Orally Administered Interferon to Prevent/Treat Foot-and-Mouth Disease

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Abstract

The Foot and Mouth (FMD) epidemic in the United States can seriously disrupt the supply of animal protein for human consumption. A similar impact could be from a planned bioterror/epidemic attack upon animal protein food supply. The epidemic response through massive livestock depopulations will produce billions of dollars of losses across multiple industries. An alternative to livestock depopulations is presented via use of interferon alpha (IFNα) in livestock feed. Such solution will minimize losses when used with or without simultaneous FMD vaccination.

Introduction

Interferons are a group of inducible cytokines that were identified originally on the basis of their antiviral activity and are now known to have many other important functions in innate and adaptive immune responses (Vilcek and Feldman 2004). The interferon alpha/beta (IFNα/β) family comprises at least 13 human genes (Vilcek and Feldman 2004, Williams 2000) while the IFNγ family includes a single gene in mammals. Other members of the family of IFN-like cytokines are IFNω, IFNκ, IFNε, IFNτ (Vilcek and Feldman 2004).

As with most cytokines, constitutive production of IFNs is either undetectable or very low. IFNα/β proteins are inducible in several cells (including specialized dendritic cells, monocytes and macrophages) by viruses, microbial products, double-stranded RNA and other substances (Vilcek and Feldman 2004, Williams 2000). IFNs are the first cytokines used in clinical therapy of more than a dozen viral diseases, malignancies, and neural disorders in 50 different countries. Until today IFNs have proven efficacy in the therapy of chronic myelogenous leukemia, Kaposi's sarcoma, lymphomas, hairy cell leukemia, multiple myeloma, gliomas, ovarian cancers, renal cell carcinoma, melanoma, and multiple sclerosis (Kalvakolanu 2003).

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Interferon Alpha effects are not Species Specific

The IFNs are now available in purified form as both naturally occurring and as recombinant molecules. An important facet of the IFNs is the fact that the IFNα family is not species-specific in action, but is better described as somewhat species-restricted. IFNα of human origin protects animal cells and human cells are protected by IFNα of animal origin. Bovine IFNα affects primate, (Tovey et al 1977) porcine and human cell cultures (Carter 1979) and Porcine IFNα affects equine, bovine and human cell cultures (Carter 1979, Carter et al. 1979). Human (Hu) IFNα affects porcine, (Carter 1979, Carter et al. 1979, Gressor et al. 1974), bovine (Carter 1979, Carter et al. 1979, Gressor et al. 1974, Branca 1986; Meister et al. 1986, Chambers et al. 1990, Pestka 1997) and feline (Desmyter and Stewart 1976) cell cultures.

Interferon Activates Many Immune Response Genes

A central intracellular pathway responding to IFN is the induction of the enzyme, 2'5'-oligoadenylate synthetase (2'5'AS) (Williams 2000). This enzyme induced by exposure to IFN is a convenient molecular marker of IFN-induced cellular activity. Increased expression of MHC class I antigen and induction of 2'5'AS (Williams 2000) are positive cellular responses to IFN. When DBA/2 mice were given oromucosal murine (Mu) IFNα/β, neither increased MHC-I nor induced 2'5'AS were detected in peripheral blood or spleen. However, MHC class I antigen expression was significantly increased in lymphoid cells harvested from the oropharyngeal cavity 24 hours after oromucosal HuIFNα 1-8 treatment (Eid et al 1999). The induction of this marker in this case may emphasize IFN action in local mucosal compartments rather than IFN systemic effects. Oromucosal MuIFNα augmented IFN response factor (IRF)-1, 2'5'AS mRNA expression levels and 2'5'AS enzyme activity in the spleen but not in cervical lymph nodes of C3H mice (Takayama et al. 1999). In contrast, HuIFNβ provided in drinking water induced 2'5'AS activity in whole blood and inhibited

the late asthmatic response in guinea pigs (Satoh et al. 1999). The concentration of HuIFNβ given to guinea pigs that induced the most of 2'5'AS was 0.1 international units (IU)/ml in drinking water with an estimated daily intake of 50 ml, or 5 IU/day (Satoh et al. 1999). Gastric administration of MuIFNα (10^2 - 10^4 IU) induced 2'5'AS in whole blood of ICR mice after 16 hours (Nakajima and Sokawa 2002). In addition to these genes and their products, numerous other genes are upregulated after oromucosal administration of IFNα. IFN-activated cells are detected four hours (but not two or eight hours) after initiation of IFN therapy (Tovey et al. 1999, Tovey et al. 2000, Dron et al. 2002). For example, the ADIR (ATP dependent IFN responsive gene) RNA transcripts were increased 5-6 fold in oropharyngeal tissue of Swiss mice four hours after oromucosal administration of 10^5 IU of MuIFNα (Dron et al 2002).

