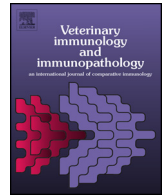




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Low-dose oral interferon modulates expression of inflammatory and autoimmune genes in cattle



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ABSTRACT

While the safety and efficacy profiles of orally administered bovine interferon (IFN) alpha have been documented, the mechanism(s) that result in clinical benefits remain elusive. One approach to delineating the molecular pathways of IFN efficacy is through the use of gene expression profiling technologies. In this proof-of-concept study, different (0, 50, 200 and 800 units) oral doses of natural bovine IFN (type I) were tested in cattle to determine if oral IFN altered the expression of genes that may be pivotal to the development of systemic resistance to viral infections such as foot-and-mouth disease (FMD). Oral IFN was administered twice: Time 0 and 8 h later. Blood was collected at 0, 8 and 24 h after the first IFN administration, and DNA isolated from peripheral blood mononuclear cells (PBMCs) was employed in quantitative polymerase chain reaction (qPCR) microarray assays. Within 8 h, 50 and 200 units of oral IFN induced significant ($P < 0.05$) changes in expression of 41 of 92 tested autoimmune and inflammatory response-associated genes. These data suggest that orally administered IFN is a viable approach for providing short-term antiviral immunity to livestock exposed to viruses such as FMD virus (FMDV) until such a time that an effective vaccine can be produced and distributed to producers.

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1. Introduction

Orally administered bovine natural interferon (IFN) is a viable candidate therapeutic agent for the control of viral diseases of livestock. Years of experience with therapeutic effects of human IFN α (HuIFN α) administered orally to livestock and companion animals indicate that oral HuIFN α is safe and efficacious (Cummins et al., 1988, 2005; Young and Cummins, 1990; Cummins and Stewart, 1991; Georgiades, 1993; Fulton et al., 1993; Georgiades and Fleischmann, 1996; Moore et al., 1996, 2004; Amadori et al., 2002). The mechanisms of action for the well-documented systemic beneficial effects of orally administered IFN are still not understood, although it has been shown that contact with the oral

cavity amplifies the IFN-mediated therapeutic effects via interactions with mononuclear cells in the oral-pharyngeal mucosa (Eid et al., 1999; Tovey and Maury, 1999; Tovey et al., 2000, 2003; Dron et al., 2001). Studies of the action of IFN-stimulated genes (ISGs) and their protein products have resulted in fundamental discoveries relevant to translational control, regulation of RNA stability and editing, and protein transport and turnover. The antiviral and cellular actions of selected potent ISGs are part of critical pathways of antiviral actions, and thus are critical to improved clinical application of IFN (Borden and Williams, 2011). The proteins encoded by ISGs individually degrade various viral RNAs, block viral transcription, inhibit translation and/or modify protein assembly steps for viral replication and release (Borden and Williams, 2011). ISGs are up-regulated in humans within a few hours after oral HuIFN α administration (Brod et al., 1999; Smith et al., 1999). In another study in cattle, cDNA microarray technology was used to help identify appropriate targets for therapeutic intervention with HuIFN α .

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Calves were given a low dose (200 units) of HuIFN α orally once daily for 5 days, and microarray analysis was performed on 8,329 genes (Namangala et al., 2006). The authors identified the profile of more than 6000 genes altered 4 h after the final oral dose of IFN. The majority of the genes had <2 fold degree of change but 8.5% had at least a 4-fold increase in expression. Low-dose oral HuIFN α at 200 units suppressed the expression of about 5% of the 6000 genes that were altered. In 15.5% of altered genes tested, oral HuIFN α altered expression of genes associated with innate immunity, antigen presentation, lymphocyte activation, immune modulation, blood coagulation, hematopoiesis and apoptosis. Non-immunologic functions were represented in 84.5% of altered genes tested (Namangala et al., 2006). The authors commented that “the observed repression of about 5% of the gene transcripts shows the pleiotropic nature of IFN, stimulating some genes while suppressing others.” It is thus reasonable to hypothesize that oral low-dose bovine IFN is capable of altering the same gene groups affected with low-dose oral HuIFN α . The purpose of the present study was to determine if low-dose oral bovine IFN would have beneficial biological effects on bovine immune response gene expression. In the cattle study reported herein, the expression of 92 immune response-related genes was analyzed. The mode of action of oral IFN on gene expression in peripheral blood mononuclear cells (PBMCs) was evaluated further by assessment of IFN effects on families of genes in the Kyoto Encyclopedia of Genes and Genomes (KEGG) cytokine–cytokine receptor interaction pathway.

2. Materials and methods

2.1. Interferon

Natural bovine IFN (type 1, alpha) was obtained from Colorado Serum Company, Denver, CO. Bovine blood was collected in the IMUFLEX WB-RP blood bag system (Turumo Corporation, Tokyo, Japan) with a filter that collected the white blood cells (WBC). The red blood cells were discarded. The filter containing WBC was incubated with an isolate (USA) of bluetongue virus (BTV) to induce IFN production by isolated WBC. Acid treatment at pH 2.0 for 60 min was used to inactivate BTV. The resultant IFN product used for the *in vivo* treatment of calves is a mixture of IFN subspecies.

2.2. IFN assay

The IFN antiviral activity titer was determined by a vesicular stomatitis virus (VSV) plaque reduction assay (Rosenquist and Loan, 1967) on cell cultures of Madin Darby Bovine Kidney (MDBK) cells. The titer of bovine IFN was calculated by a standard method (Epstein, 1976).

2.3. Preparation of control and IFN solutions

The IFN-negative control solution was 1.0% (v/v) fetal calf serum in normal saline. This solution was also used as the diluent for three IFN doses used. An IFN concentrate (1190 units/ml) was used to prepare each of three IFN treatment doses to final concentrations of 50, 200 and 800 units/calf.

2.4. Treatment of animals

Sixteen Holstein steer calves (129–193 kg body weight) were randomly allotted to control and IFN treatment groups of 50, 200 or 800 units (4 calves per group). Calves in each group were given placebo diluent or IFN dose twice (0 and 8 h) on day one of the study. Each animal received a total of 10 ml, administered into the oral cavity with a sterile disposable syringe. The calculated final amount of IFN given in each group was: 0.0, 0.3, 1.3 and 5.3 units/kg based on

the mean weight per group. Body weights and rectal temperatures were also recorded before and after the IFN treatments (0, 8 and 24 h).

2.5. Blood sample collection

Unclotted blood from each calf was collected into BD Vacutainer CPT Cell Preparation Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) at 0, 8 and 24 h for isolation of bovine RNAs by Beech Tree Laboratory (BTL, Providence, RI). Unclotted blood was also collected in both red-top BD Vacutainer tubes and EDTA tubes for complete blood counts (CBCs) and blood chemistries respectively conducted at the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL, Amarillo, TX).

