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Report Title

Interferon agonists/mimetics as therapeutics for smallpox and other respiratory viruses

ABSTRACT

We have developed small peptide mimetics of interferon (IFN) gamma that bypass the receptor extracellular binding site and interact directly downstream in the IFN signaling cascade. Importantly, unlike IFNs that are neutralized by the decoy receptors of poxviruses, the mimetics are fully active against viruses in the presence of B8R protein that blocks IFN gamma antiviral activity. 1. IFN gamma (95-132) protects C57BL/6 mice against 1e6 to 1e7 plaque-forming units (pfu) of vaccinia virus (100- to 1000- fold the lethal dose) administered intranasally or IP. Protection was complete if mimetic was administered IP at days -2, -1, and 0 relative to the virus challenge. Importantly, treatment of mice up to 2 days, after giving mice 1e6 pfu virus intranasally (approx. 100- fold the lethal dose), with 200 ug mimetic also resulted in 100% protection. 2. IFN gamma (95-132) protects mice against lethal doses of EMC virus in the presence of poxvirus B8R protein. 3. IFN gamma and IFN gamma (95-132) mimetic are involved in activation of IFN gamma inducible genes at their respective promoters. We have shown that IFN gamma, the receptor subunit IFNGR1, and the IFN gamma transcription factor STAT1? are complexed in the cytoplasm and transit as a complex to the nucleus to the promoter site of two of IFN gamma activated genes. The implication is that our IFN mimetics appear to be the best candidates thus far in prophylactic and therapeutic treatment of poxvirus infections.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

1. Johnson H.M. and Ahmed CM (2006). Gamma interferon signaling: insight to development of interferon mimetics. Cellular and Molecular Biology 52: 71-76.
2. Ahmed C.M. and Johnson H.M. (2006). IFNg and its receptor subunit IFNGR1 are recruited to the IFNg-activated sequence element at the promoter site of IFNg-activated genes: Evidence of transactivational activity in IFNGR1. J. Immunology 176: 0000-0000.

Number of Papers published in peer-reviewed journals: 2.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

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(c) Papers presented at meetings, but not published in conference proceedings (N/A for none)

Number of Papers not Published: 0.00

(d) Manuscripts

1. Ahmed C.M. and Johnson H.M. IFN Mimetic as a Therapeutic for Lethal Vaccinia Virus Infection: Possible Effects on Innate and Adaptive Immune Responses. Manuscript in preparation.
2. Mujtaba M.G., Patel C., Patel R., Burkhardt M.A., Flowers L., Ahmed, C.M. and Johnson H.M. (2006). The interferon gamma mimetic peptide prevents encephalomyocarditis virus infection, both in cell culture and in mice. Vaccine and Clinical Immunology, In press.

Number of Manuscripts: 2.00

Number of Inventions:

Graduate Students

NAME	PERCENT SUPPORTED	
Lilian Waiboci	0.50	No
FTE Equivalent:	**0.50**	
Total Number:	**1**	

Names of Post Doctorates

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FTE Equivalent:	**0.50**	
Total Number:	**1**	

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Ravi Patel	0.50	No
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Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

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FTE Equivalent:	**1.00**	
Total Number:	**2**	

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Progress Report for Agreement # W911NF-05-1-0170 to
Howard M. Johnson, University of Florida
Interferon agonists/mimetics as therapeutics for smallpox and other respiratory viruses

We have developed small peptide mimetics of interferon (IFN) gamma that bypass the receptor extracellular binding site and interact directly downstream in the IFN signaling cascade. We have synthesized these mimetics with an attached hydrophobic residue for intracellular delivery. The mimetics encompass the C-terminus of IFN gamma and unlike intact IFN gamma are species non-specific in their action. We have shown in cell culture that the IFN mimetics, like IFN, are also virus nonspecific and are highly active against the picornavirus EMC virus and the poxvirus vaccinia. Importantly, unlike IFNs that are neutralized by the decoy receptors of poxviruses, the mimetics are fully active against viruses in the presence of B8R protein that blocks IFN gamma antiviral activity.

In this final report we show the progress of testing one of the IFN mimetics, IFN gamma (95-132), against lethal vaccinia virus and EMC virus infections in mice in the presence of B8R protein. We also show the mechanism of action of the IFN mimetics at the level of gene transcription.

