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Review Article

Bovine interferon: its biology and application in veterinary medicine

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Summary

Investigations of the production and potential use of bovine interferons against viral infections have occurred since the first descriptions of interferons in other systems. The recent advent of recombinant DNA-technology has facilitated such studies and furthered our knowledge about the bovine interferon system in general. This review gives an overview of the biology, antiviral and immunomodulatory activities of bovine interferons. Areas in which the interferons are now applied or have potential application in viral diseases in cattle are described. Finally, the value of studies of the bovine interferon system with respect to comparative interferon research is discussed.

Interferon; Bovine; Antiviral effect; Immunomodulation; Recombinant interferon

Introduction

In 1958 Isaacs and Lindemann [60] discovered the reason for a phenomenon of viral interference in animals, described by a number of investigators as early as the 1930's. The factor was a protein, produced in response to a viral infection, which subsequently inhibited infection with a second, related or unrelated, virus in a non-

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specific manner. This discovery created great excitement due to the implications of having an antiviral agent with a wide spectrum of antiviral activity for use in viral therapy. During the past 28 years, many aspects of the interferon (IFN) system have been elucidated, including the finding that IFN is not one protein, but a family of proteins with a multitude of biological and physiological functions. Thus, IFNs are not only powerful antiviral agents, but also have anticellular activity (against cancer cells) as well as a plethora of effects on the immune system.

Recently there have been several comprehensive reviews, monographs and book series on the multifarious aspects of IFN biology, including its chemistry, generation, and its in vivo and in vitro biological effects [13,41,43,48,56,65,76,82,84,105, 109]. Hence, this review will be restricted specifically to the bovine IFN system, with brief references to general aspects or other animal species, where appropriate. The reader is therefore referred to the literature cited above for more general information or data specific for other animal species.

The bovine interferons (BoIFNs) probably constitute the animal IFN group on which, apart from the human and murine counterparts, most is known at the present time, regarding the genes coding for the protein and their clinical and immunological effects (summarized in Tables 1 and 2). When in addition, considerations are given to several important viral infections of cattle, which could be treated with IFN as well as being useful models for human diseases (herpes virus infections, leukosis, neonatal virus-diarrhea; for review see [23]) there is every good reason to encourage research into the various aspects of the bovine IFN system. Below we will attempt to give a brief account of the present state of knowledge relating to bovine IFN and its application in bovine medicine.

Molecular biology of bovine interferons

The genes coding for BoIFN-α can be grouped into two homologous, but distinct classes, class 1 (BoIFN-α_1) and 2 (BoIFN-α_2), respectively [31]. The BoIFN-α_1 gene family contains approximately 10–12 members, and sequence analyses of

TABLE 1

Bovine interferon types and properties.

	α	β	γ
Structure	Glycoprotein	Glycoprotein	Protein
Gene organization	No introns	No introns	Three introns
Subfamilies Class 1	10–12 Subtypes	3 Subtypes	1 Type
Class 2	15–20 Subtypes		
Size	165–172 amino acids	166	145
pH sensitivity	±	–	+
Cellular origin	Leukocytes	Fibroblasts	T lymphocytes
Biological activity	Share receptors		Distinct receptor
	Antiviral, anticellular, immunoregulatory functions		

TABLE 2

Effect of rBoIFN-α_1, and -γ on cellular activities of importance in the non-specific antimicrobial defense-mechanisms.

Cell type & function	rBoIFN-α_1		rBoIFN-γ		References
	in vitro	in vivo	in vitro	in vivo	
Alveolar macrophages					
Fc-receptor[a]	↑	=/↓	↑	↑	Bielefeldt Ohmann et al., 1984, 1986; Bielefeldt Ohmann & Babiuk, 1985d
C3b-receptor[a]	↑	↑	↑	↑	Bielefeldt Ohmann et al., 1986 and unpublished data
Bacterial phagocytosis[b]	↑	NT	↑	NT	Bielefeldt Ohmann & Babiuk, 1984
O_2^--generation[c]	↓	↓	↓	↓	Bielefeldt Ohmann & Babiuk, 1984, 1985d
H_2O_2-generation[c]	↑	NT	↑	NT	Bielefeldt Ohmann & Babiuk, 1984
Lysosomal enzymes[d]	=/↑	↑	↓	↑	Bielefeldt Ohmann & Babiuk, 1984, 1985d
Ecto-enzymes[e]	=/↑/↓	=/↑	=/↑	↑	Bielefeldt Ohmann & Babiuk, 1985[d]
PGE_2-generation	NT	↑	↑/↓	↑	Bielefeldt Ohmann et al., 1984; Bielefeldt Ohmann & Babiuk, 1985d
IFN-production[f]	↓	=	NT	=	
ADCC[g]	↑	↑	↑	↑	
Virus-plaque inhibition[g]	↑	NT	↑	NT	Bielefeldt Ohmann et al., 1984
Blood monocytes					
Migration	↓	NT	↓	NT	Bielefeldt Ohmann & Babiuk, 1984
Bacterial phagocytosis	↑	NT	↑	NT	
O_2^--generation	↓	NT	↓	NT	
NC-activity[h]	↑	↑	↑	↑	Bielefeldt Ohmann & Babiuk, 1985b
ADCC	↑	NT	↑	NT	HBO, unpublished data

TABLE 2, *continued.*

Cell type & function	rBoIFN-α_1		rBoIFN-γ		References
	in vitro	in vivo	in vitro	in vivo	
PMN					
Migration	↓	=/↑	↓	↑/↓	Bielefeldt Ohmann & Babiuk, 1984, Babiuk et al., 1985
Bacterial phagocytosis	↑	NT	↑	NT	Bielefeldt Ohmann & Babiuk, 1984
H_2O_2-generation	↑	NT	↑	NT	
O_2^--generation	↓	↑	↓	↑	Bielefeldt Ohmann & Babiuk, 1985a
Lysosomal enzymes					
ADCC	↑	NT	↑	NT	Bielefeldt Ohmann & Babiuk, 1985a
CDNC	↑	NT	↑	NT	
Virus-plaque inhibition	↑	NT	↑	NT	HBO, unpublished data

[a] Includes both rosetting and receptor-mediated phagocytosis.
[b] Opsonized *S. aureus* and opsonized *E. coli.*
[c] Following stimulation with opsonized zymosan.
[d] -glucoronidase and lysozyme.
[e] 5′ nucleotidase, alkaline phosphodiesterase-I and leucine aminopeptidase.
[f] Following BHV-1 infection.
[g] Expressed against BHV-1 infected bovine fibroblasts.
[h] Expressed against xenogeneic tumor cells and BHV-1 infected bovine fibroblasts.
↑ enhanced, ↓ suppressed, = no changes.

homology within that family of genes. This family exhibits greater homology in both their coding and flanking regions to members of the class 1 human interferon-α (HuIFN-α_1) gene family than they do to the BoIFN-α_2 gene family. Similarly the BoIFN-α_2 subfamily, which contains 15–20 members, display greater homology to the members of the class 2 HuIFN-α genes than to the BoIFN-α_1 genes. Further similarities between human and bovine IFN-α_1 and -α_2 genes are demonstrated by the fact that the class 1 genes code for a mature protein of 165–166 amino acids whereas, the class 2 genes code for a protein of 172 amino acids in length in both species.

Both classes of IFN-α genes are coordinately induced in response to a viral infection, and encode polypeptides with antiviral activity. However, whether all of the different IFN-α genes are actually expressed, i.e., translated, at any given encounter between a virus and a cell has not yet been elucidated. The indications are that depending on the virus, as well as on the cell type, BoIFN-α genes may be differentially expressed (Czarniecki et al., unpublished data). These results are in agreement with findings with human macrophage produced IFN [58], where it ap-

pears that the IFNs induced by different viruses differ in their antiviral activities, i.e., antiviral spectrum and potency [16,116]. This could reflect the ability of different viruses to depress or regulate different IFN-genes during an infection. Further elucidation of these aspects will have to await cloning and production of more IFN-α subtypes of either class. Once gene probes are available, it will be possible to quantitate production of the individual IFN-mRNA following infection with different viruses [123]. Furthermore, the availability of purified individual IFN subtypes will also allow investigation into whether any specific subtype has a different mechanism of action.

The gene-products of the BoIFN-α_1 and -α_2 genes do not appear to possess glycosylation sites [31]. At least some of the IFN-proteins encoded by class 2 genes appear to be acid labile (pH 2) (Shepard et al., unpublished data). In contrast, the class 1 gene products fit the original physico-chemical characterization of type I IFNs in that they are not acid labile [109, Shepard et al., unpublished data]. This finding may explain the reported occurrence of partial acid lability of natural BoIFN type I (α/β) induced during a primary infection of cattle, or in cell cultures from non-immune animals, i.e., under conditions where IFN-γ production could be excluded [53,66,77,115].