2.6. Isolation of PBMCs from unclotted blood

The blood-filled BD Vacutainer CPT tubes containing leukocyte separation medium (thixotropic polyester gel and Ficoll-Hypaque) were centrifuged at 3200 rpm for 30 min to separate erythrocytes from leukocytes. One-half of the plasma was removed by aspiration and the remaining plasma used to re-suspend the enriched leukocytes from the surface of the polyester gel. The leukocyte-rich plasma was diluted to 14.0 ml final volume with phosphate-buffered saline in 15 ml conical centrifuge tubes, and kept in an ice bath prior to centrifugation (1500 rpm X 10 min). After centrifugation, the supernatants were aspirated, with each cell pellet gently re-suspended in an aliquot of supernatant and then transferred to a 1.8-ml vial. Cold (4C) TRIZOL[®] reagent (Invitrogen, Carlsbad, CA) was added (0.75 ml/vial) to lyse all cells. Vials were stored at –70C until shipment on dry ice to BTL.

2.7. RNA isolation from PBMCs and RNA assessment

Two-tenths ml of reagent-grade chloroform was added to each rapidly thawed vial containing cell lysates and vortexed for 15 s. After 2–3 min, 22C, capped vials were centrifuged (12,000g, 15 min), 2–8C. The aqueous phase containing soluble RNAs was transferred to a fresh 1.8 ml vial and the RNAs were precipitated from solution with 0.5 ml isopropyl alcohol. Samples were then held at 22C, 10 min and centrifuged at 12,000g, 10 min. The RNA pellets were washed with 75% (v/v) ethanol followed by centrifugation at 7500g, 5 min; this wash was repeated and the resultant RNA pellets were air-dried for 5–10 min before dissolving in nuclease-free water. The bioanalyzer (Agilent RNA 6000 nano kit, 5067/1511; Agilent Technologies, Santa Clara, CA), is an automated bio-analytical device using microfluidics technology that provides electrophoretic separations in an automated and reproducible manner. Tiny amounts of RNA samples are separated in the channels of the microfabricated chips according to their molecular weights and subsequently detected via laser-induced fluorescence. The result is visualized as an electropherogram in which the amount of measured fluorescence correlates with the amount of RNA of a given size (Imbeaud et al., 2005).

2.8. Complementary (c) DNA synthesis and qPCR

RNA samples (0.25 μ g) were treated with genomic DNA (gDNA) elimination buffer (Qiagen, Valencia, CA) and incubated at 42C in a Multigene[™] Mini thermocycler (Labnet, Edison, NJ) for 5 min to digest any residual bovine gDNA contamination. Complementary DNA (cDNA) was synthesized from isolated bovine RNAs using an RT² First Strand Kit (Qiagen) in accordance with the protocol provided by the manufacturer. The cDNA synthesized from 0.25 μ g total RNA was combined with TaqMan[®] Gene Expression Master

Table 1
Summary of numbers of genes significantly up- or down-regulated by IFN dose and hour after the first IFN dose.

Dose (Units)	0	0	50	50	200	200	800	800
Hour	8	24	8	24	8	24	8	24
Change ^a	Up, Down	Up, Down	Up, Down	Up, Down	Up, Down	Up, Down	Up, Down	Up, Down
# Genes ^b	0, 5	1, 2	1, 21	0, 6	14, 2	2, 7	2, 3	0, 1

^a Up = Up-regulation, Down = Down-regulation of gene expression.

^b Number with significant change in gene expression ($p < 0.05$).

Mix loaded into 384-well TaqMan[®] Array Micro Fluidic Cards (Life Technologies, Carlsbad, CA).

Ninety-six inventoried bovine gene assays were selected from four different molecular pathways: Immune response (IR), inflammation (IF), cytokines (Cy) and chemokines (Ch). Four genes, ACTB, GAPDH, GUSB and 18S, were allocated as internal housekeeping controls. A real-time PCR used TaqMan[®] Array Micro Fluidic Cards to measure gene expression in cDNA libraries prepared from PBMC RNAs processed according to the manufacturer's protocols and reactions executed with an Applied Biosystems[®] ViiA[™] 7 Real-Time PCR System (Life Technologies).

2.9. Analysis of qPCR data

The cycle threshold (Ct) values obtained from the qPCR reactions were uploaded to web-based software provided by Qiagen. Ct values were normalized to housekeeping gene signals. For each gene evaluated within each treatment group, fold changes in gene expression were calculated relative to 0h control values. The resulting data reflected levels of the expression (up-regulation or down-regulation) of the various genes as a function of IFN effects versus control values for that same gene. Each calf served as its own control. Additional data evaluations and selection of genes of interest was accomplished using reviews of the scientific literature (PubMed, <http://www.ncbi.nlm.nih.gov/pubmed/>) coupled with the use of the Database for Annotation, Visualization and Integrated Discovery or DAVID program (Huang et al., 2009a, 2009b; Jiao et al., 2012).

2.10. Statistical analysis

Data were analyzed as a complete randomized design by the model $Y_{ijk} = \mu + Trt_i + Time_j + e_{ijk}$. Means were separated by protected Least Significant Differences (LSD) for treatments within time and tested for significance at $P \leq 0.05$. The change difference in gene expression at 8 and 24 h was analyzed by Chi Square Analysis. Two-tailed *t*-tests were performed to determine the statistical significance ($P < 0.05$) of the gene fold-change results. Genes were regarded as up-regulated or down-regulated when the fold change was >1.33 or <0.75 relative to controls and was statistically significant ($P < 0.05$).

3. Results

3.1. Modulation of expression of genes tested

Table 1 summarizes the expression of the 92 genes tested whose products participated within four different but overlapping molecular pathways for cellular activation (IR, IF, Cy and Ch) that were significantly ($P < 0.05$) modified by IFN at 8 and 24 h. Table 2 shows qPCR fold regulation data for individual genes whose expression was significantly ($P < 0.05$) altered by 0, 50, 200 or 800 units of IFN. Overall, of the 92 analyzed genes, 47 genes were affected by placebo or one or more concentrations of orally administered IFN, of which 41 (87%) were from the 50- or 200-unit IFN treatment groups. The

Table 2
Summary of genes significantly modified at 8 and 24 h after oral IFN at 0, 50, 200 or 800 units per dose. (Negative values = down-regulation, positive values = up-regulation).