1. IFN gamma (95-132) protects C57BL/6 mice against 10^6 to 10^7 plaque-forming units (pfu) of vaccinia virus (100- to 1000- fold the lethal dose) administered intranasally or IP. Protection was complete if mimetic was administered IP at days -2, -1, and 0 relative to the virus challenge. Protection of 80% was seen with mimetic doses as low as 15 ug. Importantly, treatment of mice up to 2 days, after giving mice 10^6 pfu virus intranasally (approx. 100- fold the lethal dose), with 200 ug mimetic also resulted in 100% protection (**Figure 1**). Treatment of mice beginning at either days 3, 4, or 5, remarkably resulted in protection of 80% of the mice against death, while mice treated at day 6 post-infection showed 40% survival. These results are particularly important in that untreated mice or mice treated with rat IFN gamma all died within 7 to 9 days at this dose of vaccinia virus.

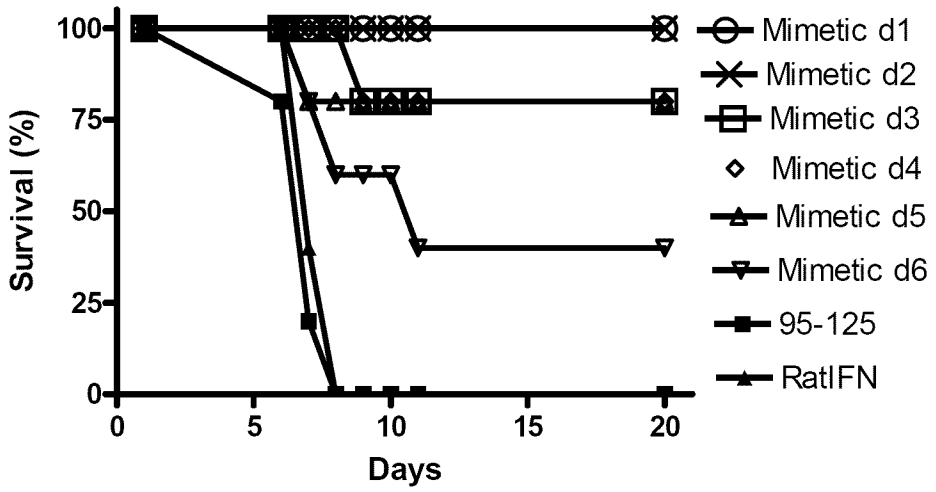

Figure 1. IFNγ(95-132) protects mice against vaccinia virus when administered late in infection. Vaccinia virus was administered to C57BL/6 mice (n=5, female 6-8 wks

old) on day 0 at 10^6 pfu per mouse (100-fold lethal dose), intranasally. IFNγ(95-132) was given at 200 ug 1 day (open circles), 2 days (marked X), 3 days (open squares), 4 days (open diamonds), 5 days (empty upward triangle), or 6 days (open inverted triangles) after initiation of infection with vaccinia virus. Control mice were injected IP on days -2, -1 and 0 with the control peptide IFNγ(95-125) (filled squares), or rat IFNγ (filled triangles). The lipophilic palmitate residue was added to all peptides to facilitate intracellular transport. As per the control peptide, IFNγ(95-125), the palmitate did not possess antiviral activity. Survival was followed for 20 days.

To our knowledge this is the most effective therapeutic known against vaccinia virus at this level of virus challenge under these conditions in this mouse model of poxvirus disease.

2. IFN gamma (95-132) protects C57BL/6 mice against lethal doses of EMC virus in the presence of poxvirus B8R protein. Similar to the vaccinia virus protection, IFN gamma (95-132) also completely protected mice against lethal doses of EMC virus when the mice were treated with poxvirus B8R protein to neutralize IFN gamma. Untreated mice or mice treated with rat IFN gamma in the presence of B8R protein were not protected. Similar to vaccinia virus infection, the EMC virus challenge IP (50 pfu) resulted in 100% mortality in 6 to 7 days. Further, rat IFN gamma was completely protective in the absence of B8R protein. Thus, B8R protein completely blocked IFN gamma protection.

3. IFN gamma and IFN gamma (95-132) mimetic are involved in activation of IFN gamma inducible genes at their respective promoters. We have shown that IFN gamma (and the mimetic), the receptor subunit IFNGR1, and the IFN gamma transcription factor STAT1α are complexed in the cytoplasm and transit as a complex to the nucleus to the promoter site of two of IFN gamma activated genes. Further, the receptor subunit IFNGR1 possessed transactivational properties at the level of gene expression.

All of the objectives of this proposal have thus been achieved. The implication is that our IFN mimetics appear to be the best candidates thus far in prophylactic and therapeutic treatment of poxvirus infections. The results of item 1 above are in preparation for publication and papers detailing the other data above are published or are in press as per the appropriate category in the OMB form.