The BoIFN-β gene family appears to comprise at least 5 homologous, yet distinct members [79]. This is in contrast to a single IFN-β gene so far detected in the human genome. DNA sequence analysis has demonstrated that the BoIFN-β exhibits only 55% homology with the HuIFN-β. This is less than the overall homology between the corresponding gene-sequences in the 2 species, and points to more recent events in gene-evolution [79,122]. Only limited studies have been conducted on the physico-chemical properties of natural BoIFN-β [2] or on recombinant produced IFN-β but preliminary data appear to suggest that it is less stable, following purification, than is BoIFN-α (unpublished data).

As in other species, only one gene encoding for IFN-γ has been detected in the bovine. The BoIFN-α gene shares similar properties with IFN-γ genes of other species in that it contains introns (in contrast to the IFN-α and β genes), and the gene product, BoIFN-γ, binds to a cell receptor distinguishable from the IFN-α/β-receptor [37, Bielefeldt Ohmann and Babiuk, unpublished data]. Nevertheless, the 3 IFN-types exhibit at least some of the same activities, although their potency or specific activity in each specific biological reaction does vary.

To date 4 of the BoIFN-α genes, 2 of the BoIFN-β genes and the BoIFN-γ gene have been cloned and expressed in *E. coli* by recombinant DNA-technology [31,32,79]. The availability of these cloned products has greatly increased the ability to conduct a more thorough analysis of the antiviral and immunomodulatory effects of each of the bovine IFNs. Furthermore, it has allowed clinical trials to be conducted with these products and their potential use as prophylactic and therapeutic compounds is being assessed.

Producer cells of interferons

The first report of IFN-production by bovine cells was published in 1959 [117]. The induction was achieved by infection of calf kidney cells in vitro with human influenza A virus. Since then a multitude of primary cell culture systems, established cell lines and organ cultures of bovine origin, and viruses, homologous as well as heterologous, have been employed for in vitro generation of IFN [1,50,51,52,53,73,78,85,95, see 92 for references prior to 1973].

The cells which produce the various IFNs in the bovine are not as well characterized as those in man and mice [for review see 65]. However, even in the latter species, the originally clear distinction between fibroblast and leukocyte-IFN, i.e. β- and α-IFN, respectively, has become more blurred as time has passed and more cell types have been studied. The distinction between IFN-types has also become more questionable since the discovery of several subspecies of IFN within each IFN-group, some of which may show overlapping physico-chemical characteristics, such as acid-lability. However, using monoclonal antibody and hybridization techniques, it should be possible to improve both the detection and identification of cells producing each class of IFN as well as to identify/classify the protein produced under different conditions [123, Shepard et al., unpublished data].

In many studies, the bovine IFN produced has in all likelihood been IFN-β, considering the cell types used, the reported acid-stability as well as other defined characteristics. However, in some cases, especially where organ cultures were employed, the IFN may have been a mixture of IFN-α and -β due to the presence of 'contaminating' macrophages (Mϕ). Bovine Mϕs are avid IFN-producers in response to in vitro virus-challenge [24,26,77,100] as well as other acellular and cellular inducers [24]. The IFN produced by bovine Mϕs is acid labile, but otherwise has the physico-chemical and antigenic characteristics of IFN-α. Recently, it has been found to belong to the class 2 IFN-α family, which, as previously mentioned, characteristically are acid labile (Shepard et al., unpublished data). When peripheral blood leukocytes (PBL) from seronegative (with respect to BHV-1) calves are exposed to BHV-1 infected fibroblasts they were found to produce IFN-α_2 (Shepard et al., unpublished data). This may largely be produced by the Mϕ population (monocytes) present in the PBL. In contrast, Fulton and Rosenquist [53] did not detect IFN-activity in PBL-cultures from non-immunized calves following infection in vitro with BHV-1. Whether this discrepancy is due to challenge dose, the way the virus was introduced into the in vitro system (i.e., as virus-infected cells or as cell-free virus suspension) or the sensitivity of the IFN-assay is presently not known. This latter finding is, however, surprising since infection of Mϕ with BHV-1 virus has been shown to rapidly induce high levels of IFN [26,47].

In PBL cultures from immune animals the major type of IFN produced upon in vitro re-exposure to the antigen, whether it be a virus, virus-infected cells, or other antigens is IFN-γ [7]. As in other species the producer cells reside in the T cell population, and the production of IFN-γ by this population is, at least partially, dependent on the presence of Mϕs as accessory cells [7].

Bovine leukocytes, other than Mϕs and lymphocytes, may also produce IFN or

IFN-like substances in response to virus exposure. Rouse et al. [99] reported that that bovine PMNs produced an IFN-like substance upon contact with BHV-1 infected cells. The finding apparently has passed rather unrecognized, and to our knowledge similar findings have not been reported for other species. Considering the potential importance of PMNs in antiviral immunity [98] it warrants further investigations regarding both the conditions for induction and secretion as well as the physico-chemical charcaterization of these antiviral molecules.

Factors affecting in vitro production of BoIFN

In vitro systems for IFN-production are liable to many variables including temperature [78], the age of the cells or their passage level, serum-factors including contaminating endotoxins and contamination with other, non-cytopathic, viruses or with mycoplasma, as well as the host cell and inducer. The influence of some of these factors on bovine IFN production has been investigated by several groups; however, no consistent results have appeared [1,42,51,73,95]. The results of Fulton and Pearson [51] suggest that considerable differences exist between bovine continuous cell lines in their ability to produce IFNs in response to viruses. In addition, the passage level of the specific cell line may also influence the results [96]. This may reflect the continuous evolution of cells in culture and alteration of gene expression or possible infection with exogenous agents. A common contaminant of bovine cells and serum is the bovine pestivirus bovine viral diarrhea-virus (BVDV). Diderholm and Dinter [42] found that an infection with non-cytopathic BVDV significantly suppressed the IFN-response to a secondary viral infection. In contrast, Rossi and Kiesel [95] found that BVDV did not affect IFN production by polyriboinosinic-polyribocytidylic acid (poly rI·poly rC). Thus, it may be that different inducers and contaminants may affect different genes or gene families differently. This could be especially important if some viral proteins or glycoproteins of BVDV affect host cell gene expression. If one isolate of BVDV is more active in this regard than another, this could easily explain the discrepancies reported. It is probable that exposure to a virus such as BVDV, which could induce various levels of IFN itself may alter subsequent IFN responses. Thus, care should be taken to insure that cultures are free of exogenous agents before they are used for the analysis of IFN responses.

The phenomenon of 'priming', which involves pretreatment of cells with IFN, to enhance the IFN-production by subsequent viral or non-viral stimulation [59,108], does also occur in bovine cells. Priming has been demonstrated to occur for all 3 IFN-types [63,95]. It is generally found that low, rather than high doses of impure IFN cause priming [108]. However, if pure interferon is used, priming can be observed at high doses [41a]. High doses of impure interferon can also induce a phenomenon designated 'blocking'. This latter phenomenon has been observed in bovine alveolar Mϕ (AM) pretreated with pure rBoIFN-α and subsequently challenged with BHV-1 [26]. These observations may have implications in the clinical use of IFNs, where the timing and doses of IFN application may alter the efficacy of its activity.

In vivo production of BoIFNs

Circulating and/or tissue IFN can be detected in cattle following natural or experimental virus infection or following stimulation with various chemical compounds. The latter group comprises poly rI·poly rC [5,62,91,111], natural double stranded or chemically modified RNA [62,112] and statolon [93]. Unfortunately, these compounds as well as other synthetic IFN-inducers, are toxic and, therefore, have not been investigated extensively in cattle [62,91,92,93]. These and other disadvantages of using synthetic IFN-inducers in preventive veterinary medicine were recently discussed by Werenne [119].

Numerous viruses have been reported to induce IFN in cattle. These include Newcastle disease virus, vaccinia virus, bovine parainfluenza-3 virus, bovine respiratory syncytial virus, bovine adenovirus, bovine enterovirus (strain LCR-4), BVDV, bovine herpesvirus-1 (BHV-1) (s. IBRV/IPV) and bovine rotaviruses [20,34,35,36,44,67,75,88,91,92,94,101,110,113,118,126]. However, the role of IFN in limiting virus replication has not been established for most of these infections with the exception of BHV-1. Several groups have found a clear relationship between replication of BHV-1 in the nasal passage and IFN-titers in nasal secretions [20,75,113]. In contrast, IFN levels in serum are usually negligible following a respiratory tract infection with BHV-1, even though BHV-1 is believed to produce systemic infections [20,113, and unpublished data]. Locally induced IFN, following an aerogenic BHV-1 infection, can confer at least partial protection against a secondary infection with either BHV-1 or an unrelated virus [34,35,36,110,113]. Whether locally produced IFN can have systemic effects and alter the outcome of the infection remains to be determined. Pretreatment of calves with cortisone enhances the IFN-generation upon BHV-1 challenge [33], probably due to a corticosteroid-induced immunosuppression with subsequent unrestricted viral replication. Experimental (oral) infection of colostrum deprived calves with bovine rotavirus, results in the production of IFN which is detectable in the intestinal content and the gut mucosa as well as in the serum [67,118]. The presence of IFN in the serum is rather surprising since the virus infection is believed to be a strictly local gut infection. This must indicate that adsorption of IFN through the mucosal surfaces is an efficient process. Following rotavirus infection, several waves of IFN production are usually detected, which correlates with virus multiplication in the gut. An inverse relationship between virus dose, IFN production and clinical disease seems to exist. With high doses of virus ($5–20 \times 10^9$ PFU), IFN is produced very early (within the first 24 h p.i.) with no clinical symptoms of infection. In contrast, with a lower dose of virus ($2–20 \times 10^4$ PFU), IFN production is delayed and the virus induces a transient diarrhea [118]. However, the role of IFN in limiting infection in the former instances must be interpreted with caution since rotavirus may, under such dose levels produce defective interfering particles which may be excellent IFN inducers, but unable to induce disease.