Genes	Conc., Units	t = 8 h		t = 24 h	
		Fold Regulation	p-value	Fold Regulation	p-value
IFNAR2	0			1.36	0.046
IRAK2	0	-1.60	0.020	-1.36	0.007
MAP3K13	0	-1.79	0.020		
MAPK14	0	-1.72	0.010	-1.36	0.049
TGFB3	0	-2.68	0.030		
TLR6	0	-2.72	0.010		
ANXA1	50	-3.59	0.005		
ANXA5	50	-2.79	0.0004	-1.57	0.027
CCL19	50	-5.87	0.009		
CCRL1	50	-3.43	0.008		
CSF1	50	-1.53	0.002		
CXCL12	50	-4.41	0.019		
FAS	50	-2.10	0.002	-1.72	0.003
IL16	50	-1.59	0.035		
IL2RA	50	-2.18	0.012		
IL6R	50	-1.71	0.008		
IRAK2	50	-1.47	0.049		
ITGAL	50	-2.56	0.012	-2.50	0.030
ITGB2	50	-1.68	0.027		
PTGDR	50	-2.56	0.015		
RAB5C	50	-1.44	0.002	-1.24	0.029
SELP	50	2.99	0.010		
TGFB3	50	-3.77	0.021		
TGFBR1	50	-2.30	0.006	-1.81	0.044
TLR4	50	-2.29	0.018		
TNFRSF1A	50	-2.62	0.009		
TNFSF13B	50	-5.58	0.0004	-1.69	0.027
WWP1	50	-1.99	0.018		
CCL19	200	2.83	0.002		
CCL8	200	1.47	0.020		
CCRL1	200			-1.67	0.026
CREBBP	200			-1.47	0.007
CXCL6	200	1.60	0.040		
CXCR2	200			6.09	0.043
EDN1	200	4.33	0.000	3.36	0.050
GRO1	200	2.67	0.046		
ICAM1	200	2.25	0.004		
IFNAR2	200	1.90	0.028		
IKBKB	200			-1.77	0.012
IL10	200	2.41	0.025		
IL10RB	200	1.43	0.022		
IL1A	200	19.90	0.000		
IL1B	200	4.88	0.004		
IL6	200	1.75	0.008		
IL6R	200	-1.62	0.014	-1.51	0.015
IRF9	200	-4.42	0.006	-3.52	0.003
ITGB2	200			-1.25	0.007
NFKBIA	200	2.68	0.004		
PTGS2	200	2.25	0.019		
SMAD4	200			-1.53	0.037
TNF	200	-2.14	0.027		
IFNG	800	-2.54	0.034		
IL10	800	2.03	0.045		
MAPK14	800	-1.30	0.037		
TGFB1	800	-1.64	0.046	-1.43	0.002
VEGFA	800	1.51	0.010		

Table 3

PBMC receptor or glycoprotein genes down-regulated by IFN (50 units, 8 h), based on DAVID enrichment analysis results for functional category.

OFFICIAL.GENE.SYMBOL	GENE NAME	Species
FAS	Fas (TNF receptor superfamily, member 6)	<i>Bos taurus</i>
ANXA1	annexin A1; similar to Annexin A1	<i>Bos taurus</i>
CCRL1	chemokine (C-C motif) receptor-like 1	<i>Bos taurus</i>
ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)	<i>Bos taurus</i>
ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	<i>Bos taurus</i>
IL2RA	interleukin 2 receptor, alpha	<i>Bos taurus</i>
PTGDR	prostaglandin D2 receptor (DP)	<i>Bos taurus</i>
TLR4	toll-like receptor 4	<i>Bos taurus</i>
TGFBR1	transforming growth factor, beta receptor 1	<i>Bos taurus</i>
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	<i>Bos taurus</i>

majority of genes affected at 8 h by 50 units of IFN were down-regulated whereas the majority of genes affected by 200 units of IFN were up-regulated.

3.2. Modulation of expression of genes by placebo and 50, 200 or 800 units of oral IFN

Modest changes were noted in six IR-related genes at 8 or 24 h in samples from placebo-treated control calves. Five genes were down-regulated at 8 h and two of these five were down-regulated at 24 h; one gene was up-regulated at 24 h. By 8 h post-first IFN treatment, 21 IR genes were significantly ($P < 0.05$) down-regulated in calves given 50 units of IFN; expression of one gene (SELP) was significantly up-regulated. The second 50-unit dose, given at 8 h, down-regulated expression of only six of these genes 16 h later.

Table 4

PBMC cytokine–cytokine receptor interaction genes down-regulated by IFN (50 units, 8 h) selected via KEGG pathway generation.

OFFICIAL.GENE.SYMBOL	GENE NAME	Species	KEGG Pathway Family
CSF1	colony stimulating factor 1 (macrophage)	<i>Bos taurus</i>	PDGF
CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	<i>Bos taurus</i>	CXC Subfamily
FAS	Fas (TNF receptor superfamily, member 6)	<i>Bos taurus</i>	TNF
IL2RA	interleukin 2 receptor, alpha	<i>Bos taurus</i>	Hematopoietins
IL6R	interleukin 6 receptor	<i>Bos taurus</i>	Hematopoietins
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	<i>Bos taurus</i>	TNF
TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b	<i>Bos taurus</i>	TNF

When calves were given 200 units of IFN, 14 genes were significantly ($P < 0.05$) up-regulated relative to controls at 8 h; only 3 genes were down-regulated. Only one of the 14 up-regulated genes at 8 h remained elevated at 24 h; two genes (IL6R and IRF9) that were down-regulated at 8 h remained down-regulated at 24 h. By 24 h, one additional up-regulated IR gene and five additional down-regulated IR genes were identified. The highest dose of IFN tested (800 units) yielded only five changes in gene expression at 8 h (two genes were up-regulated and three genes were down-regulated) and only one gene was up-regulated at 24 h. When modification of gene expression was evaluated at 24 h, 16 h after the second dose of IFN, most of the effects on gene expression noted at 8 h were not seen, regardless of the IFN dose given.

3.3. Comparison of modulation of expression of genes by 50 versus 200 units of IFN

Only one gene (CCL3) was common to the two treatment groups administered either 50 or 200 units of IFN. CCL3 was down-regulated by 50 units of IFN but up-regulated by 200 units of IFN. The observed differences in IR-related gene expression by 50 versus 200 units IFN were further evaluated using pathway analysis software, specifically, the DAVID program. Using the *Bos taurus* genome as the baseline or background control, the 21 down-regulated (50 units, 8 h) genes were analyzed with the functional annotation tool of DAVID. Two major functional categories were identified by DAVID enrichment analysis: Receptor protein genes (nine), and glycoprotein receptor genes (ten). Analyses of these aggregate genes and gene products for functional implications revealed that all of these receptor genes performed essentially identical functions. Table 3 lists the ten down-regulated genes whose products are identified as receptors and/or binding ligand glycoproteins. DAVID's gene ontology enrichment analysis program further identified six genes coding gene products involved in the IR pathway (FAS, CCL19, CXCL12, TLR4, TNFSF13B, TNFRSF1A). The gene ontology enrichment analysis for molecular functions program within DAVID yielded four genes (CCL19, CXCL12, CSF1, IL16) whose products are cytokines or molecules with cytokine-like activities and three genes (CCRL1, TGFBR1, TNFRSF1A) whose products have cytokine binding or cytokine receptor activity. Additional analyses of genes (from PBMCs of calves given 50 units of IFN) using the DAVID program to identify KEGG pathways identified seven down-regulated genes located within specific families/subfamilies of the KEGG cytokine and cytokine receptor interaction pathway including the hematopoietin, PDGF and TNF families and the chemokine CXC subfamily (Table 4). That is, seven of 21 genes/gene products down-regulated by 50 units of IFN are part of a single KEGG pathway for cytokine and cytokine receptor interactions.

The same analyses were applied to the genes/gene products altered (primarily up-regulated) in PBMCs of calves given 200 units of IFN. Functional analysis of biological processes yielded 6 genes/gene products (EDN1, IL1A, IL1B, IL10, NFKB1A,

Table 5
PBMC cytokine–cytokine receptor interaction genes up-regulated by IFN (200 units, 8 h) selected via KEGG pathway generation.

