy for control of chronic active hepatitis.

The Annual Meeting of the International Society for Interferon and Cytokine Research (ISICR-94) held in Budapest provided, for the first time, compelling evidence that oral doses of cytokines modulate immune functions and increase resistance to infectious disease. Studies were presented by investigators from a number of different laboratories. Clinical data, especially in humans, indicates that IFN, given though the oral mucosa, is capable of inducing systemic reactions and may have potential as a new therapeutic method.

Summary

A number of different laboratories reported on studies with orally administered interferons and cytokines. Their observations extend previous observations which showed that orally administered interferons and cytokines can exert both local and systemic effects. As difficult as it may be to understand how orally administered interferons and cytokines may exert both effects, the increasing number of laboratories that demonstrate biological effects with orally administered cytokines suggests that serious consideration be given to the possibility that orally administered interferons and cytokines can indeed exert effects. They also raise the possibility that these effects may have biological relevance for the treatment of human disease. Moreover, they may indicate that the nasal/oral region is a window on the environment. It is most important, however, to assure that these experiments are performed with special care to avoid presenting preliminary data that is not properly controlled. It is essential to carry out these studies with sufficient animals or patients to ascertain their significance; and to plan the studies as double-blind evaluations to avoid misinterpretations when subjective tests are used. Nevertheless, the overall data presented give one the impression of an area that should be pursued.

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