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REVIEW

Autoimmunity Is a Type I Interferon-Deficiency Syndrome Corrected by Ingested Type I IFN via the GALT System

STALEY A. BROD

ABSTRACT

Type I interferons (IFN-α/β), products of the innate immune system, can modulate immune function whereas proinflammatory IFN-γ (type II IFN), a product of the acquired immune system upregulates inflammation and enhances cell mediated immunity. We have proposed a unifying hypothesis of the origin of autoimmunity as a type I IFN immunodeficiency syndrome involving inadequate regulation of the acquired immune system product IFN-γ by the IFN-α/β innate immune system. The common theme of ingested type I IFNs in autoimmunity is inhibition of proinflammatory type II IFN systemically or at the target organ. In multiple sclerosis (MS) and insulin-dependent diabetes mellitus (IDDM) at the target organ, and in rheumatoid arthritis (RA) as a regulator of other proinflammatory cytokines, IFN-γ is the nexus of inflammation in autoimmunity. Ingested type I IFNs counteract type II IFN, overcome the relative lack of type I IFN activity, and ameliorate autoimmunity. The administration of type I IFNs (IFN-α/β) via the gut offers an exciting alternative to systemic application for overcoming the type I IFN immunodeficiency in autoimmunity. Successful use of ingested type I IFN in three separate prototypical autoimmune diseases suggests a broad antiinflammatory therapeutic profile for this technology.

THE HYPOTHESIS—THE PROTOTYPICAL AUTOIMMUNE DISEASES

ALL INTERFERONS (IFNs) display antitumor, antiviral, and antiproliferative effects. However, their immunomodulatory properties differ significantly. Type I IFNs are composed of two highly homologous protein families, the IFN-α (leukocyte IFN) and IFN-β (fibroblast IFN), whose members have similar biological properties.[1,2] IFN-α and IFN-β interact with the same cell receptor.[3,4] Type I IFNs can modulate immune function of T cells in humans when given parenterally and can increase class I major histocompatibility complex (MHC) expression and induce suppressive effects *in vitro*. In contrast, proinflammatory IFN-γ (type II IFN) upregulates the delayed-type hypersensitivity (DTH) response and inflammation, and enhances cell-mediated immune mechanisms by inhibiting immunomodulatory cell function or by promoting class II MHC expression on various cell types.[5]

We have proposed a unifying hypothesis of the origin of multiple sclerosis (MS) and other autoimmune diseases as type I IFN immunodeficiency syndrome(s).[6] The balance and mutual regulation of the innate immune system products IFN-α/β and the acquired immune system product IFN-γ action on each other are critical and may provide a key to the underlying immune dysregulation of MS and other autoimmune disease. In the absense of autoimmunity, the products of the innate immune system, IFN-α/β, can modulate the proinflammatory effect of IFN-γ of the acquired immune system, resulting in a physiological downmodulation of the total immune response. In autoimmunity, there may be a deficiency of immunomodulation on the acquired immune system by the innate immune system.

Three major disease, MS, insulin-dependent diabetes mellitus (IDDM), and rheumatoid arthritis (RA), are all thought to be autoimmune diseases characterized by T lymphocyte DTH responses, differing in their target organs—the brain, β-islet cell, and synovium, respectively. Because the pathogenic antigen is unknown in these autoimmune diseases,[7] and assuming our hypothesis is correct, supplementation with type I IFN may be a therapeutic option.

MS is a chronic demyelinating disease of the central nervous

Multiple Sclerosis Research Group, Department of Neurology, Graduate School of Biomedical Sciences in Immunology, University of Texas Health Science Center at Houston, Houston, TX 77030.

system (CNS),[8] associated with periods of disability (relapse) alternating with periods of recovery (remission) but often leading to progressive neurological disability[9] and associated with abnormalities of immunoregulation.[10] CNS white matter lesions are associated with mononuclear infiltrates that consist primarily of T cells and macrophages.[9–11] We have directly cloned T-helper I (Th1) T cells, which expressed interleukin-2 (IL-2) and IFN-γ but not IL-4 or IL-5 mRNA, from both peripheral blood and cerebrospinal fluid (CSF) of subjects with MS.[12]

IDDM is a disorder that results from presumed autoimmune destruction of the insulin-producing pancreatic islets by inflammatory cells. Many key features of human IDDM are reflected in the nonobese diabetic (NOD) mouse model, such as the development of insulinitis with infiltration of lymphocytes cytotoxic to the insulin-producing β cells and the dependence of disease pathogenesis by T cells.[13–17] Destruction of islet cells and islet graft rejection in NOD mice may be mediated by the Th1 subset of T cells producing IFN-γ[18,19] or by cells infiltrating the islets, producting proinflammatory monokines such as IL-1, IL-6, and tumor necrosis factor (TNF), causing initial tissue destruction.[20] Survival of grafts correlates with activation of Th2 subset of T cells producing IL-4 and IL-10, thereby downregulating the intraislet IFN-γ-mediated autoimmune response.[19,21,22]