Intestinal IFN produced in response to enteric virus infection is partially sensitive to pH 2, suggesting that it is a mixture of IFN-β, produced by enterocytes, and IFN-α_2, produced by leukocytes in the lamina propria/mucosa. This sugges-

tion corroborates the antigenic characterization of gut-IFN [67]. It was suggested that the serum-IFN detected in calves challenged orally with rotavirus primarily originates from adsorption of intestinal (enterocyte-produced) IFN, because the weak viremia, if occurring, seems unlikely to be able to stimulate such a pronounced IFN response [67]. However, it is also possible that blood leukocytes, which are very avid IFN-producers may produce the IFNs. It should be possible to test these alternatives by a more thorough characterization of the intestinal and serum-IFN, respectively, as well as their specific roles in preventing virus replication either directly or indirectly, in enterocytes.

Mechanisms of action

Despite the abundance of reports on possible mechanisms of antiviral activity of IFNs, no unequivocal explanation has been accepted for their action. There is no single mechanism by which treatment of cells with IFN inhibits the replication of all viruses. However, whatever the mechanism(s) at work in any particular cell type and with any particular virus type, it is generally accepted that IFNs act on the cells and change their biochemistry in such a way that virus replication is either no longer possible or, at least, significantly reduced. IFN induces a number of enzymes [14] and thereby 'prepares' the cell to resist an infecting virus, but the specific antiviral mechanism may not be activated until a virus attaches to or enters into the cell.

One of the best characterized antiviral mechanisms induced by IFN is the 2′5′-oligo(A) synthetase-RNase pathway [13,15,76]. Virus replicative intermediates such as dsRNA (viral replicative intermediates) first activate at least 2 and possibly as many as 4 synthetases, that synthesize a series of 2′5′-linked adenylate oligomers, also designated 2-5A. This activates a latent cellular endo-RNase (2-5A-dependent RNase, RNase L or F) which cleaves mRNA and rRNA. Constitutive levels of all of the enzymes in this cascade are present in variable amounts in a variety of cells and tissues, but in response to IFN the level of the 2-5A synthetase may increase $10–10^4$ fold. Thus, this enzyme can be used as a biochemical marker for the sensitivity of a cell system or animal to IFN-treatment. This has recently been applied to the bovine system [55]. Only dsRNAs containing 50 or more base pairs are effective activators of 2-5A [14]. Since dsRNA of this size is not normally found in the cell cytoplasm, the strict size-requirements ensure that the IFN-induced enzymes are not accidentally activated in uninfected cells.

The second well known mechanism of action involves an IFN-induced dsRNA-dependent protein kinase capable of phosphorylating the alpha-subunit of the eucaryotic protein synthesis initiation factor eIF2, with the subsequent inhibition of protein synthesis [72,89,125]. Whether these pathways of inhibition of protein synthesis are involved in other, or additional, mechanisms of virus inhibition such as inhibition of viral penetration [121], of glycosylation of viral glycolipids and glycoproteins [81] and, of viral assembly and/or release [27,61] is presently not known.

In addition to direct antiviral mechanisms of IFN action, the in vivo antiviral

effects of IFN may be mediated partially by the host. Thus, activation of cellular components of the immune system, such as Mϕ, natural killer (NK) cells or T cells for killing of virus-infected cells, antibody producing cells etc. can play an extremely important role in limiting virus infection and viral pathogenesis [28,41,70,102,106]. As a result of these numerous effects of IFN on cells of the immune system, it is difficult to design in vivo experiments to answer one specific question regarding the mechanism(s) of IFN action, and to verify the importance of endogenous IFN in antiviral resistance.

Gresser et al. [57] demonstrated that injection of anti-IFN-serum could reduce the resistance of mice to a variety of viruses. More recently it has also been found that genetically determined resistance of mice to herpesvirus infection correlated positively with the magnitude of the early local IFN-response [45,124], thus adding substantial support for the hypothesis that IFN does play an important role in limiting virus replication in vivo and thereby altering the cause and effects of a viral infection.

Antiviral spectrum

Regarding in vitro antiviral potency of BoIFNs, many discrepancies are apparent. This applies both to the sensitivity of the cells to the antiviral effect, and to the sensitivity of the various viruses in any given cell system [4,8,29,38,42,44,52,67–69,87,88,96,104,107,116]. However, as no standard procedure for the IFN assay has been followed by the various groups and no standard reference IFN was employed in their investigations, a direct comparison is not possible at present. This emphasizes the need for investigators to incorporate an international standard IFN in all their assays.

In most studies where a comparison of the in vitro sensitivity of bovine viruses to IFN has been performed, a relative insensitivity of BHV-1 to BoIFN, regardless of the IFN-type, has been observed [4,37,101,116]. Thus vesicular stomatitis virus (VSV) is at least 1000 fold more sensitive than is BHV-1. Recently some variability in sensitivity of different BHV-1 strains was observed but none of them were nearly as sensitive as was VSV [49]. These findings corroborate the in vivo findings, where maximal IFN-titers in nasal secretion of BHV-1 challenged calves occur simultaneously with maximal virus-titers [20,75,113], and exogenous BoIFN applied intranasally prior to nasal challenge with BHV-1 does not reduce replication to any significant degree, indicating the insensitivity of BHV-1 in vivo to the direct antiviral effects of BoIFN [4,11]. Most other viruses, reputedly involved in respiratory disease in cattle, show moderate to high sensitivity to natural and recombinant BoIFNs in vitro [4,49]. However, the in vivo results of Cummins and Rosenquist [35,36] suggest that care must be exercised when extrapolating in vitro results to what might be expected in vivo. This is probably a result of the direct and indirect effects which occur in vivo.

Several reports describe restricted activity of crude BoIFN α/β in porcine cells [3,8,35,50]. In contrast, both purified rBoIFN-α_1, and rBoIFN-β_2 have antiviral

activity in porcine cells which equals that in bovine cells [4] and BoIFN-γ was shown to be more active in porcine cells than in bovine cells [8]. However, in this context it should perhaps be reiterated that the properties of the challenge virus may influence the assay in different cell systems, as well as the cellular specificity of the IFN [83]. Rinaldo et al. [88] reported that in vivo BVDV-induced IFN had higher activity in ovine cells than in bovine, whereas rBoIFN-α has an activity in ovine and caprine cells which is slightly inferior to that seen in bovine cells [4].

Independent of the relative antiviral potential of BoIFN-α_1, -β and -γ, the kinetics of development of the antiviral state (in vitro) in sensitive cells appears to be similar [Babiuk, unpublished data, 37]. Brief exposure (1–1 5 h) of cells to BoIFN at $\geq 10^2$ units/ml results in low but reproducible levels of virus-yield inhibition. Maximum inhibition is reached after 8–24 h of treatment, depending on IFN-concentration. After removal of the BoIFN from an in vitro cell system, the antiviral state decays completely within 48 h (Babiuk, unpublished data).

Immuno-modulating effects of BoIFNs

Prominent among the non-antiviral effects of IFNs are those exerted on cells of the immune system. The majority of the studies in this area have been conducted using in vitro systems [41,65]. With the advent of pure cloned IFN in a variety of species as well as the initiation of clinical trials employing IFN, its in vivo immunomodulatory effects are now being intensively investigated both in experimental systems and clinical settings. Using these systems, it will be possible to determine whether those effects observed in vitro in isolated systems also occur in vivo where various parameters of the immune system interact and counteract [18,19]. However, valuable information must still be retrieved from the in vitro systems, where the single components of the intricate web of the immune defence can be dissected and evaluated separately as well as in combination. These in vitro studies will help us to more judiciously design in vivo experiments to produce the desired effect for specific application, i.e. specific disease situations. Once these are conducted, final confirmation of any conclusion must eventually be based on in vivo findings.

Before the advent of recombinant DNA-technology, only impure preparations of bovine IFNs were available for in vitro studies. Why this kept most laboratories concerned with veterinary immunology from conducting studies on the immunomodulatory effect of IFNs in the bovine species, when it did not do so in the murine and human field seems somewhat curious. However, up until 1983 only few reports on the immuno-modulating properties of BoIFNs emerged [9,98]. When it was found that preventive treatment of calves with rBoIFN had a very beneficial effect on the clinical performance in experimental Shipping Fever without significantly reducing virus replication in the nasal cavity [10], studies were initiated to elucidate the non-antiviral effects of BoIFNs, which might potentially contribute to these observations.

