Short Peptide Type I Interferon Mimetics: Therapeutics for Experimental Allergic Encephalomyelitis, Melanoma, and Viral Infections

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The classical canonical model of interferon (IFN) signaling focuses solely on the activation of STAT transcription factors, which limits the model in terms of specific gene activation, associated epigenetic events, and IFN mimetic development. Accordingly, we have developed a noncanonical model of IFN signaling and report the development of short type I IFN peptide mimetic peptides based on the model. The mimetics, human IFN α 1(152–189), human IFN β (150–187), and ovine IFN τ (156–195) are derived from the C-terminus of the parent IFNs and function intracellularly based on the noncanonical model. Vaccinia virus produces a decoy IFN receptor (B18R) that inhibits type I IFN, but the IFN mimetics bypass B18R for effective antiviral activity. By contrast, both parent IFNs and mimetics inhibited vesicular stomatitis virus. The mimetics also possessed anti-tumor activity against murine melanoma B16 tumor cells in culture and in mice, including synergizing with suppressor of cytokine signaling 1 antagonist. Finally, the mimetics were potent therapeutics against experimental allergic encephalomyelitis, a mouse model of multiple sclerosis. The mimetics lack toxic side effects of the parent IFNs and, thus, are a potent therapeutic replacement of IFNs as therapeutics.

Introduction

THERE IS A REMARKABLE absence of success in the rational development of mimetics to a host of biologically important proteins, including hormones, cytokines, and growth factors. Key to the current paradigm of signaling is the interaction of ligand with receptor extracellular domain, cross-linking of the receptor chains, and initiation of allosteric changes in the receptor that are responsible for all subsequent events of ligand-specific cell signaling (Brivanlou and Darnell 2002; Gough and others 2008; O'Shea and others 2013; Ivashkiv and others 2014). Gamma interferon (IFN γ) consists of a noncovalent asymmetric dimer with 2 binding sites for cross-linking of the IFN γ receptor chains (Kotenko and others 1995). Crystallographic structure studies of IFN γ and receptor subunit IFNGR1 confirmed that cross-linking occurred at least under the conditions of crystal formation, but this has not led to IFN mimetic development (Walter and others 1995). Similar crystallographic studies of type I IFNs complexed to receptor extracellular domain have also failed to result in rational development of IFN mimetics (Thomas and others 2011). The classical model of signaling by IFN γ , type I IFNs, and other cytokines involves the activation of receptor-associated tyrosine kinases, primarily of the Janus or JAK kinase family, leading to phosphorylation and dimerization of the STAT transcription

factors, which then dissociate from the receptor cytoplasmic domain and translocate to the nucleus (Brivanlou and Darnell 2002). It has recently been shown, however, that activated JAKs produced from ligand-receptor interaction also undergo nuclear translocation along with the activated STATs produced from the interaction (Lu and others 2005; Shi and others 2006; Dawson and others 2009; Griffiths and others 2011; Noon-Song and others 2011; Ahmed and Johnson 2013; Ahmed and others 2013; Johnson and others 2013). The activated JAKs, pJAK2, and pTYK2 have been shown to have epigenetic functions where they phosphorylate tyrosine 41 on histone H3 (Dawson and others 2009; Griffiths and others 2011). It is difficult to imagine that these JAK-mediated epigenetic events are random and, therefore, not linked to specific gene activation by the STATs.

There are also concerns regarding the requirement of receptor cross-linking as indicated for IFN γ . It has been shown, for example, that functionally monomeric IFN γ can also induce activation of STAT1 α (Landar and others 2000). This raises the question of whether cross-linking of IFNGR1 is the determining event in subsequent signal transduction by IFN γ .

It has been demonstrated by at least 5 different groups that intracellular types I and II IFNs possess biological activity (Fidler and others 1985; Rutherford and others 1996; Thiam and others 1998; Ahmed and others 2001, 2003). The

intracellular activities of IFNs raise the possibility that a function of extracellular binding is to induce endocytosis where internalized IFN then interacts with receptor cytoplasmic domain and induces the biological effects (Szente and others 1994; Johnson and others 2013).