RA is a common chronic autoimmune disorder involving lymphocyte-mediated tissue inflammation in synovial joints. Dexamethasone and gold treatment act in part by markedly downregulating spontaneous and/or cytokine stimulated production of IL-1β by peripheral blood mononuclear cells (PMNC).[23] IL-6 is a proinflammatory cytokine found in RA sera.[24–30] Methotrexate (MTX), dexamethasone, and gold improve RA in part by inhibiting both IL-6 and IL-8.[31,32] IL-8 recruits inflammatory cells into the joint,[33,34] is the major T cell chemoattractant in RA synovium,[35] and is secreted by T cells,[36] playing a key role in amplifying and perpetuating inflammation. Clinical trials using a chimeric anti-TNF-α antibody have shown marked clinical benefit, verifying the hypothesis that TNF-α is of primary importance in RA.[37,38] IFN-γ regulates TNF-α activity.[39,40] Therefore, we hypothesize that the primary role of type I IFNs in RA is the regulation of IFN-γ as the primary proinflammatory cytokine.

There is a Th1 cytokine profile in MS that may be susceptible to inhibition. The monokines IL-1, IL-6, and TNF may play a vital role in the initiation of inflammation in IDDM, followed by traditional Th1 cells as effector cells. Inhibiting the action of proinflammatory cytokines by using specific cytokine inhibitors or antiinflammatory cytokines is a rational basis for new therapies in autoimmune diseases.[41] All of these data suggest that cellular inflammation is a common underlying thread in these three prototypical autoimmune diseases. We have directed our experimental efforts toward demonstrating that type I IFNs are immunomodulatory and antiinflammatory in autoimmune disease in both animals models and in human autoimmune diseases.

But why ingested type I IFNs? The administration of cytokines via the gut offers an exciting alternative to systemic application for ease of dispensation in clinical and field use,[42] and patient convenience[43] and is a great step forward because gut delivery is easy, well tolerated, and inexpensive and the therapeutic index of gut IFN would seem excellent.[44] Because oral use of type I IFN triggers a cascade of events leading to activation of the immune system with little or no presence of IFN in body fluids, *oral administration could be the ideal route for the treatment.*[45] In comparison, the use of parenterally administered type I IFNs in early-relapsing, remitting MS is limited by clinical and chemical toxicities, including the generation of IL-6, a potential polyclonal B cell activator.[46,47] Furthermore, antibodies that abrogate IFN activity develop in a proportion of IFN-β-treated patients, correlating with the loss of clinical benefit.[46]

Type I IFNs are acid stable and therefore can resist preprandial stomach acidity. Fifty to two hundred high-affinity type I receptors are found on all lymphoid cells, including the gut associated lymphoid tissue (GALT).[48–50] IFNs have antiviral activity when administered orally. Stanton found that low doses of recombinant human IFN-α, or mouse IFN-α/β given orally in drinking water, protected mice from encephalitis and death following injection of Semliki forest virus. Importantly, this response was biphasic: higher levels of oral IFN were not protective, nor were high or low intraperitoneal (i.p.) doses protective.[51] Fleischmann and co-workers also demonstrated that IFNs administered by the oral route in drinking water showed a systemic effect.[52] Oral use of IFN-α/β caused neutropenia in mice without detectable levels of IFN in the blood. Circulating specific antibody to IFN blocked the neutropenic effects of i.p. IFN-β, but did not block the neutropenic effects of the orally administered IFNs. The neutropenic effect of oral use of IFN-α was transferred by injection of blood cells from donor mice treated orally to recipient mice, but not by plasma. Orally delivered IFNs exert a neutropenic effect differently from i.p. IFNS, probably involving cell-to-cell transfer of the IFN effects,[53] rather than the systemic distribution of the IFNs in the blood.[52] A question remains whether an immune response can be suppressed by ingested type I IFNs.

INGESTED TYPE I IFNs IN AUTOIMMUNE DISEASES EAE (EAN) AND MS

Is type I IFN immunoactive by ingestion?

First we examined whether ingestion of biological response modifiers (BRM) such as type I IFNs would inhibit the clinical expression of disease, decrease inflammation, and inhibit IFN-γ secretion, a Th1 cytokine, in a model of acute autoimmune disease.[54] Acute experimental autoimmune encephalomyelitis (EAE), a T cell-mediated autoimmune disease that resembles the human disease MS, provides a model to assess the ability of ingested immunoactive substances to influence the course of an experimental auto-immune disease. Lewis rats were inoculated with guinea pig myelin basic protein (GP-MBP) and complete Freund's adjuvant (CFA). Seven days preceding immunization and for 21 days thereafter, rats were fed (IFN was placed in the posterior oropharynx) either rat type I IFN-α/β (rat IFN) or mock IFN daily. There was a significant decrease in the clinical score and inflammatory foci in 5,000 units of rat IFN fed to rats compared to mock fed or rats treated with 5,000 units subcutaneously (s.c.). Ingested type I IFN inhibited mitogen proliferation in draining popliteal lymph nodes

from fed rats, the natural draining areas for s.c. administered antigens and therefore presumably the reservoir of high frequencies of sensitized MBP-specific T cells. We examined whether *in vitro* type I IFN treatment of draining popliteal lympho nodes and spleen cells from immunized but mock-treated animals would demonstrate similar effects to *in vivo* treatment. In contrast, there was no clear effect on concanavalin A (ConA) proliferation in draining popliteal lymph nodes or spleen cells exposed to IFN (1–100 units) *in vitro*, in contrast to *in vivo* IFN-α/β oral administration. These experiments demonstrate that acute EAE is more effectively inhibited by equivalent amounts of ingested in contrast to parenterally administered IFN-α. Type I IFNs are active by ingestion and have significant clinical and histological effects in acute autoimmune disease.