Effect on cellular activities involved in the non-specific defence mechanisms

In most organ systems the first line of cellular defence against extraneous microorganisms is exerted by cells of the monocyte-macrophage (Mϕ) lineage and polymorphonuclear leukocytes [neutrophils (PMN) or eosinophils (PME)]. The efficiency whereby they can combat the intruders may depend on such functions as migration, generation and release of reactive oxygen species, e.g. hydrogen peroxide (H_2O_2) and superoxide anions (o_2^-), lysosomal enzymes, expression of surface receptors for immunoglobulin (Fc-R) and complement (C-R), secretion of IFN and prostaglandins (PG), phagocytosis and cellular cytotoxicity. Most of these activities of bovine cells can be modulated by in vitro treatment of the cells with rBoIFN-α_1 or -γ (Table 2), and those functions so far examined are also subject to in vivo modulation (Table 1). However, the effect of the IFN-exposure appears to be very dependent on dose, exposure time and the health status of the animal, i.e. whether clinically healthy or infected with virus (or virus plus bacteria [17,18,19,21,22,25]. The influence of the latter is so dramatic as to give completely opposite results when, for example, migration and O_2^--generation by PMN are evaluated [18,22]. At the present time, there are no explanations to these discrepancies, but most likely a plethora of endogenous factors, released during a virus infection, will somehow change the effect of the exogenous IFN. Another explanation could be that what we consider beneficial with respect to the in vitro situation may not be beneficial in vivo. For example, although production of oxygen reactive species may be important in killing bacteria it may also lead to tissue damage and enhanced pathology. Furthermore these molecules may be a reflection of the severity of disease and therefore, there should be an inverse relationship between cellular activation and disease. No influence of age of the donor animal has so far been detected regarding sensitivity to IFN-modulation. Neither has an influence of the animals genetic make-up [40] been observed with certainty in the bovine species, although it has been suspected to play a role on some occasions (unpublished data). Further insight into this aspect may have to await better characterization of the immune response genes in the bovine species [39] or other genetic factors influencing the expression of IFN or IFN induced proteins.

Effect on natural cell-mediated cytotoxicity

Natural cell-mediated cytotoxicity is a non-specific immune defence mechanism exerted by a still unclassified effector cell (subset or subsets) which is antigen and antibody independent. The indications are that this defence-mechanism may be of importance in antiviral defence [28]. In the bovine species, cells exerting natural cytotoxicity against xenogeneic tumor cells as well as against BHV-1 infected bovine fibroblasts reside within or are more closely related to the Mϕ-lineage [24]. As in other species, their activity is highly susceptible to in vitro and in vivo modulation by IFNs [19] with responses being increased upon exposure to rBoIFN. The exact mechanism whereby IFNs modulate NC activity and whether in vitro and in

vivo modulation occurs by the same mechanisms remains to be determined. Thus, endogenous in vivo factors which are not present in vitro may modulate the effect of the exogenous BoIFNs. The in vivo enhancing effect of rBoIFNs on NC-activity may involve a direct or indirect influence on the bone marrow, i.e., the development and recruitment of effector cells [19]. Thus, although both rBoIFN-α_1 and -γ in vitro have a dose-dependent suppressive effect on the proliferation of bone marrow cells of the Mϕ-lineage, there seems to be a partially selective expansion of a certain subset of bone marrow Mϕ, which share phenotypic (i.e., antigenic) characteristics with the NC cells in peripheral blood [19, and unpublished data].

Effect of BoIFNs on lymphocyte functions

When healthy calves are treated intravenously or intramuscularly with rBoIFN-α_1 or -γ, the mitogen proliferative-response of PBL is reduced 24 h later. Addition of interleukin-2 (IL-2) to the in vitro cultures can partly or completely prevent this suppression [22]. In contrast, at later times (2–4 days after treatment) the proliferative response of PBL to Con A and PHA is actually enhanced. Although the in vivo effect of the two IFNs is similar, their effect differs when they are added to in vitro PBL cultures from normal non-treated animals. Thus, over a concentration range of rBoIFN-γ the modulation of the Con A and PHA responses varies from enhancement (low doses) to suppression (high doses). The suppressive effect can be completely reverted by addition of exogenous IL-2, which could indicate that the suppressive effect is exerted by either inhibition of mitogen stimulated IL-2 production in the cultures or by stimulating cells which compete for IL-2 binding, but without exhibiting a proliferative response. In contrast, BoIFN-α_1 if used at the same dose range as BoIFN-γ only suppresses the PHA and Con A-responses of PBL, but as with rBoIFN-γ suppression, this can be overcome by addition of IL-2 [22]. The difference between the two IFN-types may illustrate their different physiological roles. Thus, since IFN-γ is actually produced in mitogen-stimulated lymphocyte (T cells) cultures, it is tempting to speculate that IFN serves as an initial enhancer, but later as a feed-back control mechanism to shut off proliferation to prevent overreaction of the immune response. Recombinant human IFN has also been found to suppress proliferation of bovine PBL, but the effect seems somewhat capricious and dependent on serum source and the individual animal [30]. Using cloned or bulk bovine T cells, we have demonstrated that α_1 interferon can inhibit their proliferation and responsiveness to IL-2 (unpublished data).

In exploration of the mechanism(s) involved in IFN-suppression of mitogen-stimulated lymphocyte proliferation, the effect of rBoIFN-α or -γ on the Con A induced IL-2 generation was investigated. Although these results are preliminary there appeared to be enhanced IL-2 generation following in vivo exposure. In contrast, no consistent effect could be seen after in vitro exposure (unpublished data). Activation of suppressor cells by IFNs has been reported in other animal systems [6] and this possibility was therefore considered. In vitro treatment of bovine Mϕ

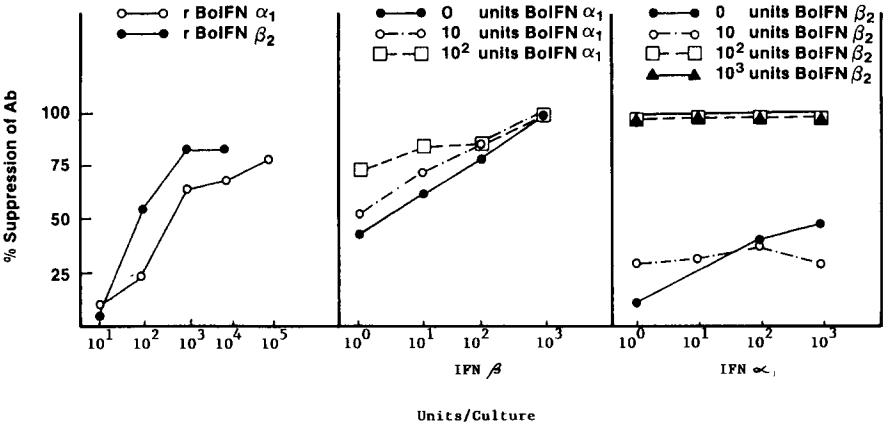

Fig. 1. In vitro effect of rBoIFN-α and -β alone or in combination on the secondary in vitro antibody response to KLH [46]. A: the two rBoIFNs added separately to the lymphocyte-cultures. B. Varying amounts of rBoIFN-α_1, added to cultures together with a constant amount of rBoIFN-β. C. Varying amounts of rBoIFN-β added to cultures together with a constant concentration of rBoIFN-α.

with either BoIFN-α or -γ did not endow them with suppressive activity. On the contrary, it enhanced their accessory cell activity, at least as applied to the mitogen response [25]. In contrast, a suppressor cell with lymphocyte characteristics was induced by high doses of rBoIFN-α_1, or -γ within a few hours of exposure [22]. It remains to be elucidated how this suppressor cell exerts its activity.

Although no inhibitory effect of rBoIFN-treatment on the antibody response to viral antigens has been detected [10], both rBoIFN-α_1, and -β have a profound effect on the in vitro secondary antibody response (Filion et al., unpublished data) (Fig. 1). The mechanism of suppression has not yet been studied in the bovine system, but in the human system it was shown to be due to a direct anti-proliferative effect of IFNs rather than via an intermediary suppressor cell mechanism [41]. It remains to be investigated what effect BoIFN-γ might have on the bovine antibody-producing cell responses. In the murine system IFN-γ was found to enhance the response by acting as a cofactor in driving the maturation of resting B cells to active immunoglobulin secretions [74,106]. If this mechanism also occurs in vivo the implications for a beneficial effect of combined immunization (vaccination) and IFN-γ treatment are considerable.

Effect on cell surface components of Mϕ

As already mentioned above, both in vivo and in vitro exposure of bovine alveolar Mϕ modulates their expression of immune receptors and ecto-enzymes, either positively or negatively (Table 2). In addition, surface antigen expression is affected [21,25], most notably the expression of a MHC-II product (Ia-like antigen). rBoIFN-γ in particular is most active in this regard. The activation of an Ia-

like antigen in combination with enhancement of the accessory cell activity may be of considerable significance for the specific immune response in animals and may actually be one of the major factors in the enhancement of immune responses following vaccination [80,114] or allowing recovery from infection.

