Review Article

Systemic effects of interferons after oral administration in animals and humans

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Induction, control, and downregulation of complex biological phenomena, such as the inflammatory response and innate and adaptive immune responses, are accomplished by the cells and substances of the hematopoietic system.[1–3] Most of the cellular sequence of events in these biological phenomena has been described. However, it is not yet clear how the combination of extracellular signaling molecules acts on these cells to deliver the appropriate precise and controlled responses needed to restore immunohomeostasis and health.[3,4]

Molecular protein and oligopeptide messengers that are collectively termed cytokines (from the Latin term for cell movement) and chemokines (chemotactic cytokines) are now known to be the chief means of cell-to-cell communication.[3–5] Cytokines are operationally defined as constitutive or induced extracellular hormones, proteins, and oligo- and polypeptides, which are released from cells after cellular activation or stimulation. Although their effects on target cells that express receptors for these molecules may be similar, cytokines are distinct from cell mediators, which are biologically active protein fragments derived from larger precursor or effector molecules.[2–5]

Cytokines activate cells via binding of the signaling molecule (ligand) to cell membrane-associated receptors.[6] The ligand-receptor binding complex is internalized or connected to intermediary cellular metabolism through systems involving second messengers (such as adenyl cyclase and others) that are operational within cells. More than 100 cytokines have been described to date; each has multiple modes of action on target cell populations, in part because the receptors for the cytokines are shared between different members of the cytokine family. Thus, redundancy and duplication of effects are the norm, not the exception, where the cytokines are concerned. This conservation of pathways is biologically efficient because diverse conditions often require similar biological responses. This concept is amply illustrated by many of the murine knockout models of cytokines, wherein the gene (or genes) encoding for a specific cytokine is experimentally deleted or inactivated during early embryogenesis. Contrary to expectations, many cytokine-knockout mice are phenotypically normal, a finding which attests to the tremendous duplication and redundancy of signaling pathways and communication systems in vivo.[3,4,6]

Biological responses may be activated or inhibited by the actions of cytokines such that the activities of a single cytokine molecule may have multiple biological effects within a mixed cellular population. Moreover, cytokines tend to act together to affect biological responses such that the proper mixture, proportions, and sequence of appearance of cytokines determine the predominant biological effect. Cytokines are antagonized by specific and nonspecific inhibitors, and their effects are conditioned by the number and distribution of cellular receptors on target cells; thus, plots of the dose-response effects of cytokines on a biological response are bell-shaped curves rather than the linear dose-response profiles characteristic of chemical or pharmaceutical interactions. That is to say, inductive effects are characteristic of low doses of cytokines acting on a cellular population, whereas high doses of cytokines often result in downregulation of or suppressive effects on biological responses (or both).[3,5–7]

The nucleotide and amino acid sequences of most of the more important cytokines are known, and recombinant molecules are now available for scientific investigation and even for clinical uses.[3,4,6] As more becomes known about the cytokines and their disparate modes of action in health and disease in humans and animals, the potential therapeutic uses of cytokines will undoubtedly receive increased attention in experimental and clinical settings.[8–11] When considering effective delivery of a cytokine to a cell population in a live host, a critical issue of concern is the route of administration. Experimentally, numerous methods have been used, including the newer approaches of target gene therapy whereby the gene encoding the cytokine of interest is transfected into cells or even administered directly into the animal. However, approaches such as these are not yet suitable for clinical applications.

To date, most in vivo studies with cytokines have involved the traditional route of parenteral administra-

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tion to achieve desired biological effects.[12] This route of administration would appear to offer distinct advantages, not the least of which is accurate and precise delivery of known quantities of the cytokine in question; however, parenteral administration may deliver too much cytokine in the peripheral tissues with resultant adverse events instead of delivering the cytokine only at the local disease site or to a portion of the immune system that will respond to the immunomodulatory effects of the cytokine. Furthermore, in animal and human experimental trials, systemic toxic effects associated with high doses of cytokines after parenteral administration are common complications, which have been demonstrated repeatedly under natural conditions of health or disease.[13–17] There is virtually no physiologic circumstance in which cytokines are generated in response to a stimulus in the quantities (ie, in the order of milligrams of protein or millions of biological units) that are required for parenteral administration. Under normal physiologic conditions, cytokines are biologically active in picogram or nanogram quantities or less,[6] a minute fraction of the doses commonly administered parenterally.

Despite these obvious caveats and the toxic effects that accompany parenteral administration of cytokines, high doses (in the order of milligrams) of cytokines are still favored and promoted as parenteral treatments by manufacturers and this approach has been approved by regulatory agencies such as the FDA.[18–26] Only a few investigators have promoted the seemingly more rational use of cytokines at doses approximating those of biological importance and by routes other than that of parenteral administration.

An ideal cytokine delivery modality would be the placement of the cytokines directly into the targeted cellular environment at doses and frequencies that most closely mimic the desired natural course of events. With current technology, this goal is rarely, if ever, met. Perhaps other routes, specifically oropharangeal delivery (into the nose or mouth so the cytokine reaches the oral and pharyngeal mucosa), might offer a means of engaging the cytokine network to foster beneficial effects in animals and humans.[27,28]

Recent reports have described the detection of literally dozens of cytokines and other signaling molecules in body fluids such as milk,[28–35] saliva,[36–40] and nasal secretions.[41–49] The amount and types of these molecules in secretions vary with the health status of the individual; therefore, in general, these signaling molecules are both a reflection and an integral component of responses in various physiologic and disease states. It is also evident that these locally produced and secreted molecules are indicative of ongoing responses to injury and have the potential to exert systemic biological effects because their concentrations vary with the state and stage of disease. Because of their presence in mucosal compartments, these secreted signaling and cytokine molecules may have biological importance and their effects may be reproduced, strengthened, or mimicked by providing these cytokines (in biologically appropriate concentrations and frequencies) to human and animal patients via the oropharynx.[27,50]

Whether the amount and types of signaling molecules in secretions can be manipulated to exert a measurable local and systemic biological effect has been a topic of research interest, and in particular, whether oral administration of cytokines can result in specific systemic biological effects. Both of these questions have been affirmed unequivocally, and in this review, it is our intent to provide a brief overview of the published data regarding oral, intragastric, or intranasal administration of recombinant and naturally occurring **interferons (IFNs)**.