OFFICIAL.GENE.SYMBOL	GENE NAME	Species	KEGG Pathway Family
IFNAR2	interferon (alpha, beta and omega) receptor 2	<i>Bos taurus</i>	Interferon
IL1A	interleukin 1, alpha	<i>Bos taurus</i>	IL-1
IL1B	interleukin 1, beta	<i>Bos taurus</i>	IL-1
IL10	interleukin 10	<i>Bos taurus</i>	IL-10
IL10RB	interleukin 10 receptor, beta	<i>Bos taurus</i>	IL-10

PTGS2) involved in regulation of cell proliferation and/or cellular localization. Molecular functional analysis identified 4 genes/gene products (CCL19, IL1A, IL1B, IL10) with cytokine activity. KEGG pathway analysis yielded 5 genes/gene products within the KEGG pathway for cytokine and cytokine receptor interactions (Table 5). This was the same KEGG pathway identified for seven genes/gene products for the 50-unit treatment group. The five up-regulated genes (IFNAR2, IL1A, IL1B, IL10 and IL10RB) were present within three specific families within this KEGG pathway: IFN, IL-10 and IL-1. A schematic picture of how KEGG cytokine–cytokine receptor interaction genes are related can be visualized at http://www.genome.jp/kegg-bin/show_pathway?scale=1.0&query=&map=bta04060&scale=1.84&auto_image=&show_description%20=%20show&multi.query=.

3.4. Altered expression of creatine kinase in serum of calves

While there were no significant changes noted in the CBCs between IFN treatment groups, the chemistry panel indicated that creatine kinase levels were decreased in calves given oral IFN compared to placebo at 8 and 24 h. However, these changes were not statistically significant (data not shown).

4. Discussion

Oral IFN markedly altered expression of genes associated with the immune response within 8 h of IFN administration. Moreover, this effect was systemic in that changes in gene expression were detected in PBMCs, which are not directly associated with the IFN-treated oral mucosa. Seven down-regulated and five up-regulated genes/gene products modulated by 50 and 200 units of orally administered IFN were shown to be part of the same KEGG pathway for cytokine and cytokine receptor interactions that are critical for the early recognition and response phases of innate and adaptive immunity. The IFN-responsive genes/gene products belong to different families of genes/products within this KEGG pathway. The effects of oral IFN on the expression of the specific cytokine/cytokine interaction genes identified in these studies merit further investigation. One concern involved the up-regulation by IFN of IL10 and IL10R. These two genes encode a cytokine and cytokine receptor, respectively, known to have Th2-type (anti-inflammatory) effects, whereas IFN, notably IFN-gamma, is thought to favor Th1-type immune responses (Kidd, 2003). Treatment of the IFN at pH 2.0 inactivated IFN-gamma and IFN-beta. However, the IFN (type I, alpha) used in these studies can have pleiotropic biological activities, including antiviral activity, antiproliferative effects, and stimulation of cytotoxic activity (Pestka, 1997; Pestka et al., 2004). In fact, type I IFN-induced production of IL-10 and its receptor has been reported in the literature (Piazzolla et al., 2000; Levings et al., 2001; Corre et al., 2013; Touzot et al., 2015). These data suggest that subspecies of IFN present in the existing formulation is/are affecting IL10 and IL10R gene expression.

Importantly, these data provide direct evidence for systemic modulation of the immune response by very low doses of orally administered IFN in an outbred domestic animal species. Systemic activation of this protective antiviral effect appears to extend into various aspects of innate and developing immune responses and can be expected to provide additional systemic beneficial effects to FMDV-challenged livestock as it has for other viral infectious diseases of animals (Cummins et al., 2005).

FMDV, a viral pathogen of ruminants and swine, is an icosahedral 25–30 nm single-stranded RNA virus, genus *Aphthovirus*, family *Picornaviridae*. Among more than 60 distinct strains, 7 serotypes are recognized (O, A, C, Asia-1 and South African Territories (SAT) 1, SAT 2, and SAT 3). The prevalence of serotypes/strains varies by geographic region; FMDV type O is the most prevalent. Depending upon the strain and species affected, morbidity may reach 100%; mortality in adults is typically less than 1%. Young animals develop FMDV-associated myocarditis and endocarditis and are more likely to die from the direct effects of FMDV infection (Alexandersen et al., 2003; Grubman and Baxt, 2004). Cattle typically present with fever, anorexia, and reduction in milk production; excessive salivation is often seen. Vesicular lesions develop on mucous membranes, interdental spaces, and coronary bands within two to three days after infection. In swine, vesicular lesions develop on coronary bands and may also be found on snout, udder, hock and elbow. Smaller ungulates (sheep and goats) develop mild vesicles in the oral cavity, bulbs of the heel and coronary bands (Alexandersen et al., 2003). Vesicles containing infectious virions rupture one to two days after formation, which facilitates spread of the infection to other animals and contamination of the environment. There is particular concern about FMDV because of its potential adverse economic impact upon livestock production and also because of embargos that will be placed upon US and Canadian meat products prepared for export should FMD occur in North America. The last FMD outbreak in the US was in 1929 and the last outbreak in Canada was in 1951 (Fenner et al., 1987). However, several recent FMDV outbreaks have occurred outside the US (OIE, 2015) and, like recent experiences with novel swine coronaviruses (Stevenson et al., 2013), could easily reach North America. While serotype-specific vaccination(s) can control FMDV, not all strains within a serotype are responsive to vaccinations and new FMDV strains may emerge by mutation(s) (USDA, 2013). Thus, FMDV control and containment time may be lost while strain-specific vaccine(s) are produced, distributed and then administered to susceptible livestock. Accordingly, an effective antiviral therapeutic agent would be useful for controlling FMDV outbreaks until vaccines are available. It is known that FMDV is inhibited by IFN (Richmond and Hamilton, 1969; Richmond and Campbell, 1973). Conversely, as a part of its intracellular replication strategy, FMDV is known to inhibit production of intracellular IFN-alpha/beta (α/β) (Chinsangaram et al., 1999) and, via its double-stranded (ds) RNA viral intermediate, block intracellular dsRNA-dependent protein kinase R (PKR), a central intracellular IFN-inducible antiviral pathway (Chinsangaram et al., 2001). A stable resistance-related calcium binding protein (sorcicin) interacts with FMDV VP1 protein to inhibit the type I IFN

response (Li et al., 2013). Thus, induction of endogenous IFN or treatment with exogenous IFN could help control FMDV under field conditions by providing IFN otherwise blocked by FMDV anti-IFN mechanisms. Both infectious bovine rhinotracheitis (IBR) herpesvirus and coital vesicular exanthema viruses inhibit FMDV infections by IFN induction (Straub and Ahl, 1976). Similarly, synthetic IFN inducers (Poly I:C and/or ICLC) inhibit FMDV in animal models of FMDV infection (Richmond and Hamilton, 1969; Richmond and Campbell, 1973) and FMDV in pigs (Cunliffe et al., 1977; Dias et al., 2012; Diaz-San Segundo et al., 2014). A related IFN induction strategy is use of recombinant replication-defective viruses containing IFN production genes within the viral genome (Chinsangaram et al., 2003; Wu et al., 2003; Moraes et al., 2003; Dias et al., 2011, 2012; Perez-Martin et al., 2012). Some chemical IFN inducers are too toxic to be useful in the management of FMDV in livestock (McVicar et al., 1973; Cunliffe et al., 1977) but Poly ICLC and Poly I:C are reported to be effective adjuvants for FMDV vaccines (Cao et al., 2013; Diaz-San Segundo et al., 2014). Regardless, toxicity is not a factor with viral IFN induction (McVicar et al., 1974).

