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(54) Title: TYPE I INTERFERON MIMETICS AS THERAPEUTICS FOR CANCER, VIRAL INFECTIONS, AND MULTIPLE SCLEROSIS

(57) Abstract: The subject invention pertains to agonist peptides of type I interferons and methods of using the peptides. These peptides are based on the amino acid sequence of the C-terminus region of the type I IFN molecules and are capable of binding to the cytoplasmic domain of type I IFN receptors. Surprisingly, these peptides were found to possess the same or similar biological activity as that associated with the full-length, mature type I IFN proteins, even though these peptides do not bind to the extracellular domain of the type I IFN receptors. In one embodiment, the peptide is a peptide of IFN α . In another embodiment, the peptide is a peptide of IFN β . The subject peptides have been shown to effect increased resistance to viral infection. Peptides of the invention can be used to treat or prevent viral infections, to treat oncological disorders, and to treat autoimmune disorders, such as multiple sclerosis.



DESCRIPTION

TYPE I INTERFERON MIMETICS AS THERAPEUTICS FOR CANCER, VIRAL
5 INFECTIONS, AND MULTIPLE SCLEROSIS

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit of U.S. Provisional Application Serial
No. 61/499,495, filed June 21, 2011, which is hereby incorporated by reference herein in
10 its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences,
or drawings.

GOVERNMENT SUPPORT

This invention was made with government support under grant
15 number R01A1056152 awarded by the NIH/NIAID. The government has certain rights
in the invention.

BACKGROUND OF THE INVENTION

Viruses are a heterogeneous group of intracellular infectious agents that depend in
20 varying degrees on the host synthetic machinery for replication. The poxviruses are large,
double-stranded DNA viruses that are assembled in the cytoplasm of infected cells
involving complex replication mechanisms (Moss, 2007). Attachment, internalization,
and disassembling of poxviruses precedes the initiation of three waves of mRNA
synthesis. The early wave codes for virus growth factors and decoy cytokine receptors.
25 Decoy receptors for both type I and type II interferons (IFNs) are produced during early
protein synthesis in poxvirus infected cells, thus blunting perhaps the most important
innate host defense system against viral infections (Moss and Shisler, 2001). A well-
known example of this is the B8R protein of vaccinia virus, which is a homolog of the
extracellular domain of the IFN γ receptor (Moss, 2007).

Encephalomyocarditis (EMC) virus is a small single-stranded RNA picornavirus of the plus strand orientation with wide host range (Racaniello, 2007). In mice, EMC virus infection is lethal, but is quite susceptible to IFN γ or an IFN γ mimetic treatment at early stages of infection (Mujtaba *et al.*, 2006). The IFN γ mimetic is also effective against
5 vaccinia virus infection even in the presence of B8R decoy receptor (Ahmed *et al.*, 2005; Ahmed *et al.*, 2007). The IFN γ mimetic is a small peptide corresponding to the C-terminus of IFN γ that functions intracellularly and thus does not interact with the extracellular domain of the IFN γ receptor (Ahmed *et al.*, 2005).

The IFN γ mimetic is also effective against another large double-stranded DNA
10 virus called herpes simplex 1 or HSV-1 that replicates in the cell nucleus (Frey *et al.*, 2009). Close relatives include the herpes Zoster virus and cytomegalovirus (Roizman *et al.*, 2007). The broad spectrum of antiviral activity of IFN γ mimetics is unique in that we are unaware of any other small antiviral that exhibits strong activity against poxviruses, picornaviruses, and herpes viruses.

15 The IFN system is regulated by an inducible endogenous tyrosine kinase inhibitor called suppressor of cytokine signaling 1 or SOCS-1 (Yoshimura *et al.*, 2007; Mansell *et al.*, 2006; Yasukawa *et al.*, 1999; Kobayashi *et al.*, 2006; Croker *et al.*, 2008). SOCS-1 is a member of a family of inducible proteins that negatively regulate IFN and other cytokine signaling via inhibition of JAK/STAT signaling (Yoshimura *et al.*, 2007). There
20 are currently eight members of the SOCS family, SOCS-1 to SOCS-7 and cytokine-inducible SH2 protein. SOCS-1 has distinct regions or domains that define the mechanism by which it inhibits the function of JAK tyrosine kinases such as JAK2 that are involved in activation of STAT transcription factors (Yoshimura *et al.*, 2007). The N-terminus of SOCS-1 contains a SH2 domain, and N-terminal to it is an extended SH2
25 sequence (ESS) adjacent to a kinase inhibitory region (KIR) (Yoshimura *et al.*, 2007). These domains or regions of SOCS-1 bind to the activation and catalytic regions of JAK2 and block its function. The C-terminus of SOCS-1 contains a domain called the SOCS box, which is involved in proteasomal degradation of JAK2. It has been shown that the KIR sequence of SOCS-1 binds to a peptide corresponding to the activation loop of
30 JAK2, pJAK2(1001-1013), and that the peptide pJAK2(1001-1013) blocked SOCS-1 activity in cells (Waiboci *et al.*, 2007). Specifically, pJAK2(1001-1013) enhances suboptimal IFN activity, blocks SOCS-1 induced inhibition of STAT3 activation,

enhances IFN γ activation site promoter activity, and enhances antigen-specific proliferation.

Influenza A virus is a segmented negative strand RNA virus that is responsible for over 30,000 deaths annually in the United States (Palese and Shaw, 2007). Pandemic influenza A virus infection can cause the deaths of millions world-wide. Type I IFNs are an important early innate immune response cytokine against influenza respiratory infections (Szretter *et al.*, 2009). Influenza virus-encoded nonstructural protein NS1 is multifunctional and is important in virus defense against IFNs by a mechanism(s) that is not fully understood but may involve induction of SOCS-1 and SOCS-3, which in turn would negatively regulate IFN signaling (Pothlichet *et al.*, 2008).

Herpes Simplex Virus (HSV) is a member of a broad class of double-stranded DNA viruses that undergo replication in the cell nucleus. Examples of other members are varicella-zoster virus (VZV) and cytomegalovirus (CMV) (Roizman *et al.*, 2007). It is estimated that HSV-1 infects 60 to 80 percent of the people throughout the world, and persists for life in the infected individuals (Diefenbach *et al.*, 2008; Koelle and Corey, 2008; Cunningham *et al.*, 2006). Primary infection commonly occurs through cells of the mucous membrane and is often asymptomatic. This is followed by uptake of virus by sensory nerve fibers and retrograde transport to the cell body of the neurons in the dorsal root or trigeminal ganglion. Here, acute infection is converted to latency and from which HSV-1 periodically migrates down the nerve tissue to again infect mucosal cells for overt disease (Roizman *et al.*, 2007; Diefenbach *et al.*, 2008; Koelle and Corey, 2008; Cunningham *et al.*, 2006).

HSV-1 infection is characterized by a strong cytokine response in infected cells, particularly the induction of type I IFNs (Cunningham *et al.*, 2006). Infection of keratinocytes, for example, results in induction of large amounts of IFN α and IFN β as well as interleukins 1, 6, and β -chemokines (Mikloska *et al.*, 1998). IFNs, macrophages, natural killer (NK) cells, and gamma/delta T cells all play an important role in host innate immune response to HSV-1 (Cunningham *et al.*, 2006). Toll-like receptor (TLR) 2 is activated on the cell surface by HSV-1, while TLR-9 is activated intracellularly by viral DNA. The latter stimulus is thought to play an important role in induction of IFN α by HSV-1 (Cunningham *et al.*, 2006).

The adaptive immune response plays an important role in confining HSV-1 and other herpes virus infections to a latent state where CD8⁺ T cells and IFN γ play critical roles (Knickelbein *et al.*, 2008; Sheridan *et al.*, 2007; Decman *et al.*, 2005). It is functionally connected to the innate immune system where NK cells can serve as a source of IFN γ , which is also produced by CD4⁺ and CD 8⁺ T cells. IFN γ can exert direct antiviral activity as well as induce upregulation of MHC class I and class II molecules on macrophages, dendritic cells, and keratinocytes (Decman *et al.*, 2005). Direct effects of IFN γ as per a mouse model suggest that this IFN prevents reactivation of HSV by inhibition of function of the key intermediate protein ICP0 (Mossman, 2005). Interaction of the antigen presenting cells with CD4⁺ T cells induces CD8⁺ T cells to control HSV-1 levels in mucosal lesions (Arduino and Porter, 2008; Patel *et al.*, 2007).

HSV-1 has developed several mechanisms to inhibit both the innate and adaptive immune responses to infection. HSV-1 downregulation of class I MHC expression occurs through high affinity binding of viral immediate early gene product ICP47 to the transporter associated with antigen processing (TAP) (Burgos *et al.*, 2006), which blocks IFN γ induction of cytotoxic CD8⁺ T cells (Goldsmith *et al.*, 1998). IFN signaling is also inhibited by blockage of JAK/STAT transcription factor phosphorylation by an unknown mechanism (Chee and Roizman, 2004). ICP0 is thought to enhance proteasome-dependent degradation of IFN stimulated genes (ISGs) (Halford *et al.*, 2006; Edison *et al.*, 2002). A recent study suggests that HSV-1 can exert an anti-interferon effect by activation of a protein called suppressor of cytokine signaling 3 (SOCS-3) (Yokota *et al.*, 2004).

Currently, there are no effective therapeutics available against HSV infection, except the nucleoside analog acyclovir (Dorsky and Crumpacker, 1987), which is known to have serious side effects. A search for a vaccine against HSV has remained elusive because of the successful adaptation to the host used by HSV (Koelle and Corey, 2008). Along with direct effects, infection with HSV has been found to increase the incidence of HIV infection, probably due to HSV-associated lesions (Wald and Link, 2002). Because of this interplay between HSV and HIV, it is conceivable that anti-HSV treatment may reduce the incidence of infection with HIV.

Type I interferons (IFNs), IFN α and IFN β have been clinically approved for the treatment of hairy cell leukemia, chronic myelogenous leukemia, melanoma, hepatitis C virus infection, and multiple sclerosis. Treatment with these IFNs is associated with severe side effects, including bone marrow suppression, depression, and fever, which has resulted in several patients dropping out of treatment programs. There remains a need in the art for type I IFN mimetics that can provide the same benefits as the parent interferons, while having less of the undesirable effects.

The classical model of cytokine signaling dominates our view of specific gene activation by cytokines such as the interferons (IFNs) (Levy and Darnell, 2002). In this model, ligand activates the cell solely via interaction with the extracellular domain of the receptor complex. This in turn results in the activation of receptor or receptor-associated tyrosine kinases, primarily of the Janus or JAK kinase family, leading to phosphorylation and dimerization of the STAT transcription factors, which then disassociate from the receptor cytoplasmic domain and translocate to the nucleus. This view ascribes no further role to the ligand, JAKs, or the receptor in the signaling process. Further, there is the suggestion that the STAT transcription factors possess intrinsic nuclear localization sequences (NLSs) that are responsible for nuclear translocation of STATs and specific gene activation (McBride *et al.*, 2000; Melen *et al.*, 2001; Begitt *et al.*, 2000).

It has recently been acknowledged that the classical model of JAK/STAT signaling was over-simplified in its original form. In the case of IFN γ , complexity beyond simple JAK/STAT activation is indicated in the relatively recent demonstration that other pathways, including MAP kinase, PI3 kinase, CaM kinase II, NF- κ B, and others cooperate with or act in parallel to JAK/STAT signaling to regulate IFN γ effects at the level of gene activation and cell phenotypes (Gough *et al.*, 2008). All of these pathways are generic in the sense that a plethora of cytokines with functions different from those of IFN γ also activate them. Thus, uniqueness of function would seem to depend on cytokine control of complex and unique qualitative, quantitative, and kinetic aspects of activation of these pathways. This uniqueness has thus far not been demonstrated.

At the STAT level, there is evidence of a functional interaction between different STATs in gene activation/suppression, which provides more insight into STAT mediation of cytokine signaling. The induction of IL-17 by activated STAT3, for example, was countered by IL-2 activation of STAT5 (Yang *et al.*, 2011). It was demonstrated by

chromatin immunoprecipitation (ChIP) sequencing that STAT3 and STAT5 bound to multiple common sites across the IL-17 gene locus, including non-coding sequences. The activation state of these STATs was not addressed. Induction of STAT5 by IL-2 resulted in more binding of STAT5 and less binding of STAT3 at these sites, whereas induction of STAT3 by IL-6 induced the opposite; the combination of the two STATs resulted in dynamic regulation of the IL-17 gene locus by the opposing effects of IL-2 (STAT5) and IL-6 (STAT3) (Yang *et al.*, 2011). A similar complementarity was observed with STAT4 and STAT6 with respect to Th1 and Th2 cell development, but with much less competition for binding sites at coding and non-coding regions of the gene (Wei *et al.*, 2010). These Yin-Yang interactions of STAT transcription factors are referred to as specification with respect to lymphocyte phenotypes. It is not clear, however, as to how these STAT interactions at the level of DNA binding translate into specific gene activation by the inducing cytokine.

There is evidence that JAK kinases, including the mutant JAK2V617F, play an important role in the epigenetics of gene activation in addition to STAT activation in the cytoplasm (Dawson *et al.*, 2009). Leukemic cells with a JAK2V617F gain-of-function mutation have constitutively active JAK2V617F in the nucleus. This leads to phosphorylation of Y41 on histone H3, which results in disassociation of heterochromatin protein 1 α , HP1 α . The heterochromatin remodeling was associated with exposure of euchromatin for gene activation. Although present in the nucleus, wild-type JAK2 was only activated when K562 cells were treated with PDGF or LIF, or when BaF3 cells were treated with IL-3. The question of how a ligand/receptor interaction resulted in the presence of activated JAK2, pJAK2, in the nucleus was not addressed, nor its targeting mechanism to discrete genomic sites and specific promoters.

It has been shown in the case of IFN γ that receptor subunit IFNGR1 is associated with pJAK2 and phosphorylated histone H3Y41 at the promoter of the IRF1 gene, while the β -actin gene is unaffected, since it is not acted on by IFN γ (Noon-Song *et al.*, 2011). Activated TYK2, pTYK2, in the nucleus and at promoters of genes activated by type I IFNs. TYK2 is also activated by other cytokines such as IL-12 and IL-23, which have biological effects different from IFN (Jones and Vignali, 2011; Duvallet *et al.*, 2011). We were therefore particularly interested in whether there was an association between pTYK2 and type I IFN receptors at the promoters and chromatin of genes activated by

these IFNs and whether such association provided insight into pTYK2 induced specific epigenetic events in genes activated by the IFNs. The findings provide insight into the mechanism of specific gene activation by type I IFNs, including the associated epigenetic events.

5

BRIEF SUMMARY OF THE INVENTION

The subject invention pertains to agonist peptides of type I interferons and methods of using the peptides. These peptides are based on the amino acid sequence of the C-terminus region of the type I IFN molecules and are capable of binding to the cytoplasmic domain of type I IFN receptors. Surprisingly, these peptides were found to possess the same or similar biological activity as that associated with the full-length, mature type I IFN proteins, even though these peptides do not bind to the extracellular domain of the type I IFN receptors. In one embodiment, the peptide is a peptide of IFN α . In another embodiment, the peptide is a peptide of IFN β . The subject peptides have been shown to effect increased resistance to viral infection. Peptides of the invention can be used to treat or prevent viral infections, to treat oncological disorders, and to treat autoimmune disorders, such as multiple sclerosis.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. IFN α mimetic protects against EMC virus infection. L929 cells (40,000 per well) were seeded in a microtiter plate and grown overnight. Treatment with IFN α peptide (5 and 1 μ g), or the control peptide (R9, 5 μ g) was for 4 hr. Cells were infected with EMC virus (0.01 moi) for 24 hr, followed by staining with crystal violet.

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Figure 2. IFN α mimetic protects against vaccinia virus infection. BSC-40 cells were seeded in a 12 well plate and grown overnight. Treatment with IFN α peptide (10 μ M and 2 μ M), or the control peptide (R9, 5 μ M) was for 4 hr. Cells were infected with vaccinia virus (0.01 moi) for 1 hr, followed by growth for 48 hr. Cells were stained with crystal violet and plaques were counted.

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Figure 3. Therapeutic efficacy of type I IFN mimetic in protection of mice against relapsing/remitting EAE. SJL/J mice (n=5), were injected i.p. with PBS (●), control peptide (□, 15 μ g/mouse), or IFN α mimetic (▲, 15 μ g/mouse) every other day

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starting from day 12 post-immunization with MBP. 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.

Figures 4A-4C. Activated JAKs and receptor subunits are present in the nucleus of cells treated with type I IFNs. WISH cells were incubated with or without 1,000 U/ml of IFN α 2 (Figure 4A) or IFN (Figure 4B) for the indicated times and their nuclei were purified and solubilized (see MATERIALS and METHODS). Nuclear and cytoplasmic samples were subjected to Western blotting against indicated antibodies. (Figure 4C) WISH cells were similarly treated with IFN τ and the nuclear fraction was Western blotted with antibodies to IFNAR2 and IFNAR1. -tubulin (cytoplasm) and -lamin (nucleus) blots were performed to confirm the purity of nuclear fraction.

Figures 5A-5C. Nuclear translocation of IFN τ , IFNAR1, and IFNAR2 as determined by confocal microscopy. GFP fusion constructs of IFN τ , IFNAR1, and IFNAR2 were used to separately transfect WISH cells (see MATERIALS and METHODS). IFN τ -GFP transfected cells measured nuclear translocation of IFN τ (Figure 5A). In the case of IFNAR1-GFP (Figure 5B) and IFNAR2-GFP (Figure 5C), cells were treated with 1,000 U/ml of IFN τ and receptors were found to translocate to nuclei as seen by confocal microscopy.