Of the cytokines studied to date, the IFN family is the cytokine group most widely applied to animals and humans by the oral, intranasal, or intragastric routes. Not only were the IFNs the first family of cytokines described,[51] but also the IFNs are now available in purified form as naturally occurring or recombinant molecules. Another facet of the IFNs is the fact that the α family of IFNs is not species-specific in its action but rather best described as species-restricted. That is, IFNα of human origin interacts with cells of animal origin, and human cells are modulated by IFNα of animal origin. For example, bovine IFNα is active on primate,[52] porcine, or human cell cultures.[53] Porcine IFNα is active on equine,[53] bovine, and human cell cultures.[53,54] **(HU)IFNα** is active on porcine,[53–55] bovine,[53–59] and feline[60] cell cultures. When injected, feline IFNω protects dogs from parvovirus[61] and protects Japanese pearl oysters from akoya-virus infection.[62]

Furthermore, it has been determined that the IFNs are nontoxic after administration into the nose or mouth (oromucosal route) or the stomach (intragastric route) at different doses and under different administration conditions. The resultant patterns of IFN activity are also useful as a guide to understanding the actions of other cytokines of interest.

Laboratory Animal Species

Physiologic, immunologic, and pharmacologic effects of IFNs administered via the oromucosal or intragastric routes in rodents—Because the IFNs are proteins, it is not surprising that few (if any) molecules of IFN remain intact after passage into the gastrointestinal tract. Experimental biodistribution data support this assumption. The activity of HuIFNα was not detected in serum samples collected after high doses of HuIFNα were given orally or via gavage to animals.[63–65] Rabbits that weighed 3.3 to 3.6 kg were administered 2.5 to 6×10^6 units of HuIFNα in a 5-mL volume[63]; in other similar experiments, dogs (weight range, 9.6 to 14.6 kg) and African Green monkeys were given HuIFNα (3×10^6 U/kg[64] or 6×10^6 U/kg,[65] respectively). After oral administration of HuIFNα1-8 radiolabeled with iodine 125 to Swiss mice, HuIFNα1-8 could not be detected in a biologically active form in the sera obtained from those animals.

In all of these experiments, the failure to detect HuIFNα activity in the serum after oral administration is attributable to proteolytic digestion of HuIFNα in the gastrointestinal tract.[66] The results of these studies are often cited as the primary reason why the parenteral route of administration of IFNα is favored over other routes. The assumption made is that IFNs must enter

into the circulation to exert an effect at a site distant from the site of application in the gastrointestinal tract. However, this assumption has neglected interactions of the IFNs with host cells and tissues prior to entry into the stomach. Recently, data have become available that challenged this assumption and have focused on the interactions of IFNs within the oropharyngeal cavity as the key to understanding the molecular basis of action of IFNs that are administered orally and the apparent paradox of the development of a systemic effect in the absence of detectable IFN in peripheral circulation compartments.

Critical evidence for local actions of IFN in the oral cavity—The first indication that local actions of IFN in the oral mucosa may be the key to understanding the systemic effects of IFN after oral administration was achieved through in vivo labeling studies. After oromucosal administration, IFN radiolabeled with sulfur 35 was retained in areas proximal to lymphoid regions, including the posterior aspect of the nasal cavity, posterior aspect of the tongue, small intestine, and rectum of C57BL/6 mice.[67] A central intracellular pathway for responsiveness to IFN is the induction of the enzyme **2'5'-oligoadenylate synthetase (2'5'AS)**.[68] This enzyme is induced only by IFN; thus, it is a specific molecular marker of IFN-induced cellular activation. In vitro, cellular response to IFN is measured by increased expression of **major histocompatability complex (MHC)** class I antigen and induction of 2'5'AS in target cells.[68] In DBA/2 mice that were administered **murine IFN (Mu)**IFNα and β via the oromucosal route, MHC class I antigen expression was not increased and induced intracellular 2'5'AS activity was not detected in samples of peripheral blood or spleen but the MHC class I antigen expression was markedly increased in lymphoid cells harvested from the oropharyngeal cavity 24 hours after MuIFNα and β treatment.[66] The induction of MHC class I antigen highlights IFN action within local mucosal compartments.

However, local intracellular induction of 2'5'AS is not necessarily an indication that systemic activation of 2'5'AS has also occurred. In BALB/c mice that received 200 and 20,000 units of either MuIFNα and β or HuIFNα1-8 orally, there was no effect on 2'5'AS activity in splenic lymphocytes at any of the evaluations within 10 days from the start of treatment.[69] In contrast, MuIFNβ administered oromucosally augmented IFN response factor-1 and 2'5'AS mRNA expression levels and intracellular 2'5'AS enzyme activity in the spleen but not in the cervical lymph nodes of C3H mice.[70] In a guinea pig model of asthma, HuIFNβ administered in drinking water induced 2'5'AS activity in cells of whole blood samples; at a concentration of 1 U of HuIFNβ/mL, the treatment suppressed the asthma-associated increase in respiratory resistance, and at a concentration of 10 units of HuIFNβ/mL, the treatment suppressed eosinophil infiltration into the trachea and lungs.[71] In that experiment, the concentration of HuIFNα that induced the highest intracellular 2'5'AS activity was 0.1 U/mL of drinking water with an estimated daily intake of 50 mL or 5 units.[71]