Is type I IFN immunoactive by ingestion in inflammatory experimental allergic neuritis?

IFN-β can be used to treat Guillain-Barre syndrome successfully.[55] We investigated the effect of ingested type I IFN in experimental allergic neuritis (EAN) in Lewis rats immunized with bovine peripheral nerve myelin. Starting at 7 days preceding immunization, rats were fed daily until sacrifice either with 5,000 units of rat IFN-α/β or mock IFN. The clinical severity of EAN was reduced significantly in IFN-α/β-fed rats. Demyelination, but not inflammation, was decreased in IFN-α/β-fed rats at day 20 after immunization. *In situ* IFN-γ production and inflammation were reduced when evaluated by immunocytochemistry at day 13 after immunization. Spleen cells from IFN-α/β-fed EAN rats showed significantly reduced proliferation to stimulation with ConA or peripheral nerve myelin. IFN-γ production in draining lymph node cells was significantly reduced after stimulation with bovine peripheral nerve myelin. Ingested IFN-α/β reduces the severity of EAN by a reduction in IFN-γ production. [56]

Can ingested type I IFNs suppress an ongoing immune response?

A question remained whether an established ongoing immune response can be suppressed by ingested type I IFNs. CR-EAE is a chronic inflammatory autoimmune process of the CNS that more closely resembles the human disease MS than does acute monophasic EAE because animals cycle through multiple relapses.[57–59] Therefore, we examined whether ingestion of the BRM type I IFN could suppress the clinical expression of relapses, decreased DTH response, and *in vitro* proliferation to MBP in murine CR-EAE. After inoculation and following recovery from the first attack, SJL/J mice were fed varying doses of natural human IFN-α (nHuIFN-α), natural murine IFN-α/β (nMuIFN-α/β), or mock IFN three times per week for 6 weeks. The type I IFN was directly delivered to the distal esophagus, stomach, and proximal small intestine using a syringe fitted with a 20-gauge ball point needle as determined experimentally by injecting Evans blue during routine feeding and subsequent sacrifice. Using nHuIFN-α, the DTH response was significantly decreased in the IFN group compared to the mock controlled group (previously unpublished). Ingested MuIFN-α/β suppressed clinical relapse disease, proliferation to GP-MBP, and *Mycobacterium tuberculosis* (MT) in draining lymph nodes and diminished inflammation in the CNS. Ingested IFN-α/β altered the cytokine profile of ConA-activated spleen cells by decreasing IL-2 and IFN-γ. These results suggest that type I IFNs are active by ingestion, have significant clinical and immunomodulatory effects, and can decrease an established and ongoing immune response to sensitized antigens. Ingested type I IFNs, lacking a protective matrix to prevent protein digestion in the alimentary canal, are capable of producing immunomodulation to previously sensitized antigens.[60]

Is type I IFN the immunoactive component in IFN preparations?

A question remained whether a component other than the IFN may contribute to the immunomodulating activity of IFN preparations. Available natural murine type I IFN are manufactured by viral induction in murine fibroblasts and could contain non-IFN immunoactive proteins other than IFN that could be responsible for disease modification. Human IFNs demonstrate a wide range of antiviral activity (1–50%) in murine systems[61,62] and human recombinant IFNs do not contain extraneous immunoactive proteins. Therefore, suppression of actively induced relapses with pure human preparations would eliminate the possibility that non-IFN murine proteins modify disease, but might require higher doses relative to murine IFN to show effects. Accordingly, we examined whether ingestion of rHuIFN-α and murine species-specific IFN-α could suppress actively induced relapses.

CR-EAE was induced in SJL/J mice and following recovery from the initial attack, animals were fed varying doses of rHuIFN-α or MuIFN-α, or mock IFN three times per week. Oral administration of human or murine IFN-α suppressed relapse in actively immunized animals, decreased mitogen/antigen PLP 139-151 proliferation and IFN-γ secretion.[63] Lewis rats were inoculated with GP-MBP and CFA and fed hrIFN-α or mock IFN as described above. We utilized pure human preparations to eliminate the possibility that non-IFN murine proteins might modify disease. There was a significant decrease in the clinical score and inflammatory foci in 5,000 IU rHuIFN-α fed rats compared to mock-fed or rats treated with 5,000 units s.c. These data document that ingested murine species-specific IFN-α and rHuIFN-α can inhibit acute EAE[54] and suppress relapses of EAE.[63]

Can type I IFN ingestion in donors prevent adoptive transfer of EAE?