Figure 6. Type I IFN stimulation induces the association of IFNAR1, TYK2, STAT1 α , and H3pY41 with the ISRE at the OAS1 promoter by ChIP assay. WISH cells were treated with 1,000 U/ml of IFN τ for 1 hr, then treated with 1% formaldehyde for 10 min. Details of ChIP assay are in MATERIALS and METHODS and described previously (Noon-Song *et al.*, 2011). Abbrev: AR1, IFNAR1; pYH3, phosphorylated tyrosine 41 on histone H3.

Figure 7. Association of TYK2, pSTAT1 α , and H3pY41 with IFNAR1 in the nucleus of cells treated with a type I IFN. WISH cells were treated with 1,000 U/ml of IFN α 2 for 1 hr, after which a solubilized extract from the isolated nuclei was immunoprecipitated with antibodies to IFNAR1 and Western blotted with the indicated antibodies (see MATERIALS and METHODS).

Figures 8A-8B. Type I IFN treatment induces histone H3K9 demethylation/acetylation as well as H3Y41 phosphorylation at the ISRE of the promoter region of the OAS1 gene. (Figure 8A) WISH cells were treated with 1,000 U/ml of IFN τ

for the indicated time and ChIP assays were performed as in Figure 6 using antibodies to H3K9ac, H3K9me3, and H3pY41. (Figure 8B) Western blot for H3pY41 in WISH cells treated with IFN τ as indicated in Figure 8A. Abbrev: H3K9ac, acetylated lysine 9 in histone H3; H3pY41, phosphorylated tyrosine 41 in histone H3; H3K9me3, trimethylated lysine 9 in histone H3.

Figures 9A-9C. N-terminal truncated IFN α 1(69-189)R9 or IFN β (100-187)R9 possessed antiviral activity and IFN α 1(69-189)R9 protected against relapsing/remitting EAE in SJL/J mice. IFN α 1(69-189)R9 (Figure 9A), or IFN β (100-187)R9 (Figure 9B), or the control peptides without the R9 plasma membrane penetration sequence were added to L929 cells (40,000 per well) and treated for 4 hr. Cells were infected with EMC virus (moi = 0.01) for 24 hr, followed by staining with crystal violet. (Figure 9C) SJL/J mice (n = 5), were injected i.p. with PBS (●), IFN α mimetic, IFN α 1(69-189)R9 (▲, 15 μ g/mouse), or the control peptide, IFN α 1(69-189) (□, 15 μ g/mouse), every other day starting from day 12 post-immunization with MBP. 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.

Figure 10. Weight loss comparison. Mice (C57BL/6, n = 3) were injected i.p. with IFN β (Δ, 10³ U/mouse), IFN β (100-179)R9 (○), 2 x 10³ U (200 μ g), or 2 x 10³ U (200 μ g) of IFN α (69-189)R9 (□), i.p. on alternate days. Activity refers to the antiviral activity assessed by cytopathic effect of EMCV 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 in IFN β treated mice is not seen with IFN β mimetic. On day 11, the difference between the IFN β or IFN β mimetic showed a n < 0.05.

Figure 11. Lack of apoptosis in type I IFN mimetics in comparison with intact IFN α 2. WISH cells (150,000) were seeded in a 6 well plate and grown overnight. They were treated with type I IFN mimetics (100 U/ml), or IFN α 2 (100 U/ml) for 4 days. Cells were doubly stained with Annexin V and propidium iodide (PI) and analyzed by flow cytometry to measure the extent of apoptosis. The data shown indicate the percentage of apoptosis based on cells staining for both Annexin V and PI from the analysis of 10,000 cells.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is a peptide mimetic of human IFN α 1.

SEQ ID NO:2 is a peptide mimetic of human IFN β 1.

5 **SEQ ID NO:3** is a peptide mimetic of **SEQ ID NO:1** that further comprises a lipophilic sequence on the N-terminus of the peptide.

SEQ ID NO:4 is a peptide mimetic of **SEQ ID NO:2** that further comprises a lipophilic sequence on the N-terminus of the peptide.

SEQ ID NO:5 is a peptide mimetic of human IFN α 2.

SEQ ID NO:6 is a peptide mimetic of human IFN α 4.

10 **SEQ ID NO:7** is the amino acid sequence of a peptide designated herein as MuIFN γ (95-132).

SEQ ID NO:8 is the amino acid sequence of a peptide designated herein as huIFN γ (95-134).

SEQ ID NO:9 is the amino acid sequence of a peptide designated herein as Tkip.

15 **SEQ ID NO:10** is the amino acid sequence of a peptide designated herein as SOCS1-KIR.

SEQ ID NO:11 is the full-length precursor human IFN α 1 amino acid sequence.

SEQ ID NO:12 is the full-length precursor human IFN β 1 amino acid sequence.

SEQ ID NO:13 is a histone H3 peptide.

20 **SEQ ID NO:14** is a primer for amplifying human OAS1 promoter region.

SEQ ID NO:15 is a primer for amplifying human OAS1 promoter region.

SEQ ID NO:16 is a primer for amplifying human β -actin promoter region.

SEQ ID NO:17 is a primer for amplifying human β -actin promoter region.

SEQ ID NO:18 is a nuclear localization sequence of IFNAR2.

25 **SEQ ID NOs:19-37** are cell-penetrating peptides that can be used in accordance with the subject invention.

DETAILED DESCRIPTION OF THE INVENTION

30 The subject invention pertains to agonist peptides of type I interferons and methods of using the peptides. These peptides are based on the amino acid sequence of the C-terminus region of the type I IFN molecules and are capable of binding to the

cytoplasmic domain of type I IFN receptors and activating the receptor. Surprisingly, these peptides were found to possess the same or similar biological activity as that associated with the full-length, mature type I IFN proteins, even though these peptides do not bind to the extracellular domain of the type I IFN receptors. In one embodiment, the peptide is a peptide of IFN α protein (*e.g.*, a human IFN α protein). In another embodiment, the peptide is a peptide of IFN β protein (*e.g.*, a human IFN β protein). The subject peptides have been shown to effect increased resistance to viral infection. Peptides of the invention can be used to treat or prevent viral infections, to treat oncological disorders, and to treat autoimmune disorders, such as multiple sclerosis. Peptides of the invention generally lack the side effects associated with use of full-length type I IFNs.

In an exemplified embodiment, the huIFN α 1 (69-189) peptide (SEQ ID NO:1) based on human IFN α 1 has an amino acid sequence corresponding to amino acid residues 69 through 189 of the full-length human IFN α 1 protein (SEQ ID NO:11). The huIFN β 1 (100-187) peptide (SEQ ID NO:2) based on human IFN β 1 has an amino acid sequence corresponding to amino acid residues 100 through 187 of the full-length human IFN β 1 protein (SEQ ID NO:12). In one embodiment, a peptide of the invention comprises a lipophilic sequence or moiety that facilitates penetration through a cell membrane for entry into a cell. In one embodiment, a peptide of the invention comprises one or more arginine or lysine amino acids at one or both termini of the peptide. In a specific embodiment, a peptide of the invention comprises one or more arginine amino acids at the N-terminus of the peptide. In an exemplified embodiment, a peptide of the invention has the amino acid sequence shown in SEQ ID NO:3 or SEQ ID NO:4. In another embodiment, a peptide of the invention has the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:6. In one embodiment, a peptide of the invention comprises a nuclear localization sequence (NLS). Peptides of the invention can be provided in purified or isolated form.

The discovery of peptide agonists of type I interferons is highly unexpected. Use of synthetic peptide agonists rather than the full-length type I IFN molecules offers advantages such as targeting of specific cells and immune system components. Also, specific amino acid residues of the peptides can be easily and rapidly modified to allow for generation of more effective agonists or antagonists.

As those skilled in the art can readily appreciate, there can be a number of variant sequences of a protein found in nature, in addition to those variants that can be artificially created by the skilled artisan in the lab. The peptides of the subject invention encompasses those specifically exemplified herein, as well as any natural variants thereof, as well as any variants which can be created artificially, so long as those variants retain the desired biological activity.

The peptides contemplated in the subject invention include the specific peptides exemplified herein as well as equivalent peptides which may be, for example, somewhat longer or shorter than the peptides exemplified herein. For example, using the teachings provided herein, a person skilled in the art could readily make peptides having from 1 to about 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70 or more amino acids added to, or removed from, either end of the disclosed peptides using standard techniques known in the art. In one embodiment, amino acids are removed from the N-terminus of a peptide of the invention. In a specific embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 50, 60, 70 or more amino acids can, independently, be removed from either or both ends of a peptide of the invention. Preferably, any added amino acids would be the same as the corresponding amino acids of a mature full-length type I IFN protein. The skilled artisan, having the benefit of the teachings disclosed in the subject application, could easily determine whether a variant peptide retained the biological activity of the specific peptides exemplified herein. Such a longer or shorter peptide would be within the scope of the subject invention as long as said peptide does not encompass the entire full-length IFN protein and said longer or shorter peptide retains substantially the same relevant biological activity as the peptides exemplified herein. For example, a longer or shorter variant of the huIFN α 1 (69-189) (SEQ ID NO:1) peptide would fall within the scope of the subject invention if the variant peptide had the ability to increase cellular resistance to viral infection.

Also within the scope of the subject invention are peptides which have the same amino acid sequences of a peptide exemplified herein except for amino acid substitutions, additions, or deletions within the sequence of the peptide, as long as these variant peptides retain substantially the same relevant biological activity as the peptides specifically exemplified herein. For example, conservative amino acid substitutions

within a peptide which do not affect the ability of the peptide to, for example, to increase cellular resistance to viral infection would be within the scope of the subject invention. Thus, the peptides disclosed herein should be understood to include variants and fragments, as discussed above, of the specifically exemplified sequences.

5 The subject invention further includes nucleotide sequences which encode the peptides disclosed herein. These nucleotide sequences can be readily constructed by those skilled in the art having the knowledge of the protein and peptide amino acid sequences which are presented herein. As would be appreciated by one skilled in the art, the degeneracy of the genetic code enables the artisan to construct a variety of nucleotide
10 sequences that encode a particular peptide or protein. The choice of a particular nucleotide sequence could depend, for example, upon the codon usage of a particular expression system.

 The subject invention contemplates the use of the peptides described herein in pharmaceutical compositions for administration to an animal or human for the treatment
15 of clinically important disease conditions that are amenable to treatment with full-length interferon. For example, using the teachings described herein, the skilled artisan can use the subject invention to modulate or stimulate the immune response of an animal or human. Similarly, the subject peptides can be used to treat certain viral infections, as well as to treat certain forms of cancer or tumors. The peptides of the subject invention can be
20 prepared in pharmaceutically acceptable carriers or diluents for administration to humans or animals in a physiologically tolerable form. Materials and methods for preparing such compositions are known in the art.

 The peptides of the subject invention can be administered using a variety of techniques that are known in the art. The peptides can be encapsulated in liposomes that
25 are targeted to specific cells or tissues and the liposome-encapsulated peptides delivered to the cells or tissue either *in vitro*, *in vivo*, or *ex vivo*. Procedures for preparing liposomes and encapsulating compounds within the liposome are well known in the art. See, for example, U.S. Patent No. 5,252,348, which issued to Schreier *et al.* Peptides can also be conjugated or attached to other molecules, such as an antibody, that targeted a
30 specific cell or tissue. Peptides can also be administered using a drug delivery system similar to that described in U.S. Patent No. 4,625,014, which issued to Senter *et al.*

As described herein, the peptide sequences of the subject invention can also be the basis for producing peptides that act as type I IFN antagonists. These antagonists are also within the scope of the subject invention. Inhibition or antagonism of interferon function without agonist activity can be accomplished through the use of anti-peptide antibodies or
5 modification of residues within the peptide itself. An especially productive means for generation of peptide antagonists has been substitution of L-amino acids with D-amino acids. The efficacy of this approach has been well characterized in the generation of arginine vasopressin analogs with selectively enhanced antidiuretic antagonism by appropriate substitution of L-amino acids with D-amino acids (Manning *et al.*, 1985).
10 Further, not only can antagonism be produced with D-amino acid substitutions, but this antagonism can be directed toward a specific function. Production of potent antagonist peptides can be of value in specifically manipulating immune function.

A further aspect of the claimed invention is the use of the claimed peptides to produce antibodies, both polyclonal and monoclonal. These antibodies can be produced
15 using standard procedures well known to those skilled in the art. These antibodies may be used as diagnostic and therapeutic reagents. For example, antibodies that bind to the human IFN α 1 (69-189) (SEQ ID NO:1) peptide may be used as an antagonist to block the function of IFN α . Antibodies that are reactive with the peptides of the subject invention can also be used to purify type I IFN protein or peptides from a crude mixture. In one
20 embodiment, an antibody binds specifically to the human IFN α 1(69-189) (SEQ ID NO:1) peptide. In another embodiment, an antibody binds specifically to the human IFN β 1(100-187) (SEQ ID NO:2) peptide.

An antibody that is contemplated by the present invention can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv,
25 Fab, and similar fragments, as well as a single chain antibody that includes the variable domain complementarity determining regions (CDR), and similar forms, all of which fall under the broad term "antibody," as used herein.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include
30 Fab, Fab', F(ab')₂ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily.

Pepsin treatment of an antibody yields an $F(ab')_2$ fragment that has two antigen binding fragments, which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "antigen binding fragment" with respect to antibodies, refers to, for example, Fv, F(ab) and $F(ab')_2$ fragments.

Antibody fragments can retain an ability to selectively bind with the antigen or analyte are contemplated within the scope of the invention and include:

(1) Fab is the fragment of an antibody that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

(2) Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

(3) $(Fab')_2$ is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction. $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds.

(4) Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H - V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain (V_L), the variable region of the heavy chain (V_H), linked by a suitable polypeptide linker as a genetically fused single chain

molecule. Such single chain antibodies are also referred to as “single-chain Fv” or “sFv” antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv fragments, see Pluckthun in The Pharmacology of
5 Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds. Springer-Verlag, N.Y., pp. 269 315 (1994).

Antibodies within the scope of the invention can be of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and
10 IgA2 subtypes.

Antibodies of the subject invention can be genus or species specific to a target. Antibodies of the invention can be prepared using standard techniques known in the art. Antibodies useful in the invention can be polyclonal or monoclonal antibodies. Monoclonal antibodies can be prepared using standard methods known in the art (Kohler
15 *et al.*, 1975). Antibodies of the invention can be mammalian antibodies, including mouse, rat, goat, rabbit, pig, dog, cat, monkey, chimpanzee, ape, or human.

The subject peptides can also be used in the design of new drugs that bind to the cytoplasmic domain of a type I IFN receptor. Knowledge of peptide sequences that induce type I IFN biological activity upon binding of the peptide to a localized region on
20 the type I IFN receptor enables a skilled artisan to develop additional bioactive compounds using rational drug design techniques. Thus, the skilled artisan can prepare both agonist and antagonist drugs using the teachings described herein.

The subject invention also concerns compositions comprising one or more peptides or polynucleotides of the invention. In one embodiment, a composition further
25 comprises a suitable carrier, diluent, or buffer. Compositions contemplated within the scope of the invention can comprise one or more peptides or polynucleotides of the invention and, optionally, one or more other antiviral compounds. For example, a peptide of the invention can be provided in a composition with one or more of IFN α , IFN β , IFN γ , acyclovir (Zovirax), zidovudine (AZT), lamivudine (3TC), zanamivir (Relenza),
30 oseltamivir (Tamiflu), valacyclovir (Valtrex), amantadine (Symmetrel), rimantadine (Flumadine), cidofovir (Vistide), foscarnet (Foscavir), ganciclovir (Cytovene), ribavirin (Virazole), nelfinavir (Viracept), ritonavir (Norvir), rifampin (Rifadin), and famciclovir

(Famvir). In one embodiment, the composition comprises a peptide of the invention in a pharmaceutically or physiologically acceptable carrier, buffer, or diluent. Compositions of the invention can also comprise additional peptides such as an IFN γ mimetic. Examples of IFN γ mimetic peptides are described in U.S. Patent Nos. 5,770,191 and 6,120,762. In one embodiment, the IFN γ mimetic peptide comprises the amino acid sequence shown in SEQ ID NO:7 (MuIFN γ (95-132)) or SEQ ID NO:8 (huIFN γ (95-134)), or a fragment or variant thereof that exhibits antiviral activity. In one embodiment, a composition of the invention can also comprise one or more peptides comprising the amino acid sequence shown in SEQ ID NO:9 (Tkip peptide), or a fragment or variant thereof that exhibits antiviral activity, and/or a peptide comprising the amino acid sequence shown in SEQ ID NO:10 (SOCS1-KIR), or a fragment or variant thereof that exhibits antiviral activity.

The methods of the invention contemplate that a peptide, polynucleotide, composition, or other agent of the invention is administered to the person or animal prior to infection by a virus. Also contemplated within the scope of the methods is that a peptide, polynucleotide, composition, or other agent of the invention is administered at the time of infection or after the person or animal has been infected. In one embodiment, a person or animal to be treated is one that has previously been vaccinated against infection by a virus, such as a poxvirus. In another embodiment, the person or animal has not been previously vaccinated against the virus.

In one embodiment, peptides and polynucleotides of the invention are modified so as to enhance uptake into a cell. In one embodiment, a lipophilic group is attached to a peptide, polynucleotide, or other agent of the invention. In one embodiment, a palmitic acid is attached to a peptide of the invention. In a specific embodiment, a palmitoyl-lysine group is attached to the peptide, for example at the N-terminus of the peptide. Other methods for enhancing uptake of a peptide or polynucleotide into a cell are known in the art and are contemplated within the scope of the invention.

Peptides, polynucleotides, compositions, and other agents of the invention can also be delivered into cells by encapsulation of the peptide, polynucleotide, and other agents of the invention within a liposome. Methods for encapsulation of peptides, polynucleotides, and other agents of the invention within liposomes are well known in the art.