Normal, nude, and SCID mice given recombinant MuIFNα in their drinking water for 3 days all had intracellular 2'5'AS activity in the liver and whole blood. Normal and sham-operated mice, but not hypophysectomized or adrenalectomized mice, had intracellular 2'5'AS activity in the liver and whole blood after recombinant MuIFNα was given in the drinking water for 3 days. The authors concluded that the induction of 2'5'AS by oral MuIFNα was not mediated by the T cell system but possibly via the hypothalmic-pituitary-adrenal axis in mice.[a]

Sixteen to 24 hours after intragastric administration of MuIFNα (10^2 to 10^4 units) or ovine IFNτ (10^2 to 10^5 units), induced 2'5'AS was detected in cells of whole blood samples obtained from ICR mice.[72] In addition to that of 2'5'AS, other genes are upregulated after oromucosal administration of IFNα.[73,b–d] For example, the amount of RNA transcripts of the ATP-dependent IFN, responsive gene was increased 6-fold in oropharyngeal tissue of Swiss mice 4 hours after oromucosal administration of MuIFNα (10^5 units), compared with the amount in untreated mice.[73]

Oral administration of IFNα has been shown to affect systemic phenotypic expression of lymphocyte populations. Interferon-activated natural killer cells, B cells, and subpopulations of T cells are detected in the peripheral circulation of mice with tumors as early as 4 hours after the initiation of oromucosal treatment with IFNα. In addition, oromucosal treatment with IFN also induced trafficking of cells from both the spleen and peripheral lymph nodes to the site of tumor cell replication. Other genes that are upregulated after oral administration of IFNα include genes for Crg2 (and other chemokines) and proteases associated with antigen processing and those involved in lymphocyte activation, apoptosis, and protein degradation.[d]

In mice, the effects of IFN on antibody responses to IFNs have also been determined. Oral pretreatment of mice once a day for 7 days with HuIFNα or β significantly inhibited specific IgM and IgG antibody responses to SC injection of HuIFNα or β at 21 and 28 days. In Swiss or BALB/c mice, tolerance to the immunogenic proteins of HuIFNα or β (assessed via parenteral administration of those IFNs at high doses) was induced after oromucosal administration of HuIFN? at doses of 10^3 to 10^6 units or HuIFNα at doses of 10^3, 10^6, or 10^7 units.[74] In an animal model for sensitization to ragweed pollen, compared to placebo, oromucosal administration of recombinant MuIFNα or natural MuIFNα and β during the allergic sensitization (days 0 to 6), the hypersensitive response (days 11 and 12), or both periods caused a significant dose-dependent reduction in allergen-specific IgE production and allergen-induced eosinophil recruitment in sensitized BALB/c mice sensitized to ragweed pollen. Treatment during the hypersensitive response period alone appeared to be most effective. Oromucosal treatment was as effective as IP treatment, with maximum inhibition of both allergen-specific IgE production and allergen-induced eosinophil recruitment observed at a dose of 1,000 units of IFNα.[69]

The in vivo immunomodulating potential of the oral administration of natural MuIFNα was also evaluated through antibody production in BALB/c mice with induced tolerance.[68] Ovalbumin was administered IP to

induce systemic antibody production on day 0 when ovalbumin feeding was initiated; ovalbumin was fed every 2 to 3 days for a total of 14 doses to suppress serum antibody concentrations. Oral administration of MU IFNα was initiated on day 0 and was continued for 5 consecutive days weekly for 5 weeks. On every sampling date (days 10, 17, 24, and 32), specific antibody concentrations in the groups treated with 1 or 10 U of natural MuIFNα/dose were significantly higher than those in the control group; in the IFNα-treated groups, tolerance to ovalbumin was blocked. Altogether, it is suggested that oral administration of IFNα can elicit immunomodulating actions (eg, influence serum antibody concentrations) by affecting the systemic immune system.[68]

Compared with mice treated with placebo, treatment of BALB/c mice with either MuIFNα or MuIFNα and β at doses ranging from 10^3 to 10^5 units via either the oromucosal or IP route resulted in almost complete eosinopenia. The number of eosinophils present in samples of bronchial alveolar lavage fluid obtained from IFN-treated mice was similarly reduced.[69] In ovalbumin-sensitized and ovalbumin-challenged guinea pigs, the dose of HuIFNβ administered in drinking water for 3 days that had the most suppressive effect on eosinophil counts was approximately 500 U/d, whereas 50,000 U/d had no effect.[71] In drinking water, doses of 5 to 50,000 units of MuIFNα and β, MuIFNβ, MuIFNγ, and HuIFNαA/D suppressed peripheral leukocyte counts in mice.[75–77] These effects were not blocked by antibodies that neutralize IFN activity and could be transferred to recipient mice by use of spleen cell suspensions, but not plasma.[76] These data suggest that IFN administered orally may have a therapeutic role in the management of allergic diseases in both animals and humans.

Effects of oromucosal administration of IFN in rodents with infectious diseases—In a study[78] in which mice received an oral challenge with vesicular stomatitis virus (1 LD_{50}), mice that received milk supplemented with MuIFNα and β (maximum concentration, 500 U/mL) had a significant reduction in mortality rate, compared with that among the control mice. Those investigators did not propose a mechanism of action, but reported that < 1% of rabbit IFNα and β reached the circulation after oral administration.