This is indeed the case for IFN γ where we showed that short peptides which correspond to IFN γ C-terminus were sufficient to activate STAT1 α , induce an antiviral state, and up-regulate MHC I expression (Szente and others 1994, 1995). This was done in the context of a noncanonical JAK/STAT model that we developed for both types I and II IFN signaling (Ahmed and others 2013; Johnson and others 2013). The noncanonical model bears a striking resemblance to steroid hormone/steroid receptor signaling and addresses the issues raised earlier with regard to the shortcomings of the classical model of JAK/STAT signaling for the IFNs. We test an attractive aspect of the noncanonical model of IFN signaling with regard to the development of type I IFN mimetics. The model predicts that only a part of the C-terminus of the IFN molecule is necessary to carry out biological activity if that region can be internalized.

Materials and Methods

Peptide synthesis

Ovine IFN τ (158–195), scrambled IFN τ , and suppressors of cytokine signaling 1 (SOCS1) antagonist peptide were synthesized using fluorenylmethyloxycarbonyl chemistry, as previously described (Szente and others 1994). A lipophilic group (palmitoyl-lysine) for cell penetration was added to the N-terminus as a last step of synthesis, using a semi-automated protocol. Human lipo-IFN α 1(152–189) and lipo-IFN β (150–187) were synthesized by GenScript (Piscataway, NJ). Peptides were dissolved in DMSO followed by dilution in phosphate-buffered saline (PBS).

Cell culture and antiviral assays

BSC40 and L929 cells were grown in MEME with 10% fetal bovine serum (FBS) and antibiotics. B16F10 cells were grown in DMEM with 10% FBS. BSC40 cells were grown to confluency in a 12-well plate, treated with the IFN mimetic peptides, control peptide, or intact IFN for 4 h, and infected with vaccinia virus (VV) for 1 h. Cells were washed, fresh growth medium was added, and at the end of 48 h plaques were counted. Antiviral assays with vesicular stomatitis virus (VSV) were performed by using a cytopathic effect (CPE) assay. L929 cells (40,000 per well in a microtiter dish) were grown overnight. IFN mimetics, the control peptide, or parent IFN were added to cells at the concentrations indicated for 4 h, followed by infection with VSV (multiplicity of infection=0.01) for 24 h. Cells were stained with crystal violet and read in a microtiter plate at 550 nm.

Induction of melanoma and treatment with peptides

The Institutional Animal Care and Use Committee at the University of Florida approved all of the animal protocols mentioned here. Female C57BL/6 mice (6 to 8 weeks old, $n=5$) were injected i.p. with 10^4 B16F10 cells. Peptides were administered i.p. in a volume of 100 μ L. Survival of mice was followed over a period of 40 days.

Induction of experimental allergic encephalomyelitis, evaluation of symptoms, and administration of peptides

Female SJL/J mice (6 to 8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in standard SPF facilities. On day 1, SJL/J mice were injected with 300 μ g/mouse bovine myelin basic protein (Invitrogen) that was emulsified in Complete Freund's Adjuvant with 8 mg/mL H37Ra *Mycobacterium tuberculosis* (Sigma-Aldrich) and injected subcutaneously into 2 sites at the base of the tail along with 400 ng/mouse of pertussis toxin (List Biological Laboratories, Inc.) in PBS i.p. (Jager and others 2011). On day 3, the pertussis toxin injection was repeated. Beginning on day 12 postimmunization, after lymphocyte infiltration of the CNS had begun, mice were administered the following treatments or peptides every other day via an i.p. injection in 100 μ L final volume: PBS, human IFN α 1, human IFN β , and ovine IFN τ mimetics (50 μ g/mouse).

Results

We recently showed that N-terminal truncations of human IFN α 1, IFN α 1(69–189), and IFN β , IFN β (100–187), resulted in loss of biological function (Ahmed and others 2013). However, if the truncated IFNs were produced with an N-terminal sequence of 9 arginines (R9) for cell penetration, they possessed antiviral activity and were potent therapeutics against experimental allergic encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS). In addition, the functional truncations lacked toxicity of weight loss and lymphocyte suppression that is associated with the corresponding IFNs at the same antiviral activity.