Peptides having substitution of amino acids other than those specifically exemplified in the subject peptides are also contemplated within the scope of the present invention. For example, non-natural amino acids can be substituted for the amino acids of a peptide of the invention, so long as the peptide having substituted amino acids retains substantially the same activity as the peptide in which amino acids have not been substituted. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ -amino butyric acid, ϵ -amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ -butylglycine, τ -butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby a peptide having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the peptide having the substitution still retains substantially the same biological activity as a peptide that does not have the substitution. Table 1 below provides a listing of examples of amino acids belonging to each class.

Table 1.	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Single letter amino acid abbreviations are defined in Table 2.

Table 2.	
Letter Symbol	Amino Acid
A	Alanine
B	Asparagine or aspartic acid
C	Cysteine
D	Aspartic Acid
E	Glutamic Acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
W	Tryptophan
Y	Tyrosine
Z	Glutamine or glutamic acid

The peptides of the present invention can be formulated into pharmaceutically-acceptable salt forms. Pharmaceutically-acceptable salt forms include the acid addition salts and include hydrochloric, hydrobromic, nitric, phosphoric, carbonic, sulphuric, and organic acids like acetic, propionic, benzoic, succinic, fumaric, mandelic, oxalic, citric, tartaric, maleic, and the like. Pharmaceutically-acceptable base addition salts include sodium, potassium, calcium, ammonium, and magnesium salts. Pharmaceutically-acceptable salts of the peptides of the invention can be prepared using conventional techniques.

The subject invention also concerns polynucleotide expression constructs that comprise a polynucleotide of the present invention comprising a nucleotide sequence

encoding a peptide of the present invention. In one embodiment, the polynucleotide encodes a peptide comprising the amino acid sequence shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a fragment or variant thereof that exhibits antiviral activity.

5 As used herein, the term “expression construct” refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term “operably linked” refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in
10 contiguous relation.

Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in, for example, bacterial host cells, yeast host cells, plant host cells, insect host
15 cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a peptide of the invention.
20 Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted
25 without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. If the cells are mammalian cells, then promoters such as, for example, actin promoter,
30 metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early promoter and SV40 late promoter, Lck promoter, BMP5 promoter, TRP-1 promoter, murine mammary tumor

virus long terminal repeat promoter, STAT promoter, or an immunoglobulin promoter can be used in the expression construct. The baculovirus polyhedrin promoter can be used with an expression construct of the invention for expression in insect cells. Promoters suitable for use with an expression construct of the invention in yeast cells include, but are not limited to, 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase promoter, metallothionein promoter, alcohol dehydrogenase-2 promoter, and hexokinase promoter.

For expression in prokaryotic systems, an expression construct of the invention can comprise promoters such as, for example, alkaline phosphatase promoter, tryptophan (trp) promoter, lambda P_L promoter, β -lactamase promoter, lactose promoter, phoA promoter, T3 promoter, T7 promoter, or tac promoter (de Boer *et al.*, 1983).

If the expression construct is to be provided in a plant cell, plant viral promoters, such as, for example, the cauliflower mosaic virus (CaMV) 35S (including the enhanced CaMV 35S promoter (see, for example U.S. Patent No. 5,106,739)) or 19S promoter can be used. Plant promoters such as prolifera promoter, Ap3 promoter, heat shock promoters, T-DNA 1'- or 2'-promoter of *A. tumefaciens*, polygalacturonase promoter, chalcone synthase A (CHS-A) promoter from petunia, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter (Xu *et al.*, 1993), maize Wip1 promoter, maize trpA gene promoter (U.S. Patent No. 5,625,136), maize CDPK gene promoter, and RUBISCO SSU promoter (U.S. Patent No. 5,034,322) can also be used. Seed-specific promoters such as the promoter from a β -phaseolin gene (of kidney bean) or a glycinin gene (of soybean), and others, can also be used. Constitutive promoters (such as the CaMV, ubiquitin, actin, or NOS promoter), tissue-specific promoters (such as the E8 promoter from tomato), developmentally-regulated promoters, and inducible promoters (such as those promoters that can be induced by heat, light, hormones, or chemicals) are contemplated for use with the polynucleotides of the invention.

Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, signal peptide sequence, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient

termination. Signal peptides are a group of short amino terminal sequences that encode information responsible for the relocation of an operably linked peptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting a peptide to an intended cellular and/or extracellular destination through the use of operably linked signal peptide sequence is contemplated for use with the peptides of the invention. Chemical enhancers are cis-acting elements that increase gene transcription and can also be included in the expression construct. Chemical enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, cytomegalovirus (CMV) early promoter enhancer element, and the SV40 enhancer element. DNA sequences which direct polyadenylation of the mRNA encoded by the structural gene can also be included in the expression construct.

Unique restriction enzyme sites can be included at the 5' and 3' ends of the expression construct to allow for insertion into a polynucleotide vector. As used herein, the term "vector" refers to any genetic element, including for example, plasmids, cosmids, chromosomes, phage, virus, and the like, which is capable of replication when associated with proper control elements and which can transfer polynucleotide sequences between cells. Vectors contain a nucleotide sequence that permits the vector to replicate in a selected host cell. A number of vectors are available for expression and/or cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).

Polynucleotides, vectors, and expression constructs of the subject invention can be introduced into a cell by methods known in the art. Such methods include transfection, microinjection, electroporation, lipofection, cell fusion, calcium phosphate precipitation, and by biolistic methods. In one embodiment, a polynucleotide or expression construct of the invention can be introduced *in vivo* via a viral vector such as adeno-associated virus (AAV), herpes simplex virus (HSV), papillomavirus, adenovirus, and Epstein-Barr virus (EBV). Attenuated or defective forms of viral vectors that can be used with the subject invention are known in the art. Typically, defective virus is not capable of infection after the virus is introduced into a cell. Polynucleotides, vectors, and expression constructs of the invention can also be introduced *in vivo* via lipofection (DNA transfection via liposomes prepared from synthetic cationic lipids) (Felgner *et al.*, 1987). Synthetic

cationic lipids (LIPOFECTIN, Invitrogen Corp., La Jolla, CA) can be used to prepare liposomes to encapsulate a polynucleotide, vector, or expression construct of the invention. A polynucleotide, vector, or expression construct of the invention can also be introduced *in vivo* as naked DNA using methods known in the art, such as transfection, microinjection, electroporation, calcium phosphate precipitation, and by biolistic methods.

Polynucleotides and peptides of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. See NCBI/NIH website.

The subject invention also contemplates those polynucleotide molecules (encoding peptides of the invention) having sequences which are sufficiently homologous with the polynucleotide sequences encoding a peptide of the invention so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis, T. *et al.*, 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature (T_m) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A. *et al.*, 1983):

$T_m = 81.5^\circ\text{C} + 16.6 \log[\text{Na}^+] + 0.41(\%G+C) - 0.61(\% \text{ formamide}) - 600/\text{length of duplex in base pairs.}$

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).

(2) Once at $T_m - 20^\circ\text{C}$ for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

As used herein, the terms “nucleic acid” and “polynucleotide sequence” refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. The polynucleotide sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The polynucleotide sequences include both full-length sequences as well as shorter sequences derived from the full-length sequences.

It is understood that a particular polynucleotide sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell. The polynucleotide sequences falling within the scope of the subject invention further include sequences which specifically hybridize with the sequences coding for a peptide of the invention. The polynucleotide includes both the sense and antisense strands as either individual strands or in the duplex.

The subject invention also concerns methods for inducing an antiviral state in a cell. In one embodiment, a cell is contacted with an effective amount of a peptide, polynucleotide, or a composition of the invention. In one embodiment, the cell is not infected with a virus prior to contact with a peptide, polynucleotide, or composition of the invention. In another embodiment, the cell is already infected with a virus prior to contact with a peptide, polynucleotide, or composition of the invention. In one embodiment, the peptide has the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a fragment or variant thereof that exhibits antiviral activity. In one embodiment, the composition comprises a peptide of the invention and an antiviral drug and/or a IFN mimetic. The cell can be a human or mammalian cell. In one embodiment, the cell can be a keratinocyte, a fibroblast, a macrophage, or a lymphocyte. Peptides, polynucleotides, compositions,

and/or other agents of the invention can be delivered to a cell either through direct contact of peptide, *etc.* with the cell or via a carrier means. Carrier means for delivering compositions to cells are known in the art and include encapsulating the composition in a liposome moiety, and attaching the peptide or polynucleotide to a protein or nucleic acid that is targeted for delivery to the target cell. Published U.S. Patent Application Nos. 20030032594 and 20020120100 disclose amino acid sequences that can be coupled to another peptide, protein, or nucleic acid and that allows the peptide, protein, or nucleic acid to be translocated across biological membranes. Published U.S. Patent Application No. 20020035243 also describes compositions for transporting biological moieties, such as peptides and proteins across cell membranes for intracellular delivery. Peptides can also be delivered using a polynucleotide that encodes a subject peptide. In one embodiment, the polynucleotide is delivered to the cell where it is taken up and the polynucleotide is transcribed into RNA and the RNA is translated into the encoded peptide. Antiviral activity can be induced in a cell against viruses such as vaccinia virus, EMC virus, influenza virus, herpes simplex virus (*e.g.*, HSV-1), cytomegalovirus, herpes zoster virus, and other herpes viruses, poxvirus, coxsackie virus, lentivirus (*e.g.*, HIV), and picornavirus. Methods of the invention can be conducted *in vitro* or *in vivo*.

The subject invention also concerns methods for preventing or treating a viral infection and/or a viral associated disorder in a patient. In one embodiment, the disorder is hepatitis (*e.g.*, caused by hepatitis B or hepatitis C virus). In one embodiment, an effective amount of a peptide, polynucleotide, and/or composition of the present invention is administered to a patient having a viral infection and who is in need of treatment thereof. In another embodiment, the patient is not yet infected with a virus or does not yet have a viral associated disorder. Optionally, the patient is a person or animal at risk of virus infection or at risk of developing a viral associated disorder. In one embodiment, the peptide has the amino acid sequence in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a fragment or variant thereof that exhibits antiviral activity. Methods of the invention can also further comprise administering one or more compounds useful for treating a viral infection or viral associated disorder. Such compounds can be administered prior to, in conjunction with, and/or subsequent to administration of a peptide, polynucleotide, and/or composition of the present invention. The patient can be a human or other mammal, such as a dog, cat,

or horse, or other animals having the disorder. Means for administering and formulating peptides and polynucleotides for administration to a patient are known in the art, examples of which are described herein. Peptides, polynucleotides, and/or compositions of the invention can be delivered to a cell either through direct contact of peptide, polynucleotide, or composition with the cell or via a carrier means. In one embodiment, a peptide, polynucleotide, or composition of the invention comprises an attached group that enhances cellular uptake of the peptide. In one embodiment, the peptide, polynucleotide, or composition is attached to an antibody that binds to a targeted cell. In another embodiment, the peptide, polynucleotide, or composition is encapsulated in a liposome. Peptides can also be delivered using a polynucleotide that encodes a subject peptide. Any polynucleotide having a nucleotide sequence that encodes a peptide of the invention is contemplated within the scope of the invention. In one embodiment, the polynucleotide is delivered to the cell where it is taken up and the polynucleotide is transcribed into RNA and the RNA is translated into the encoded peptide. Examples of viruses whose replication can be inhibited using the present invention include, but are not limited to, herpes viruses, poxviruses, and picornaviruses, such as vaccinia virus, EMC virus, influenza virus, herpes zoster virus, cytomegalovirus, and herpes simplex virus (*e.g.*, HSV-1).

For the treatment of viral infections, the peptides, polynucleotides, and compositions of this invention can be administered to a patient in need of treatment in combination with other antiviral substances. These other antiviral substances may be given at the same or different times as the peptides, polynucleotides, and compositions of this invention. For example, the peptides, polynucleotides, and compositions of the present invention can be used in combination with one or more viral inhibitors such as interferons, and/or other drugs or antibodies, such as IFN α , IFN β , IFN γ , acyclovir (Zovirax), zidovudine (AZT), lamivudine (3TC), zanamivir (Relenza), oseltamivir (Tamiflu), valacyclovir (Valtrex), amantadine (Symmetrel), rimantadine (Flumadine), cidofovir (Vistide), foscarnet (Foscavir), ganciclovir (Cytovene), ribavirin (Virazole), nelfinavir (Viracept), ritonavir (Norvir), rifampin (Rifadin), and famciclovir (Famvir).

The subject invention also concerns methods for treating an oncological disorder or an autoimmune disorder in a patient. In one embodiment, an effective amount of a peptide, polynucleotide, or composition of the present invention that is an agonist of a

type I IFN protein is administered to a patient having an oncological disorder or an autoimmune disorder and who is in need of treatment thereof. The subject invention also concerns methods for inhibiting the growth of a cancer cell by contacting the cell *in vitro* or *in vivo* with an effective amount of a peptide, polynucleotide, or composition of the present invention. The subject invention also concerns methods for activating an immune cell (*e.g.*, T cell, NK cell, macrophage, *etc.*), and/or upregulating antigen presentation to lymphocytes, and/or upregulating major histocompatibility complex (MHC) molecules, and/or activating a JAK/STAT pathway, and/or activating TYK2 in a cell by contacting the cell *in vitro* or *in vivo* with an effective amount of a peptide, polynucleotide, or composition of the present invention. In one embodiment, the peptide has the amino acid sequence in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a fragment or variant thereof that exhibits anticancer activity. Methods of the invention can also further comprise administering or contacting a cell with one or more compounds for treating an oncological or autoimmune disorder. Such compounds can be administered prior to, in conjunction with, and/or subsequent to administration of a peptide, polynucleotide, and/or composition of the present invention. Methods of the invention can optionally include identifying a patient who is or may be in need of treatment of an oncological or autoimmune disorder. The patient can be a human or other mammal, such as a primate (monkey, chimpanzee, ape, *etc.*), dog, cat, cow, pig, or horse, or other animals having an oncological disorder. Means for administering and formulating peptides, polynucleotides, or compositions of the invention for administration to a patient are known in the art, examples of which are described herein. Autoimmune disorders within the scope of the invention include, but are not limited to, multiple sclerosis and rheumatoid arthritis. In one embodiment, a huIFN β 1(100-187) (SEQ ID NO:2) peptide, or a polynucleotide encoding the peptide, is used to treat a person or animal having multiple sclerosis. Oncological disorders within the scope of the invention include, but are not limited to, cancer and/or tumors of the anus, bile duct, bladder, bone, bone marrow, bowel (including colon and rectum), breast, eye, gall bladder, kidney, mouth, larynx, esophagus, stomach, testis, cervix, head, neck, ovary, lung, mesothelioma, neuroendocrine, penis, skin, spinal cord, thyroid, vagina, vulva, uterus, liver, muscle, pancreas, prostate, blood cells (including lymphocytes and other immune system cells), and brain. Specific cancers contemplated for treatment with the present invention include

carcinomas, Kaposi's sarcoma, melanoma, mesothelioma, soft tissue sarcoma, pancreatic cancer, lung cancer, leukemia (hairy cell, acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myeloid, and other), and lymphoma (Hodgkin's and non-Hodgkin's), and follicular lymphoma, and multiple myeloma. In one embodiment, a
 5 huIFN α 2 (SEQ ID NO:5) peptide, or a polynucleotide encoding the peptide, is used to treat a person or animal having a melanoma.

Examples of cancers that can be treated according to the present invention are listed in Table 3.