Oromucosal or IP administration of MuIFNα and β or oromucosal administration of individual recombinant MuIFNα, IFNβ, IFNγ species, or HuIFNα1-8 exerted marked antiviral activity in Swiss mice challenged systemically with lethal doses of encephalomyocarditis virus, vesicular stomatitis virus, or varicella zoster virus.[79] Intranasal or sublingual administration of 1,000 units of MuIFNα and β resulted in similar survival benefit after a lethal encephalomyocarditis virus challenge. The effects of a single oromucosal administration of 10^4 units once daily for 4 days or 10 individual doses of 10^3 units of MuIFNα and β administered over 60 minutes once daily for 4 days were equivalent to each other, and both treatments resulted in 70% survival to an encephalomyocarditis virus challenge, which was fatal to all untreated control mice; even a dose as low as 2 units of MuIFNα and β for 4 days resulted in 25% survival rate among treated mice. Thirty percent of Swiss mice given MuIFNα and β (10^4 units) oromucosally once per day for 4 days survived for 100 days after administration of 100 LD_{50} of vesicular stomatitis virus, compared to survival of < 10 days for control or untreated mice. Oromucosal MuIFNα and β also inhibited varicella zoster virus replication in the spleen, lungs, and brains of BALB/c mice after intranasal administration of varicella zoster virus.[79]

In Swiss mice, a single oromucosal dose of HuIFNα1-8 (140,000 units) or a single IP injection of MuIFNα and β (60,000 units) given 1 hour after administration of 100 LD_{50} of encephalomyocarditis virus resulted in equal survival rates (20%) among the IFN-treated mice (none of the control mice survived). This protection occurred even though oromucosal administration of HuIFNα1-8 did not induce detectable intracellular 2'5'AS activity, whereas as little as 20 units of MuIFNα injected IP resulted in a marked increase in intracellular 2'5'AS activity.[66]

Compared with findings in control mice, Swiss mice at 1 research facility that were given 20,000 units of MuIFNα via the oromucosal route once daily for 4 days had significantly greater survival rate (40%) and mean survival time (12 ± 2.5 days after challenge with 44 LD_{50} of encephalomyocarditis virus), compared with 5% survival rate in controls with a mean survival time of 6.1 ± 0.4 days. Swiss mice given 20,000 units of MuIFNα did not survive at a significantly greater rate when the challenge inoculation of encephalomyocarditis virus was 88, 220, or 440 LD_{50}. At a different research facility, Swiss mice from the same supplier as those used in the aforementioned study received the same dose of MuIFNα (derived from the same source) and were challenged with 100 LD_{50} of encephalomyocarditis virus; in this study,[80] IFN-treated mice had a significantly greater survival rate and survival time than did the control mice.

Oromucosal administration of MuIFNα once daily for a week significantly reduced replication of murine cytomegalovirus in the spleen and liver of BALB/c mice, compared with findings in mice that did not receive IFN treatment.[67] In BALB/c mice, an oromucosal dose of 10 units of MuIFNα and β given daily for 1 week prior to murine cytomegalovirus challenge was optimal for reduction of early replication of the virus in spleen and liver (compared with virus activity in mice given saline solution orally) and produced results comparable to those achieved with administration of 2×10^4 units of MuIFNα and β given IP 6 hours before challenge.[81] Moreover, in another study[82] involving BALB/c mice, 10 units of MuIFNα and β administered via the oromucosal route once daily for 7 days prior to murine cytomegalovirus challenge was as effective as administration of a single IP injection of 2×10^4 units of MuIFNα and β 6 hours before challenge in significantly suppressing the inflammatory response in both the acute and chronic phases of murine cytomegalovirus-induced myocarditis. Oromucosal administration of MuIFNα and β in 10-unit doses once daily for 7 days significantly altered spleen cell populations (particularly splenic B cells) in BALB/c,

CBA/CaH, and Swiss mice, compared with those populations in control mice; results of a dose-response study[83] indicated that 1 unit of MuIFNα and β was the optimal dose to effect changes in spleen cell populations. In C3H/HeN mice infected with vaccinia virus, oromucosal administration of MuIFNα (1, 10, or 100 U/body/d for 6 days) significantly increased the number of virus-specific cytotoxic T cells in the spleen; administration of MuIFNα at 1, 10, or 100 U/body/d for 15 days significantly reduced the number of pocks on the tails of vaccinia virus-infected C3H/HeN mice, compared with findings in control mice.[84] Protection from a lethal challenge of Semliki Forest virus was observed when low levels (10 to 100 U/mL) but not high levels of HuIFNα A/D or MuIFNα and β were added directly to drinking water of mice.[e] Cotton rats were given HuIFNα in drinking water before and after challenge with human respiratory syncytial virus; in those rats, administration of HuIFNα reduced the severity and the amount of recoverable respiratory syncytial virus infection in the lung, compared with rats that did not receive IFN.[f] In that study, the lowest dose of HuIFNα evaluated (0.2 U/mL of drinking water) was most effective. In another study,[85] a low dose (7×10^3 U/d) of MuIFNγ or tumor necrosis factor-α (various doses) was provided in drinking water to adult HAM/ICR mice starting 1 day prior to inoculation with *Salmonella* serovar Typhimurium; the low dose of MuIFNγ but not tumor necrosis factor-α reduced the penetration of salmonellae into intestinal epithelial cells, development of bacteremia, and the mortality rate and prolonged survival times, compared with findings in control mice. In an experiment[g] to investigate the effect of oral administration of IFN against systemic infection with *Listeria monocytogenes* in mice, animals fed 20 units of HuIFNα daily for 12 days (starting 6 days before bacterial challenge) in a feed formulation had a significantly lower concentration of the organism in spleen tissue than did control animals 5 days after challenge.