Synthesis of short C-terminal peptide candidates as type I IFN mimetics

We asked the question based on our experience with IFN γ mimetics (Ahmed and others 2013) whether we could make more severe truncations from N-terminal to C-terminal for several different type I IFNs and still observe antiviral activity if the C-terminal peptides were internalized. We, thus, synthesized N-terminal truncated human IFN α 1, human IFN β , and ovine IFN τ as represented by IFN α 1(152–189), IFN β (150–187), and ovine IFN τ (156–195), respectively, with an attached palmitate (lipo-) for cell penetration. Ovine IFN τ as well as its bovine counterpart is a pregnancy recognition hormone that is produced by the placenta (Ott and others 1991). It is as potent as the other type I IFNs and functions through the same type I IFN receptor complex. All 3 truncations as well as a scrambled version of IFN τ , scrIFN τ (156–195) (Table 1), were tested for antiviral activity against VV and VSV.

Short C-terminal type I IFN mimetic peptides possess antiviral activity

As shown in Fig. 1A, all 3 truncated peptides inhibited VV growth in a dose-dependent manner on BSC-40 monkey cells. Lipo-scrIFN τ (156–195), however, lacked antiviral activity against VV. A mimetic peptide lacking the palmitate group for cell penetration also failed to inhibit virus replication. VV decoy receptor B18R blocked the antiviral activity of one of the parent IFNs, IFN α 1, under the same

TABLE 1. AMINO-ACID SEQUENCE OF TYPE I IFN MIMETICS, SOCS1 ANTAGONIST (pJAK2), AND IFN AND JAK2 CONTROL PEPTIDES

Lipo-IFN α 1(152–189):	LYLTEKKYSPCAWEVVRAEIM RSLSLSTNLQERLRRKE
Lipo-IFN β (150–187):	ILHYLKAKEYSHCAWTIVRVEIL RNFYFINRLTGGLRN
Lipo-IFN τ (156–195):	EKGYS DCAWEIVRVE MMRAL TSSTT LQKRLTKTGG DLNSP
Lipo-scrambled:	MAVKLGTNLNGMYVWLCKSRSTTCG SEDIEVKLEPARQMDTR
Lipo-pJAK2:	¹⁰⁰¹ LPQDKEpYYKVKEP
Lipo-pJAK2, scrambled:	PpYVLDKYQKKEP

Lipo refers to the palmitic acid residue attached to the N terminus for the purpose of gaining entry across the plasma membrane. IFN α 1 and IFN β mimetic sequences are from human IFNs. IFN τ mimetic sequence is from ovine IFN τ . The scrambled peptide is derived by rearranging the sequence of IFN τ mimetic. pJAK2 peptide spans the amino acids 1001–1013 from the activation loop of JAK2. Its inactive control is the scrambled pJAK2 peptide. IFN, interferon.

conditions where the corresponding internalized mimetic inhibited VV. B18R corresponds to the extracellular domain of the type I IFN receptor and competes with receptor for binding to type I IFNs (Alcami and others 2000). The data suggest that the type I IFN mimetics possess biological activity when internalized and that the cytoplasmic domain of the receptor plays a role in mimetic function.

IFNs are virus nonspecific and, thus, have broad antiviral activity. VSV is a single-stranded negative sense RNA virus and should, thus, be considered a suitable test for the broad antiviral activity of the peptide mimetics. As shown in Fig. 1B, all 3 peptide mimetics, lipo-IFN α 1(151–189), lipo-IFN β (150–187), and lipo-IFN τ (156–195), inhibited VSV CPE on mouse L cell fibroblasts in a dose-dependent manner, similar to their effects on VV growth. The scrambled version of the IFN τ mimetic lacked antiviral activity and intact IFN α 1, in contrast to no effect on VV, was a potent inhibitor of VSV growth. All of these type I IFN short peptide mimetics, thus, possess the broad antiviral activity that is characteristic of their parent IFNs. This is a property that would be expected of a type I IFN mimetic. Significantly, it is demonstrated for 3 different type I IFN mimetics.