Modification of adoptive transfer of EAE from type I IFN-fed donor immunized mice would demonstrate that ingested type I IFN acts on potential encephalitogenic cells in the donor animal. Accordingly, we examined whether ingestion of rHuIFN-α and MuIFN-α would modify adoptive transfer of EAE by using ConA-activated spleen cells from actively immunized mice.[64–66] After relapse, in the CR-EAE mice described above ingesting either rHuIFN-α and MuIFN-α, ConA-activated spleen cells were transferred adoptively from mock-fed or IFN-fed donors into naïve recipient mice. Activated donor cells from mice ingesting IFN-α were less effective in transfering clinical disease compared to cells from mock-fed mice. In contrast, ConA-activated spleen cells from

mock-fed mice treated with rHuIFN-α *in vitro* as opposed to *in vivo* did not prevent adoptive disease transfer. Ingested IFN-α acts by modifying the encephalitogenicity of donor spleen T cells.[63]

Is ingested type I IFN more effective than parenteral type I IFN in EAE?

Studies of parenterally administered hrIFN-β in relapsing remitting MS demonstrated decreased relapses,[67] decreased activity on serial magnetic resonance imaging (MRI),[68] decreased spontaneous *in vitro* IFN-γ production,[69] and a reduction of clinical progression, relapse rate, and gadolinium-defined inflammatory activity on MRI.[70] However, their use may be limited because 40% of IFN-β_{1b}-treated patients generated neutralizing antibodies that are frequently found in those patients who appear to lose both clinical benefits and MRI-defined responses.[46] Previously, we have demonstrated that ingested type I IFNs inhibit clinical attacks at doses equivalent to ineffective parenteral doses in acute rat EAE.[54] Therefore, we examined the optimal clinical ingested dose of MuIFN-α for suppression of relapse attacks and compared it to s.c. IFN-α in a dose–response experiment in the CR-EAE model.

We first determined if 0.1, 1, 10, 100, or 1,000 units of ingested MuIFN-α would suppress relapse attacks. The optimal clinically effective dose for suppression of EAE of ingested MuIFN-α was 10 units (0.1, 1, and 1,000 units were not clinically effective) and for s.c. administration the dose was 100 units, although the optimal ingested dose was much more clinically effective than the optimal s.c. dose. ConA and MT-induced spleen cell proliferation was inhibited by ingested IFN-α, as was ConA-induced IL-2 secretion, but s.c. IFN-α did not inhibit the ConA-induced proliferation in spleen cells. Ingested IFN-α inhibited the mitogen-induced production of IL-2 and IFN-γ but s.c. IFN-α increased MT-induced IFN-γ and IL-6 secretion in spleen cells and ConA-induced IL-6 and MT-induced IL-2 and IL-6 in lymph node cells.[71] Similarly, in patients with MS, mitogen-mediated IL-6 secretion was increased after initiating IFN-β_{1b} treatment, correlating with systemic side effects.[47] These data are consistent with our previous data that demonstrated decreases in IL-2 or IFN-γ secretion (Th1-like cytokines) in IFN-α-fed animals.[54,60]

Does adoptive transfer of T cell or T cell subsets from mice ingesting type I IFN protect against active disease?

To examine whether splenocytes from IFN-fed donors were anergized or "suppressor-like" populations, donor CR-EAE SJL/J mice were immunized and fed with mock IFN-α or with IFN-α every other day for at least 4 weeks after initial clinical attack. Recipients of adoptively transferred $CD8^+$ T cells from mock-fed donors showed no clinical improvement compared to actively immunized controls. In contrast, recipients of adoptively transferred $CD8^+$ T cells from IFN-α-fed donors demonstrated decreased clinical disease compared to recipients of mock-fed $CD8^+$ T cells.[72] To examine the mechanism of protection by donor $CD8^+$ T cells and to determine if ingested IFN-α activates natural immunomodulatory cell populations, we used the acute EAE model and naive-fed donor animals as sources of T and $CD8^+$ T cells. ConA-activated donor spleen T and $CD8^+$ T cells from naive nonimmunized MuIFN-α-fed donors inhibited actively induced disease in recipients and demonstrated decreased IFN-γ and TNF-α proinflammatory secretion, important in the induction and pathogenesis of EAE.[72–74] Type I IFNs can decrease TNF-α secretion[45,75,76] and increase antiinflammatory TNFsRp55 levels levels.[77] This result suggests, whatever the underlying immunomodulatory mechanism of T cell populations from IFN-fed donors, that the inhibition of proinflammatory cytokine secretion, in particular TNF-α, in recipients is paramount.

Is ingested IFN-α a biological response modifier in the prototypical human autoimmune disease MS?