The precise cellular and molecular mechanisms of action of IFN against FMDV are not all known but a correlation is reported between protection and specific ISG up-regulation and tissue-specific infiltration of dendritic cells and natural killer cells (Moraes et al., 2007; Diaz-San Segundo et al., 2010). Furthermore, studies note that IFN induction may also regulate expression of immunoregulatory-, cytokine-, chemokine- and IFN-stimulated genes that contribute to preventing or decreasing the duration and severity of FMDV infections and FMDV shedding into the environment (de los Santos et al., 2012; Diaz-San Segundo et al., 2013).

The administration of exogenous IFN to at-risk livestock populations in the face of an FMDV outbreak represents an attractive and inexpensive alternative to induction of endogenous IFN by chemical, biological or recombinant means. Based on the data from this study, benefits occur within 8 h and not days (as in the case of viral inducers) to be effective. The IFNs are now widely available in purified form as both naturally-occurring and also as purified recombinant molecules. An important attribute of the IFN family is that, aside from IFN gamma, the other IFNs, particularly IFN α , are not species-specific in their biological actions but rather are variably species-restricted, presumably because the cellular receptors for IFN α subfamilies are largely conserved across species. For example, human cells are responsive to animal-origin IFN and many animal cells are responsive to natural or recombinant HuIFN α (Cummins et al., 1999a). Bovine-origin IFN α is active *in vitro* on primate (Tovey et al., 1977), porcine (Carter et al., 1979; Gressor et al., 1974) and human cell cultures (Gressor et al., 1974; Carter, 1979). Porcine IFN α is active on equine, bovine and human cell cultures (Carter, 1979; Carter et al., 1979). HuIFN α is active on porcine, bovine (Gressor et al., 1974; Carter 1979; Carter et al., 1979; Branca, 1986; Meister et al., 1986; Chambers et al., 1990; Pestka, 1997), and feline (Desmyter and Stewart, 1976) cell cultures.

In addition to activity against FMDV, oral HuIFN α is beneficial when given to cattle with shipping fever pneumonia complex or to cattle challenged with virulent IBR virus or with the hemotropic protozoan parasite, *Theileria parva* (Young et al., 1990; Cummins and Hutcheson, 1993; Georgiades, 1993; Cummins et al., 1999b). In all four of these studies, the beneficial oral doses of HuIFN α were 30–500 IU per calf, which compare favorably with the active doses in the present study.

A central question raised in all of these studies is as follows: How does a low dose of IFN given orally exert such diverse systemic beneficial immunomodulatory effects? Available data suggests that orally administered IFN α is bound to the tonsils and associated lymphoid and dendritic cell aggregates in the oral and pharyngeal cavity (Beilharz et al., 1997). From here, a cascade of secondary cytokine and chemokine signals are released which have

pleiotropic systemic effects. For example, IFN α or IFN β given orally up-regulates antiviral Mx gene activity in the murine splenocytes and human PBMCs (Brod et al., 1999). Two-prime-five-prime (2'5') adenylyl synthetase (AS), a well-characterized inducible intracellular enzyme in the IFN-induction pathway (Johnson et al., 1980) is activated systemically, not just in the oral cavities of mice and guinea pigs given oral IFN (Satoh et al., 1999). Other human (Smith et al., 1999) and murine (Eid et al., 1999; Tovey et al., 2000, 2003; Dron et al., 2001) genes are also up-regulated by orally or oronasally administered murine IFN.

Is it possible that something other than IFN may be causing the modulation(s) of gene expression documented in this study? This possibility was considered but rejected because the placebo control solution was also used as the diluent, and because multiple peer-reviewed scientific reports indicate that low doses but not higher doses of oral IFN have beneficial effects upon different diseases in cattle (Cummins et al., 1993), horses (Moore et al., 1996, 2004), swine (Cummins et al., 1995), mice (Tanaka-Kataoka et al., 1999), cats (Cummins et al., 1988), poultry (Fulton et al., 1993) and humans (Ship et al., 1999). In the present study, the data show that 800 units of bovine IFN had negligible effects on gene expression. This finding is consistent with clinical observations that higher doses of oral HuIFN α do not provide additional beneficial immunomodulatory effects and may, in fact, have adverse consequences in animals treated with these higher HuIFN α doses.

In this study, oral IFN markedly altered expression of genes associated with the immune response within 8 h of IFN administration. Moreover, this effect was systemic in that changes in gene expression were detected in PBMCs, which are remote to the oral mucosa. It is not yet known if the down-regulation of genes at 50 units and up-regulation of genes at 200 units represent a biphasic dose response, or if the gene expression effects are temporal in nature. A similar study employing the two doses with samples being collected at multiple time points between 0 and 8 h could yield more information on this matter. The results also show little effects of IFN on gene expression in samples collected at 24 h (i.e., 16 h after a second IFN dose administered at 8 h). It is not known, however, whether the second dose of IFN was ineffective, as samples were not collected to match with the 8-h time point used for sample collection after the first dose. Other studies note relatively rapid effects of oral dosing. ISGs were up-regulated in humans within a few hours after oral HuIFN α administration (Brod et al., 1999; Smith et al., 1999). A 15 kDa ISG protein was up-regulated in human buccal epithelial cells both *in vivo* and *in vitro*, with peak levels of ISG-15 occurring two hours after oral HuIFN α exposure (Smith et al., 1999). In short, the data suggest that the dose of IFN is critical for modulating gene expression and that there is a relatively narrow window for therapeutic effectiveness. Thus, it becomes important to ascertain whether the dose of oral IFN supplied to cattle in water or feed to cattle can be adequately controlled.

It is difficult, expensive and time-consuming to catch and restrain livestock to administer parenteral anti-FMDV vaccines or medications. Given in the feed or water, IFN may be a fast and inexpensive aid in FMD control. Because the effects of oral IFN were manifested strongly by 8 but not 16 h after treatment, it will probably be necessary to provide IFN in feed or water 2 or more times per day. The livestock industry has a history of adapting to any new useful technology. Automatic systems for the production of balanced rations for animals and food production consumption are in wide use for delivery of micro-ingredients (amino acids, mycotoxin binders, organic acids, vitamins and trace minerals) into batching processes to improve accuracy of delivery of micro-ingredients to increase efficiency of production. These systems are used widely in manufacturing many beef, dairy, swine and poultry feeds. The system is operated by a control unit that delivers specified amounts/types of micro-ingredients (milligrams

or micrograms per head per day) by weighing the premix of the product into the ration based on intake in PPMs. Different micro-machines are approved by FDA for mixing FDA-approved drugs for disease or improvement of animal efficiency. It would be a simple matter to determine if the expected feed intake per animal can be balanced with an effective dose of IFN. Automated delivery trials for IFN have already been tested. One study delivered HuIFN α in hog feed to control porcine reproductive and respiratory syndrome and post-weaning multisystemic wasting syndrome (Amadori et al., 2002). Other studies have used water to deliver IFN to poultry, mice and fish (Fleischmann et al., 1991, 1992; Fulton et al., 1993; Jarosinski et al., 2001; Watanuki et al., 2009).