Application of IFNs in veterinary medicine

The use of IFNs in the control of bovine viral diseases was considered at a time when IFN research was still in its infancy [92]. It was suggested at that time that bovine respiratory disease, especially Shipping Fever, might be the model of choice for evaluating the usefulness of IFN in disease control of cattle [85,92]. This model is especially attractive because of its experimental reproducibility, its predictable yearly recurrence in the field, the number of viruses (conjecturally) involved and last, but not least, its economic importance for the cattle industry and the consumers.

Three different strategies for IFN-therapy can be employed: induction of endogenous IFN by (1) an avirulent viral agent, or (2) a suitable non-toxic, chemical compound, or (3) local or systemic application of IFN from an exogenous source. As previously mentioned, it has proven virtually impossible to optimize a system using chemical/synthetic inducers, because of problems with toxicity, individual variations in responsiveness, etc. [92,120]. The studies conducted to date on the endogenous IFN-induction by challenge with an avirulent virus have also been equally discouraging. Thus, although some protection has been obtained after in vivo IFN-induction with a supposedly avirulent BHV-1 strain (vaccine strain) against food and mouth disease virus [110], virulent BHV-1 [113] bovine rhinovirus, parainfluenza-3 and adenovirus [34,35,36], protection was only partial.

The use of exogenous IFN seems to be the strategy of choice for treatment of animals. However, until the advent of recombinant DNA-technology, and the cloning of IFN-genes into bacteria with subsequent large scale production and refined purification methods, the production of BoIFNs for clinical use in cattle appeared to require almost heroic efforts and certainly was not economically feasible [85]. The present availability of recombinant *E. coli*-derived IFNs, should insure their evaluation and application, as a control measure against bovine viral disease within the foreseeable future [11,119].

Recombinant HuIFN-α_2 has been tested in cattle against experimental vaccinia virus infection [103] and against BHV-1 induced respiratory disease [90]. In both instances multiple injections of IFN were given and although some protection was obtained, individual variations in sensitivity to the treatment were notable. Moreover, in respiratory disease models, toxic effects, such as CNS-depression and hyperthermia, resulting from IFN-treatment were evident, though the authors tended to neglect this aspect [90].

In contrast to the effects seen with the HuIFN, cattle appear to tolerate extremely high doses of rBoIFN-α_1, with the only detectable 'toxic' effect being hyperthermia of brief duration (less than 8 h). Doses of 10^6 units/kg body weight of

rBoIFN-γ may induce diarrhea in some animals in addition to the hyperthermia and transient changes in blood leukocyte numbers [22]. However, this dose far exceeds the dose necessary for a prophylactic effect of rBoIFN-γ against bovine respiratory disease (unpublished data). This dose may be further reduced if it is found that different BoIFNs act synergistically. Thus, this adverse effect can be easily avoided under therapeutic or prophylactic situations.

Pharmaco-kinetically the 2 rBoIFNs also differ, in that maximum serum levels of IFN obtained with rBoIFN-γ are less than 20% of that seen with rBoIFN-α regardless of whether it is administered intravenously or intramuscularly. Whether this reflects the kinetics of binding to cells remains to be elucidated. If so, then BoIFN-γ may prove to be effective more rapidly than BoIFN-α_1. Preliminary results suggest that this may indeed be the case (unpublished data). Based on these observations, studies need to be conducted with respect to timing of treatment as well as dose or dose combinations. This may be especially important in such infections as bovine respiratory diseases where different effects would be needed for induction of activity to the viruses and bacteria that are involved in this disease complex. This is especially true when BHV-1 is employed as in vivo test virus, because of the apparent negligible effect of IFN on viral replication in the nasal cavity [10,11,34–36,110,113]. Thus, other parameters such as the overall clinical performance (i.e., severity and duration of symptoms) of the animal may prove a better measure of the value of IFN-therapy [11], at least as it applies to bovine respiratory disease.

A single treatment with either rBoIFN-α_1 or -γ appears to be just as effective as multiple applications in prevention of Shipping Fever symptoms, providing the treatment is given 36–48 h prior to virus-exposure [11 and unpublished data]. More effective and longer protection may be required under field conditions where timing of infection may not always be known. It is possible that various delivery systems, including slow release mechanisms may have to be developed. Whether this will lead to a refractory state or have an adverse effect on the overall immune system needs careful consideration.

A second economically important disease where active immunization is not feasible is in neonatal diarrhea. In this instance the disease occurs within the first 2–3 wk of life. This disease is caused by a wide variety of different agents including rotavirus, coronavirus, breda virus, astroviruses, caliciviruses, parvoviruses, and possibly other unidentified viral agents, as well as enterotoxigenic *E. coli*. The only feasible methods of controlling these agents involve oral feeding of specific antibody containing milk or monoclonal antibodies against either the bacteria or the specific viruses [6b]. Since there are so many different viruses presently identified as agents involved in induction of neonatal diarrhea, specific chemotherapeutic methods do not appear to have any potential role in inhibiting virus replication. Thus IFN, with its broad spectrum of antiviral activity, appears to be the ideal candidate for making cells refractory to virus infection and therefore, could dramatically reduce economic losses due to neonatal diarrhea. At present there is at least one report [120] wherein intramuscular treatment of calves with human IFN-α decreased symptoms of rotavirus diarrhea, but did not alter virus excretion. These

initial observations appear promising and hopefully improvements in efficacy could be achieved by better administration methods and doses. Since all of the agents causing neonatal calf diarrhea are generally restricted to the intestinal epithelium of the gastrointestinal tract, it is possible that administration of the IFN into the intestinal tract to provide a refractory antiviral state, would even prove to be more efficacious than systemic administration. This is especially important since all of these viruses produce diarrhea very rapidly and the disease process is very localized. However, before these types of studies can be accepted in the field, considerable experimentation will have to be conducted to determine whether IFN application locally in the intestine will alter gut physiology and whether replication of the crypt cells will be affected [86]. If this occurs, IFN may itself induce diarrhea or alter adsorption as well as influence the immune system. Another consideration that must be taken into account in the treatment of calves with IFN against enteric infections, is that the refractory state will have to be maintained for approximately 3 wk, until a time when most calves have reached an age where their susceptibility to these enteric infections is reduced. In order to achieve this continued refractory state, sufficient levels of IFN will have to be maintained in the gut for extended periods. It also remains to be determined whether the IFN can withstand the proteolytic enzymes within the jejunum and ileum, the site where the majority of the viruses replicate. If the proteolytic enzymes do degrade IFN, then novel approaches of systemic release and transport to the intestinal surface over extended periods will be required. Thus, it is evident that many questions still need to be addressed before the application of IFN in neonatal diarrhea can be used with guarantee for efficacy and security.

The majority of enteric viruses which cause neonatal diarrhea in calves are not easy to cultivate in vitro; therefore, very little information regarding their potential susceptibility to IFN is available. At present, there is evidence that bovine rotavirus show little susceptibility to the direct antiviral effects of IFN [4,38,69]. However, it appears that in vivo treatment of calves with IFN is efficacious against rotaviruses. Thus, there may not always be a direct correlation between in vitro sensitivity of viruses to IFN and the clinical effect of IFN. This has already been clearly demonstrated with BHV-1 [11]. Hence, it appears that all of these individual viruses will have to be tested in in vivo situations before any concrete statements can be made about the potential value of IFN treatment in reduction of the wide spectrum of viruses causing neonatal diarrhea in calves. However, the availability of sufficient quantities of IFN and animal model systems reproducing neonatal diarrhea in calves, should make such studies feasible in the near future.

Epilogue

Investigations covering all aspects of the bovine IFN system are now well under way, and the results obtained within the last few years with respect to the use of rBoIFNs in the control of some diseases appear encouraging. However, these trials should now also be extended to include other important infections of cattle. In ad-

dition to the viral infections, with or without secondary bacterial infections, rBoIFNs may prove useful against infections due to chlamydia, rickettsia or other intracellular parasites [63,64,97]. Infections in cattle with these agents also have potentials as animal models for corresponding human diseases. The studies of the mechanisms whereby the rBoIFNs confer protection, including the immuno-modulating effects, should of course continue in parallel with clinical evaluations and could by themselves contribute significantly to the knowledge concerning IFN activity in general.

Other therapeutic strategies may also be considered for evaluation. In vitro it has been shown that IFN and virus-specific antibody (VAb) can act synergistically to inhibit various virus types [71]. The effect is probably related to 1) the ability of VAb to retard extracellular spread of virus and to reduce the multiplicity of infection, thus allowing more time for IFN to act, and 2) the ability of IFN to protect uninfected cells and reduce virus yields from infected cells, thus making even low levels of VAb protective. Different virus types may differ in their sensitivity to the VAb-IFN combinations, depending on the IFN type [71]. However, with the two new tools, monoclonal antibodies and recombinant DNA produced IFNs, investigations of various permutations should be possible. Thus, this strategy certainly lends itself for exploitation not only for prevention, but also for therapy of virus infections.