Effects of oral administration of IFNα in mice with tumors—Administration of IFNα via the oral route is associated with beneficial effects against experimental neoplastic diseases in rodents. Oromucosal administration of MuIFNα and β (10^5 units) twice daily to DBA/2 mice resulted in a 50% survival rate after challenge with 20,000 LD_{50} of **Friend Leukemia cells (FLCs)**; compared with control mice that did not receive IFN treatment, the IFN-treated mice had a greater survival rate after challenge with L1210 lymphoma or EL4 tumor cells.[79] Similarly, oromucosal administration of 10^4 units of MuIFNα and β increased the survival time of DBA/2 mice challenged with FLCs, compared with that of control mice[86]; administration of anti-IFN antibodies blocked that increase in survival time in mice given MuIFNα and β orally and challenged with FLC.[86] Oral administration of MuIFNα and β was as effective as parenteral administration of IFN in protecting against development of FLC-associated tumors,[79,86] which further suggests the importance of contact of IFNα and β with cells of the oropharyngeal cavity.

In C57B1/6 mice, antitumor activity against parenteral challenge of B16 melanoma cells was achieved via administration of 5,000 units of HuIFNα A/D in drinking water.[87] Furthermore, in those mice, HuIFNαadministered oromucosally interacted synergistically with MuIFNγ but not with HuIFNα that was administered IP.[87]

Effects of oromucosal or intragastric administration of IFN in rodents with autoimmune disease—The effects of IFN treatment on clinicopathologic manifestations of different experimentally induced diseases of suspected and proven autoimmune etiologies have been studied in rodents. Mixed cryoglobulinemia thymic stromal lymphopoietin-deficient transgenic mice develop mixed cryoglobulinemia with glomerulonephritis that closely resembles mixed cryoglobulinemia (an autoimmune disease) in humans. In 1 study,[88] such transgenic mice were administered 500 units of universal type I IFN or placebo PO daily for 21 days. Three variables (mean glomerular tuft area, mean glomerular areas occupied by macrophages, and mean number of inflammatory cells per glomerulus) were decreased in IFNα-treated mice, compared with values in control mice.

Effects of IFNs on experimentally induced allergic encephalomyelitis and allergic neuritis in rodents—In 1 investigation,[h] spleen cells that were harvested from mice (SJL/J strain) fed MuIFNα 3 times/wk for 6 weeks and stimulated in vitro secreted less IFNγ than spleen cells from mock-fed mice. Furthermore, activated spleen cells from mice fed 100 units of MuIFNα 3 times/wk for 6 weeks have a significantly decreased ability to passively transfer **experimentally induced allergic encephalomyelitis (EAE)**.[i] Spleen cell proliferation induced by mitogens and mitogen-induced production of **interleukin (IL)**-2 and MuIFNγ in SJL/J mice were inhibited by MuIFNα administered via the intragastric route (ie, a ballpoint needle was used for oral delivery to the distal portion of the esophagus and stomach). Clinical relapses of EAE in SJL/J mice were significantly suppressed to a greater extent by intragastric administration of MuIFNα at a dose of 10 units given 3 times/wk for 15 weeks than by higher doses of MuIFNα administered SC.[89] Clinical outcome of those mice given 0.1, 1, or 1,000 units was significantly worse than the clinical outcome of mice given 10 units.[90] In SJL/J mice, splenic T cells and CD8+ T cells had upregulated mRNA for Mx proteins (members of the dynamin superfamily[68]) after the mice ingested 10 or 100 units (but not 1,000 or 5,000 units) of MuIFNα.[91] Results of 2 studies to investigate IFN administration via the intragastric route in SJL/J mice with EAE have indicated that significant suppression of EAE clinical relapse occurred. When MuIFNα was given at a dosage of 100 units 3 times/wk for 6 weeks[92] or given at a dosages of 10 or 100 units but not 1,000 units 3 times/wk or when HuIFNα was given at dosages of 100 or 1,000 units 3 times/wk, EAE relapses were significantly suppressed,[j] compared with findings in EAE-affected mice that were not treated with IFN. Donor spleen cells collected from mice given MuIFNα via the intragastric route for 7

days inhibited acute EAE in recipient mice.[k] Nelson et al[93] reported that intragastric administration of 5,000 units of rat IFNβ enhanced the suppressive effects of intragastric administration of myelin antigens in Lewis rats with EAE. In another study,[94] Lewis rats with EAE had a significant decrease in clinical score (determined by a blinded observer scoring tail and hind limb weakness) and a decrease in the number of inflammatory foci after receiving 5,000 units of rat IFNα and β or 5,000 units of HuIFNα via intragastric administration once daily for 28 days, compared to placebo-treated rats; in contrast, SC administration of 5,000 units of HuIFNα or intragastric administration of 1,000 units of HuIFNα was not beneficial.

Oral administration of ovine-origin IFNτ has been evaluated in animals as a treatment for multiple sclerosis, a chronic neurologic disease of humans thought to be the result of an autoimmune T cell-mediated response to CNS myelin proteins.[l] In mice with EAE (used as a model of multiple sclerosis in humans), intragastric administration of ovine IFNτ has the same effect (ie, induction of suppressor cells) as those achieved with oromucosal administration of MuIFNα and β and can prevent development of acute or chronic EAE.[95,96,l] Intragastric or IP administration of ovine IFNτ affected the cytokine profile in sera from EAE-affected SJL/J mice and appeared to synergize with intragastric administration of myelin basic protein, resulting in induction of IL-4 and increased production of IL-10 (compared with controls).[m] Intragastric administration of 10^2 to 10^5 units of ovine IFNτ increased intracellular activity of 2'5'AS from baseline values in 5 different strains of mice.[72] Finally, intragastric administration of rat IFNα and β that was initiated 7 days before experimental induction of allergic neuritis immunization in Lewis rats resulted in a reduction in severity of the disease, compared with placebo-treated control rats.[90]