The next question was whether ingested IFN-α is a BRM in autoimmune disease in humans. rHuIFN-α (Roferon, Roche Pharmaceuticals, Nutley, NJ), diluted in 5 ml of saline solution, was aliquoted and stored at $-70°C$. Estimated dose equivalency of ingested IFN-α in humans was based by a direct extrapolation on a unit/kg basis of the most effective dose of MuIFN-α in murine EAE[71] of 10 units/20 mg mouse ($\approx$30,000 units/60 kg human). Each dose was thawed, placed in the mouth, and immediately swallowed with at least 150 ml of water. Ten subjects with RRMs were administered rHuIFN-α for 2 weeks at each dose with at least 2 weeks washout without rHuIFN-α between the first dosing cycle (10,000 units), the second dosing cycle (30,000 units), and the third dosing cycle (100,000 units). Ingested rHuIFN-α showed no toxicity in normal volunteers or patients with relapsing-remitting MS (RRMS) at doses ranging from 300 to 100,000 units. We did not detect IFN-α serum levels 1 h after ingestion. In subjects with RRMS, a significant decrease in ConA-mediated proliferation and serum-soluble intercellular adhesion molecule-1 (sICAM-1), a surrogate measure for disease activity in MS, was found after ingesting 10,000 and 30,000 units rHuIFN-α. The RRMS subjects also showed decreased IL-2 secretion after ingesting 10,000 units of IFN-α, and decreased IFN-γ, TGF-β, and IL-10 production after ingesting 30,000 units of IFN-α. The decreased secretion of IFN-γ and IL-2 suggests that ingested IFN-α may cause a functional inhibition of Th1-like T helper cells in RRMS, a potential site of intervention at the level of effector T cells in MS, supporting the use of ingested IFN-α as a BRM in humans.[78] Currently, we are running a phase II parallel-designed, randomized, placebo-controlled clinical study assessing the biological activity of ingested IFN-α on the frequency of active gadolinium-enhancing lesions as a marker for paraclinical efficacy.[79]

The NOD mouse and IDDM

Can ingested type I IFNs inhibit insulinitis and diabetes in the NOD mouse? The NOD mouse is a model of IDDM.[80–82] Because ingested type I IFNs are BRMs in EAE and MS, and ingested type I IFN suppresses Th1 T helper cell and other proinflammatory cytokines in EAE (IL-1, IL-6, TNF-α), we determined whether ingested MuIFN-α administered to NOD mice inhibits insulinitis and suppresses IDDM. Ten units of ingested MuIFN-α, administered daily directly into the stomach, decreased islet inflammation and suppressed diabetes. Ingestion of MuIFN-α increased the mitogen-induced production of IL-4, IL-10, and IFN-γ secretion in spleen cells from treated mice.[83] Th2-like cytokines can protect against the develop-

ment of IDDM in the NOD mouse.[22,84–89] Although IFN-γ may itself by immunosuppressive under certain conditions,[90,91] the actions of intraislet IFN-γ (Th1-like) are in general diabetes promoting.[92–96] The ratio of intraislet counterregulatory antiinflammatory IL-4 or IL-10 to IFN-γ may be critically important in determining the balance between disease and protection in the NOD mouse.[87]

Can type I IFN ingestion in donors prevent spontaneous onset of DM in recipients? Adoptive transfer of unstimulated splenocytes from the mice described above that secrete IL-4 and IL-10 from MuIFN-α-fed donors suppresses spontaneous DM in recipients. The protective effect of adoptively transferred unstimulated splenocytes demonstrates the presence of ingested IFN-α-activated regulatory splenic cell populations that may work via increased IL-4 or IL-10 production.[83]

Can ingested IFN-α prolong the 'honeymoon' period in newly diagnosed IDDM? In the NOD mouse model, we treated mice before they became manifestly diabetic. Because of the difficulties in screening for high-risk IDDM patients, we decided to treat newly diagnosed IDDM patients. The natural history of IDDM after diagnosis includes a brief partial remission ('honeymoon') in most patients,[97] with mean insulin dose decreasing during the first months after diagnosis, to a nadir at 3 months, corresponding to the average 'honeymoon' period, and thereafter increasing in the vast majority of patients.[98] The incidence of spontaneous remission is reported to be 3%,[99] 5% in the French cyclosporine trial,[100,101] one in eleven in azathioprine trials,[102] and 10% in the Canadian-European cyclosporin trial.[98,103] Therefore, remission is an unlikely event, and β-cell function, as indicated by the plasma concentration of C-peptide, is ultimately lost and islet cell antibodies disappear.[104,105] Interventions prolonging the honeymoon in newly diagnosed patients, indicative of the reversal of the disease, are considered positive.[106]

We treated newly diagnosed type I diabetics with ingested 30,000 IU hrIFN-α every other day and examined baseline and Sustacal®-induced C-peptide responses at 0, 3, 6, 9, and 12 months in an open label pilot phase I study. Preliminary results demonstrate preservation of 'honeymoon' at 12 months after initiation of treatment. Ingested IFN-α may induce remission in recent onset IDDM and may prevent the onset of IDDM (submitted for publication).

Although the optimal therapeutic approach to human disease is the prediction and prevention of disease, only 10–15% of IDDM is predictable,[107] and therefore therapeutic interventions that permanently prolong the 'honeymoon' period in newly diagnosed IDDM may in themselves have an major impact on IDDM, a disease category for which there is no known safe and reliable preventive treatment.