5. Conclusions

Collectively, extensive *in vivo* data have demonstrated that orally administered low-dose IFN has rapid and systemic beneficial biological effects in animals and humans. In the present study, it was demonstrated that oral IFN statistically modulated the expression of 12 genes within a specific KEGG pathway (cytokine–cytokine receptor interactions). In turn, these data suggest that low-dose oral IFN exerts systemic and largely beneficial anti-viral effects by up- or down-regulation of specific immune response genes and their products likely needed for recovery from any viral disease, including FMDV. The accumulated evidence from this and other studies suggests that adoption of orally administered bovine IFN α or HuIFN α as a therapeutic modality to suppress viral replication in livestock at risk for FMDV exposure is an inexpensive, easily dispensed and widely available alternative and adjunct to vaccinations for FMDV. In addition to the obvious antiviral effects of oral IFN, systemic immune modulating effects are also part of the overall beneficial responses to IFN and it is the latter that may well provide prolonged systemic beneficial effects in virus-susceptible livestock populations. The results are intended to stimulate further testing using delivery in feed or water, potentially helping the livestock industry manage viral respiratory disease and protect against an FMDV outbreak.

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References

- Alexandersen, S., Zhang, Z., Donaldson, A.I., Garland, A.J.M., 2003. The pathogenesis and diagnosis of foot-and-mouth disease. *J. Comp. Pathol.* 129, 1–36.
- Amadori, M., Candotti, P., Begni, B., Rota Nodari, S., Nigrelli, A., 2002. Somministrazione orale di alfa interferone nel suinetto per il controllo delle sindromi PRSS e PMWS. *L'Osservatorio*–Agosto, pp. 2–4.
- Beilharz, M., McDonald, W., Watson, M., Heng, J., McGeachie, J., Lawson, C., 1997. Low-dose Type I interferons reduce early virus replication of murine cytomegalovirus *in vivo*. *J. Interferon Cytokine Res.* 17, 625–630.
- Borden, E., Williams, B.R., 2011. Interferon-stimulated genes and their protein products: what and how? *J. Interferon Cytokine Res.* 31, 1–5.
- Branca, A., 1986. High-affinity receptors for human interferon in bovine lung and human placenta. *J. Interferon Res.* 6, 305–311.
- Brod, S.A., Nelson, L., Jin, R., Wolinsky, J.S., 1999. Ingested interferon alpha induces Mx RNA. *Cytokine* 11, 492–499.
- Cao, Y., Lu, Z., Li, Y., Sun, P., Li, D., Li, P., Bai, X., Fu, Y., Bao, H., Zhou, C., Xie, B., Chen, Y., Liu, Z., 2013. Poly(I:C) combined with multi-epitope protein vaccine completely protects against foot-and-mouth disease virus challenge in pigs. *Antivir. Res.* 97, 145–153.
- Carter, W.A., Davis, L.R., Chadha, K.C., 1979. Porcine leukocyte interferon and antiviral activity in human cells. *Mol. Pharmacol.* 15, 685–690.
- Carter, W.A., 1979. Mechanisms of cross-species activity of mammalian interferons. *Pharmacol. Ther.* 7, 245–252.
- Chambers, P.J., Saltis, J., Alin, P., Wright, A., Linnane, A.W., Cheetham, B.F., 1990. Receptors for human interferon α on bovine cells: specificity and tissue distribution. *Immunopharmacol. Immunotoxicol.* 12, 513–525.
- Chinsangaram, J., Piccone, M.E., Grubman, M.J., 1999. Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. *J. Virol.* 73, 9891–9898.
- Chinsangaram, J., Koster, M., Grubman, M.J., 2001. Inhibition of L-depleted foot-and-mouth disease virus replication by alpha/beta interferon involved double stranded RNA-dependent protein kinase. *J. Virol.* 75, 5498–5503.
- Chinsangaram, J., Moraes, M.P., Koster, M., Grubman, M.J., 2003. Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease. *J. Virol.* 77, 1621–1625.
- Corre, B., Perrier, J., El Khouri, M., Cerboni, S., Pellegrini, S., Michel, F., 2013. Type I T interferon potentiates T-cell receptor mediated induction of IL-10-producing CD4 cells. *Eur. J. Immunol.* 43, 2730–2740.
- Cummins, J.M., Stewart, W.E.I.I., 1991. A review article A review article. Interferon administration by the oral route. *Clin. Microbiol.* 18, 57–61.
- Cummins, J.M., Guthrie, D., Hutcheson, D.P., Krakowka, S., Rosenquist, B.D., 1999a. Natural human interferon- α administered orally as a treatment of bovine respiratory disease complex. *J. Interferon Cytokine Res.* 19, 907–910.
- Cummins, J.M., Beilharz, M.W., Krakowka, S., 1999b. Oral use of interferon. *J. Interferon Cytokine Res.* 19, 853–858.
- Cummins, J.M., Tompkins, M.B., Olsen, R.G., Tompkins, W.A., Lewis, M.G., 1988. Oral use of human alpha interferon in cats. *J. Biol. Response Modif.* 7, 513–523.
- Cummins, J.M., Mock, R.E., Shive, B.W., Krakowka, S., Richards, A.B., Hutcheson, D.P., 1995. Oral treatment of transmissible gastroenteritis with natural human interferon alpha: a field study. *Vet. Immunol. Immunopathol.* 45, 355–360.
- Cummins, J.M., Hutcheson, D.P., Cummins, M.J., Georgiades, J.A., Richards, A.B., 1993. Oral therapy with human interferon alpha in calves experimentally injected with infectious bovine rhinotracheitis virus. *Arch. Immunol. Ther. Exp.* 41, 193–197.
- Cummins, J.M., Krakowka, G.S., Thompson, C.G., 2005. Systemic effects of interferons after oral administration in animals and humans. *Am. J. Vet. Res.* 66, 164–176.
- Cunliffe, H.R., Richmond, J.Y., Campbell, C.H., 1977. Interferon inducers and foot-and-mouth disease vaccines: influence of two synthetic polynucleotides on antibody response and immunity in guinea pigs and swine. *Can. J. Comp. Med.* 41, 117–121.
- Desmyter, J., Stewart, W.E., 1976. Molecular modification of interferon: attainment of human interferon in a conformation active on cat cells but inactive on human cells. *Virology* 70, 451–458.
- Dias, C.C., Moraes, M.P., Diaz-San Segundo, F., Pacheco, J.M., Artz, J., Grubman, M.J., de los Santos, T., 2011. Porcine Type I Interferon rapidly protects swine against challenge with multiple serotypes of foot-and-mouth disease virus. *J. Interferon Cytokine Res.* 31, 227–236.
- Dias, C.C., Moraes, M.P., Weiss, M., Diaz-San Segundo, F., Perez-Martin, E., Salazar, M., de los Santos, T., Grubman, M.J., 2012. Novel antiviral therapeutics to control foot-and-mouth diseases. *J. Interferon Cytokine Res.* 32, 462–474.
- Diaz-San Segundo, F., Moraes, M.P., de los Santos, T., Dias, C.C., Grubman, M.J., 2010. Interferon-induced protection against foot-and-mouth disease virus infection correlates with enhanced tissue-specific innate immune cell infiltration and interferon-stimulated gene expression. *J. Virol.* 84, 2063–2077.
- Diaz-San Segundo, F., Dias, C.C., Moraes, M.P., Weiss, M., Perez-Martin, E., Owens, G., Custer, M., Kamrud, K., de los Santos, T., Grubman, M.J., 2013. Venezuelan Equine Encephalitis replicon particles can induce rapid protection against foot-and-mouth disease virus. *J. Virol.* 87, 5447–5460.
- Diaz-San Segundo, F., Dias, C.C., Moraes, M.P., Weiss, M., Perez-Martin, E., Salazar, A.M., Grubman, M.J., de los Santos, T., 2014. Poly ICLC increases the potency of a replication-defective human adenovirus vectored foot-and-mouth disease vaccine. *Virology* 468–470, 283–292.
- Dron, M., Meritet, J.-F., Dandoy-Dron, F., Meyniel, J.-Ph., Tovey, M.G., 2001. Protein related to the tocsins. *J. Interferon Cytokine Res.* 21 (Suppl. 1), S65.
- Eid, P., Meritet, J.F., Maury, C., Lasfar, A., Weill, D., Tovey, M.G., 1999. Oral interferon therapy: pharmacokinetics and pharmacodynamics. *J. Interferon Cytokine Res.* 19, 157–169.
- Epstein, L., 1976. Assay of human immune interferon from lymphocyte-macrophage cultures by a virus plaque reduction method. In: Rose, N.R., Friedman, H. (Eds.), *Manual of Clinical Immunology*. ASM Press, Washington (DC), pp. 120–128.
- Fenner, F., Bachmann, P.A., Gibbs, E.P.J., Murphy, F.A., Studdert, M.J., White, D.O., 1987. Picornaviridae. In: *Veterinary Virology*. Academic Press, Inc., San Diego, CA, pp. 421–443 (Chapter 23).
- Fleischmann, W.R., Fields, E.E., Wang, J.L., Hughes, T.K., Stanton, G.J., 1991. Modulation of peripheral leukocyte counts in mice by oral administration of interferons. *Proc. Soc. Exp. Biol. Med.* 197, 424–430.
- Fleischmann, W.R., Koren, S., Fleischmann, C.M., 1992. Orally administered interferons exert their white blood cell suppression effects via a novel mechanism. *Proc. Soc. Exp. Biol. Med.* 201, 200–207.
- Fulton, R.W., Teeter, R.G., Cummins, J.M., Georgiades, J.A., Hutcheson, D.P., 1993. The use of interferon modulates the negative effects of heat stress on poultry production. *Arch. Immunol. Ther. Exp. (Warsz)* 41, 209–212.
- Georgiades, J.A., Fleischmann, W.R., 1996. Oral applications of cytokines. *J. Biotherapy* 8, 205–212.
- Georgiades, J.A., 1993. Effect of low dose natural human interferon alpha given into the oral cavity on the recovery time and death loss in feedlot hospital pen cattle: a field study. *Arch. Immunol. Ther. Exp.* 41, 205–207.