10 Table 3. Examples of Cancer Types

Acute Lymphoblastic Leukemia, Adult	Hairy Cell Leukemia
Acute Lymphoblastic Leukemia, Childhood	Head and Neck Cancer
Acute Myeloid Leukemia, Adult	Hepatocellular (Liver) Cancer, Adult (Primary)
Acute Myeloid Leukemia, Childhood	Hepatocellular (Liver) Cancer, Childhood (Primary)
Adrenocortical Carcinoma	Hodgkin's Lymphoma, Adult
Adrenocortical Carcinoma, Childhood	Hodgkin's Lymphoma, Childhood
AIDS-Related Cancers	Hodgkin's Lymphoma During Pregnancy
AIDS-Related Lymphoma	Hypopharyngeal Cancer
Anal Cancer	Hypothalamic and Visual Pathway Glioma, Childhood
Astrocytoma, Childhood Cerebellar	
Astrocytoma, Childhood Cerebral	
Basal Cell Carcinoma	Intraocular Melanoma
Bile Duct Cancer, Extrahepatic	Islet Cell Carcinoma (Endocrine Pancreas)
Bladder Cancer	
Bladder Cancer, Childhood	Kaposi's Sarcoma
Bone Cancer, Osteosarcoma/Malignant	Kidney (Renal Cell) Cancer
Fibrous Histiocytoma	Kidney Cancer, Childhood
Brain Stem Glioma, Childhood	
Brain Tumor, Adult	Laryngeal Cancer
Brain Tumor, Brain Stem Glioma, Childhood	Laryngeal Cancer, Childhood
Brain Tumor, Cerebellar Astrocytoma, Childhood	Leukemia, Acute Lymphoblastic, Adult
Brain Tumor, Cerebral	Leukemia, Acute Lymphoblastic, Childhood
Astrocytoma/Malignant Glioma, Childhood	Leukemia, Acute Myeloid, Adult
Brain Tumor, Ependymoma, Childhood	Leukemia, Acute Myeloid, Childhood
Brain Tumor, Medulloblastoma, Childhood	Leukemia, Chronic Lymphocytic
Brain Tumor, Supratentorial Primitive	Leukemia, Chronic Myelogenous
	Leukemia, Hairy Cell
	Lip and Oral Cavity Cancer
	Liver Cancer, Adult (Primary)
	Liver Cancer, Childhood (Primary)

Neuroectodermal Tumors, Childhood Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood Brain Tumor, Childhood Breast Cancer Breast Cancer, Childhood Breast Cancer, Male Bronchial Adenomas/Carcinoids, Childhood Burkitt's Lymphoma Carcinoid Tumor, Childhood Carcinoid Tumor, Gastrointestinal Carcinoma of Unknown Primary Central Nervous System Lymphoma, Primary Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma/Malignant Glioma, Childhood Cervical Cancer Childhood Cancers Chronic Lymphocytic Leukemia Chronic Myelogenous Leukemia Chronic Myeloproliferative Disorders Colon Cancer Colorectal Cancer, Childhood Cutaneous T-Cell Lymphoma, see Mycosis Fungoides and Sézary Syndrome Endometrial Cancer Ependymoma, Childhood Esophageal Cancer Esophageal Cancer, Childhood Ewing's Family of Tumors Extracranial Germ Cell Tumor, Childhood Extragonadal Germ Cell Tumor Extrahepatic Bile Duct Cancer Eye Cancer, Intraocular Melanoma Eye Cancer, Retinoblastoma Gallbladder Cancer Gastric (Stomach) Cancer Gastric (Stomach) Cancer, Childhood Gastrointestinal Carcinoid Tumor Germ Cell Tumor, Extracranial,	Lung Cancer, Non-Small Cell Lung Cancer, Small Cell Lymphoma, AIDS-Related Lymphoma, Burkitt's Lymphoma, Cutaneous T-Cell, see Mycosis Fungoides and Sézary Syndrome Lymphoma, Hodgkin's, Adult Lymphoma, Hodgkin's, Childhood Lymphoma, Hodgkin's During Pregnancy Lymphoma, Non-Hodgkin's, Adult Lymphoma, Non-Hodgkin's, Childhood Lymphoma, Non-Hodgkin's During Pregnancy Lymphoma, Primary Central Nervous System Macroglobulinemia, Waldenström's Malignant Fibrous Histiocytoma of Bone/Osteosarcoma Medulloblastoma, Childhood Melanoma Melanoma, Intraocular (Eye) Merkel Cell Carcinoma Mesothelioma, Adult Malignant Mesothelioma, Childhood Metastatic Squamous Neck Cancer with Occult Primary Multiple Endocrine Neoplasia Syndrome, Childhood Multiple Myeloma/Plasma Cell Neoplasm Mycosis Fungoides Myelodysplastic Syndromes Myelodysplastic/Myeloproliferative Diseases Myelogenous Leukemia, Chronic Myeloid Leukemia, Adult Acute Myeloid Leukemia, Childhood Acute Myeloma, Multiple Myeloproliferative Disorders, Chronic Nasal Cavity and Paranasal Sinus Cancer Nasopharyngeal Cancer Nasopharyngeal Cancer, Childhood Neuroblastoma Non-Hodgkin's Lymphoma, Adult Non-Hodgkin's Lymphoma, Childhood Non-Hodgkin's Lymphoma During Pregnancy Non-Small Cell Lung Cancer
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Childhood Germ Cell Tumor, Extragonadal Germ Cell Tumor, Ovarian Gestational Trophoblastic Tumor Glioma, Adult Glioma, Childhood Brain Stem Glioma, Childhood Cerebral Astrocytoma Glioma, Childhood Visual Pathway and Hypothalamic Skin Cancer (Melanoma) Skin Carcinoma, Merkel Cell Small Cell Lung Cancer Small Intestine Cancer Soft Tissue Sarcoma, Adult Soft Tissue Sarcoma, Childhood Squamous Cell Carcinoma, see Skin Cancer (non-Melanoma) Squamous Neck Cancer with Occult Primary, Metastatic Stomach (Gastric) Cancer Stomach (Gastric) Cancer, Childhood Supratentorial Primitive Neuroectodermal Tumors, Childhood T-Cell Lymphoma, Cutaneous, see Mycosis Fungoides and Sézary Syndrome Testicular Cancer Thymoma, Childhood Thymoma and Thymic Carcinoma Thyroid Cancer Thyroid Cancer, Childhood Transitional Cell Cancer of the Renal Pelvis and Ureter Trophoblastic Tumor, Gestational Unknown Primary Site, Carcinoma of, Adult Unknown Primary Site, Cancer of, Childhood Unusual Cancers of Childhood Ureter and Renal Pelvis, Transitional Cell Cancer Urethral Cancer Uterine Cancer, Endometrial	Oral Cancer, Childhood Oral Cavity Cancer, Lip and Oropharyngeal Cancer Osteosarcoma/Malignant Fibrous Histiocytoma of Bone Ovarian Cancer, Childhood Ovarian Epithelial Cancer Ovarian Germ Cell Tumor Ovarian Low Malignant Potential Tumor Pancreatic Cancer Pancreatic Cancer, Childhood Pancreatic Cancer, Islet Cell Paranasal Sinus and Nasal Cavity Cancer Parathyroid Cancer Penile Cancer Pheochromocytoma Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors, Childhood Pituitary Tumor Plasma Cell Neoplasm/Multiple Myeloma Pleuropulmonary Blastoma Pregnancy and Breast Cancer Pregnancy and Hodgkin's Lymphoma Pregnancy and Non-Hodgkin's Lymphoma Primary Central Nervous System Lymphoma Prostate Cancer Rectal Cancer Renal Cell (Kidney) Cancer Renal Cell (Kidney) Cancer, Childhood Renal Pelvis and Ureter, Transitional Cell Cancer Retinoblastoma Rhabdomyosarcoma, Childhood Salivary Gland Cancer Salivary Gland Cancer, Childhood Sarcoma, Ewing's Family of Tumors Sarcoma, Kaposi's Sarcoma, Soft Tissue, Adult Sarcoma, Soft Tissue, Childhood Sarcoma, Uterine Sézary Syndrome Skin Cancer (non-Melanoma) Skin Cancer, Childhood
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Uterine Sarcoma	
Vaginal Cancer	
Visual Pathway and Hypothalamic Glioma, Childhood	
Vulvar Cancer	
Waldenström's Macroglobulinemia	
Wilms' Tumor	

For the treatment of oncological disorders, the peptides, polynucleotides, and compositions of this invention can be administered to a patient in need of treatment in combination with other antitumor or anticancer substances and/or with radiation and/or photodynamic therapy and/or with surgical treatment to remove a tumor. These other substances or treatments may be given at the same as or at different times from the peptides, polynucleotides, and compositions of this invention. For example, the peptides, polynucleotides, and compositions of the present invention can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosphamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies, such as, for example, GLEEVEC (Novartis Pharmaceuticals Corporation) and HERCEPTIN (Genentech, Inc.), respectively. Peptides, polynucleotides, and compositions of the invention can be used in combination with proteasome inhibitors, including, but not limited to, Bortezomib, Carfilzomib, and Salinosporamide A. The subject invention also concerns methods for inhibiting the growth of a cancer cell by contacting the cell *in vitro* or *in vivo* with an effective amount of a peptide, polynucleotide, or composition of the present invention.

Many tumors and cancers have viral genome present in the tumor or cancer cells. For example, Epstein-Barr Virus (EBV) is associated with a number of mammalian malignancies. The peptides, polynucleotides, and compositions of the subject invention can also be used alone or in combination with anticancer or antiviral agents, such as ganciclovir, azidothymidine (AZT), lamivudine (3TC), *etc.*, to treat patients infected with

a virus that can cause cellular transformation and/or to treat patients having a tumor or cancer that is associated with the presence of viral genome in the cells.

The methods of the present invention can be used with humans and other animals. The other animals contemplated within the scope of the invention include domesticated,
5 agricultural, or zoo- or circus-maintained animals. Domesticated animals include, for example, dogs, cats, rabbits, ferrets, guinea pigs, hamsters, pigs, monkeys or other primates, and gerbils. Agricultural animals include, for example, horses, mules, donkeys, burros, cattle, cows, pigs, sheep, and alligators. Zoo- or circus-maintained animals include, for example, lions, tigers, bears, camels, giraffes, hippopotamuses, and
10 rhinoceroses.

In one embodiment, one or more of the peptides of the subject invention can be provided in the form of a multiple peptide construct. Such a construct can be designed so that multiple peptides are linked to each other by intervening moieties wherein the intervening moieties are subsequently cleaved or removed following administration of the
15 multiple peptide construct to a patient. Methods for constructing multiple peptide constructs are known in the art. For example, peptides of the present invention can be provided in the form of a multiple antigenic peptide (MAP) construct. The preparation of MAP constructs has been described in Tam (1988). MAP constructs utilize a core matrix of lysine residues onto which multiple copies of an immunogen are synthesized. Multiple
20 MAP constructs, each containing different peptides, can be prepared and administered in accordance with methods of the present invention. In another embodiment, a multiple peptide construct can be prepared by preparing the subject peptides having at least one metal chelating amino acid incorporated therein, preferably at the amino and/or carboxy terminal of the peptide as described, for example, in U.S. Patent No. 5,763,585. The
25 peptides are then contacted with a solid support having attached thereto a metal ion specific for the metal chelating amino acid of the peptide. A multiple peptide construct of the invention can provide multiple copies of the exact same peptide, including variants or fragments of a subject peptide, or copies of different peptides of the subject invention.

Therapeutic application of the subject peptides, polynucleotides, and compositions
30 containing them, can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. The peptides and polynucleotides can be administered by any suitable route known in the art including, for

example, oral, nasal, rectal, parenteral, subcutaneous, or intravenous routes of administration. Administration of the peptides and polynucleotides of the invention can be continuous or at distinct intervals as can be readily determined by a person skilled in the art.

5 Compounds and compositions useful in the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the
10 subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive peptide or polynucleotide is combined with a suitable carrier in order to facilitate effective administration of the composition. The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as
15 tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the subject peptides and
20 polynucleotides include, but are not limited to, water, saline, oils including mineral oil, ethanol, dimethyl sulfoxide, gelatin, cyclodextrans, magnesium stearate, dextrose, cellulose, sugars, calcium carbonate, glycerol, alumina, starch, and equivalent carriers and diluents, or mixtures of any of these. Formulations of the peptide or polynucleotide of the invention can also comprise suspension agents, protectants, lubricants, buffers,
25 preservatives, and stabilizers. To provide for the administration of such dosages for the desired therapeutic treatment, pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 45%, and especially, 1 and 15% by weight of the total of one or more of the peptide or polynucleotide based on the weight of the total composition including carrier or diluent.

30 The peptides, polynucleotides, and compositions of the subject invention can also be administered utilizing liposome technology, slow release capsules, implantable pumps,

and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time.

The subject peptides and polynucleotides can also be modified by the addition of chemical groups, such as PEG (polyethylene glycol). PEGylated peptides typically generate less of an immunogenic response and exhibit extended half-lives *in vivo* in comparison to peptides that are not PEGylated when administered *in vivo*. Methods for PEGylating proteins and peptides known in the art (see, for example, U.S. Patent No. 4,179,337). The subject peptides and polynucleotides can also be modified to improve cell membrane permeability. In one embodiment, cell membrane permeability can be improved by attaching a lipophilic moiety, such as a steroid, to the peptide or polynucleotide. In another embodiment, peptides and polynucleotides of the invention comprise a cell-penetrating peptide (CPP). CPPs are typically short peptides that are highly cationic and typically include several arginine and/or lysine amino acids. CPPs can be classified as hydrophilic, amphiphilic, or periodic sequence. In one embodiment, a CPP is provided at the terminus of a peptide or polynucleotide. Examples of CPPs include, but are not limited to penetratin or antenapedia PTD (RQIKWFQNRRMKWKK) (SEQ ID NO:19), TAT (YGRKKRRQRRR) (SEQ ID NO:20), SynB1 (RGGRLSYSRRRFSTSTGR) (SEQ ID NO:21), SynB3 (RRLSYSRRRF) (SEQ ID NO:22), PTD-4 (PIRRRKKLRLK) (SEQ ID NO:23), PTD-5 (RRQRRTSKLMKR) (SEQ ID NO:24), FHV Coat-(35-49) (RRRRNRTRNRRRVR) (SEQ ID NO:25), BMV Gag-(7-25) (KMTRAQRRAAARRNRWTAR) (SEQ ID NO:26), HTLV-II Rex-(4-16) (TRRQRTRRRARRNR) (SEQ ID NO:27), D-Tat (GRKKRRQRRRPPQ) (SEQ ID NO:28), R9-Tat (GRRRRRRRRRPPQ) (SEQ ID NO:29), Transportan (GWTLSAGYLLGKINLKALAALAKKIL) (SEQ ID NO:30) chimera, MAP (KLALKLALKLALALKLA) (SEQ ID NO:31), SBP (MGLGLHLLVLAALQGAWSQPKKKRKV) (SEQ ID NO:32), FBP (GALFLGWLGAAGSTMGAWSQPKKKRKV) (SEQ ID NO:33), MPG (ac-GALFLGFLGAAGSTMGAWSQPKKKRKV-cya) (SEQ ID NO:34), MPG^(ΔNLS) (ac-GALFLGFLGAAGSTMGAWSQPKSKRKV-cya) (SEQ ID NO:35), Pep-1 (ac-KETWWETWWTEWSQPKKKRKV-cya) (SEQ ID NO:36), and Pep-2 (ac-KETWFETWFTEWSQPKKKRKV-cya) (SEQ ID NO:37). Other CPPs can have only arginine (R) or only lysine (K) amino acids, *e.g.*, having a formula (R)_n or (K)_n, where

n = an integer from 3 to 20. Other groups known in the art for providing for cell membrane permeability can be linked to peptides and polynucleotides of the present invention.

The subject invention also concerns a packaged dosage formulation comprising in one or more containers at least one peptide, polynucleotide, and/or composition of the subject invention formulated in a pharmaceutically acceptable dosage. The package can contain discrete quantities of the dosage formulation, such as tablet, capsules, lozenge, and powders. The quantity of peptide and/or polynucleotide in a dosage formulation and that can be administered to a patient can vary from about 1 mg to about 5000 mg, or about 1 mg to about 2000 mg, or more typically about 1 mg to about 500 mg, or about 5 mg to about 250 mg, or about 10 mg to about 100 mg.

The subject invention also concerns kits comprising one or more peptides, polynucleotides, compositions, compounds, or molecules of the present invention in one or more containers. In one embodiment, a kit contains a peptide, polynucleotide, and/or composition of the present invention. In a specific embodiment, a kit comprises a peptide comprising the amino acid sequence shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or a fragment or variant of the peptide that exhibits antiviral activity. A kit of the invention can also comprise one or more antiviral compounds, biological molecules, or drugs and/or one or more type I IFN peptide mimetics. In one embodiment, the biological molecule is one or more of IFN α , IFN β , or IFN γ . In one embodiment, in addition to a peptide, polynucleotide, composition, or compound of the invention, a kit also comprises one or more peptides of SEQ ID NO:7 (MuIFN γ (95-132)) and/or SEQ ID NO:8 (huIFN γ (95-134)), and/or SEQ ID NO:9 (Tkip peptide), and/or SEQ ID NO:10, or a fragment or variant thereof that exhibits antiviral activity. In one embodiment, a kit comprises one or more of IFN α , IFN β , IFN γ , acyclovir (Zovirax), zidovudine (AZT), lamivudine (3TC), zanamivir (Relenza), oseltamivir (Tamiflu), valacyclovir (Valtrex), amantadine (Symmetrel), rimantadine (Flumadine), cidofovir (Vistide), foscarnet (Foscavir), ganciclovir (Cytovene), ribavirin (Virazole), nelfinavir (Viracept), ritonavir (Norvir), rifampin (Rifadin), and famciclovir (Famvir). In another embodiment, a kit comprises one or more of mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosphamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea,

DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies, such as, for example, GLEEVEC (Novartis Pharmaceuticals Corporation) and HERCEPTIN (Genentech, Inc.),
5 respectively.

In one embodiment, a kit of the invention includes instructions or packaging materials that describe how to administer a peptide, polynucleotide, compositions, compounds, or molecules of the kit. Containers of the kit can be of any suitable material, *e.g.*, glass, plastic, metal, *etc.*, and of any suitable size, shape, or configuration. In one
10 embodiment, a peptide, polynucleotide, compositions, compounds, or molecules of the invention is provided in the kit as a solid, such as a tablet, pill, or powder form. In another embodiment, a peptide, polynucleotide, compositions, compounds, or molecules of the invention is provided in the kit as a liquid or solution. In one embodiment, the kit comprises an ampoule or syringe containing a peptide, polynucleotide, compositions,
15 compounds, or molecules of the invention in liquid or solution form.

The subject invention also concerns methods for inhibiting type I IFN cell activation and/or intracellular signaling. In one embodiment, an inhibitor of an IFNAR is provided in a cell. In a further embodiment, the IFNAR is IFNAR1 or IFNAR2. The cell can be a mammalian cell, such as a human cell. In one embodiment, the cell is infected
20 with a virus. Any suitable inhibitor that can inhibit the function of an IFNAR is contemplated within the scope of the invention. Examples of inhibitors include, but are not limited to, antibodies (and antigen binding fragments thereof) and other compounds or agents that bind to an IFNAR.

25 MATERIALS AND METHODS FOR FIGURES 1-3

Cells and virus

L929 and BSC-40 cells were obtained from ATCC and propagated on DMEM with 10 % FBS. Cells were grown at 37 °C in a humidified atmosphere with 5 % CO₂. Vaccinia virus was a gift from Dr. Richard Condit (University of Florida, Gainesville,
30 FL). Vaccinia virus was grown and titrated on BSC-40 cells, and purified on sucrose gradient.

Antiviral assay for encephalomyocarditis (EMC) virus were carried out on L929 cells. L929 cells were seeded in a microtiter plate at 40,000 cells per well and allowed to grow overnight. These cells were treated with IFN α (69-189) at 5 μ g or 1 μ g per ml for 1 hour, followed by infection with EMCV (0.01 moi). Cells were allowed to grow for one day, stained with crystal violet and absorption was recorded in Bio-Tek plate reader.