IFN mimetics inhibit melanoma in cell culture as well as in tumorigenic mice

IFN mimetics lipo-IFN α 1(152–189) and lipo-IFN β (150–187) were tested for inhibition of B16F10 melanoma cells in culture. As shown in Fig. 2A, both mimetics showed dose-dependent inhibition of B16F10 melanoma cells when incubated with the cells for 72 h with ~70% inhibition at 50 μ M. A scrambled version of one of the mimetics (see Table 1) failed to have any antiproliferative effect on the B16F10 cells.

Lipo-IFN α 1(152–189) also showed significant protection against lethal B16 melanoma in C57BL/6 mice in a proof-of-concept experiment. Mice were injected intraperitoneally (IP) with 10,000 B16 cells at day 0 and lipo-IFN α 1(152–189) along with a scrambled peptide were injected IP starting at day 1 at 100 μ g every other day for a period of 14

FIG. 1. Inhibition of vaccinia virus (VV) and vesicular stomatitis virus (VSV) replication by type I IFN mimetics. **(A)** BSC40 cells were grown to confluence and treated with the C-terminal peptide mimetics of IFNs α , β , and τ for 2 h. Cells were then infected with 0.01 m.o.i. of VV for 1 h, followed by washing and addition of fresh medium. Forty eight hours later, cells were stained with crystal violet and plaques were counted. Percent inhibition was calculated with the plaques in media-only cells versus the various treatments. Intact IFN α 1 added at 5,000 U/mL did not protect against VV infection. A scrambled IFN τ peptide was used as a negative control. Nonpalmitic refers to the peptide that was not conjugated with palmitic acid and, thus, does not gain entry across the plasma membrane. See Table 1 for mimetics of α , β , τ , and τ scrambled mimetic sequences of the corresponding IFNs. **(B)** L929 cells were seeded in a microtiter plate and incubated with the IFN mimetics of Table 1, including scrambled IFN τ peptide, or IFN α 1 (5,000 U/mL) at the concentrations indicated for 2 h. Cells were then infected with 0.1 m.o.i. of VSV for 24 h. Cells were then stained with crystal violet, and the absorbance was read. IFN, interferon; m.o.i., multiplicity of infection.

days. As shown in Fig. 2B, IFN α 1 mimetic protected 40% of the mice for 40 days when the experiment was terminated. Scrambled peptide and PBS injected mice were not protected, and all the mice died by day 22.

IFN mimetic and an SOCS1 antagonist are additive/synergistic against B16 melanoma in mice

SOCS1 and 3 play an important role in the regulation of both innate and adaptive immune responses that are mediated by cytokines such as the IFNs (Inagaki-Ohara and

FIG. 2. Antimelanoma effects of the type I IFN mimetics. **(A)** Cell culture. B16F10 cells (20,000 in 0.1 mL) were incubated in microtiter plates with an equal volume of the IFN α 1 and IFN β mimetics as well as a scrambled (scr) mimetic (see Table 1) for 3 days, after which the cells were counted and compared with untreated cells. **(B)** IFN mimetic protection of C57BL/6 mice from B16F10 cells in the presence of suppressors of cytokine signaling 1 (SOCS1) antagonist. Female C57BL/6 mice ($n=5$) were injected i.p. with 10^4 B16F10 cells. Starting from day 1, mice were injected i.p. with PBS, lipo-IFN α 1(156–189) (100 μ g), SOCS1 antagonist lipo-pJAK2(1001–1013) (100 μ g), or a combination of the 2 on alternate days over a period of 14 days. As a control, scrambled peptide (scr) corresponding to the IFN mimetic was used at 100 μ g per mouse. The survival of mice was followed over 40 days. A comparison of the combined treatment against the PBS injection on day 40 gave a P value of 0.001. PBS, phosphate-buffered saline.

others 2013; Linossi and others 2013). These inducible intracellular regulatory proteins play a key role in regulating the extent of the immune response, similar to the role played by regulatory T cells (Larkin and others 2013).