Hundreds of genes are regulated by IFNα, IFNβ and IFNγ (Der et al 1998). Gene expression analysis using specific ̀cover the biological basis of diseases, enhance our understanding of host-pathogen interactions and provide avenues for immunotherapy (Der et al. 1998, Ren et al. 2000, Shoemaker et al. 2001, Dhiman et al. 2001, Huang et al. 2001). The microarrays identify which genes are activated in cells by detecting the presence or absence of a corresponding messenger RNA (mRNA). When stimulated, cells convert genetic information into proteins, by first forming the corresponding intermediate product, the mRNAs. Therefore, if the mRNA corresponding to a gene is found in cells it is likely that the specific protein is also produced by cells (Dhiman et al 2001).

In addition to 2'5' AS, orally administered IFNα has been shown to affect systemic phenotypic expression of lymphocyte populations. IFN activated NK1.1, CD11b+, CD19/CD20 B-cells, and subpopulations of T-cells are present in the peripheral circulation of tumor bearing mice as early as four hours after the initiation of oromucosal IFNα therapy. In addition, oromucosal IFN therapy also induced trafficking of cells from both the spleen and peripheral lymph nodes to the site of tumor cell replication. Other genes upregulated by oral IFNα include Crg2 and other chemokines, proteases associated with antigen processing and genes involved in lymphocyte activation, apoptosis and protein degradation (Tovey et al. 2003).

FMD Virus Shuts Down Host's Interferon Production

As shown by scientists at ARS, the FMD virus has the ability to overcome several key host defenses. FMD virus inhibits production of IFNα/β (Chinsangaram et al. 1999) and blocks a key IFN-inducible, antiviral pathway, i.e.-double-stranded RNA (dsRNA)-dependent protein kinase R (PKR) (Chinsangaram et al. 2001). The PKR is a ubiquitously expressed serine/threonine protein kinase induced by IFN and activated by dsRNA, cytokines, growth factors and stress signals (Williams 2000). The antiviral activity of PKR is mediated by its phosphorylation of the subunit of initiation factor eIF2. Phosphorylation of eIF2 prevents recycling of eIF2:GDP2 to eIF2:GTP, trapping the recycling factor eIFa and resulting in rapid inhibition of translation (Williams 2000). PKR also mediates programmed cell death (apoptosis) induced by dsRNA (Williams 2000, Williams 1999, Tan and Katze 1999). Both inhibition of protein synthesis and induction of apoptosis restrict viral replication (Williams 2000). *Since the FMD virus controls host cells through suppression of IFNα/β production and blocking of the PKR effect then host treatment with exogenous IFNα will supplement endogenous IFNα production in the event of FMD viral infection.*

Interferon Blocks FMD Virus

Cattle given coital vesicular exanthema virus and infected with FMD virus had a milder form of FMD and developed later than control calves infected with FMD virus alone (Kubin 1961). A protective effect was noted in cattle infected with FMD virus and given multiple injections of yeast RNA (Thely et al. 1963). Presumably, the induction of IFN by virus and RNA, respectively, was responsible for the protection noted in these studies (Kubin 1961, Thely et al. 1963).

IFNα inhibits FMD virus (subtype A5) (Straub and Ahl 1976), IBR virus (Todd et al. 1972), bovine rhinovirus (Cummins and Rosenquist 1980), parainfluenza virus, type 3 (Cummins and Rosenquist 1982a) and bovine adenovirus type 3 (Cummins and Rosenquist 1982b).

IFN induced in the nasal secretions (NS) with intranasal vaccination with infectious bovine rhinotracheitis (IBR) virus provided protection against FMD viral challenge (Straub and Ahl 1976). Calves were challenged with FMD virus one or two days after intranasal vaccination with IBR virus. IFN was detected in the NS within 24 hours of vaccination and persisted at high levels for six additional days and at low levels through the tenth day after IBR virus vaccination. Vaccinated calves had a milder course of FMD and more than a 99% reduction in FMD virus titers in the NS (Staub and Ahl 1976).

In studies of FMD virus transmission from carrier to susceptible cattle, carriers of FMD virus were inoculated intranasally with IBR virus in an effort to create a stress, which could increase excretion of FMD virus from carrier cattle. As a result FMD virus disappeared from the esophageal-pharyngeal fluid of two carrier animals one day after IBR virus inoculation and was not detected again during the four-week sampling period (McVicar et al 1974). IFN was not assayed in this experiment, but it would have been induced by IBR virus and probably inhibited the FMD virus in carrier animals. IFN is readily induced in the NS of feedlot calves by IBR viral vaccine (Todd et al. 1972, Cummins and Hutcheson 1983).