Antiviral assay for vaccinia virus was done on BSC-40 cells. Cells were seeded to near confluence in a 12 well plate, and treated with 10 μ M or 2 μ M of IFN α (69-189)R9 or a control peptide without R9, for 1 hour, followed by infection with vaccinia virus (0.01 moi) for 1 hour. Cells were washed and grown overnight. Cells were stained with crystal violet and the plaques were counted.

Peptide expression in pET30 vector

IFN α (69-189)R9 or IFN α (69-189) coding sequence was inserted in the bacterial expression plasmid pET30a(+). *E. coli* (Rosetta strain) was transformed with these plasmids. Induction was with 200 μ M IPTG for 4 hours. Proteins were separated on a Sephadex G-50 column. Purity of the peptide was ascertained after SDS-PAGE and staining.

EAE in mice

Experimental allergic encephalomyelitis (EAE) is the mouse model for multiple sclerosis (MS). IACUC at the University of Florida approved the animal protocol mentioned herein. Female SJL/J mice (6 to 8 weeks) were purchased from Jackson Laboratories (Bar Harbor, ME). On day 1, mice were injected with 300 μ g per mouse bovine myelin basic protein (Invitrogen, Carlsbad, CA) emulsified in complete Freund's adjuvant with 8 mg/ml H37Ra Mycobacterium tuberculosis, subcutaneously into two sites at the base of the tail and 400 ng/mouse of pertussis toxin was delivered i.p. On day 3, the pertussis injection was repeated. On day 12, treatment with IFN α (69-189)R9 or the control peptide (15 μ g per mouse, i.p.) on alternate days was started. Mice were monitored daily for signs of EAA and graded as follows: 0, normal; 1, loss of tail tone, 2, hind limb weakness; 3, paraparesis; 4, paraplegia; 5, moribund, 6, death.

MATERIALS AND METHODS FOR EXAMPLES 1-6

Cell culture and Abs

WISH and Daudi cells were purchased from American Type Culture Collection (ATCC) and were grown in MEME and RPMI (Sigma-Aldrich), respectively, with 10% FBS and antibiotics. For all experiments, cells were serum starved for at least 4 hours, washed twice with PBS and then given serum free media with or without 1,000 U/ml IFN α 2 (Calbiochem) or IFN. The following polyclonal antisera were purchased from Santa Cruz Biotech: IFNAR1, IFNAR2, STAT1, pSTAT1, STAT2, pSTAT2, TYK2, pTYK2, normal rabbit IgG, β -Tubulin, β -Lamin, and Histone H3. The following polyclonal antisera were purchased from Active Motif: H3K9ac and H3K9me3. Additional Abs to TYK2 and IFNAR1 were also purchased from BD Bioscience and Epitomics, respectively. We produced the antibody to tyrosine phosphorylated histone H3 by immunization of rabbits with histone H3 peptide, ³³GGVKKPHRpYRPGTVALREIR (SEQ ID NO:13), with a phosphate at tyrosine 41. We tested antibodies to some proteins from different sources to monitor the specificity.

Chromatin immunoprecipitation (ChIP) assay

WISH cells were treated or not with type I IFN for 1 hr. Cells were then washed twice with cold PBS and treated with 1% formaldehyde for 10 min at 37°C. The rest of the procedure was conducted using the ChIP kit from Millipore, as per the manufacturer's protocol. Sonication was conducted to get DNA fragments of ~500 bp. Control IgG, or different Abs, were used for each immunoprecipitation as indicated. DNA fragments eluted were used for PCR with the following primers that spanned the ISRE element in their promoters. Human OAS1 promoter region was amplified with the primers 5'-CATTGACAGGAGAGAGAGTG-3' (SEQ ID NO:14) (-147 to -133) and 5'-TCAGGGGAGTGTCTGATTTG-3' (SEQ ID NO:15) (-17 to +3). As a control, PCR was conducted with the primers from the human β -actin promoter 5'-CTCGCTCTCGCTCTTTTTTTTTTTC-3' (SEQ ID NO:16) (-967 to -941) and 5'-CTCGAGCCATAAAAGGCAACT-3' (SEQ ID NO:17) (-844 to -864). The PCR conditions were as follows: heating at 94°C for 5 min, followed by 35 cycles at 94°C for 15 sec, 60°C for 30 sec, and 68°C for 15 sec. This was followed by annealing at 68°C for

5 min. Following ChIP with the indicated Abs, the DNA protein complex was used to elute the associated proteins by boiling with the electrophoresis buffer and was analyzed by Western blotting as mentioned before (Noon-Song *et al.*, 2011).

5 *Isolation of nuclei*

IFN treated WISH cells were washed twice in cold PBS, removed by scraping in lysis buffer (10 mM HEPES pH 7.9, 100 mM KCl, 1% Triton X-100, 1 mM NaF, 1 mM Na₃VO₄, 2 mM MgCl₂, 1 mM DTT, and 1 mM PMSF), and pelleted by low speed centrifugation. The supernatant was saved as cytoplasmic fraction. The pellet containing
10 intact nuclei, was gently resuspended in lysis buffer. The centrifugation, re-suspension, and decanting was then repeated twice more. Isolated nuclei were confirmed by trypan blue staining.

Western Blot analysis and immunoprecipitation

15 Cells were washed with PBS and harvested in lysis buffer (10 mM HEPES pH 7.9, 100 mM KCl, 1% Triton X-100, 1 mM NaF, 1 mM Na₃VO₄, 2 mM MgCl₂, 1 mM DTT, and 1 mM PMSF). Whole cell lysate was generated via sonication on ice and insoluble material removed via centrifugation at 14k rpm for 10 min at 4°C. Protein concentration was measured using 660 nm protein assay reagent (Pierce). Protein (10 µg
20 each) was electrophoresed on an acrylamide gel, transferred to PVDF membrane, and probed with the indicated Abs. HRP-conjugated secondary Abs were then added and detection was conducted by chemiluminescence (Pierce). Immunoprecipitation was conducted by incubating specific Abs with equal amounts of lysate, followed by incubation with Protein A-Agarose (Santa Cruz Biotech) for at least 2 hours. Precipitated
25 material was sedimented and washed thrice with PBS. Pellets were taken in electrophoresis buffer, boiled and loaded on an acrylamide gel, transferred and probed with antibodies indicated.

Expression and purification of type I IFN mimetics

30 Type I IFN mimetics were expressed as follows. The coding sequence for human IFNα1, IFNα1(69-189), preceded by nine arginine (R9) residues (for cell penetration) was inserted in the bacterial expression vector, pET30a+. The coding sequence for human

IFN β , IFN β (100-187) preceded by R9 was similarly inserted into pET30a+. As controls, the human IFN α 1(69-189) or IFN β (100-187) without the R9 were also inserted in pET30a+. *E. coli* BL21 (DE3) Rosetta strain was used to transform the expression sequence in pET30a+. After the bacterial growth had reached the mid-log phase, induction with 0.5 mM IPTG was carried out and growth continued for 4 hours. The proteins were purified by using the Ni-NTA His Bind Resin (Novagen). The His tag was removed by digesting with enterokinase. The purity of the protein was assessed by SDS-PAGE analysis and coomassie blue staining.

10 *GFP fusion constructs and microscopy*

The coding sequence from IFN τ , IFNAR1, or IFNAR2 was used to generate a PCR product that was fused in frame with the C terminus of humanized rGFP in the plasmid pHRGFPII-C (Stratagene). WISH cells that were grown on coverslips to near 30% confluency in a 35 mm dish were transfected using lipofectamine (Invitrogen Life Technologies), with 3 μ g of the empty vector or the IFN τ fused GFP vector. IFNAR1 or IFNAR2 sequences fused to GFP were similarly transfected. Where indicated, IFN τ was added at 1,000 U/ml. Next day, cells were fixed with 2% paraformaldehyde in PBS, mounted on a slide, and viewed in a Zeiss Axiovert Zoom confocal microscope using LSM Pascal software, as described before (Ahmed and Johnson, 2006).

20

Antiviral assay

Antiviral assays were performed by using a cytopathic effect (CPE) reduction assay using encephalomyocarditis (EMC) virus. WISH cells (40,000 per well in a microtiter dish) were grown overnight. IFN α 1(69-189)R9, IFN β (100-187)R9, or their controls without the R9 sequence were added to cells at the concentrations indicated for 4 hr, followed by infection with EMCV (moi = 0.01). Virus was washed after one hr and cells were grown overnight. Cells were stained with crystal violet and read in a microtiter plate at 550 nm.

30 *Induction of EAE, evaluation of clinical disease, 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 µg/mouse bovine myelin basic protein (Invitrogen) emulsified in Complete Freund's Adjuvant with 8 mg/ml H37Ra *Mycobacterium tuberculosis* (Sigma-Aldrich) and injected subcutaneously into two sites at the base of the tail along with 400 ng/mouse pertussis toxin (List Biological Laboratories Inc) in PBS i.p. On day 3, the
5 pertussis toxin injection was repeated (Jager *et al.*, 2011). Beginning on day 12 post-immunization, after lymphocyte infiltration of the CNS had begun, mice were administered the following treatments or peptides every other day via i.p. injection in 100 µl final volume: PBS, IFNα1(69-189)R9 (15 µg/mouse), or IFNα1(69-189) (15 µg/mouse). The mice were monitored daily for signs of EAE and graded according to the
10 following scale: 0, normal; 1, loss of tail tone; 2, hind limb weakness; 3, paraparesis; 4, paraplegia; 5, moribund; and 6, death. The Institutional Animal Care and Use Committee at the University of Florida approved all of the animal protocols mentioned here.

All patents, patent applications, provisional applications, and publications referred
15 to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and
20 all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Activated TYK2, JAK1, and interferon alpha/beta receptor subunits (IFNAR1 and IFNAR2) in the nucleus of type I IFN treated cells

We have recently shown that treatment of WISH cells with IFNγ resulted in the
25 presence of activated JAK1 and JAK2 in the nucleus (Noon-Song *et al.*, 2011). We thus treated WISH cells with type I IFNs IFNα2 and IFNτ, which have similar specific antiviral activities, but IFNα2 with much more potent apoptosis activity (Subramaniam *et al.*, 1995). The focus was on the presence of activated TYK2, JAK1, and receptor subunits IFNAR1 and IFNAR2 in the nucleus of cells treated with type I IFNs. At a
30 concentration of 1000 U/ml, IFNα2 treatment resulted in the presence of both phosphorylated (activated) JAKs, pJAK1 and pTYK2, in the nucleus (Figure 4A). Non-phosphorylated TYK2 was constitutively present in the nucleus of untreated cells. Non-

phosphorylated JAK1 was also present in the nucleus (data not shown). IFN τ treatment similarly resulted in the presence of pTYK2 in the nucleus (Figure 4B). Both phosphorylated STAT1 α and STAT2 were detected in the nucleus only after treatment of cells with the IFNs (Figures 4A and 4B). To ascertain the purity of nuclear fractionations, β -tubulin and β -lamin were used as markers of nuclear and cytoplasmic fractions, respectively (Figures 4A, 4B, and 4C).

Focus on activated JAKs in the nucleus without context, although of some interest, provides little insight into their role in specific gene activation. Thus, we also examined the movement of type I IFN receptor subunit IFNAR1 into the nucleus of WISH cells treated with the IFNs. For both IFN α 2 (Figure 4A) and IFN τ (Figure 4B), IFN treatment resulted in the presence of IFNAR1 in the nucleus. There were relatively low or trace amounts of IFNAR1 in the nucleus of untreated cells, which increased several fold after treatment with IFN α 2 or IFN τ . This is consistent with a low constitutive endogenous level of IFN β in untreated cells (Takoka *et al.*, 2000; Taniguchi and Takaoka, 2008). This constitutive IFN β has been shown to be important for priming cells for both induction of type I IFNs and in enhancement of the cellular response to both IFN γ and type I IFNs. We also determined that IFNAR2 similarly underwent nuclear translocation in IFN τ treated WISH cells (Figure 4C). The movement of IFNAR1 and IFNAR2 into the nucleus along with the JAKs suggests an association of the two events. Consistent with these results, we have identified a functional nuclear localization sequence in IFNAR1 (Subramaniam and Johnson, 2004) and IFNAR2 (²⁸³RKKK (SEQ ID NO:18); unpublished observation) and a putative NLS in TYK2 (Ragimbeau *et al.*, 2001).

To further verify the movement of IFNAR1 and IFNAR2 into the nucleus of IFN treated cells as well as to determine if the type I IFN similarly underwent nuclear import, we carried out confocal microscopy with GFP fusion proteins. Specifically, WISH cells were transfected separately with CMV promoter driven constructs of IFN τ , IFNAR1, or IFNAR2 fused to GFP. As control, WISH cells were also transfected with vector containing only GFP. As shown in Figure 5A, IFN τ -GFP treated cells showed an increased presence of IFN τ -GFP in the nucleus, while control GFP was present throughout the cell. In IFNAR1-GFP transfected cells, treatment with IFN τ caused nuclear translocation, while untreated cells showed no preference of IFNAR1-GFP for the nucleus as shown in Figure 5B. IFNAR2-GFP was similarly driven into the nucleus of

cells treated with IFN τ as shown in Figure 5C. Thus, the type I IFN IFN τ and receptor subunits IFNAR1 and IFNAR2 all undergo increased nuclear translocation in cells treated with the IFN. Possible small amounts of IFNAR1 and IFNAR2 in the nucleus of untreated cells would probably be due to the constitutive endogenous IFN β (Takoka *et al.*, 2000; Taniguchi and Takaoka, 2008). These findings differ from the IFN γ system in that IFNGR1 translocated to the nucleus, while the IFNGR2 receptor subunit remained in the plasma membranes after IFN γ treatment of cells where it provided JAK2 to IFNGR1 (Szente *et al.*, 1995). Thus, although similar in nuclear events, the type I and type II IFN systems differ in terms of receptor movement.

Example 2 – Recruitment of IFNAR1, TYK2, and STAT1, along with phosphorylation of histone H3Y41 (H3pY41) at the ISRE in the promoter region of the OAS1 gene of cells treated with type I IFN

To determine if the type I IFN players of Figure 4 were specifically recruited to the promoter region of a gene activated by IFN α 2 in cells, we performed ChIP assays. WISH cells were treated with 1000 U/ml of IFN τ for 1 hour and analyzed by ChIP of sonicated chromatin of approximately 500-bp fragments of DNA, followed by PCR. Chromatin fragments were immunoprecipitated with antibodies to IFNAR1, histone H3 tyrosine 41 (H3pY41), TYK2, and STAT1, followed by PCR of the OAS1 promoter region extending from nucleotides -147 to 3. As a control, PCR product for the promoter of β -actin gene, -967 to -844, was chosen for ChIP analysis. IgG did not interact with the promoter containing complex as a control for non-specific binding. As shown in Figure 6, IFNAR1, H3pY41, TYK2, and STAT1 were associated with the ISRE element of the OAS1 promoter in IFN α 2 treated cells. The β -actin gene is not activated by type I IFNs and ChIP analysis showed that receptor subunit IFNAR1, H3pY41, JAKs, and STAT1 were not associated at its promoter after IFN τ treatment. Studies of IFN signaling have tended to focus extensively on STATs when examining the promoter region of genes activated by IFN as well as by other factors that signal via the JAK/STAT pathway (Levy and Darnell, 2002). The ChIP data here provide insight into the mechanism of specific gene activation as well as the associated H3pY41 epigenetic event of the type I IFN signaling and suggest that STAT is but one player in these complex events.

Example 3 – TYK2 associates with IFNAR1 in the nucleus of cells treated with type I IFN

The demonstration of activated JAK2V617F and cytokine activated JAK2 in the nucleus of cells and their phosphorylation of H3Y41 (H3pY41) in the chromatin did not address the fact that epigenetic events such as this must involve some mechanism of specificity (Dawson *et al.*, 2009). JAK2V617F, for example, is associated with specific myeloproliferative disorders and cytokine activation of wild type nuclear JAK2 is associated with the specific biological effect of the particular cytokine. We showed in Figure 6 above that TYK2 and H3pY41 were specifically associated with the OAS1 promoter in cells treated with a type I IFN. Since IFNAR1 is specific to type I IFN signaling and was present along with TYK2 at the OAS1 promoter, we asked the question as to whether TYK2 and IFNAR1 were associated in the nucleus of cells treated with a type I IFN, as this would suggest a basis of specificity. Accordingly, the human fibroblast cells were treated with 1000 U/ml of IFN α 2 for 30 min, after which the cells were lysed and nuclear and cytosolic fractions were isolated. The nuclear and cytoplasmic fractions were IPed with antibody to IFNAR1 and Western blotted with antibodies to IFNAR1, TYK2, activated STAT1 α (pSTAT1 α), and H3pY41. As can be seen in Figure 7, nuclear TYK2, pSTAT1 α , and H3pY41 showed increased binding to IFNAR1 in IFN α 2 treated cells. IgG treated control cells did not show similar association in whole cell extracts (data not shown). This is evidence that TYK2 as well as pSTAT1 α do not function alone or independently of the cytokine system whose function they are associated with in the nucleus at the level of gene activation.