Thus, we are probably far from having tapped all the resources of the IFN system. Other potentials may still await discovery, and exciting and useful results may be ahead of us in the control of infectious diseases in man and animals which we have not envisaged at present.

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References

1 Ahl, R. (1982) Production and regulation of synthesis of interferon in bovine kidney cell cultures. Dev. Biol. Standard 50, 159–166.
2 Ahl, R. and Gottschalk, M. (1984) Purification and characterization of bovine interferon β. In: Adjuvants, Interferon and Non-specific Immunity (F.M. Cancellotti and D. Galassi eds.), pp. 31–37. Comm. Eur. Commun. (Eur 8675EN).
3 Ahl, R. and Rump, A. (1976) Assay of bovine interferons in cultures of the porcine cell line IB-RS-2. Infect. Immun. 14, 603–606.
4 Anderson, K., Czarniecki, C., McCracken, J., Bielefeldt Ohmann, H. and Babiuk, L.A. (1985) The potential uses of bovine interferon for bovine respiratory disease. Proc. 4th Vet. Resp. Symp., Anaheim, CA, pp. 39–54.

5 Angulo, A.B. and Savan, M. (1970) Preliminary studies in interferon induction in the respiratory tract of cattle. Proc. 74th Annu. Meet. U.S. Anim. Health Assoc. pp. 577–583.
6 Aune, T.M. and Pierce, C.W. (1982) Activation of a suppressor T-cell pathway by interferon. Proc. Natl. Acad. Sci. U.S.A. 79, 3808–3812.
6a Bakiuk, L.A. (1984) Virus-induced gastroenteritis in animals. Appl. Virol., Academic Press, New York, pp. 349–363.
7 Babiuk, L.A. and Rouse, B.T. (1976) Immune interferon production by lymphoid cells role in the inhibition of herpesviruses. Infect. Immun. 13, 1567–1578.
8 Babiuk, L.A. and Rouse, B.T. (1977) Bovine type II interferon: activity in heterologous cells. Intervirology 8, 250–256.
9 Babiuk, L.A. and Rouse, B.T. (1978) Interactions between effector cell activity and lymphokines: implications for the recovery from herpesvirus infections. Int. Arch. Allergy Appl. Immunol. 57, 62–73.
10 Babiuk, L.A. and Bielefeldt Ohmann, H. (1984) Effect of levamisole and bovine interferonα_1, on bovine immune responses and susceptibility to bovine herpesvirus-1. Prog. Clin. Biol. Res. 161, 433–442.
11 Babiuk, L.A., Bielefeldt Ohmann, H., Gifford, G., Czarniecki, C.W., Scialli, V.T. and Hamilton, E.B. (1985) Effect of bovineα_1, interferon on bovine herpes type-1-induced respiratory disease. J. Gen. Virol. 66, 2383–2394.
12 Babiuk, L.A., Sabara, M. and Hudson, G.R. (1985) Rotavirus and coronavirus infections in animals. Prog. Vet. Microbiol. Immunol. Vol. 1, 80–120.
13 Baglioni, C. (1979) Interferon-induced enzymatic activities and their role in the antiviral state. Cell 17, 255–264.
14 Baglioni, C., Benvin, S., Maroney, P.A., Minks, M.A., Nilsen, T.W. and West, D.K. (1980) Interferon-induced enzymes: activation and role in the antiviral state. Ann. N.Y. Acad. Sci. 350, 497–509.
15 Baglioni, C. and Nilsen, T.W. (1981) The action of interferon at the molecular level. Sci. Am. 69, 392–399, 1981.
16 Bell, D.M., Roberts, N.J. and Hall, C.B. (1983) Different antiviral spectra of human macrophages interferon activities. Nature (London) 305, 319–21.
17 Bielefeldt Ohmann, H. and Babiuk, L.A. (1984) Effect of bovine recombinant alpha-1 interferon on inflammatory responses of bovine phagocytes. J. Interferon Res. 4, 249–263.
18 Bielefeldt Ohmann, H. and Babiuk, L.A. (1985a) Alteration of neutrophil function after in vitro and in vivo exposure to recombinant bovine interferon alpha or -gamma. J. Leuk. Biol. 38, 165 (abstract).
19 Bielefeldt Ohmann, H. and Babiuk, L.A. (1985b) In vitro and systemic effects of recombinant bovine interferons on natural cell-mediated cytotoxicity in healthy and bovine herpesvirus-1 infected cattle. J. Interferon Res. 5, 551–564.
20 Bielefeldt Ohmann, H. and Babiuk, L.A. (1985c) Viral-bacterial pneumonia in calves: effect of bovine herpesvirus-1 on immunologic functions. J. Infect. Dis. 151, 937–947.
21 Bielefeldt Ohmann, H. and Babiuk, L.A. (1986) Alteration of alveolar macrophage functions by in vivo treatment with recombinant interferons alpha-1 and gamma. Proc. 1st Intern. Vet. Immunol. Symp., Guelph, Ontario, p. 71.
22 Bielefeldt Ohmann, H. and Babiuk, L.A. (1986) Alteration of some leukocyte functions following in vivo and in vitro exposure to recombinant bovine interferon α_1, and γ. J. Interferon Res. 6, 123–136.
23 Bielefeldt Ohmann, H. and Babiuk, L.A. (1986) Viral infections of domestic animal as models for studies of viral immunology and pathogenesis. J. Gen. Virol. 67, 1–25.
24 Bielefeldt Ohmann, H., Davis, W.C. and Babiuk, L.A. (1985) Functional and phenotypic characteristics of bovine natural cytotoxicity cells. Immunobiology 169, 503–519.
25 Bielefeldt Ohmann, H., Davis, W.C. and Babiuk, L.A. (1986) Surface antigen expression by bovine alveolar macrophages: functional correlation and influence of interferons in vivo and in vitro. Immunobiology 171, 125–142.
26 Bielefeldt Ohmann, H., Gilchrist, J.E. and Babiuk, L.A. (1984) Effect of recombinant DNA-produced bovine interferon alpha (BoIFN-α) on the interaction between bovine alveolar macrophages and bovine herpesvirus type 1. J. Gen. Virol. 65, 1487–1495.

27 Billiau, A., Heremans, H., Allen, P.T., DeMaeyer-Guignard, J. and DeSomer, P. (1976) Trapping of coronavirus particles at the surface of interferon-treated cells. Virology 73, 537–542.
28 Biron, C.A. and Welsh, R.M. (1982) Activation and role of natural killer cells in virus infections. Med. Microbiol. Immunol. 170, 155–172.
29 Bouffard, A. and Derbyshire, J.B. (1981) Effects of some components of the humoral immune response on the replication of bovine herpesvirus type 1 in tracheal organ cultures. Vet. Microbiol. 6, 129–141.
30 Brochier, B., Werenne, J., Goossens, A., Vanden Broecke, C., Derbove, G. and Pastoret, P.P. (1984) In vitro effect of bacterially produced human interferon (Hu-IFN-α_2) on non-specific blastogenesis in the bovine species. In: Adjuvants, Interferon and Non-specific Immunity. (F.M. Cancellotti and D. Galassi eds.). pp. 59–63, Comm. Eur. Comm. (Eur 8675EN).
31 Capon, D.J., Shepard, H.M. and Goeddel, D.V. (1985) Two distinct families of human and bovine interferon-α genes are coordinately expressed and encode functional polypeptides. Mol. Cell. Biol. 5, 768–779.
32 Cerretti, D.P., McKereghan, K., Larsen, A., Cosman, D., Gillis, S. and Baker, P.E. (1986) Cloning, sequence and expression of bovine interferon-γ. J. Immunol. 136, 4561–4564.
33 Cummins, J.M. and Rosenquist, B.D. (1979) Leukocyte changes and interferon production in calves injected with hydrocortisone and infected with infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 40, 239–240.
34 Cummins, J.M. and Rosenquist, B.D. (1980) Protection of calves against rhinovirus infection by nasal secretion interferon induced by infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 41, 161–165, 1980.
35 Cummins, J.M. and Rosenquist, B.D. (1982) Temporary protection of calves against adenovirus infection by nasal secretion interferon induced by infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 43, 955–959.
36 Cummins, J.M. and Rosenquist, B.D. (1982) Partial protection of calves against parainfluenza-3 virus infection by nasal secretion interferon induced by infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 43, 1334–38.
37 Czarniecki, C.W., Hamilton, E.B., Fennie, C.W. and Wolf, R.L. (1986) In vitro biological activities for *Escherichia coli*-derived bovine interferons and interferons -α_1, -β and γ. J. Interferon Res. 6, 29–37.
38 Dagenais, L., Pastoret, P.P., van den Broecke, C. and Werenne, J. (1981) Susceptibility of bovine rotavirus to interferon. Arch. Virol. 70, 377–379.
39 Davis, W.C. (1984) The use of monoclonal antibodies to define the bovine lymphocyte antigen system BoLA leukocyte differentiation antigens, and other polymorphic antigens. In: Characterization of the bovine immune system and the genes regulating expression of immunity with particular reference to their role in disease resistance. Proc. Symp. May 1–5. Honolulu, Hawaii. Ed. by W.C. Davis, J.N. Shelton and C.W. Weems.
40 DeMaeyer, E. and DeMaeyer-Guignard, J. (1980) Host genotype influences immunomodulation by interferon. Nature 284, 173–175.
41 DeMaeyer, E. and DeMaeyer-Guignard, J. (1982) Immunomodulating properties of interferons. Philos. Trans. R. Soc. London, Ser. B 299, 77–90.
41a DeMaeyer-Guignard, J.D., Cachard, A. and DeMaeyer, E. (1980) Electrophoretically pure mouse interferon has priming but not blocking activity in poly (I.C.)-induced cells. Virology 102, 222–225.
42 Diderholm, H. and Dinter Z. (1966) Interference between strains of bovine virus diarrhea virus and their capacity to suppress interferon of a heterologous virus. Proc. Soc. Exp. Biol. Med. 121, 976–980.
43 Dziewanowska, Z.E. and Pestka, S. (1982) The human interferons. Med. Res. Rev. 2, 325–353.
44 El Azhary, M.A.S.Y., Silim, A. and Roy, R.S. (1981) Interferon, fluorescent antibody and neutralizing antibody responses in sera of calves inoculated with bovine respiratory syncytial virus. Am. J. Vet. Res. 42, 1378–82.
45 Engler, H., Zawatzky, R., Kirchner, H. and Armerding, D. (1982) Experimental infection of inbred mice with herpes simplex virus. IV. Comparison of interferon production and natural killer cell activity in susceptible and resistant adult mice. Arch. Virol. 74, 239–247.
46 Filion, L., Bielefeldt Ohmann, H., Owen, P.W. and Babiuk, L.A. (1984) Characterization of the bovine secondary in vitro antibody response. Vet. Immunol. Immunopathol. 7, 19–32.