We show in Fig. 2B that IFN mimetic lipo-IFN α 1(152–189) is additive/synergistic with an SOCS1 peptide antagonist, lipo-pJAK2(1001–1013), to enhance protection to 80%; whereas each peptide alone resulted in 40% protection. The antagonist peptide is derived from the pJAK2 binding site of SOCS1 (Waiboci and others 2007). This additive/synergistic effect is consistent with that which we previously showed for IFN γ and SOCS1 antagonist treatment of VV infected cells (Ahmed and others 2010). The effect of SOCS antagonist is also consistent with reports of increased SOCS1 protein in cells of individuals with metastatic melanoma cancer (Li and others 2004; Fojtova and others 2007).

Surviving mice, whether treated with IFN mimetic alone or in combination with the SOCS antagonist, were visually

FIG. 3. Lipo-IFN α 1(152–189) and lipo-JAK2(1001–1013) SOCS1 antagonist-protected mice show absence of peritoneal B16 tumors. Evidence of B16 tumors throughout the peritoneum of scrambled peptide/PBS-treated mice (*top left*) and absence of obvious tumors in IFN mimetic/SOCS1 antagonist-treated mice (*top right*). Also shown is a liver of scrambled peptide/PBS-treated mice (*bottom left*) with tumor nodules, which are absent from the combined mimetic/antagonist treatment of mice (*bottom middle*). Enlarged spleen with tumor nodules is seen in control mice (*bottom right, left side of frame*) but not in the combined treatment (*bottom right, right side of frame*).

free of B16 tumors in the peritoneum as shown in Fig. 3; whereas the internal organs of scrambled/PBS-treated mice had an extensive spread of B16 tumors. The type I IFN mimetics, thus, possess antiproliferative and antitumor effects similar to the parent IFNs.

It should be noted that the mimetics lacked the toxicity in mice which is associated with the parent IFNs as illustrated in Fig. 4. Mice injected IP with 5,000 U/mL lipo-IFN α 1(152–189) every other day for 13 days gained weight, while mice injected with 5,000 U/mL of IFN α 1 had a 15% weight loss over 13 days. Scrambled peptide-injected mice also showed weight gain. The antitumor effect of the IFN mimetics, thus, was not associated with toxicity.

IFN mimetics are effective therapeutics against EAE

A type I IFN, IFN β , is the FDA therapeutic of choice in the treatment of MS (Castro-Borrero and others 2012). We tested all 3 type I IFN mimetics, lipo-IFN α 1(152–189), lipo-IFN β (150–187), and lipo-IFN τ (156–195), as well as the scrambled version of the IFN τ mimetic (Table 1) for their ability to protect against ongoing EAE in SJL/J mice. Mice were injected i.p. with 50 μ g (2,500 U) mimetic at day 12 of immunization with MBP and every other day thereafter. Lymphocyte infiltration of the central nervous system has begun by day 12 (Jager and others 2011). PBS and scrambled mimetic peptide-injected mice showed minimal paralysis early, which increased to paraplegia by day 45 (Fig. 5). By contrast, all of the type I IFN mimetics controlled the

FIG. 4. Type I IFN mimetic lipo-IFN α 1(152–189) lacked toxicity under conditions where the intact IFN was toxic. Mice (C57BL/6, $n=3$) were injected i.p. with murine IFN α 1 (Δ , 5×10^3 U/mouse), Lipo-IFN α 1(152–189), 5×10^3 U (100 μ g, $\blacksquare$), or PBS ($\circ$), i.p. on alternate days. IFN activity refers to the antiviral activity assessed by the cytopathic effect of encephalomyocarditis virus on L cells. Body weight was measured daily. The average body weight is presented as a percentage of initial weight, and the standard deviation is shown. The weight loss seen with IFN α 1-treated mice was not seen with the IFN α 1 mimetic-treated mice. On day 11, the difference between the IFN α 1 and IFN α 1 mimetic showed a significance of $P < 0.05$ by the Mann-Whitney rank test.

paralysis such that it was kept at a minimum or was not observed. This level of therapeutic efficacy was as effective as that of the type I IFNs in EAE (Burks 2005).