Example 4 – Specific epigenetic changes at the OAS1 promoter of cells treated with a type I IFN

We showed by ChIP analysis that IFN τ treatment of cells resulted in specific binding of IFNAR1, STAT1 α , and TYK2 to the ISRE of the promoter of the OAS1 gene. We examine here associated epigenetic changes by similar ChIP analysis at the OAS1 promoter. Figure 8A shows decreased trimethylated lysine 9 on histone H3, H3K9me3, in the OAS1 promoter region of cells treated with 1000 U/ml of IFN τ over 40 minutes. Acetylation of H3K9, H3K9ac, occurred concomitantly over the same time span. Demethylation/acetylation of H3K9 is associated with gene activation (Berger, 2007;

Mehta *et al.*, 2011). Related to this, phosphorylation of H3 at Y41, H3pY41, increased as H3K9me3 decreased over the same time period. Phosphorylation of H3Y41 was confirmed by Western blot (Figure 8B). By comparison, the constitutively activated β -actin gene, which is not affected by IFN, showed constitutive H3K9ac, no H3pY41, and no H3 K9me3. The presence of activated JAKs at the OAS1 region of type I IFN treated cells may be related to H3Y41 phosphorylation which in turn could play a role in the demethylation and acetylation of H3K9 at the promoter region of the gene. These observations suggest that the receptor/transcription factor/JAK complex of type I IFN treated cells plays a key role in specific gene activation, including the associated events of heterochromatin modification.

Example 5 – N-terminal truncated type I IFNs lose extracellular activity while retaining intracellular activity. Evidence of cytoplasmic binding

In development of the IFN γ mimetics, we found that N-terminal truncations of IFN γ to IFN γ (95-132) for mouse and IFN γ (95-134) for human IFN γ resulted in loss of recognition of extracellular receptor (Szente *et al.*, 1996; Ahmed *et al.*, 2005). These truncated IFNs were, however, active when introduced intracellularly via a palmitate group with full antiviral activity (Ahmed *et al.*, 2005). In order to determine if IFN α 1 and IFN β possessed similar C-terminus function intracellularly while losing extracellular function, we expressed truncated IFN α 1(69-189)R9 and IFN β (100-187)R9 with nine arginines (R9) for cell penetration in a bacterial expression system and purified the polypeptides. As controls, we also expressed these truncations without R9. Both IFN α 1(69-189)R9 (Figure 9A) and IFN β (100-187)R9 (Figure 9B) possessed antiviral activity against EMC virus, while the same constructs without R9 for cell penetration lacked antiviral activity. R9 alone also lacked antiviral activity (data not shown). This is consistent with previous studies that showed that intracellularly expressed IFN α possessed antiproliferative and antiviral activity (Ahmed *et al.*, 2001). The truncation studies, however, are not subject to the argument that somehow the intracellular IFN may have leaked out of the cell and interacted with the extracellular receptor domains, since the truncations were not functional in terms of extracellular induced antiviral activity.

Type I IFN is the treatment of choice for relapsing/remitting multiple sclerosis (MS) (National Multiple Sclerosis Society Bulletin, 2012), so we tested IFN α 1(69-

189)R9 for its ability to therapeutically treat SJL/J mice in experimental allergic encephalomyelitis (EAE), a mouse model of MS. Immunization of mice with bovine myelin basic protein (MBP) where cellular infiltration into the CNS has occurred by day 12 was used to test the truncated IFNs (Jager *et al.*, 2011). IFN α (69-189)R9 administration beginning at day 12 and every other day thereafter remitted with essentially complete therapeutic efficacy, while IFN α 1(69-189) and PBS treated mice developed paraplegia (Figure 9C).

The results presented here for induction of antiviral activity and EAE therapy by cell-penetrating truncated type I IFNs are inexplicable in the context of a model where the type I IFN exerts its effect solely by extracellular interaction with the receptor. The data are compatible with our IFN γ model where IFN after binding to receptor extracellular domain goes on to bind to the cytoplasmic domain of receptor in conjunction with endocytic events (Subramaniam and Johnson, 2002). The complex formation and the functional cytoplasmic activity of IFN truncations thus show similarities to steroid signaling (Johnson *et al.*, 2012).

Example 6

Specific gene activation by cytokines such as the IFNs is attributed solely to the activated STATs (Levy and Darnell, 2002). In the case of IFN γ signaling, interaction of IFN γ with receptor results in autoactivation of JAK1 and JAK2, which in turn activate STAT1 α in conjunction with receptor subunit IFNGR1. Activated STAT1 α forms a homodimer, dissociates from IFNGR1, and undergoes active nuclear transport via an unconventional nuclear localization sequence (NLS) that associates with the importin α/β proteins (Johnson *et al.*, 2004). The fact that STAT1 α is activated by other cytokines in addition to IFN γ would suggest that STATs do not intrinsically contain the mechanism for specific gene activation by a particular cytokine (Johnson *et al.*, 2012; Johnson *et al.*, 2004; Johnson and Ahmed, 2006). This is reinforced by the fact that there are just seven STATs that function mostly as homodimeric transcription factors for over 60 different cytokines, growth factors, and hormones (Johnson *et al.*, 2012; Johnson *et al.*, 2004; Johnson and Ahmed, 2006).

Recently, nuclear JAK2 has been shown to play an important role at the epigenetic level in gene activation. Mutant activated JAK2, JAK2V617F, was shown to be

constitutively present in the nucleus of effector cells of myeloproliferative disorders (Dawson *et al.*, 2009). JAK2V617F was shown to phosphorylate tyrosine 41 on histone H3 (H3Y41), which is associated with gene activation. The constitutive activation of JAK2V617F required association with hematopoietic receptors such as that for erythropoietin (EPOR) at the cytoplasmic domain (Lu *et al.*, 2008). The mechanism of how JAK2V617F underwent nuclear translocation as well as possible involvement of other proteins such as EPOR was not addressed. It was also shown that wild-type JAK2 was constitutively present in the nucleus of nonmyeloproliferative cell lines, but was only activated after treatment of K562 cells with PDGF or LIF or treatment of BaF3 cells with IL-3 (Dawson *et al.*, 2009). As with JAK2V617F, the mechanism by which nonphosphorylated and phosphorylated JAK2 entered into the nucleus was not addressed.

Phosphorylation of H3Y41 by activated nuclear JAK2 assigns a previously unknown function to a JAK kinase. The presence of JAKs in the nucleus, phosphorylated and unphosphorylated, was however previously known. JAK1, JAK2, and TYK2 have all previously been shown to be constitutively present in the nucleus (Ragimbeau *et al.*, 2001; Zouein *et al.*, 2011; Nilsson *et al.*, 2006). Activated JAK2 was shown to be present in growth hormone treated CHO cells that had been transfected with growth hormone receptor (Lobie *et al.*, 1996). These observations may not have received much attention as they were not obviously explainable in the context of the classical model of JAK/STAT signaling.

We have previously shown that IFN γ and one of its receptor subunits, IFNGR1, are translocated to the nucleus, together with activated STAT1 α as one macromolecular complex via the classical importin-dependent pathway (Ahmed and Johnson, 2006). We have further shown that IFN γ and IFNGR1 are recruited to the IFN γ -activated genes (Noon-Song *et al.*, 2011; Ahmed and Johnson, 2006). The direct association of IFNGR1 with the promoter region of IFN γ -activated genes suggested a transcriptional/cotranscriptional role for IFNGR1 as well as its possible role in determining the specificity of gene activation by IFN γ .

The role of activated JAKs in specific gene activation of IFN γ was addressed in the context of the above macromolecular complex. ChIP followed by PCR in IFN γ treated WISH cells showed association of activated JAK1 (pJAK1) and JAK2 (pJAK2) with the IFN γ /IFNGR1/pSTAT1 α complex on the same DNA sequence of the 1RF-I gene

promoter (Noon-Song *et al.*, 2011). The β -actin gene, which is not activated by IFN γ , did not show this association. Activated JAKs in the nucleus were associated with phosphorylation of H3Y41 in the GAS region of the IRF-1 promoter (Noon-Song *et al.*, 2011). Unphosphorylated JAK2 was constitutively present in the nucleus and was capable of undergoing activation in IFN γ treated cells, most likely via nuclear IFNGR1. The IFN γ studies of activated JAK2 in the nucleus suggest that it functions in the context of the IFN γ /IFNGR1/pSTAT1 α complex. This in turn provides a mechanism for controlling or identifying specific chromatin regions for pJAK2 activated epigenetic effects.

Our results here provide insight into type I IFN signaling in terms of IFN/receptor/STAT/TYK2 nuclear complexes. We showed that nonphosphorylated TYK2, like JAK2, is constitutively present in the nucleus. TYK2 is activated (pTYK2) in the nucleus only after interaction of type I IFN with the receptor complex, and, like pJAK2, phosphorylated H3Y41 at a gene (OASI) that is activated by type I IFNs, while absent from an unrelated gene (β -actin). Thus, the epigenetic event of H3Y41 phosphorylation is not unique to any particular JAK, but probably involves the JAKs that are associated with the stimulating cytokine.

Both IFNAR1 and IFNAR2 underwent nuclear translocation in type I IFN treated cells. This is in contrast to IFN γ where IFNGR2 remained associated with the cell membrane while IFNGR1 underwent nuclear translocation as part of a complex as indicated above (Ahmed and Johnson, 2006; Ahmed *et al.*, 2003). We showed that IFNGR2 provided JAK2 to IFNGR1 via IFN γ induced increased binding affinity for IFNGR1 (Szente *et al.*, 1995). For type I IFNs, TYK2 is associated with IFNAR1 while JAK1 is associated with IFNAR2 (Stark *et al.*, 1998). After type I IFN treatment, pTYK2 and probably pJAK1 undergo nuclear translocation as a part of macromolecular complex that contains IFNAR1 and IFNAR2. Similar to IFN γ , we also observed nuclear translocation of type I IFN, IFN τ , by confocal microscopic analysis. Nuclear translocation of type I IFN has been known for some time (Kushnaryov *et al.*, 1986). This observation again cannot be explained by the classical model of JAK/STAT signaling.

Since pTYK2 involvement in phosphorylation of H3Y41 was specific for a gene that is induced by type I IFNs, the question arises as to whether histone associated demethylation and acetylation show similar specificity. Focusing on trimethylated histone H3 lysine 9 (H3K9me3), we observed that in type I IFN treated cells H3K9me3

underwent demethylation in association with acetylation (H3K9ac) at the region of the OAS1 promoter. These changes in H3K9 are associated with gene activation (Berger, 2007; Mehta *et al.*, 2011). The association of IFN receptors with pSTAT1 α , pTYK2, and probably other factors in the region of genes activated by IFN provides insight into the mechanism of specific gene activation, including associated phosphorylations, methylations, demethylations, and acetylations.

In a search for precedent, it seems that our study of both type I and type II IFN signaling shares similarities to that of steroid receptor (SR) signaling. SRs are a major subset of nuclear receptors (Stanisic *et al.*, 2010). Basically, synthesis of steroid hormones (SHs) occurs in the adrenal cortex and in gonads (Stanisic *et al.*, 2010). Broadly, the current model of SH signaling is as follows. In the absence of hormone, cytoplasmic SR monomers are associated with heat shock proteins (HSPs) and usually possess some basal level of phosphorylation (Stanisic *et al.*, 2010). Upon binding of hormone, SRs dissociate from HSPs, dimerize, and translocate to the nucleus where they bind to hormone receptor elements (HREs) at genes that are activated by SHs. The complex of SH/SR recruits a series of coactivators to both regulate target gene transcription as well as the associated epigenetic events that accompany gene expression. Site-specific phosphorylation of receptors occurs subsequent to hormone binding with varied kinetics, depending on the kinase and the target in the receptor complex. The kinases, while not the only components of the receptor associated co-activator complexes, are important for their action on members of the receptor complex as well as for specific epigenetic events of gene activation and thus act on histones as well as on members of the receptor complex.

Unlike SH/SR interaction, both type I and II IFN signaling initiates with ligand binding to the receptor extracellular domain. However, we have shown that IFN γ also binds to the cytoplasmic domain of receptor subunit IFNGR1 during the process of endocytosis (Szente *et al.*, 1995; Szente *et al.*, 1996). We showed that the N-terminus of IFN γ played the key role in recognition of IFNGR1 extracellular domain, while the C-terminus played the key role in binding to the cytoplasmic domain. This in turn led to development of IFN γ mimetics based on the C-terminus (Szente *et al.*, 1996; Ahmed *et al.*, 2007). We showed here that N-terminus truncations of IFN α and IFN β resulted in loss of signaling via extracellular receptor interaction, while the same truncated IFNs with R9

attached for cell penetration possessed antiviral activity and anti-autoimmune function in EAE. These results would suggest that type I IFNs also interact with receptor cytoplasmic domain. Type I IFN cytoplasmic receptor interaction is probably more complex than that of IFN γ where only the receptor subunit IFNGR1 undergoes endocytosis, while both IFNAR1 and IFNAR2 undergo endocytosis in type I IFN signaling. The demonstration of extracellular receptor interaction for IFNs is essentially the extra step in signaling compared to SHs, which interact directly with the cytoplasmic SR. In both systems we have ligand/receptor/coactivator complexes that undergo nuclear translocation. The receptor complexes bind to promoter regions of genes that they specifically activate. Thus, the results of this and previous studies with IFN γ suggest that signaling by cytokines such as the IFNs is but a variation of steroid/steroid receptor signaling.

Example 7 – Type I IFN mimetics lack toxicity under conditions where intact IFNs are toxic

We compared mouse IFN β with human IFN β (100-187)R9 and IFN α 1(69-189)R9 mimetics for relative toxic effects against C57BL/6 mice as reflected by weight loss (Figure 10). Injection of mice I.P. on alternate days with 1,000 U per mouse of IFN β resulted in approximately 15% weight loss by day 10, while mice injected with 2,000 U per mouse of IFN mimetic gained weight as would be expected under normal growth conditions (see Figure 10). Lymphocyte counts showed a similar pattern of toxicity with 24% reduction in IFN β injected mice and 4-9% loss in IFN mimetic treated mice (Table 4). A comparison of human IFN α 2 with the mimetics on toxicity/apoptosis in WISH cells showed that IFN α 2 had toxicity of approximately 14% above controls, while the mimetics showed toxicity at the level of untreated cells (Figure 11). Thus, the IFN mimetics lacked toxicity of weight loss, lymphopenia, and cellular toxicity under conditions where the intact type I IFNs were toxic.

Table 4. Lymphocyte suppression seen with IFN is not observed with IFN mimetics.

Lymphocyte count in mice under different treatments.

Treatment	Lymphocytes (%)	% Reduction	Significance
PBS	78 ± 4		
IFN α (69-189)R9	75 ± 3	4	NS
IFN β (100-187)R9	72 ± 5	9	NS
IFN β	59 ± 6	24	< 0.01

- 5 Mice (C57BL/6, n = 3) were injected i.p. with PBS, IFN mimetics (2×10^3 U in 200 μ g per mouse), or IFN β (10^3 U/mouse) on alternate days for ten days. On day 11, mice were bled. Blood smears were stained and lymphocytes were counted.

10 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any
15 combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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CLAIMS

We claim:

1. An agonist peptide of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor.

2. The peptide according to claim 1, wherein the peptide comprises the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity, or the peptide comprises an amino acid sequence having 60% or greater sequence identity with the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity.

3. The peptide according to claim 2, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or more amino acids are, independently, removed from one or both termini of the peptide.

4. The peptide according to claim 1, wherein the peptide comprises a protein or nucleic acid that is attached to the peptide and that targets delivery to the cell and/or that provides for translocation of the peptide across a biological membrane of the cell.

5. The peptide according to claim 1, wherein a lipophilic group is attached to the peptide.

6. The peptide according to claim 5, wherein the lipophilic group is a palmitoyl-lysine group.

7. The peptide according to claim 5, wherein the peptide comprises one or more arginine amino acids at one or both termini of the peptide.

8. The peptide according to claim 5, wherein the peptide comprises one or more arginine amino acids at the N-terminus of the peptide.

9. The peptide according to claim 1, wherein the peptide comprises a nuclear localization sequence (NLS).

10. A composition comprising i) a peptide according to any of claims 1-9, 84, 85, or 86, or ii) a polynucleotide that encodes a peptide according to any of claims 1-9, 84, 85, or 86; and a suitable carrier, diluent, or buffer.

11. The composition according to claim 10, wherein the composition further comprises i) one or more antiviral compounds, and/or ii) one or more anticancer or antitumor compounds, and/or one or more compounds for treating autoimmune disorders.

12. The composition according to claim 11, wherein the one or more antiviral compound is IFN α , IFN β , IFN γ , acyclovir (Zovirax), zidovudine (AZT), lamivudine (3TC), zanamivir (Relenza), oseltamivir (Tamiflu), valacyclovir (Valtrex), amantadine (Symmetrel), rimantadine (Flumadine), cidofovir (Vistide), foscarnet (Foscavir), ganciclovir (Cytovene), ribavirin (Virazole), nelfinavir (Viracept), ritonavir (Norvir), rifampin (Rifadin), or famciclovir (Famvir).

13. The composition according to claim 11, wherein the one or more antiviral compounds is an interferon-gamma (IFN- γ) peptide mimetic.

14. The composition according to claim 13, wherein the IFN- γ peptide mimetic comprises the amino acid sequence shown in SEQ ID NO:7 or SEQ ID NO:8, or a fragment thereof that exhibits antiviral activity.

15. The composition according to claim 10, wherein the one or more anticancer or antitumor compound is taxol, vinblastine, cyclophosphamide, ifosfamide, 5-fluorouracil, hydroxyurea, adriamycin, bleomycin, etoposide, camptothecin, angiostatin, tamoxifen, GLEEVEC, HERCEPTIN, Bortezomib, Carfilzomib, or Salinosporamide A.

16. The composition according to claim 10, wherein the composition further comprises a peptide comprising the amino acid sequence shown in SEQ ID NO:9 and/or SEQ ID NO:10, or a fragment thereof.

17. The composition according to claim 10, wherein the peptide or polynucleotide is encapsulated in a liposome.

18. A kit comprising in one or more containers:

- i) a peptide according to any of claims 1-9, 84, 85, or 86;
 - ii) a polynucleotide encoding a peptide according to any of claims 1-9, 84, 85, or 86;
- and/or
- iii) a composition according to any of claims 10-17.