Effects of IFNs on diabetes in rodents— **Nonobese diabetic (NOD)** mice are used as a model for studies of insulin-dependent diabetes mellitus in humans. Recombinant HuIFNαA/D bgl II (10^5 units) administered IP 3 times/wk for 28 weeks significantly prevented development of this form of diabetes in 86% of recipients versus 30% of control mice.[97] Intragastric administration of MuIFNα (10-unit dose) every other day from 9 to 24 weeks of age suppressed type 1 diabetes in NOD mice, possibly because IFNα administered oromucosally activates regulatory splenic cell populations.[98] Tanaka-Kataoka et al[99] confirmed that intragastric administration of MuIFNα 3 times/wk from 6 to 38 weeks of age (100 U/dose) delayed the onset of diabetes mellitus in NOD mice. In those 2 studies, the intragastric dose of 10 units of MuIFNα[98] and the intragastric dose of 100 units of MuIFNα[99] resulted in the development of diabetes in significantly fewer mice. The reduction in development of diabetes by intragastric administration of MuIFNα was as good as the reduction achieved by the most effective IP dose of HuIFNα (10^5 units)[97] in the NOD-mouse model. In C3H mice, rejection of transplanted islet cells was significantly delayed by intragastric administration once daily for 21 days (starting 7 days before induction of diabetes with streptozotocin) with 10 or 100 but not 1,000 units of MuIFNα, compared with findings in mice that were not treated with IFN.[100]

Effects of IFNs on adjuvant arthritis in rodents— Adjuvant arthritis is an autoimmune disease that develops in rats after intradermal injection of type II native articular cartilage collagen. In a study[101] of collagen-induced arthritis in rats, intragastric administration of rat IFNα and β at 1,000, 5,000, or 25,000 units reduced joint inflammation scores in a dose-dependent manner if given daily for 5 consecutive days before but not after induction of arthritis via injection of type II collagen. In another study[102] of adjuvant arthritis in Lewis rats, these same doses of rat IFNα and β administered intragastrically suppressed mean joint scores in a dose-dependent manner if given before but not after the injections to induce arthritis. Furthermore, the mean joint scores in Lewis rats with adjuvant arthritis were significantly reduced by intragastric administration of collagen or 5,000 units of rat IFNα and β before immunization, compared with placebo-treated rats; fed together, the collagen and IFNα were more effective than either alone.[103]

Overall assessment of oromucosal administration of IFNs in laboratory animals—Oral administrations of IFNs of murine, ovine, or human origin have been shown to have beneficial systemic effects in rodents with various infectious, autoimmune, or experimentally induced neoplastic diseases. Available data strongly suggest that these beneficial effects associated with oromucosal administration of IFN are not mediated directly by absorption of IFN into the circulation but rather via interactions between IFN and mucosal lymphoid tissues that are local to the site of administration; through these interactions, the effects of IFN are relayed from the oral or pharyngeal mucosa to systemic sites of action. In studies[68,73,b–d] of oromucosal administration of IFN, a consistent finding is induction of 1 or more indices of IFN-mediated cellular activation such as 2'5'AS in these sites. Thus, a cascade mechanism is initiated wherein the effects of small doses of IFN administered to sites with preexistent receptors for these molecules are amplified into systemic effects over time. Indeed, as little as 1 unit of MuIFNα and β placed in the oral cavity alters numbers of splenic WBCs in mice.[83]

Importantly, adverse consequences of oral administration of IFNs, such as systemic toxicoses or enhancement of disease, have not been documented; it appears that use of this route of administration for this cytokine family is accompanied by the appropriate cellular and molecular regulatory processes, such that the net response is physiologically beneficial and not pharmacologically harmful. It is this latter feature that is most attractive for clinical applications and has prompted investigation of the use of IFNs in both domestic animal species and humans.

Domestic Animal Species

Cats and dogs—Clinical signs resolved in ill cats with naturally occurring feline leukemia after they were given bovine natural fibroblast IFN

(7,000 units).[104,105] Compared with cats that did not receive treatment with IFN, cats given HuIFNα (0.5 U/d)[106] oromucosally had improved survival after subsequent challenge with FeLV. However, findings in at least 2 reports[107,108] indicate that oral administration of IFN was not effective in modulating established FeLV disease.

In dogs with keratoconjunctivitis sicca, oromucosal administration of HuIFNα (< 250 U/d) improved tear production[109] and may provide a therapeutic alternative to surgical correction or the use of artificial tears to treat this common ophthalmic condition in dogs. In a blinded placebo-controlled study, 5 dogs with idiopathic recurrent superficial pyoderma given recombinant HuIFNα (1,000 units) orally once daily for 18 weeks generally responded better than 6 placebo-treated dogs, as measured by mean clinical scores and a decreased requirement for antimicrobials.[110] Clinical improvement in 2 dogs with pigmented epidermal plaques coincided with treatment of concurrent hypoglobulinemia and oral administration of HuIFNα (1,000 units) once daily for 21 days followed by no treatment for 7 days and then treatment with HuIFNα for another 21 days.[111]

Horses—Oromucosal administration of 50 and 150 units of HuIFNα (but not 450 units or placebo) daily for 5 days relieved clinical signs (determined by endoscopic examination) of inflammatory airway disease and reduced the cell count collected by the bronchioalvealar lavage in Standardbred racehorses.[112] The significant benefit in horses given 50 units of HuIFNα was noted 3 and 10 days after HuIFNα treatment ceased. It appears that IFN administered oromucosally is useful in restoring the appropriate cellular and tissue control mechanisms in horses with inflammatory airway disease. In another blinded placebo-controlled study, 34 Standardbred racehorses with inflammatory airway disease were given natural HuIFNα (50 units), recombinant HuIFNα (90 units), or placebo once daily for 5 consecutive days. Significantly fewer horses given HuIFNα relapsed within 2 weeks of treatment, compared with control horses. Seventeen of 22 horses given HuIFNα were cough free 4 weeks after treatment, compared with only 4 of 12 horses given placebo.[113] However, no apparent benefit was obtained when IFN was used to treat horses with respiratory disease associated with equine herpesvirus infection.[114]