- Gressor, I., Bandu, M.T., Brouty-Boye, D., Tovey, M., 1974. Pronounced antiviral activity of human interferon on bovine and porcine cells. *Nature* 251, 543–545.
- Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. *Clin. Microbiol. Rev.* 17, 465–493.
- Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009a. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protoc.* 4, 44–57.
- Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009b. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* 37, 1–13.
- Imbeaud, S., Graudens, E., Boulanger, V., Barlet, X., Zaborski, P., Eveno, E., Mueller, O., Schroeder, A., Auffray, C., 2005. Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. *Nucleic Acids Res.* 33 (6), e56.
- Jarosinski, K.W., Jia, W., Sekellick, M.J., Marcus, P.I., Schat, K.A., 2001. Cellular responses in chickens treated with IFN- α orally or inoculated with recombinant Marek's disease virus expressing IFN- α . *J. Interferon Cytokine Res.* 21, 287–296.
- Jiao, X., Sherman, B.T., Huang da, W., Stevens, R., Baseler, M.W., Lane, H.C., Lempicki, R.A., 2012. DAVID-WS: a stateful web service to facilitate gene/protein list analysis. *Bioinformatics* 28, 1805–1806.
- Johnson, M.I., Zoon, K.E., Friedman, R.M., DeClercq, E., Torrance, P.F., 1980. Oligo (2'5') adenylate synthetase in human lymphoblastoid cells. *Biochem. Biophys. Res. Commun.* 97, 375–383.
- Kidd, P., 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern. Med. Rev.* 8, 223–246.
- Levings, M.K., Sangregorio, R., Galbiati, F., Squadrone, S., de Waal Malefyt, R., Roncarolo, M.G., 2001. IFN- α and IL-10 induce the differentiation of human type 1T regulatory cells. *J. Immunol.* 166, 5530–5539.
- Li, X., Wang, J., Liu, J., Li, Z., Wang, Y., Xue, Y., Li, X., Cao, H., Zheng, S.J., 2013. Engagement of soluble resistance calcium binding protein (sorcin) with foot-and-mouth disease virus (FMDV) VP1 inhibits Type I interferon response in cells. *Vet. Microbiol.* 166, 35–46.
- McVicar, J.W., Richmond, J.Y., Campbell, C.H., 1973. Observation of cattle: goats and pigs after administration of synthetic interferon inducers and subsequent exposure to foot and mouth disease virus. *Can. J. Comp. Med.* 37, 362–368.
- McVicar, J.W., McKercher, P.O., Graves, J.M., 1974. The influence of infectious bovine rhinotracheitis virus in the foot-and-mouth disease carrier state. *Proc. 80th Ann. Meeting US Animal Health Assoc.*, 254–261.
- Meister, A., Uze, G., Mogensen, K.E., Gressor, I., Tovey, M.G., Grutter, M., Meyer, F., 1986. Biological activities and receptor binding of two human recombinant interferon and their hybrids. *J. Gen. Virol.* 67, 1633–1643.
- Moore, B.R., Krakowka, S., Cummins, J.M., Robertson, J.T., 1996. Changes in airway inflammatory cell populations in standardbred racehorses after interferon-alpha administration. *Vet. Immunol. Immunopathol.* 49, 347–358.
- Moore, I., Horney, B., Day, K., Lofstedt, J., Cribb, A.E., 2004. Treatment of inflammatory airway disease in young standardbreds with interferon alpha. *Can. Vet. J.* 45, 594–601.
- Moraes, M.P., Chinsangaram, J., Brum, M.C., Grubman, M.J., 2003. Immediate protection of swine from foot-and-mouth disease: a combination of adenoviruses expressing interferon alpha and a foot-and-mouth disease virus subunit vaccine. *Vaccine* 22, 268–279.
- Moraes, M.P., de los Santos, T., Koster, M., Turecek, T., Wang, H., Andreyev, V.G., Grubman, M.J., 2007. Enhanced antiviral activity against foot-and-mouth disease virus by using a combination of Type I and II porcine interferons. *J. Virol.* 81, 7124–7135.
- Namangala, B., Inoue, N., Kohara, J., Kuboki, N., Sakurai, T., Hayashida, K., Sugimoto, 2006. Evidence for the immunostimulatory effect of low-dose orally delivered human IFN- α in cattle. *J. Interferon Cytokine Res.* 26, 675–681.
- OIE, 2015. Office International Des Epizooties, www.oie.int.
- Perez-Martin, E., Weiss, M., Diaz-San Segundo, F., Pacheco, J.M., Arzt, J., Grubman, M.J., de los Santos, T., 2012. Bovine type III interferon significantly delays and reduces the severity of foot-and-mouth disease in cattle. *J. Virol.* 86 (8), 4477–4487.
- Pestka, S., Krause, C.D., Sarkar, D., Walter, M.R., Shi, Y., Fisher, P.B., 2004. Interleukin-10 and related cytokines and receptors. *Annu. Rev. Immunol.* 22, 929–979.
- Pestka, S., 1997. The human interferon- α species and hybrid proteins. *Semin. Oncol.* 24 (3 Suppl. 9) S9–4–S9–17.
- Piazzolla, G., Tortorella, C., Schiraldi, O., Antonaci, S., 2000. Relationship between interferon-gamma, interleukin-10, and interleukin-12 production in chronic hepatitis C and in vitro effects of interferon-alpha. *J. Clin. Immunol.* 20, 54–61.