19. The kit according to claim 18, wherein the kit further comprises one or more antiviral compounds, biological molecules, or drugs.

20. The kit according to claim 18, wherein the kit further comprises instructions or packaging materials that describe how to administer the peptide, polynucleotide, or composition.

21. A method for treating or preventing infection by a virus in a human or animal or treating or preventing a viral associated disorder in a human or animal, said method comprising administering to the human or animal an effective amount of

i) a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or

ii) a polynucleotide that encodes a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or

iii) a composition comprising a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but

does bind to the cytoplasmic domain of a type I IFN receptor, or a polynucleotide that encodes a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor.

22. The method according to claim 21, wherein the peptide comprises the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity or the peptide comprises an amino acid sequence having 60% or greater sequence identity with the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity.

23. The method according to claim 21, wherein the virus is a herpes virus, poxvirus, or picornavirus.

24. The method according to claim 21, wherein the virus is a vaccinia virus, encephalomyocarditis (EMC) virus, influenza virus, herpes simplex virus (HSV), cytomegalovirus (CMV), herpes zoster virus, and other herpes viruses, poxvirus, coxsackie virus, lentivirus, hepatitis virus (hepatitis A, B, C, D, and E), or picornavirus.

25. The method according to claim 24, wherein the influenza virus is an influenza A virus.

26. The method according to claim 25, wherein the influenza A virus is serotype H1N1.

27. The method according to claim 21, wherein the peptide, polynucleotide, or composition is administered to the person or animal prior to infection by the virus.

28. The method according to claim 21, wherein the peptide, polynucleotide, or composition is administered after the human or animal is infected by the virus.

29. The method according to claim 21, wherein the peptide, polynucleotide, or composition is administered prior to, in conjunction with, or subsequent to administration of one or more antiviral compounds.

30. The method according to claim 29, wherein the antiviral compound is IFN α , IFN β , IFN γ , acyclovir (Zovirax), zidovudine (AZT), lamivudine (3TC), zanamivir (Relenza), oseltamivir (Tamiflu), valacyclovir (Valtrex), amantadine (Symmetrel), rimantadine (Flumadine), cidofovir (Vistide), foscarnet (Foscavir), ganciclovir (Cytovene), ribavirin (Virazole), nelfinavir (Viracept), ritonavir (Norvir), rifampin (Rifadin), or famciclovir (Famvir).

31. The method according to claim 21, wherein the peptide, polynucleotide, or composition is administered in conjunction with an interferon-gamma (IFN- γ) peptide mimetic.

32. The method according to claim 31, wherein the IFN- γ peptide mimetic comprises the amino acid sequence shown in SEQ ID NO:7 or SEQ ID NO:8, or a fragment thereof that exhibits antiviral activity.

33. The method according to claim 21, wherein the peptide, polynucleotide, or composition are provided in a carrier means for delivering the peptide or polynucleotide to a cell and, optionally, facilitating transport of the peptide or polynucleotide into the cell.

34. The method according to claim 33, wherein the carrier means comprises liposome encapsulating the peptide, polynucleotide, or composition.

35. The method according to claim 33, wherein the carrier means comprises a protein or nucleic acid that is attached to the peptide, polynucleotide, or composition and that targets delivery to the cell and/or that provides for translocation of the peptide, polynucleotide, or composition across a biological membrane of the cell.

36. The method according to claim 21, wherein a lipophilic group is attached to the peptide.

37. The method according to claim 36, wherein the lipophilic group is a palmitoyl-lysine group.

38. The method according to claim 36, wherein the peptide comprises one or more arginine amino acids at the N-terminus of the peptide.

39. The method according to claim 21, wherein the method further comprises identifying a human or animal who is or who may be in need of treatment or prevention of infection by a virus.

40. A method for inducing an antiviral state in a cell against a virus or inhibiting the growth of a cancer cell, comprising contacting the cell *in vitro* or *in vivo* with an effective amount of

i) a peptide that is an agonist of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or

ii) a polynucleotide that encodes a peptide that is an agonist of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or

iii) a composition comprising a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or a polynucleotide that encodes a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor.

41. The method according to claim 40, wherein the peptide comprises the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity or the peptide comprises

an amino acid sequence having 60% or greater sequence identity with the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity.

42. The method according to claim 40, wherein the virus is a herpes virus, poxvirus, or picornavirus.

43. The method according to claim 40, wherein the virus is a vaccinia virus, encephalomyocarditis (EMC) virus, influenza virus, herpes simplex virus (HSV), cytomegalovirus (CMV), herpes zoster virus, and other herpes viruses, poxvirus, coxsackie virus, lentivirus, hepatitis virus, or picornavirus.

44. The method according to claim 43, wherein the influenza virus is an influenza A virus.

45. The method according to claim 44, wherein the influenza A virus is serotype H1N1.

46. The method according to claim 40, wherein the peptide, polynucleotide, or composition is contacted with the cell prior to infection by the virus.

47. The method according to claim 40, wherein the peptide, polynucleotide, or composition is contacted with the cell after infection by the virus.

48. The method according to claim 40, wherein the peptide, polynucleotide, or composition is contacted with the cell in conjunction with one or more antiviral compounds.

49. The method according to claim 48, wherein the antiviral compound is IFN α , IFN β , IFN γ , acyclovir (Zovirax), zidovudine (AZT), lamivudine (3TC), zanamivir (Relenza), oseltamivir (Tamiflu), valacyclovir (Valtrex), amantadine (Symmetrel), rimantadine (Flumadine), cidofovir (Vistide), foscarnet (Foscavir), ganciclovir (Cytovene), ribavirin

(Virazole), nelfinavir (Viracept), ritonavir (Norvir), rifampin (Rifadin), or famciclovir (Famvir).

50. The method according to claim 40, wherein the peptide, polynucleotide, or composition is administered in conjunction with an interferon-gamma (IFN- γ) peptide mimetic.

51. The method according to claim 50, wherein the IFN- γ peptide mimetic comprises the amino acid sequence shown in SEQ ID NO:7 or SEQ ID NO:8, or a fragment thereof that exhibits antiviral activity.

52. The method according to claim 40, wherein the peptide, polynucleotide, or composition are provided in a carrier means for delivering the peptide or polynucleotide to a cell and, optionally, facilitating transport of the peptide or polynucleotide into the cell.

53. The method according to claim 52, wherein the carrier means comprises liposome encapsulating the peptide, polynucleotide, or composition.

54. The method according to claim 52, wherein the carrier means comprises a protein or nucleic acid that is attached to the peptide, polynucleotide, or composition and that targets delivery to the cell and/or that provides for translocation of the peptide, polynucleotide, or composition across a biological membrane of the cell.

55. The method according to claim 40, wherein a lipophilic group is attached to the peptide.

56. The method according to claim 55, wherein the lipophilic group is a palmitoyl-lysine group.

57. The method according to claim 55, wherein the peptide comprises one or more arginine amino acids at the N-terminus of the peptide.

58. A method for treating an oncological disorder or an autoimmune disorder in a person or animal, comprising administering to the person or animal an effective amount of i) a peptide that is an agonist of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or ii) a polynucleotide that encodes a peptide that is an agonist of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or iii) a composition comprising a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or a polynucleotide that encodes a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor.

59. The method according to claim 58, wherein the peptide comprises the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity or the peptide comprises an amino acid sequence having 60% or greater sequence identity with the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a fragment thereof that exhibits antiviral activity.

60. The method according to claim 58, wherein the peptide, polynucleotide, or composition is administered prior to, in conjunction with, or subsequent to administration of one or more anticancer or antitumor compound for treating an oncological disorder.

61. The method according to claim 60, wherein the anticancer or antitumor compound is taxol, vinblastine, cyclophosphamide, ifosfamide, 5-fluorouracil, hydroxyurea, adriamycin, bleomycin, etoposide, camptothecin, angiostatin, tamoxifen, GLEEVEC, HERCEPTIN, Bortezomib, Carfilzomib, or Salinosporamide A.

62. The method according to claim 58, wherein the oncological disorder is cancer and/or tumors of the anus, bile duct, bladder, bone, bone marrow, bowel (including colon and

rectum), breast, eye, gall bladder, kidney, mouth, larynx, esophagus, stomach, testis, cervix, head, neck, ovary, lung, mesothelioma, neuroendocrine, penis, skin, spinal cord, thyroid, vagina, vulva, uterus, liver, muscle, pancreas, prostate, blood cells (including lymphocytes and other immune system cells), and brain; carcinomas, Kaposi's sarcoma, melanoma, mesothelioma, soft tissue sarcoma, pancreatic cancer, lung cancer, leukemia (hairy cell, acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myeloid, and other), lymphoma (Hodgkin's and non-Hodgkin's), follicular lymphoma, or multiple myeloma.

63. The method according to claim 58, wherein the peptide, polynucleotide, or composition is provided in a carrier means for delivering the peptide or polynucleotide to a cell and, optionally, facilitating transport of the peptide, polynucleotide, or composition into the cell.

64. The method according to claim 63, wherein the carrier means comprises liposome encapsulating the peptide, polynucleotide, or composition.

65. The method according to claim 63, wherein the carrier means comprises a protein or nucleic acid that is attached to the peptide, polynucleotide, or composition and that targets delivery to the cell and/or that provides for translocation of the peptide, polynucleotide, or composition across a biological membrane of the cell.

66. The method according to claim 58, wherein the autoimmune disorder is multiple sclerosis or rheumatoid arthritis.

67. The method according to claim 58, wherein a lipophilic group is attached to the peptide.

68. The method according to claim 67, wherein the lipophilic group is a palmitoyl-lysine group.

69. The method according to claim 67, wherein the peptide comprises one or more arginine amino acids at the N-terminus of the peptide.

70. The method according to claim 58, wherein the peptide or polynucleotide is administered prior to, in conjunction with, or subsequent to administration of one or more compounds for treating an autoimmune disorder.

71. The method according to claim 58, wherein the method further comprises identifying a patient who is or who may be in need of treatment of an oncological and/or an autoimmune disorder.

72. A polynucleotide encoding a peptide according to any of claims 1-9, 84, 85, or 86.

73. A polynucleotide expression construct comprising a polynucleotide encoding a peptide according to any of claims 1-9, 84, 85, or 86.

74. The polynucleotide expression construct according to claim 73, wherein said expression construct comprises one or more regulatory elements.

75. The polynucleotide expression construct according to claim 74, wherein said one or more regulatory elements are promoters, transcription termination sequences, translation termination sequences, enhancers, or polyadenylation elements.

76. The polynucleotide expression construct according to claim 73, wherein said expression construct provides for expression in bacterial cells, yeast cells, plant cells, insect cells, or mammalian cells.

77. The polynucleotide expression construct according to claim 73, wherein said expression construct provides for expression in human cells.

78. An antibody, or an antigen binding fragment thereof, that binds to a peptide according to any of claims 1-9, 84, 85, or 86.

79. The antibody according to claim 78, wherein the antibody is a monoclonal antibody.

80. The antibody according to claim 78, wherein the antibody binds specifically to huIFN α 1(69-189) (SEQ ID NO:1) peptide.

81. The antibody according to claim 78, wherein the antibody binds specifically to huIFN β 1(100-187) (SEQ ID NO:2) peptide.

82. A method for inhibiting type I IFN cell activation and/or intracellular signaling, comprising providing in a cell an effective amount of an inhibitor of an interferon alpha/beta receptor (IFNAR) subunit.

83. The method according to claim 82, wherein the inhibitor is an antibody that binds to the IFNAR.

84. The peptide according to claim 1, wherein the peptide comprises a cell-penetrating peptide (CPP).

85. The peptide according to claim 84, wherein the CPP comprises the amino acid sequence of SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; or SEQ ID NO:37.

86. The peptide according to claim 84, wherein the CPP comprises only arginine (R) or only lysine (K) amino acids.

87. The method according to claim 21, wherein the peptide is the peptide according to any of claims 2-9, 84, 85, or 86; or the polynucleotide encodes a peptide according to any of claims 2-9, 84, 85, or 86; or the composition is a composition according to any of claims 10-17.

88. The method according to claim 40, wherein the peptide is the peptide according to any of claims 2-9, 84, 85, or 86; or the polynucleotide encodes a peptide according to any of claims 2-9, 84, 85, or 86; or the composition is a composition according to any of claims 10-17.

89. The method according to claim 58, wherein the peptide is the peptide according to any of claims 2-9, 84, 85, or 86; or the polynucleotide encodes a peptide according to any of claims 2-9, 84, 85, or 86; or the composition is a composition according to any of claims 10-17.

90. A method for activating an immune cell (*e.g.*, T cell, NK cell, macrophage, *etc.*), and/or upregulating antigen presentation to lymphocytes, and/or upregulating major histocompatibility complex (MHC) molecules, and/or activating a JAK/STAT pathway, and/or activating TYK2 in a cell, comprising contacting the cell *in vitro* or *in vivo* with an effective amount of i) a peptide that is an agonist of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or ii) a polynucleotide that encodes a peptide that is an agonist of a type I interferon (IFN) wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or iii) a composition comprising a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor, or a polynucleotide that encodes a peptide that is an agonist of a type I interferon wherein the peptide does not bind to the extracellular domain of a type I IFN receptor but does bind to the cytoplasmic domain of a type I IFN receptor.

91. The method according to claim 90, wherein the peptide is the peptide according to any of claims 2-9, 84, 85, or 86; or the polynucleotide encodes a peptide according to any of claims 2-9, 84, 85, or 86; or the composition is a composition according to any of claims 10-17.