Unlike their parent IFNs, however, the mimetics lacked toxicity in mice as illustrated for lipo-IFN α 1(152–189) in

FIG. 5. Type I IFN mimetics protect mice against the symptoms of EAE. SJL/J mice ($n=5$) were immunized with myelin basic protein. Twelve days later, they were injected i.p. with PBS ($\bullet$) or 50 μ g (2,500 U) each of lipo-IFN α 1(152–189) ($\blacktriangle$), lipo-IFN β (150–187) ($\blacklozenge$), lipo-IFN τ (156–195), ($\circ$) or scrambled lipo-IFN τ ($\square$) on alternate days. Mice were followed daily. The mean daily severity of disease was graded as follows. 0, normal; 1, loss of tail tone; 2, hind leg weakness; 3, paraparesis; 4, paraplegia; 5, moribund; and 6, death. Statistics were performed using an unpaired Student's t -test. EAE, experimental allergic encephalomyelitis.

Fig. 4. Mice injected with 100 μ g (5,000 U) of the mimetic, twice the dose used for EAE, gained weight usually over 12 days; while the parent IFN caused a 15% loss of weight over the same time period. The type I IFN mimetics are, thus, potential candidates for replacement of IFN β as therapeutics for MS and possibly other autoimmune diseases, particularly so, because of their lack of toxicity.

IFN mimetics activate TYK2 and STAT1 α

Since type I IFN mimetic should activate signal transduction similar to the parent IFNs, we treated WISH cells with lipophilic IFN α 1(152–189), IFN β (150–187), and IFN τ (156–195) for 10 min, assessed cellular extracts for phosphorylated TYK2 (pTYK2), and phosphorylated STAT1 α (pSTAT1 α). As shown in Fig. 6A, all of the mimetics

FIG. 6. Intracellular signaling of IFN mimetic and SOCS1 antagonist. (A) IFN mimetics signal through TYK2 and STAT1 α phosphorylation. WISH cells were treated with the peptides indicated, or the parent IFN for 10 min. Cell extracts were Western blotted with phospho-TYK2 or phospho-STAT1 α . Membranes were stripped and re-probed with total TYK2 or STAT1 α . The results are representative of at least 2 experiments. (B) B16F10 cells show a reduction in SOCS1 levels after treatment with SOCS1 antagonist. B16F10 cells were treated with the SOCS1 antagonist peptide, pJAK2, or its scrambled control peptide for 10 min. Cell extracts were Western blotted with SOCS1 antibody. Numbers under the lanes represent the relative intensities of bands as measured by the Image J program (NIH). Membrane was stripped and re-probed with Lamin B1, to ascertain equal loading of proteins.

as well as IFN α 1 activated TYK2 and STAT1 α ; while a scrambled version of one on the mimetics was inactive. The data demonstrate that the IFN mimetics activate a relevant JAK and STAT similar to the IFNs.

SOCS1 antagonist, pJAK2(1001–1013), decreased the basal level of SOCS1 in B16F10 melanoma cells

We showed that SOCS1 antagonist synergized with IFN α 1(152–189) in the treatment of mice against B16F10 melanoma tumor cells (Fig. 2B). Consistent with our previous observation (Ahmed and others 2010), lipophilic pJAK2(1001–1013) reduced intracellular SOCS1 levels in B16F10 tumor cells by more than 60% as shown in Fig. 6B. There are reports of increased SOCS1 protein in cells of individuals with metastatic melanoma cancer (Li and others 2004; Fojtova and others 2007). Thus, the synergistic effect of the SOCS1 antagonist appears to be due to a reduction in intracellular levels of SOCS1 in B16F10 cells, which, in turn, should enhance the IFN mimetic therapeutic effects.

Discussion

The classical model of IFN signaling is particularly challenged by the recent discovery that activated JAK2 undergoes nuclear translocation where it functions epigenetically by phosphorylation of tyrosine 41 on histone H3 (H3pY41) (Ahmed and Johnson 2013; Johnson and others 2013). The activation of the JAK2 can be constitutive as is the case of the gain-of-function mutant JAK2V617F or it can occur by growth factor or cytokine activation of wild-type JAK2 (Lu and others 2005; Shi and others 2006; Dawson and others 2009; Griffiths and others 2011). The presence of H3pY41 resulted in the dissociation of heterochromatin protein 1 α (HP1 α) from H3. The resultant heterochromatin remodeling was associated with exposure of chromatin to gene activation. It seems reasonable that nuclear pJAK2 activity should be coordinated with activated STAT in the nucleus, but such coordination is currently not considered in the classical model.