47 Forman, A.J. and Babiuk, L.A. (1982) Effect of infectious bovine rhinotracheitis virus infection on bovine alveolar macrophage function. Infect. Immun. 35, 1041–1047.
48 Friedman, R.M. (1981) Interferon: A Primer. Acad. Press, N.Y.
49 Fulton, R.W., Downing, M.M. and Cummins, J.M. (1984) Antiviral effects of bovine interferons on bovine respiratory tract viruses. J. Clin. Microbiol. 19, 492–497.
50 Fulton, R.W. and Pearson, N.J. (1980) Interferon production by bovine tracheal organ cultures infected with bovid herpesvirus-1 strains. Can. J. Comp. Med. 44, 447–452.
51 Fulton, R.W. and Pearson, N.J. (1982) Interferon induction in bovine and feline monolayer cultures by four bluetongue virus serotypes. Can. J. Comp. Med. 46, 100–102.
52 Fulton, R.W. and Root, S.K. (1978) Antiviral activity in interferon-treated bovine tracheal organ cultures. Infect. Immun. 21, 672–673.
53 Fulton, R.W. and Rosenquist, B.D. (1976) In vitro interferon production by bovine tissues: induction with infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 37, 1497–1502.
54 Gillespie, J.H., Robson, D.S., Scott, F.W. and Schiff, E.I. (1985) In vitro protective effect of bacteria-derived bovine alpha interferon Ii against selected bovine viruses. J. Clin. Microbiol. 22, 912–914.
55 Goossens, A., van den Broecke, C., Schwers, A., Dagenais, L., Maenhoudt, M., Pastoret, P.P. and Werenne, J. (1984) In vitro antiviral efficiency of bacterially produced human interferon (HuIFNα_2) against several bovine viruses. In: Adjuvants, Interferon and Non-specific Immunity (Cancellotti, F.M. and D. Galassi eds.), pp. 37–44. Comm. Eur. Comm. (EUR 8675EN).
56 Gresser, I. (1979-00) (Ed.) Interferon. Acad. Press, London.
57 Gresser, I., Tovey, M.G., Maury, C. and Bandu, M.T. (1976) Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease and influenza viruses. J. Exp. Med. 144, 1316–1327.
58 Hiscott, J., Cantell, K. and Weissman, C. (1984) Differential expression of human interferon genes. Nucleic Acids Res. 12, 3727–46.
59 Isaacs, A. and Burke, D.C. (1958) Mode of actions of interferon. Nature 182, 1073–74.
60 Isaacs, A. and Lindenmann, J. (1957) Virus interference. I. The interferon. Proc. R. Soc. London, Ser. B. 147, 258–267.
61 Jay, F.T., Dawood, M.R. and Friedman, R.M. (1983) Interferon induces the production of membrane protein deficient and infectivity defective vesicular stomatitis virions through interference in the virion assembly process. J. Gen. Virol. 64, 707–712.
62 Knight, D.J. and Ashton, R.J. (1980) Induction of circulating interferon in calves with 3 different types of double stranded ribonucleic acid. Res. Vet. Sci. 28, 386–388.
63 Kiderlen, A.F., Kaufmann, S.H.E. and Lohmann-Matthes, M.L. (1984) Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. Eur. J. Immunol. 14, 964–967.
64 Kierszenbaum, F. and Sonnenfeld, G. (1984) β-interferon inhibits cell infection by *Trypanosoma cruzi*. J. Immunol. 132, 905–908.
65 Kirchner, H. (1984) Interferons, a group of multiple lymphokines. Spring Sem. Immunopathol. 7, 347–374.
66 Kono, Y. (1967) Rapid production of interferon in bovine leukocyte cultures. Proc. Soc. Exp. Biol. Med. 124, 155–160.
67 La Bonnardiere, C., Cohen, J. and Contrepois, M. (1981) Interferon activity in rotavirus infected newborn calves. Ann. Rech. Vet. 12, 85–91.
68 La Bonnardiere, C. and Laude, H. (1983) Interferon induction in rotavirus and coronavirus infections: A review of recent results. Ann. Rech. Vet. 14, 507–511.
69 La Bonnardiere, C., De Vaureix, C., L'Haridon, R. and Scherber, R. (1980) Weak susceptibility of rotavirus to bovine interferon in calf kidney cells. Arch. Virol. 64, 167–170.
70 Landolfo, S., Cafano, F., Giovarelli, M., Prat, M., Cavallo, G. and Forni, G. (1985) Inhibition of interferon-gamma may suppress allograft reactivity by T lymphocytes in vitro and in vivo. Science 229, 176–179.
71 Langford, M.P., Villarreal, A.L. and Stanton, G.J. (1983) Antibody and interferon act synergistically to inhibit enterovirus, adenovirus and herpes simplex virus infection. Infect. Immun. 41, 214–218.