Rheumatoid arthritis

RA is a third cellular-mediated autoimmune disease. *In vitro* or parenteral type I IFNs increase IL-1 receptor antagonist (IL-1Ra)[75,108–110] and inhibit IL-1 induction.[109–111] Therefore, we determined the safety, clinical effects on joint disease, and potential modulation of proinflammatory cytokine secretion in subjects with RA in an open-label pilot phase I study with ingested IFN-α. Patients (Table 1) with clinically stable RA had complete rheumatologic examination, routine blood tests, and PMNC mitogen-induced IL-1β, IL-6, IL-8, and TNF-α secretion before (entry) and after 8 weeks (exit) of 30,000 units of rHuIFN-α ingested QOD. Ingested rHuIFN-α was not toxic. Overall, of the 24 possible clinical and laboratory disease indices measured in 4 patients, there were 14 indices that improved by at least 20% and four that worsened by 20%, all the worsening in 1 patient (Table 2). Ingested IFN-α significantly decreased PMNC CD3-induced IL-1 and demonstrated a decreased trend in IL-6 and IL-8 secretion (Fig. 1). There were no consistent alterations of TNF-α. Early treatment of RA with ingested IFN-α is nontoxic and reduces the secretion of IL-1, a proinflammatory cytokine.[112]

Transduction of ingested type I IFN signal across the gastrointestinal wall

Systemic effects may be achieved with IFN-α administered directly to the upper gastrointestinal (GI) tract in experimental animal models of autoimmune disease and human autoimmune disease. However, oral administration of IFN-α in mice (>5,000 units/mouse),[53] rabbits (6×10^6 units),[113] dogs (3×10^6 units/kg),[114] monkeys (6×10^6 units/kg),[115] or humans ($1{,}350 \times 10^6$ units)[43] does not result in detectable levels of IFN-α in the blood. Up to 48 h after 10^9 units of oral IFN-β was administered, serum IFN, β_2-microglobulin, neopterin, or 2-5A synthetase were not increased compared to pretreatment levels.[43] But how does ingested type I IFN transduce immunomodulatory signals across the gut?

Mx is a type 1 IFN-specific induced mRNA/protein, thus providing a marker indicating type I IFN/type I IFN receptor interaction.[50] Induction of Mx mRNA is found in the absence of detectable serum IFN activity and demonstrates that MxA gene expression is a good marker for detecting minute quantities of biologically active type I IFN.[116] Ingested type I IFNs must act through type I IFN receptor to transduce signals to im-

TABLE 1. PATIENT DEMOGRAPHICS

Patient	*Age*	*Sex*	*Years RA*	*Present Rx*	*Past Rx*
C.W.	67	Female	7	Prednisone (10 mg/d), Tolectin (400 qd)	SSZ, MTX, AZA, minocycline
J.N.	55	Female	7	Aspiron 3.9 (g/d)	SSZ, HQ, oral gold, MTX
A.S.	36	Female		Prednisone (10 mg/d), ibuprofen (2,400 qd)	
P.L.	49	Female	13	Prednisone (7.5 mg/d), ibuprofen (800 tid)	MTX, DMSO, oral gold

TABLE 2. CLINICAL AND LABORATORY EVALUATION

Patient	*Study point*	*ESR*	*Patient global assess*	*Physician global assess*	*Painful joints*	*Swollen joints*	*Morning stiffness*
C.W.	Entry	21	23	24	17	22	45
	Exit	55+	72+	53+	7	28+	30*
J.N.	Entry	57	25	28	5	17	15
	Exit	61	16*	6*	0*	14	2*
A.S.	Entry	49	ND	25	5	11	ND
	Exit	29*	ND	6*	4*	8*	ND
P.L.	Entry	61	28	34	27	30	60
	Exit	48*	28	22*	13*	13*	60

Examination at entry into the trial and exit from the trial was performed that included standard painful (PJ) and swollen joint (SJ) counts, both patient and physician global assessment of disease activity, duration of morning stiffness (MS), and erythrocyte sedimentation rate (ESR) (Westergren method). A 20% decrease in scores from entry to exit is designated by asterisk (*) and 20% increase in scores is designated by cross (+) from entry to exit.

munomodulatory cells.[3] We examined the relative levels of the Mx mRNA signal using semiquantitative reverse transcription (RT)-PCR on splenocytes from mice and PMNC from humans after IFN-α ingestion. Both mice and humans demonstrated inducible levels of Mx mRNA after ingesting IFN-α. Murine spleen T cells and $CD8^+$ T cells also demonstrated upregulation of Mx mRNA. Murine whole splenocytes demonstrated upregulation of Mx mRNA after IFN-α ingestion of 10 and 100 units, clinically effective doses, but not after 0, 1,000, or 5,000 units, clinically ineffective doses.[71] Ingested IFN-α acts via established pathways of type I IFN signaling.[117]

The above data demonstrate molecular evidence of type I IFN/type IFN receptor interaction after ingestion of IFN-α, an intrinsically immunoactive protein in humans and mouse. IFN-α was administered directly to the stomach and small bowel in mice or immediately swallowed with at least 5 ounces of water in humans to deliver the IFN to the small bowel. These maneuvers bypass the oral cavity and deliver the IFN directly to a dense concentration of lymphocytes in the upper small bowel. T cells and the $CD8^+$ T cell subsets demonstrate increased Mx expression, both of which can transfer protection against EAE from naive donor mice ingesting MuIFN-α.[72]