Swine—Oromucosal administration of HuIFNα (5.0 U/d) or HuIFNα added to milk (1,670 U/L) and fed to pigs resulted in significant weight gain in pigs challenged with or naturally exposed to porcine enteric rotavirus, compared with placebo-treated control pigs.[115] In an outbreak of transmissible gastroenteritis virus disease in piglets, those piglets ≥ 1 day old that received 1 to 20 units of HuIFNα via the oromucosal route had a significantly greater survival rate than did placebo-treated piglets.[116]

Cattle—Feedlot calves with either naturally acquired or experimentally induced upper respiratory tract disease (shipping fever pneumonia) that were administered HuIFNα oromucosally at a dose of approximately 1 U/kg had a significantly greater survival rate[117] and greater weight gain[118,119] than did placebo-treated affected cattle. Oromucosal administration of HuIFNα (1 U/kg) has been shown to control experimentally induced infection with *Theileria parva* in cattle.[120] Veal calves fed HuIFNα (500 U/d) in milk replacer had a significantly shorter duration and decreased incidence of diarrhea and significantly lower incidence of bacterial otitis media, compared with placebo-treated calves.[121]

Chickens—In 1 study,[122] broiler chickens were given HuIFNα in their drinking water (0.01 to 1.0 U/mL) for 21 days; results indicated that the lowest dose of HuIFNα significantly improved the feed-to-gain ratio in heat-stressed birds. In chickens, recombinant **chicken (Ch)IFNα** administered for 11 days in drinking water (10 to 1,000 U/mL) exerted an antiviral effect against Newcastle disease virus.[123] Intragastric administration of recombinant ChIFNα (10^3 units) or natural spleen cell-derived ChIFNγ (200 units) protected chicks against a challenge by infectious bronchitis virus; doses of 100 or 10^4 units of ChIFNα were less effective than 1,000 units.[124] In chickens given ChIFNα in drinking water (2,000 U/mL), the replication of Marek's disease herpesvirus was significantly decreased.[125]

Overall assessment of oral, oromucosal, and intragastric administration of IFNs in domestic animals—Numerous animal studies have investigated the use of IFNα, β, γ, or τ to achieve beneficial effects in the treatment of various disease conditions. Importantly, in many of these studies, an increased dose of IFNα or β did not increase the beneficial effects of the IFNs administered.[70,71,81,83,90,99,100,106,109,112,116,118] These data indicate that, in general, low doses (1 to 5 U/kg) of IFN generate a greater clinical benefit than do high doses (> 10 U/kg). Further, it appears that the dose must be adjusted with each specific disease so that the maximal clinical benefit can be achieved.

Finally, it appears that in most instances, maximum benefits are attained if IFN is given prior to the onset of disease. This is particularly true for infectious diseases. For autoimmune disorders, cessation or downregulation of the ongoing inflammatory response is achieved via oral administration of IFN; the degree of clinical improvement appears to be closely linked to the amount of tissue damage present in the tissue or organ prior to the start of IFN treatment. For example, the degree of restoration of lacrimal function in dogs with keratoconjunctivitis sicca or in humans affected with Sjögren's syndrome after IFN treatment is probably a function of the amount of lacrimal tissue that remains after the inflammatory response has been ablated by the action of IFN. Nevertheless, oromucosal administration of IFN is not of benefit in the treatment of all disorders in animals. Depending on the dose of IFNα or β, the target disease, and the target species, oromucosal administration of IFNα and β is reported to be ineffective in cattle with protozoan infections,[126] horses with equine herpesvirus infection,[114] or cats with active feline leukemia-related disease.[107,108]

Humans

Physiologic, immunologic, and pharmacologic aspects of oromucosal or intragastric administration of IFN in humans—In general, parenteral administration of IFN, although highly toxic, has beneficial effects in humans.[18–26] In this regard, it is possible that IFNα administered parenterally has its beneficial effect in part because some of the injected IFNα tracks back into the oropharyngeal cavity and activates local mucosal IFNα receptors. In support of this, Diez et al[127] reported that in humans given IFNα radiolabeled with iodine 123 IV, IFNα was detected in the saliva, oral cavity, nose, and paranasal sinuses; this may represent mucosal binding in these regions analogous to that described in mice.[80]

Following oromucosal administration, HuIFNα upregulates expression of aquaporin-5 in human parotid glands in vitro[128] and stimulates IFN-stimulated gene-15 transcription and production[129] and HLA-DR expression in human buccal epithelial cells.[130] Because IFN-stimulated gene-15 is known to induce IFNγ, IFNα administered oromucosally may result in enhanced IFNγ production and increased natural killer cell activity.[129] Both inhibition and promotion of IFNγ activity by IFNα and β have been detected, depending on the experimental circumstances.[131]

Twenty human volunteers were given placebo or HuIFNα orally at doses of 10^3, 10^5, or 10^7 units once daily for 7 days; the 1-mL doses of placebo or HuIFNα were held in the mouth for 3 minutes before swallowing. Changes in lymphocyte counts, plasma β-2 microglobulin concentrations, and natural killer cell activity led the investigators to conclude that the lower doses of HuIFNα were immunostimulating and the higher doses were immunosuppressive, compared with findings in the individuals receiving placebo.[n]

In another study,[132] 20 human volunteers were given placebo or HuIFNα orally (150 or 450 units) 2 or 3 times daily for 1 or 5 days; doses of placebo or HuIFNα solutions were held in the mouth for 2 minutes before swallowing. Individuals given HuIFNα but not placebo had increases in percentages or absolute values of CD3+, CD4+, CD8+, CD25+, or DR+ lymphocytes after treatment, compared with findings in the control group.