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FIG. 1

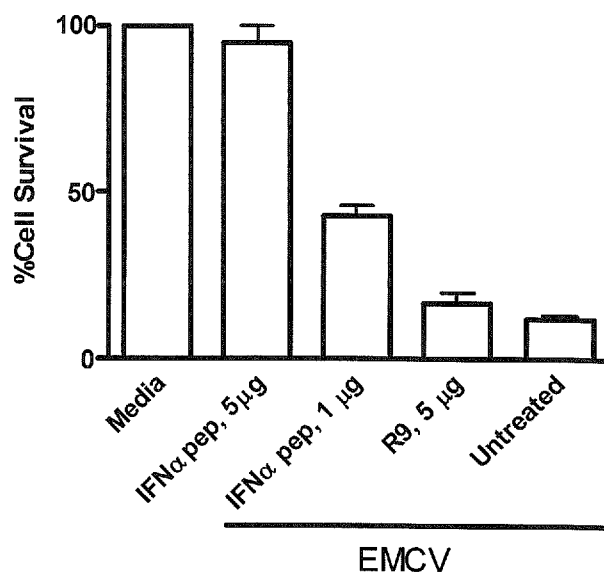


FIG. 2

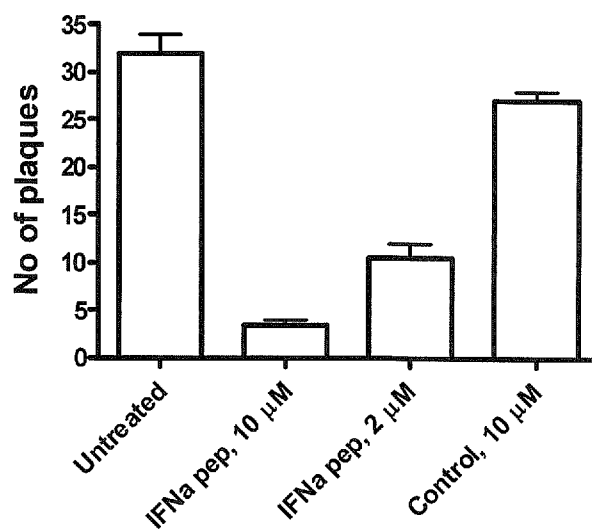


FIG. 3

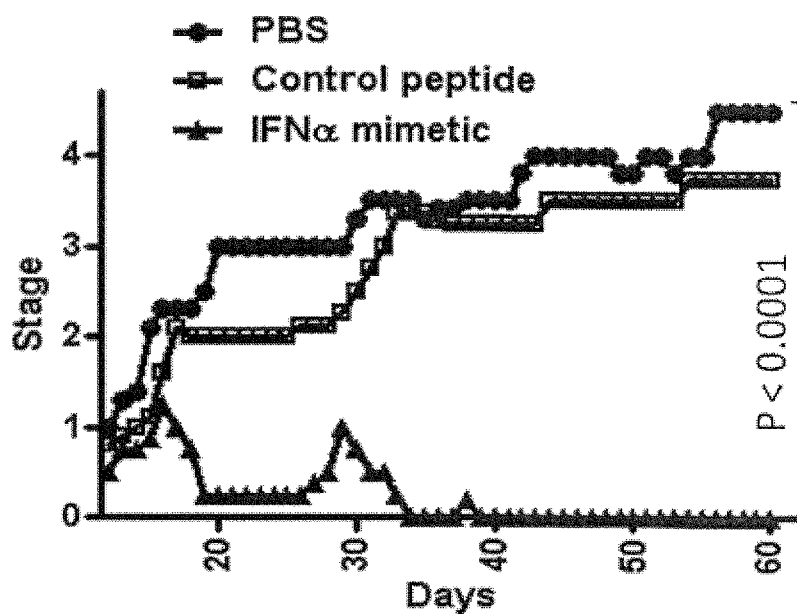


FIG. 4A

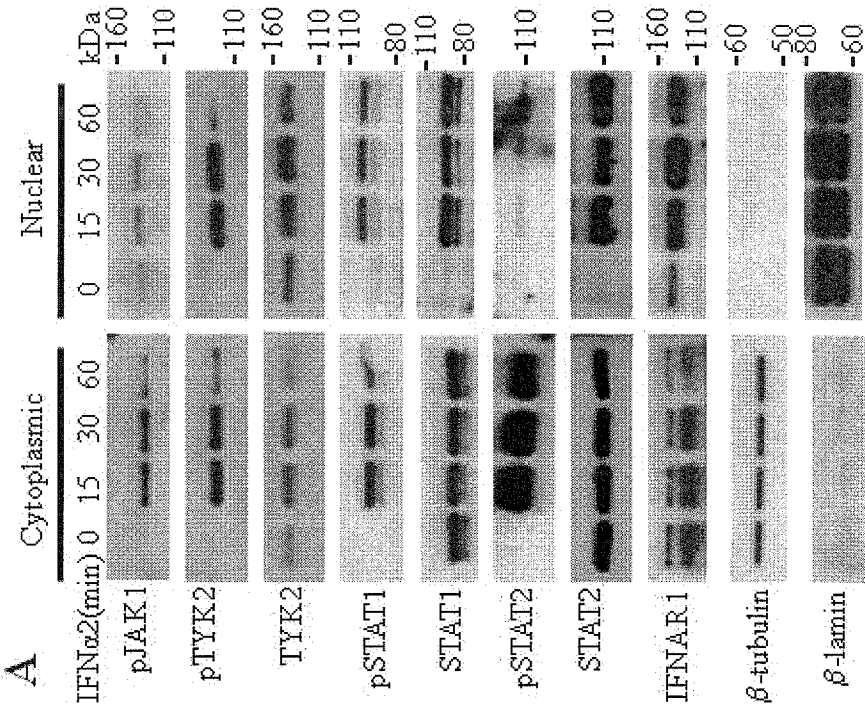


FIG. 4B

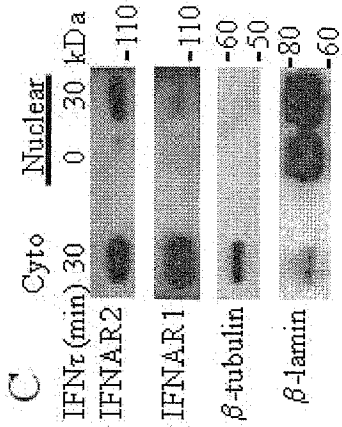
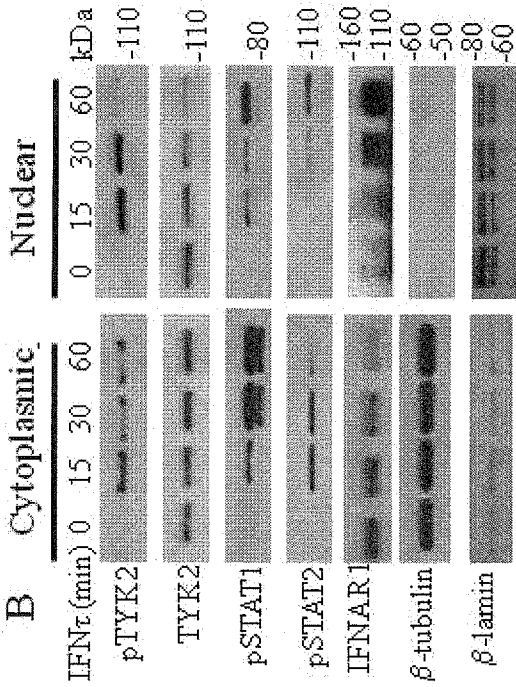
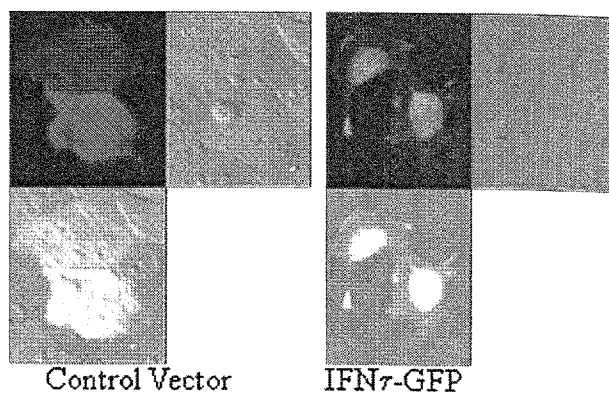
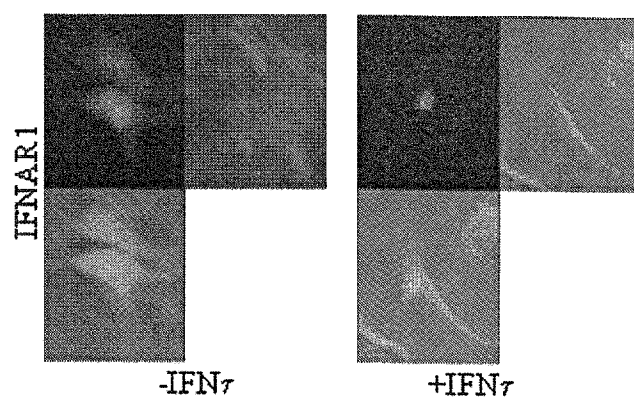
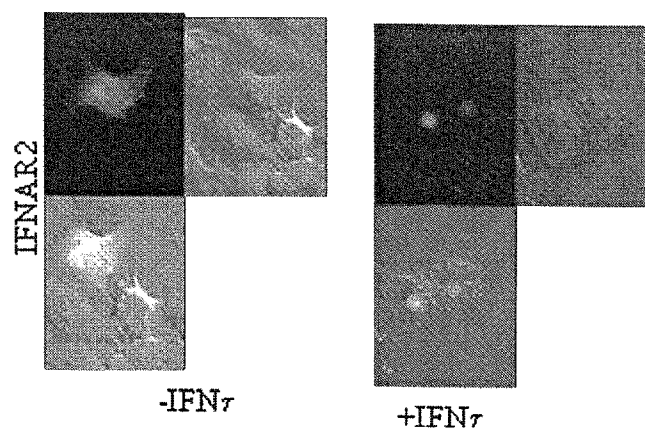


FIG. 4C

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**FIG. 5A****FIG. 5B****FIG. 5C**

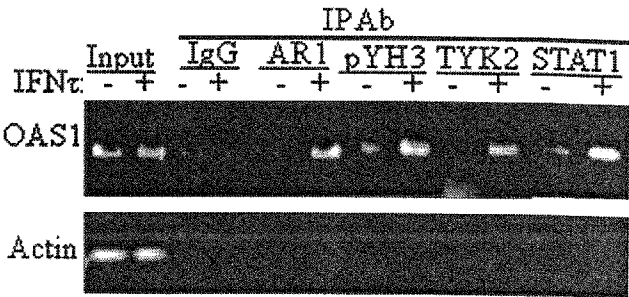


FIG. 6

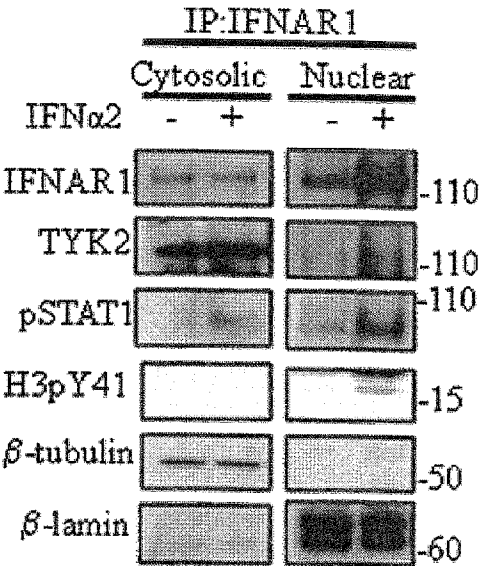


FIG. 7

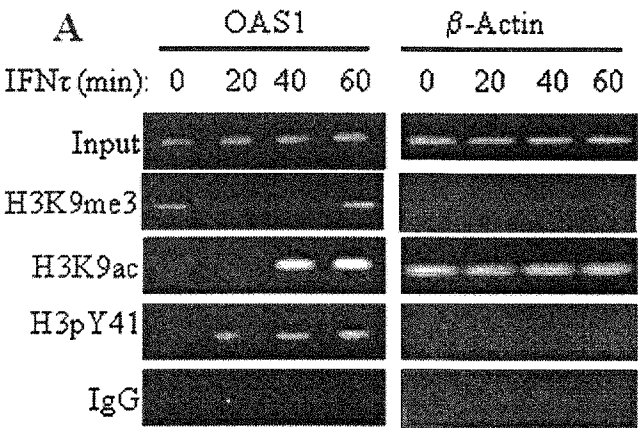


FIG. 8A

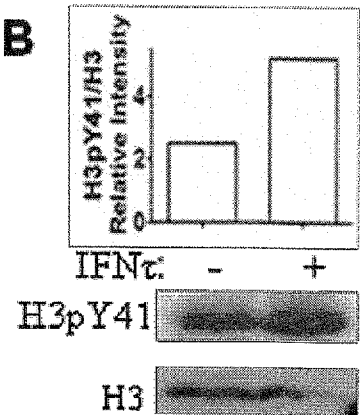


FIG. 8B

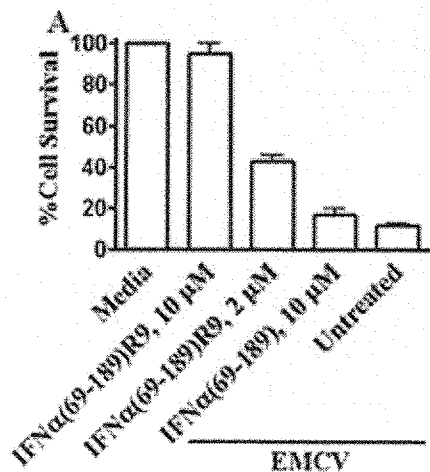


FIG. 9A

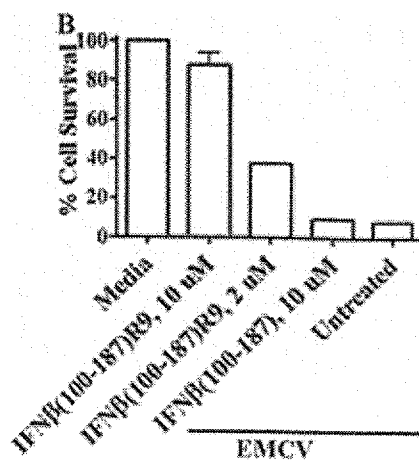


FIG. 9B

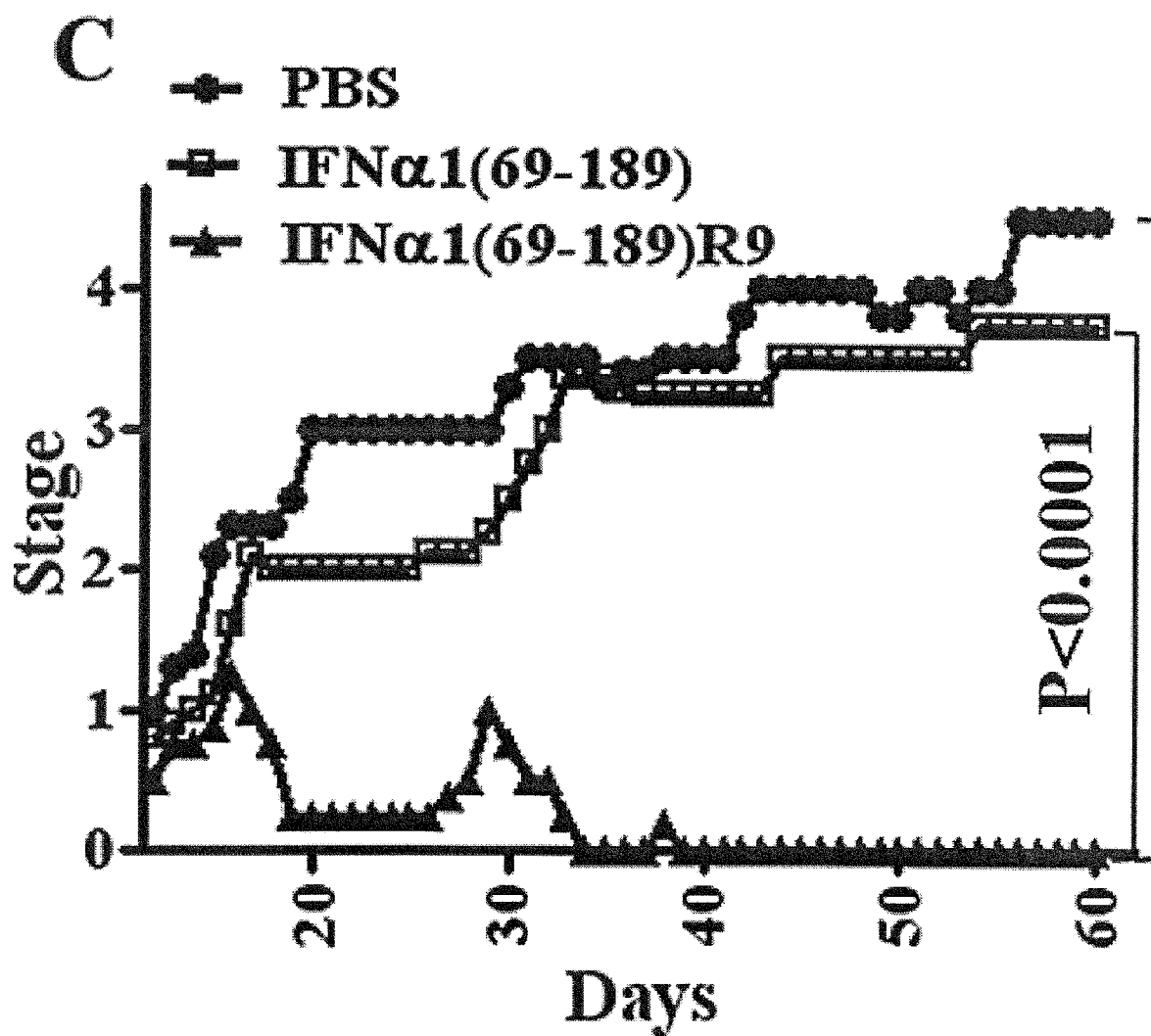


FIG. 9C

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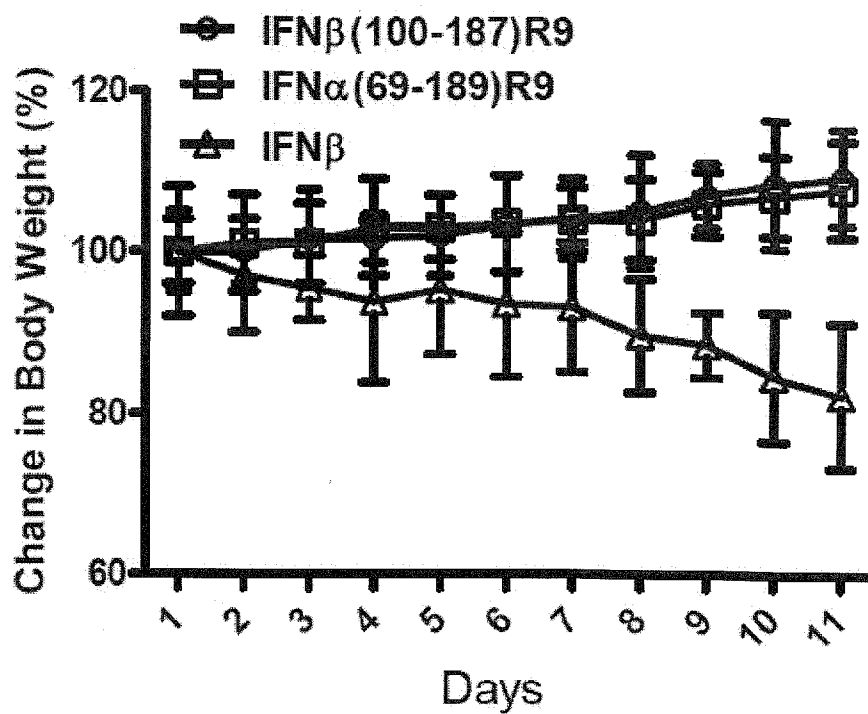


FIG. 10

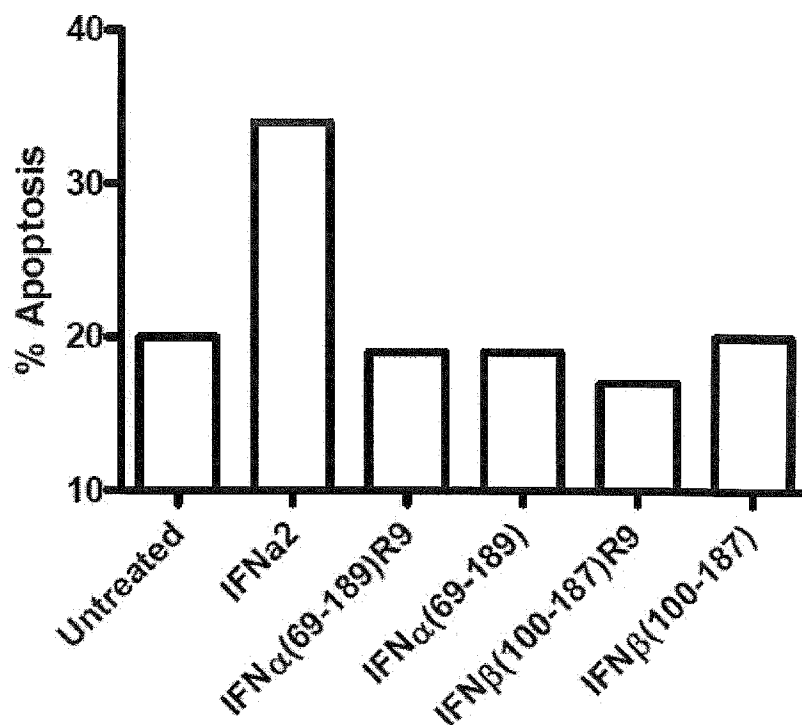


FIG. 11