Ingestion of an immunoactive protein utilizes the largest regional immune system in the body (GALT). The GALT has multiple types of constituent immune cells. It consists of lymphoid nodules termed Peyer's patches (PP).[118] PP contain T and B lymphocytes, macrophages, dendritic cells, and a germinal center with B lymphocytes. T lymphocytes in PP are predominantly composed of the $CD4^+$ Th1 and Th2 phenotypes, whereas parafollicular cells are both $CD4^+$ and $CD8^+$.[119,120] PP are the site where regulatory cells can be generated.[121–123] Therefore, there are diverse cell populations in the GALT that may potentially become immunoregulatory via ingested type I IFN and suppress proinflammatory cytokines.

These findings strongly suggest that the biologic effect does not require transit of intact IFN across the bowel. The absence of increases in biological protein markers (β_2-microglobulin, neopterin or 2,5-oligoadenylate synthetase) after oral administration,[43] but their presence with subcutaneous or intravenous IFN-β[114,115,124,125] and the data above all suggest that ingested IFN acts through a different mechanism. The neutropenic effect of orally administered IFN can be transferred by injection of blood cells, but not by serum, to recipient animals, demonstrating that IFN acts through cellular immune mechanisms.[53]

Activated monocytes and lymphocytes, by virtue of their ability to circulate through the body, potentially can transfer their biological activities widely in the absence of circulating cytokines after contacting IFN or IFN-induced cells in the GALT.[126–130] At their destination (lymph node?spleen? brain?pancreas?sy novium?), type I IFN-activated cells may release counterregulatory antiinflammatory immunomodulators that are able to inhibit neighboring inflammatory cells. This possibility suggests that BRMs such as ingested type I IFNs act outside the realm of classical pharmacology and may suppress inflammation by activating a unique natural immune system originating in the GALT.

What ingested type I IFN is and what it is not!

The GALT has a long and winding path, from the Ring of Waldeyer downward. There appear to be distinct portions of the GALT that may generate either immunity or immunosuppression, depending on the point of contact of type I IFN with the GALT. Ingested type I IFNs delivered to the small intestine suppress clinical relapses (suppress immunity), diminish inflammation (decrease immunity), and inhibit proliferation of activated cells, decrease (proinflammatory) IL-1, IL-2, IL-6, TNF-α, and IFN-γ secretion in EAE and MS.[60,63,71,78] In the NOD mouse model, ingested type I IFNs delivered to the small intestine can increase counterregulatory antiinflammatory IL-4 and IL-10 secretion.[83]

In contrast, interactions of type I IFN receptors with IFN-α in the oral cavity result in a generalized elevation of immunocompetence in the host, generating antiviral activity.[131–137] The oral epithelium has been touted as the primary portal of entry for antiviral defense by natural human IFN-α that specifically targets oral epithelium.[138] Recent studies demonstrate the antiviral activity of oral IFN-α against murine cytomegalovirus, vaccinia virus, and measles.[139–141] Low-dose oral delivery of HuIFN-α (150 IU) can improve salivary function in Sjögren's syndrome and decrease lymphocytic infiltration. However, this antiinflammatory effect may be due to di-

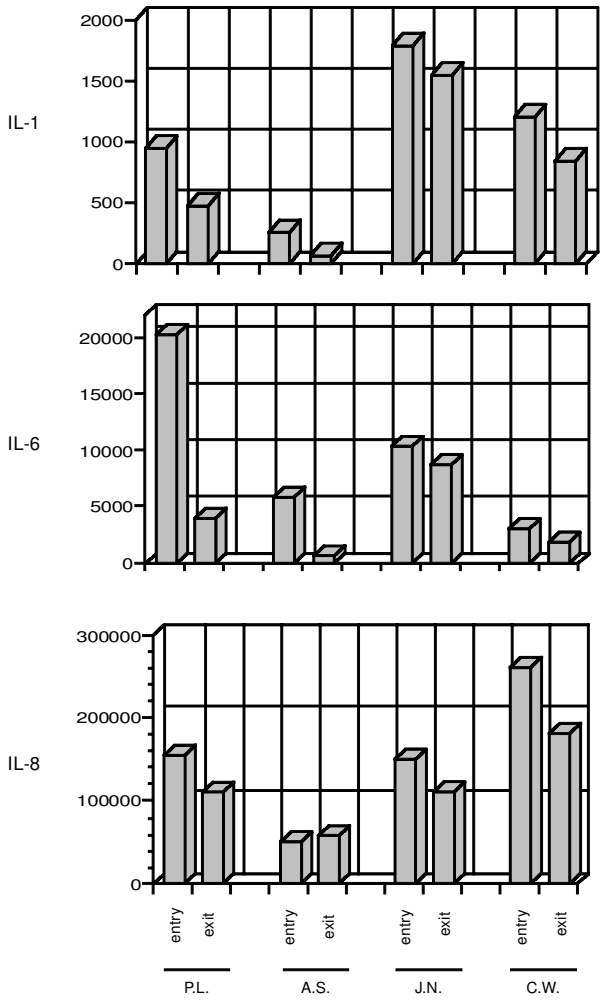

FIG. 1. Subjects with RA demonstrate decreased CD3-activated IL-1β (top) ($p < 0.03$ with Bonferroni correction) and a trend for decreased Il-6 (middle) and IL-8 (bottom) PMNC secretion after ingesting 30,000 units of rHuIFN-α. PMNC from RA subjects were taken immediately before initiation of treatment (entry) and 1 h after the last dose of ingested IFN-α after 8 weeks on treatment (exit). CD3 (black bars) are shown for all 4 patients. Results are expressed as pg/ml with mean values expressed for entry and exit.

