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-\(\gamma\) 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 IFN\(\alpha\) on HLA-DR expression in human buccal epithelial cells in culture**

The mechanism by which orally administered IFN-\(\alpha\) 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 fluorescence studies to show that IFN-\(\alpha\) 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 mediated 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 antifungal 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 (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 [Tonkony 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