For the type I IFN signaling, we found that activated TYK2(pTYK2), similar to JAK2, possessed epigenetic activity and caused the formation of H3pY41 specifically at genes that were activated by the IFN (Noon-Song and others 2011; Ahmed and others 2013). In fact, nuclear immunoprecipitations and ChIP analysis showed that pTYK2 was associated with activated STAT1 (pSTAT1) at the promoter of the genes which were activated by the IFNs. When we looked at the various components of type I IFN signaling in addition to pTYK2 and pSTAT1, we found by ChIP-qPCR and nuclear immunoprecipitation that IFNAR1, IFNAR2, pTYK2, pSTAT1, and H3pY41 were present in the nucleus at the oligoadenylate synthetase promoter, but not at the β -actin promoter, a gene that is not directly affected by type I IFNs (Ahmed and others 2013). The IFN protein by virtue of the C-terminus function was also present in the nucleus. This is basically our noncanonical model of IFN signaling, which is remarkably similar to that of steroid hormone/steroid receptor signaling (Stanisic and others 2010).

Our noncanonical model of IFN γ signaling suggested that the C-terminus of IFN γ , if internalized, played a key

role in IFN signaling (Ahmed and Johnson 2013; Ahmed and others 2013). The 3 different short C-terminus peptides of IFN α , IFN β , and IFN τ (Table 1), thus, provide a test for the generality of IFN signaling via the noncanonical modeling. In this regard, we showed here that the mimetics possessed antiviral, antitumor, and ant-EAE activity. For the antiviral studies, we focused on VV for the following reason. This virus produces a decoy receptor (B18R) that competes with the type I IFN extracellular domain for intact type I IFN. Thus, acting intracellularly, all of the mimetics induced an antiviral state against VV, while IFN α 1 was ineffective, which is consistent with previous observations on IFN γ mimetic and B8R decoy receptor of VV (Ahmed and others 2013). By comparison, the mimetics and IFN α 1 possessed antiviral activity against VSV, which unlike VV, lacks a type I IFN decoy receptor. It should be noted that a scrambled version (Table 1) of one of the mimetics lacked antiviral activity. Further, the mimetics had to be internalized to be functional, as their activity occurs through the IFNAR receptor cytoplasmic domains. Translationally, these type I IFN mimetics should be candidates for the treatment of poxvirus infections.

IFN α has been the most effective treatment of advanced metastatic melanoma, but its use has been limited by undesirable side effects of high doses (Francis and others 2003; Schwid and others 2005). The IFN α mimetic possessed antitumor activity against B16F10 melanoma in an established mouse model. Further, it synergized with our SOCS1 antagonist and enhanced antimelanoma activity. We previously showed that IFN toxicity/apoptosis was associated with an extracellular interaction with the receptor (Subramaniam and others 1995). The anti-melanoma effect of IFN α 1(152–189) suggests that IFN toxicity and efficacy in cancer are not necessarily operating through the same mechanism. In addition, the limitations that the toxic side effects place on IFN therapy in cancer can potentially be overcome via the IFN mimetics.

IFN β is the FDA-approved treatment of choice against relapsing/remitting MS, but its use is also limited by toxic side effects (Francis and others 2003; Schwid and others 2005). All 3 of the IFN mimetics were highly effective as therapeutics in our EAE model of MS without the undesirable side effects of precipitous weight loss. Thus, these mimetics should also have potential as improved therapeutics for MS.

We have focused here on the therapeutic aspects of the type I IFN mimetics that were developed based on our noncanonical model of IFN signaling (Ahmed and Johnson 2013; Ahmed and others 2013). The noncanonical model has been constructed on a platform of connected studies which have not shown any internal contradictions, that is, there is a consistency such that we predicted that the mimetics presented here could, in fact, be constructed based on such a model (Ahmed and Johnson 2013; Ahmed and others 2013). It is worth noting that the mimetics readily lend themselves to testing by others. We are not aware of any evidence-based refutation.

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Author Disclosure Statement

No competing financial interests exist.

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