rect contact of the IFN on salivary glands.[142] The orally administered IFN-α probably acts locally after contact with salivary glands. There is no evidence from these experiments that the IFN-α acts systemically. Delivery directly to the stomach completely avoids involvement of any potential resistance mechanism(s) that would be activated by IFNs in the oral epithelium. Ingestion or delivery directly to the stomach is not 'oral' administration, but rather bypasses the oral cavity. The immunosuppressive effects of ingested type I IFNs demonstrated in animal models of autoimmune diseases, and human autoimmune diseases MS and IDDM is in contrast with the antiviral immunity generated by type I IFN in the oral cavity.

Administration via nasal or deep-lung inhalation,[143] accessing readily available lymphoid tissue, such as the bronchial-associated lymphoid tissue (BALT), may also appear attractive to interventionists. Patients with advanced, previously treated non-small cell lung cancer inhaled 60×10^6 IU natural IFN-α from a dosimeter-equipped jet nebulizer, resulting in measurable levels of circulating IFN. IFN, given by inhalation, can penetrate into the bloodstream, but also causes systemic side effects such as temperature rise, headache, and malaise similar to those described after systemic IFN administration.[144] There may be other significant problems associated with inhalation delivery of immunoactive proteins, including bronchospasm and hypersensitivity pneumonitis.[144–147] Notwithstanding the side effects associated with inhalation, the high levels of absorption of protein or peptides through the deep lung into the bloodstream without the need for enhancers may be counterproductive.[143,148] Optimal *ad*sorption in the BALT might activate potential beneficial immunomodulatory lymphoid circuits. Maximal *ab*sorption via the lung would bypass the BALT, negating the potential advantage of inhaled delivery. Gut administration of type I IFNs avoids both problems of systemic side effects and local tissue hyperreactivity. Because high-affinity type I receptors are found on all lymphoid cells, the lymphocytes could act as a filter eliminating absorption and thereby avoiding the potential problems of absorption, systemic side effects, and neutralizing antibody formation. GI hyperreactivity, manifested by abdominal symptoms or diarrhea to the endogenous IFN-α protein, also does not appear to be a clinical concern.

Other type I IFNs, confined to the suborder Ruminantia,[149] such as ovine IFN-τ, have demonstrated activity in EAE,[150] and ovine IFN-τ has activity via oral feeding at high dosages (10^5 U/dose).[151] However, anti-IFN-τ antibodies blocked the effect of the orally administered IFN-τ, suggesting that IFN-τ is absorbed. Absorption of IFN-τ, probably due to low affinity of IFN-τ for type I IFN receptors on GALT lymphocytes,[152] generates the potential for systemic neutralizing antibody formation. The absence of the IFN-τ (and IFN-δ, another type I IFN in pigs)[149] gene in the human genome makes IFN-τ a transspecies protein in humans, and therefore less attractive for human autoimmune diseases.

The human genome codes for another type I IFN, IFN-omega with high antiviral, antitumor, and antiproliferation activity. Its therapeutic potential will have to await clinical trials.[153]

THE FUTURE

The ingestion of BRMs such as IFN-α potentially provides a continuous means of generating immuno regulation that is convenient and active at lower doses with minimal side effects, and may provide enhanced efficacy via unique and potent immunoregulatory circuits in the GALT. IFN-α, an endogenous protein that possesses intrinsic immunoactivity, would have easy access to lymphoid cells with receptors for type I IFN that allow activation of T and other cells with subsequent systemic migration. As we have demonstrated above, ingested type I IFNs can produce target organ-blind immunomodulation without respect to sensitized antigen. Although we have not measured any possible antibody response elicited by ingested rHuIFN-α, the oral route may circumvent potential therapeutic limitations associated with neutralizing antibodies to injected cytokines in subjects with neurologic and other autoimmune

diseases.[47,154] Ingested type I IFNs can inhibit proinflammatory Th1 cytokines and proinflammatory monokines IL-1, IL-6 and TNF-α or increase IL-4 and IL-10 antiinflammatory cytokines. Successful use of ingested type I IFN-activated immunomodulatory T cells suggest a broad antiinflammatory therapeutic profile for this technology.

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Address reprint requests to:
Dr. Staley A. Brod
Department of Neurology
University of Texas Health Science Center at Houston
6431 Fannin Street, Suite 7044
Houston, TX 77030

Tel: 713-500-7046
Fax: 713-500-7041

E-mail: sbrod@neuro.med.uth.tmc.edu

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