Oromucosal administration of IFN in humans with infectious diseases, autoimmune diseases, cancer, and diseases of unknown origin—Despite the results of some studies[133–141,o,p] to the contrary, most data indicate that oromucosal administration of IFN is safe or beneficial in the treatment of human diseases caused by viruses,[142–159,q–aa] cancer,[160,161,bb] or autoimmunity[162–167,cc,dd] and those of unknown etiology.[168–171,ee]

Oral Administration of IFN as a Treatment Modality

Many reports have indicated that oromucosal or intragastric administration of IFN can induce systemic beneficial effects in animals and humans. It is also evident that additional research is needed to more clearly delineate the sites and mechanisms of action of IFN after oromucosal or intragastric administration, determine optimal doses and dosing schedules, and identify disease indications and circumstances in which beneficial effects can be most reliably achieved.

At present, the best available data suggest that beneficial effects of orally administered IFNα are mediated by local interactions between the administered IFN and certain populations of regulatory cells present in the oropharyngeal mucosa. This interferon-cellular interaction is translated into systemic effects by amplification phenomena secondary to this interaction. Within the oral mucosa, a common intracellular event appears to be induction of 2'5'AS enzyme activity[68,70–72] and upregulation of MHC class I proteins[68] on cells exposed to IFN. Finally, it must be emphasized again that all available data indicate that the oromucosal route of administration has notable systemic activity without the troublesome and serious adverse effects of high-dose parenteral treatment.

An emerging concept is that the beneficial effects of oral administration of IFN are also critically dependent on the timing of administration with regard to the stage of the immune or inflammatory stimulus. In general, IFN given to humans and animals prior to their encounter with immunogen suppresses immunoglobulin production and class switching by B cells. This is particularly striking in several animal species used as models of asthma,[69,70,112,113] wherein IFN pretreatment suppresses the IgE allergen response and inhibits systemic and local eosinophilia characteristic of allergic disease. Similar seemingly protective effects are detected when IFN is administered to experimental animals prior to challenge with infectious, particularly viral, organisms. It is not known if this protective effect is mediated by IFN-enhanced immune responses or by other cytokine-mediated mechanisms.

In contrast, when IFN is administered during ongoing autoimmune and inflammatory diseases of uncertain etiology, IFN-mediated induction of immune suppressor effects are observed in which suppressor T cells are induced and the activity of cytotoxic T cells and the cytokine products of cytotoxic T cells (eg, IFNγ) are reduced. The net effect of this action is to dampen harmful and progressive inflammatory disease and thus reestablish tissue equilibrium in the affected hosts. This effect is particularly striking in the suppression of relapsing EAE in various animal species,[89–94,96,h–l] sialoadenitis and lacrimitis characteristic of Sjögren's syndrome in humans,[162–164] and keratoconjunctivitis sicca in dogs.[109] These data suggest that for immune-mediated diseases, the progression of clinical disease can be downregulated by oral administration of IFNα.

The antiviral effects of orally administered IFN are also striking and have been demonstrated for both DNA and RNA viruses and in both naturally acquired and experimentally induced diseases.[78-82,84,104–106,115–119,123–125,142–151,154-159,e,f,q–s,va,aa] It is not known whether the administered IFN exerts its effects directly on virus-infected cells or indirectly via interactions with the immune system.

Parenteral administration of IFNα is approved by the FDA for treatment of humans with hepatitis B, hepatitis C, genital warts, and various cancers[19,22–24,26]; IFNβ is FDA-approved for treatment of humans with multiple sclerosis,[20,21,25] and IFNα is approved for treat-

ment of chronic granulomatous disease.[18] The recommended parenteral dose of IFNα for these conditions is typically 3 million units. Adverse physiologic and psychologic events, including suicidal behavior, in patients receiving IFNs are a major impediment to widespread acceptance of this treatment by both patients and physicians. In contrast, the lozenge dose of IFNα in clinical trials for Sjögren's syndrome is 150 units every 8 hours, which is approximately 6,700 times less than the amount of IFNα contained in a parenteral injection dose. In contrast to the experiences with IFNs administered parenterally, oromucosal administration of IFNα in humans has the distinct advantages that it is not associated with toxic effects and is easy to perform. Despite the adverse effects, parenteral administration of cytokines is regarded as a viable therapeutic option for selected human diseases. We welcome and encourage clinical testing of the administration of low doses of IFNs and other cytokines to define the efficacy and safety of these materials for use in treatment of human and animal clinical disease.

The purpose of this article was to review the veterinary and human medical literature on the benefits and uses of the oral route of administration of IFNs in humans and animals. It is hoped that through this endeavor, practitioners will gain a better understanding of the challenges and benefits of use of this interesting and important class of signaling molecules in clinical medicine. Regulatory approval was granted in July 2004 by the Ministry of Agriculture, Forestries and Fisheries of Japan for low-dose HuIFNα for the oromucosal treatment of rotavirus diarrhea in calves < 30 days old. The approval dose of HuIFNα is 0.5 U/kg of body weight, once daily, for 5 consecutive days. The product was launched in August of 2004 to veterinarians and livestock owners in Japan.[ff] In the future, it appears likely that IFN will be made available to veterinarians worldwide in a form and formulation uniquely adapted to their patients.

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