The use of a viral inducer of IFN in cattle with FMD is in agreement with the successful use of oral synthetic IFN in-

ducers, which protected mice from infection with FMD virus (Richmond and Campbell 1973). One oral IFN inducer protected mice if given 2, 24 or 48 hours before FMD virus inoculation. Another inducer protected mice when it was given 18 hours or less before infection with FMD virus (Richmond and Campbell 1973). A single injection of mice with 150 µg of the synthetic IFN inducer polyriboinosinic: polyribocytidylic acid (PolyI:C) 18 hours before a challenge with 100 LD_{50} of FMD virus (strain Asia-10) was 100% protective (Richmond and Hamilton 1969). However, PolyI:C is toxic when tested in cattle (Rosenquist 1971, Theil et al. 1971, Angulo and Savan 1970).

Human Interferon Given Orally is Effective in Cattle

The oral delivery of the human IFNα (HuIFNα) has been beneficial to cattle undergoing shipping fever and in cattle challenged with virulent IBR virus or *Theileria parva* (Georgiades 1993, Cummins and Hutcheson 1993, Cummins et al. 1999, Young et al. 1990). In studies involving 7,000 feeder cattle a single dose of orally administered HuIFNα at the time of diagnosis of respiratory disease given with antibiotics reduced mortality significantly ($p<0.001$) when compared to feeder calves given placebo and antibiotics (Georgiades 1993). The clinical effects of a virulent IBR virus challenge in feeder calves were significantly reduced by oral HuIFNα therapy (Cummins and Hutcheson 1993). In studies of naturally occurring shipping fever oral HuIFNα given for three days before shipping or once after animal's arrival reduced respiratory illness and improved weight gain (Cummins et al. 1999). In a challenge study of calves given *Theileria parva*, the causative agent of East Coast Fever, some calves given oral HuIFNα survived an otherwise fatal challenge (Young et al. 1990). In the four studies cited above (Georgiades 1993, Cummins and Hutcheson 1993, Cummins et al. 1999, Young et al. 1990) the beneficial oral dose of HuIFNα was less than 500 IU per calf.

USDA, ARS Scientists Develop Interferon Based Defense Against FMD

In January 2003, scientists at ARS on Plum Island reported that a recombinant replication-defective human adenovirus type 5 vector containing porcine IFNα (Ad5-pIFNα) was constructed (Chinsangaram et al. 2003). When the Ad5-pIFNα was injected into swine the resulting IFNα production completely protected three swine given FMD virus. Swine given Ad5-pIFNα showed no signs of FMD, did not develop viremia and did not develop antibodies against viral nonstructural proteins. However, when Ad5-pIFNα or this same vector modified to carry bovine IFNα was injected into cattle, only Ad5-pIFNα provided partial *in vivo* protection by delaying viremia one day and decreasing vesicle formation (Wu et al. 2003). In December 2003, ARS scientists reported that Ad5-pIFNα given one, three or five days (but not seven days) before challenge with FMD virus resulted in complete protection. ARS scientists concluded, "It was interesting to note that animals challenged seven days after Ad5-pIFNα administration had no detectable IFNα in their plasma for 2-3 days prior to challenge; yet they had delayed and lower levels of viremia and vesicular lesions as compared to the control animals. This data implies that although complete resistance to virus replication may only last 1-2 days after clearance of IFNα, IFN 'activated' cells can still reduce the rate of virus replication for an additional 1-2 days" (Moraes et al. 2003).

Summary

It is prudent to consider alternatives to animal depopulations in case of FMD epidemic in the United States and other parts of the world. Vaccination against FMD outside of the U.S. has been practiced with success. However, FMD protective immunity takes time to develop. In the past, challenge studies were conducted at least four weeks after vaccination to test for FMD immunity (McKercher and Giordan 1967, McKercher and Farris 1967, McKercher 1967, McKercher and Bachrach 1976, Morgan and Moore 1990). Newer vaccine methods provide protection more quickly and protective immunity may develop in four to seven days instead of weeks (Moraes et al. 2002, Beard et al. 1999, Doel 2003, Cedillo-Barron et al. 2001, Barnett and Carabin 2002). The combined use of new vaccines for long-term immunity and oral livestock dosing with HuIFNα for short-term (in days) immune modulation may be a reasonable and economical alternative to massive animal depopulations in the face of a multi-regional FMD outbreak. It is proposed that HuIFNα provided to livestock in feed may sufficiently boost animal's immune defenses against FMD virus by up-regulating key immune response genes and their protein products. Use of HuIFNα in animal feed is cost efficient, safe, easy to administer and effective against FMD and other diseases in multiple livestock and animal species. The proposed oral, in-feed use of HuIFNα can be implemented as a broad-spectrum FMD treatment and prevention solution.

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