72 LeBleu, R., Sen, G.C., Shaila, S., Cabrer, B. and Lengyel, P. (1976) Interferon, dsRNA and protein phosphorylation. Proc. Natl. Acad. Sci. U.S.A. 73, 3107–3111.
73 Lefkowitz, S.S. and Luna, V.E.R. (1984) Priming of bovine interferons. Intervirology 21, 221–223.
74 Leibson, H.J., Gefter, M., Zlotnik, A., Marrack, P. and Kappler, J.W. (1984) Role of γ-interferon in antibody producing responses. Nature (London) 309, 799–801.
75 LeJan, C. and Asso, J. (1981) The local and systemic immune response of calves following experimental infection with IBR virus. Adv. Exp. Med. Biol. 137, 677–692.
76 Lengyel, P. (1982) Biochemistry of interferons and their actions. Ann. Rev. Biochem. 51, 251–282.
77 Letchworth, G.J. and Carmichael, L.E. (1983) Production of interferon by bovine peripheral blood monocytes infected with bovid herspesvirus 2. Vet. Microbiol. 8, 69–80.
78 Letchworth, G.J. and Carmichael, L.E. (1984) The effect of temperature on production and function of bovine interferons. Arch. Virol. 82, 211–221.
79 Leung, D.W., Capon, D.J. and Goeddel, D.V. (1984) The structure and bacterial expression of three distinct bovine interferon β genes. Biotechnology (May) 458–464.
80 Lipscomb, M.P., Toews, G.B., Lyons, G.R. and Uhr, J.W. (1981) Antigen presentation by guinea pig alveolar macrophages. J. Immunol. 126, 286–291.
81 Maheshwari, R.K., Demsey, A.E., Mohanty, S.B. and Friedman, R.M. (1980) Interferon treated cells release vesicular stomatitis virus particles lacking glycoprotein spikes: correlation with biomedical data. Proc. Natl. Acad. Sci. U.S.A. 77, 2287–87.
82 Merigan, T.C., Friedman, R.M. and Fox, C.F. (eds.) (1982) Interferons UCLA Symp. Mol. Cell Biol. 25.
83 Nilsen, T.W., Wood, D.L. and Baglioni, C. (1980) Virus-specific effects of interferon in embryonal orcinoma cells. Nature (London) 286, 178–180.
84 Pestka, S. (1983) The human interferons – from protein purification and sequence to cloning and expression in bacteria: before, between and beyond. Arch. Biochem. Biophys. 221, 1–37.
85 Peterhans, E., Charrey, J., Richoz, Y. and Wyler, R. (1976) Production of large quantities of interferon with calf testicular cells and leukocytes. Res. Vet. Sci. 20, 99–100.
86 Preble, O.T. and Friedman, R.M. (1983) Biology of disease. Interferon-induced alterations in cells: relevance to viral and non-viral diseases. Lab. Invest. 49, 4–18.
87 Ransohoff, R.M., Maroney, P.A., Nayak, D.P., Chambers, T.M. and Nilsen, T.W. (1985) Effect of human alpha A interferon on influenza virus replication in MDBK cells. J. Virol. 56, 1049–52.
88 Rinaldo, C.R., Isackson, D.W., Overall, J.C., Glasgow, L.A., Brown, T.T., Bistner, S.I., Gillespie, J.H. and Scott, F.W. (1976) Fetal and adult bovine interferon production during bovine viral diarrhea virus infection. Infect. Immun. 14, 660–666.
89 Roberts, W.K., Hovanessian, A., Brown, R.E., Clemens, M.J. and Kerr, B.M. (1976) Interferon-mediated protein kinase and low-molecular weight inhibitor of protein synthesis. Nature (London) 264, 477–480.
90 Roney, C.S., Rossi, C.R., Smith, P.C., Lauerman, L.C., Spano, J.S., Hanrahan, L.A. and William, J.C. (1985) Effect of human leukocyte A interferon on prevention of infectious bovine rhinotracheitis virus infection in cattle. Am. J. Vet. Res. 46, 1251–55.
91 Rosenquist, B.D. (1971) Polyriboinosinic-polyribocytidylic acid induced interferons in calves. Am. J. Vet. Res. 32, 35–39.
92 Rosenquist, B.D. (1973) Induction and biological significance of interferon with particular reference to cattle. Am. Vet. Med. Assoc. 163, 821–824.
93 Rosenquist, B.D. and Loan, R.W. (1969) Production of circulating interferon in the bovine species. Am. J. Vet. Res. 30, 1293–1303.
94 Rosenquist, B.D. and Loan, R.W. (1969) Interferon induction in the bovine species by infectious bovine rhinotracheitis. Am. J. Vet. Res. 30, 1305–1312.
95 Rossi, C.R. and Kiesel, G.K. (1980) Factors affecting the production of bovine type I interferon on bovine embryonic lung cells by polyriboinosinic-polyribocytidylic acid. Am. J. Vet. Res. 41, 557–560.
96 Rossi, C.R., Kiesel, G.K. and Hoff, E.J. (1980) Factors affecting the assay of bovine type I interferon on bovine embryonic lung cells. Am. J. Vet. Res. 41, 552–556.
97 Rothermel, C.D., Rubin, B.Y. and Murray, H.W. (1983) γ-interferon is the factor in lymphokine that activates human macrophages to inhibit intracellular chlamydia psittaci replications. J. Immunol. 131, 2542–44.

98 Rouse, B.T. (1981) Role of neutrophils in antiviral immunity. Adv. Exp. Med. Biol. 137, 263–278.
99 Rouse, B.T., Babiuk, L.A. and Henson, P.M. (1979) Neutrophils in antiviral immunity. Induction of an interferon-like inhibitor by herpesviruses in bovine neutrophils. J. Infect. Dis. 141, 223–232.
100 Rudenko, V.I., Smorodintsev, A.A., Aksenov, O.A. and Lyabina, L.M. (1970) The nature of interferon-producing cells. Vopr. Virusol. 15, 52–56.
101 Savan, M., Angulo, A.B. and Derbyshire, J.B. (1979) Interferon, antibody responses and protection induced by an intranasal infectious bovine rhinotracheitis vaccine. Can. Vet. J. 20, 207–210.
102 Schellekens, H., Weimar, M., Cantell, K. and Stitz, L. (1979) Antiviral effect of interferon in vivo may be mediated by the host. Nature (London) 278, 742.
103 Schwers, A., van den Broecke, C., Goossens, A., Maenhoudt, M., Bugyaki, L., Pastoret, P.P. and Werenne, J. (1982) Administration of human interferon to young calves: circulating antiviral activity and biological response of the host. Arch. Int. Physiol. Biochem. 90, B214–15.
104 Sellers, R.F. and Fitzpatrick, M. (1962) An assay of interferon produced in rhesus monkey and calf kidney tissue culture using bovine enterovirus M6 as challenge. Brit. J. Exp. Pathol. 43, 674–683.
105 Sehgal, P.B. (1982) The interferon genes. Biochim. Biophys. Acta 695, 17–33.
106 Sidman, C.L., Marshall, J.D., Shultz, L.D., Gray, P.W. and Johnson, H.H. (1984) γ-interferon is one of several direct B cell maturing lymphokines. Nature (London) 309, 801–804.
107 Smorodintsev, A.A. (1968) The production and effect of interferon in organ cultures of calf trachea. Br. J. Exp. Pathol. 49, 511–515.
108 Stewart, W.C., Gosser, L.B. and Lockhart, R.Z. (1971) Priming: A non-viral function of interferons. J. Virol. 7, 792–801.
109 Stewart, W.E. II (ed. 1979/1981) The Interferon System (Second ed.) Springer Verlag, New York.
110 Straub, O.C. and 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 Klauenseuche-virus. Abl. Vet. Med. B. 23, 470–482.
111 Theil, K.W., Mohanty, S.B. and Hetrick, F.M. (1971) Effect of poly I:C on infectious bovine rhinotracheitis virus infection in calves. Proc. Soc. Exp. Biol. Med. 137, 1176–79.
112 Thely, M., Choay, J., Dhennin, L. and Dhennin, L. (1963) Virostase induite in vivo par des acides ribonucléiques non-infectieux. C. R. Acad. Sci. 256, 1048–50.
113 Todd, J.D., Volenec, F.J. and Paton, I.M. (1972) Interferon in nasal secretions and sera of calves after intranasal administration of avirulent infectious bovine rhinotracheitis virus: association of interferon in nasal secretions with early resistance to challenge with virulent virus. Infect. Immun. 5, 699–706.
114 Toews, G.B., Vial, W.C. and Dunn, M.M. (1984) The accessory cell function of human alveolar macrophages in specific T cell proliferation. J. Immunol. 132, 181–186.
115 Tovey, M.G., Bandu, M.-T., Began-Lours, J., Brouty-Boye, D. and Gresser, I. (1977) Antiviral activity of bovine interferons on primate cells. J. Gen. Virol. 36, 341–344.
116 Trueblood, M.S. and Manjara, J. (1972) Response of bovine viruses to interferon. Cornell Vet. 62, 3–12.
117 Tyrrell, D.A.J. (1959) Interferon produced by cultures of calf kidney cells. Nature (London) 184, 452–453.
118 van den Broecke, C., Schwers, A., Dagenais, L., Goossens, A., Maenhoudt, M., Pastoret, P.P. and Werenne, J. (1984) Interferon response in colostrum-deprived newborn calves infected with bovine rotavirus: Its possible role in the control of the pathogenicity. Ann. Rech. Vet. 15, 29–34.
119 Werenne, J. (1984) Use of exogenous interferon in man and animals. In quest of an antiviral of broad veterinary use. In: Adjuvant Interferon and Non-specific Immunity (F.M. Cancellotti and D. Galassi eds.), pp. 17–30. Comm. Eur. Commun. (EUR 8675EN).
120 Werenne, J. (1985) Interferon in veterinary medicine: the present position and future prospects. Outlook on Agriculture, Pergamon Press, London, U.K.
121 Whitaker-Dowling, P.A., Wilcox, D.K., Widnell, C.C. and Younger, J.S. (1983) Interferon-mediated inhibition of virus penetration. Proc. Natl. Acad. Sci. U.S.A. 80, 1083–1086.
122 Wilson, V., Jeffreys, A.J., Barrie, P.A., Boseley, P.G., Slocombe, P.M., Easton, A. and Burke, D.C. (1982) A comparison of vertebrate interferon gene families detected by hydridization with human interferon DNA. J. Mol. Biol. 166, 457–475.

123 Zawatzky, R., DeMaeyer, E. and DeMaeyer-Guignard, J. (1985) Identification of individual interferon-producing cells by in situ hybridization. Proc. Natl. Acad. Sci. U.S.A. 82, 1136–40.
124 Zawatzky, R., Hilfenhaus, J., Marcucci, F. and Kirchner, H. (1981) Experimental infection of inbred mice with herpes simplex virus. I. Investigation of humoral and cellular immunity and of interferon induction. J. Gen. Virol. 53, 31–38.
125 Zilberstein, A., Federman, P., Shulman, L. and Revel, M. (1976) Specific phosphorylation in vitro of a protein associated with ribosomes of interferon-treated mouse L-cells. FEBS Lett. 68, 119–124.
126 Zygraich, N. (1984) Nasale Interferonbildung nach Impfung mit einem temperaturspecifischen Parainfluenza-3-virus. Tieraerztl. Umsch. 39, 127–128.