- Richmond, D.Y., Campbell, C.H., 1973. Foot-and-mouth disease virus: protection induced in mice by two orally administered interferon inducers. *Arch. Ges. Virusforsch.* 42, 102–105.
- Richmond, J.Y., Hamilton, L.D., 1969. Foot-and-mouth disease virus inhibition induced in mice by synthetic double stranded RNA (polyriboinosinic and polyribocytidylic acids). *Proc. Natl. Acad. Sci. U. S. A.* 64 (1), 81–86.
- Rosenquist, B.D., Loan, R.W., 1967. Interferon production with strain SF-4 in bovine species. *Am. J. Vet. Res.* 28, 619–628.
- Satoh Y. I. Kasama, K., Kuwabara, M., Diao, H.-Y., Nakajima, H., Kohanawa, M., Minagawa, T., 1999. Suppression of late asthmatic response by low-dose oral administration of interferon- β in the guinea pig model of asthma. *J. Interferon Cytokine Res.* 19, 887–894.
- Ship, J.A., Fox, P.C., Michalek, J.E., Cummins, M.J., Richards, A.S., 1999. Treatment of primary Sjogren's syndrome with low-dose natural human interferon- α administered by the oral mucosal route: a phase II clinical trial. *J. Interferon Cytokine Res.* 19, 943–951.
- Smith, J.K., Siddiqui, A.A., Krishnaswamy, A., Dykes, R., Berk, S.L., Magee, M., Joyner, W., Cummins, J., 1999. Oral use of interferon- α stimulates ISG-15 transcription and production by human buccal epithelial cells. *J. Interferon Cytokine Res.* 19, 923–928.
- Stevenson, G.W., Hoang, H., Schwartz, K.J., Burrough, E.R., Sun, D., Madson, D., Cooper, V.L., Pillatzki, A., Gauger, P., Schmitt, B.J., Koster, L.G., Killian, M.L., Yoon, K.J., 2013. Emergence of porcine epidemic diarrhoea virus in the United States: clinical signs, lesions, and viral genomic sequences. *Vet. Diagn. Invest.* 25, 649–654.
- Straub, O.C., Ahl, R., 1976. Lokale interferonbildung beim rind nach intranasaler infektion mit avirulentem IBR/IPV-virus und deren wirkung auf eine anschließende infektion mit Maul-und klausenseuche-virus. *Zbl. Vet. Med.* 23, 470–482.
- Tanaka-Kataoka, M., Kunikata, T., Takayama, S., Iwaki, K., Fujii, M., Ohashi, K., Ikeda, M., Kurimoto, M., 1999. Oral use of interferon- α delays the onset of insulin-dependent diabetes mellitus in nonobese diabetes mice. *J. Interferon Cytokine Res.* 19, 877–879.
- Touzot, M., Cacoub, P., Bodaghi, B., Soumelis, V., Saadoun, D., 2015. IFN- α induces IL-10 production and tilt the balance between Th1 and Th17 in Behçet disease. *Autoimmun. Rev.* 14, 370–375.
- Tovey, M.G., Maury, C., 1999. Oromucosal interferon therapy: marked antiviral and antitumor activity. *J. Interferon Cytokine Res.* 19, 145–155.
- Tovey, M.G., Bandu, M.T., Begon-Lours, J., Brouty-Boye, D., Gressor, I., 1977. Antiviral activity of bovine interferons on primate cells. *J. Gen. Virol.* 36, 341–344.
- Tovey, M.G., Meritet, J.F., Dron, M., Meyniel, J-Ph., Marionnet, D., Lallemand, C., Baouz, S., Mimaud, V., Monese, R., Maury, C., 2000. Oral interferon therapy: mechanisms of action. *Eur. Cytokine Netw.* 11 (special issue) 154.
- Tovey, M., Lallemand, C., Meritet, J.-F., Dron, M., Meyniel J-Ph, Marionnet, D., Baouz, S., Mimaud, V., Maury, C., 2003. Oromucosal interferon therapy: mechanism(s) of action. In: Presentation at Annual Meeting ISICR, Cairns, Australia.
- United States Department of Agriculture, 2013. FAD Prep Ready Reference Guide: Overview of FAD Vaccine Issues Version 3.0: G1–G2.
- Watanuki, H., Chakraborty, G., Korenaga, H., Kono, T., Shivappa, R.B., Sakai, M., 2009. Immunostimulatory effects of natural human interferon-alpha (hulFN- α) on carps *Cyprinus carpio* L. *Vet. Immunol. Immunopathol.* 131, 273–277.
- Wu, Q., Brum, M.C., Caron, L., Koster, M., Grubman, M.J., 2003. Adenovirus-mediated Type 1 interferon expression delays and reduces disease signs in cattle challenged with foot-and-mouth disease virus. *J. Interferon Cytokine Res.* 23, 359–368.
- Young, A.S., Cummins, J.M., 1990. The history of interferon and its use in animal therapy. *East Afr. Med. J.* 67 (7 Suppl. 2), SS31–SS63.
- Young, A.S., Maritim, A.C., Kariuki, D.P., Stagg, D.A., Wafula, J.M., Mutugi, J.P., Cummins, J.M., Richards, A.B., Burns, C., 1990. Low-dose oral administration of human interferon alpha can control the development of *Theileria parva* infection in cattle. *Parasitology* 101 (Pt 2), 201–209.
- de los Santos, T.B., Zhu, J.J., Diaz-San Segundo, F., Grubman, M.J., Koster, M.J., 2012. Antiviral activity of Bovine Type III interferon against foot-and-mouth disease virus. US Patent Application Number 201 201 64171, issued June 28.