RESPONSE OF CATTLE TO BOVINE HERPESVIRUS-1 VACCINE AFTER *IN VIVO* ADMINISTRATION OF INTERFERON

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CHAPTER I

INTRODUCTION

The marketing and management practices of the cattle industry in the United States impose tremendous distresses on cattle (Horton, 1984). This, along with the anatomy and physiology of the bovine lung (Robinson et al, 1984), set the stage for pneumonic disease, better known as the shipping fever complex. Respiratory disease is the most important malady of stocker and feedlot cattle (Perino, 1985). Thus, a variety of biologic and therapeutic interventions have been used to reduce the incidence of respiratory disease. Among the biologic interventions have been a host of viral vaccines, both modified live and killed viruses. Administration of exogenous interferon (IFN) has also been explored as an aid in reducing the impact of the shipping fever complex. If the Food and Drug Administration approves IFN for use in cattle, then the logical time to administer it would be when cattle are marketed or during the handling that occurs routinely after arrival at new facilities. It is during marketing and arrival handling that vaccines are also administered, thus concurrent administration of IFN and viral vaccines would be a likelihood. There is little information in the literature concerning the effects of

concurrent administration of IFN and vaccines on the immune response to viral vaccines. However, the study of both endogenous and exogenous IFN is difficult because IFN is present in serum for a very short time. Thus, a sensitive indicator of the presence of IFN would be of value. The IFN-induced enzyme 2',5'-oligoadenylate (2',5'-oligo(A)) synthetase is a candidate for such an indicator in cattle.

The purpose of these studies was to determine: 1) information on the normal concentrations of 2',5'-oligo(A) synthetase in peripheral blood mononuclear leukocytes (PBML) in cattle, the relationship between amount of IFN administered and 2',5'-oligo(A) synthetase levels in PBML, and the duration of elevation of 2',5'-oligo(A) synthetase after IFN administration; 2) the effects of a modified live virus (MLV) vaccine and/or IFN on 2',5'-oligo(A) synthetase levels in PBML; and 3) the modulatory effects of IFN on the immune response of cattle to a MLV vaccine.

CHAPTER II

REVIEW OF THE LITERATURE

Interferon

In 1957, Isaacs and Lindenmann incubated heat inactivated influenza virus with chick chorioallantoic membrane (Isaacs and Lindenmann, 1957) in order to study the phenomenon of viral interference that had been described in the 1930s (Hoskins, 1935). Attempts to decrease the effect of the medium to inhibit virus multiplication by adsorbing the interference causing viral particles resulted in increased antiviral activity. Isaacs and Lindenmann named the soluble factor that was responsible for this effect "interferon". Since its discovery, IFN has been found to possess not only antiviral effects, but also effects on a multiplicity of cellular functions including cellular growth and differentiation and immune function. IFN may be thought of as a hormone-like messenger which, after release, diffusion, and binding to its receptor, induces metabolic changes in the cell. However, nonspecific antiviral activity in at least homologous cells remains a hallmark of IFN (Stewart et al, 1980).

Classification

During the purification and characterization of IFN, it was discovered that there exist not one but several types and subtypes. IFN may be classified by various schemes based on its biochemical, genetic or immunologic characteristics as well as cell of origin. The three classes of IFN are alpha (α), beta (β), and gamma (γ). IFN-α and IFN-β are collectively called Type I IFN and are produced predominantly by leukocytes and fibroblasts, respectively. IFN-γ, also called Type II or immune IFN, is produced by mitogen-stimulated nonsensitized T lymphocytes or antigen-stimulated sensitized T lymphocytes (Epstein, 1981).

IFN-α is a group of glycoproteins composed of two homologous but distinct subfamilies, class 1 and 2. In cattle, there are approximately 10 to 12 class 1 and 15 to 20 class 2 IFN-α genes. These genes are on chromosome U18 (Adkison et al, 1988), and none of the genes have been shown to contain introns. The class 1 genes code for a 166 amino acid long protein with a 23 amino acid leader sequence, whereas the class 2 genes code for a protein 172 amino acids in length. The molecular weight of IFN-α varies depending upon the degree of glycosylation but is approximately 17 KDaltons. Examination of the amino acid sequence of IFN-α reveals a lack of glycosylation sites. Thus, the small amounts of carbohydrate detected are due to O-glycosylation. The presence of the carbohydrate moieties does not appear to

be required for the molecule to be functional (Knight, 1984) but may affect its pharmacokinetics (Satoh et al, 1984).

IFN-α was originally characterized based upon its remarkable stability in harsh biochemical conditions. It is stable not only at a pH of 2 at 4°C , but also in the presence of sodium dodecyl sulfate (SDS) (Steward et al, 1974). However, some of the IFN-α class 2 gene products appear to be acid labile (Bielefeldt-Ohmann and Babiuk, 1987). IFN-β is also a group of glycoproteins. In cattle there are 5 IFN-β genes on chromosome U18 (Adkinson et al, 1988), in contrast to the single IFN-β1 gene in humans on chromosome 9 (Bielefeldt-Ohmann and Babiuk, 1987). The genes code for proteins composed of 166 amino acids. Additionally, IFN-β shares several characteristics with IFN-α including: genes have no introns, stable at a pH of 2 at 4°C and in the presence of SDS.

The third class of IFN, IFN-ϖ, is distinct from the other two classes. In cattle, as in humans, there is only one IFN-ϖ gene. This gene contains introns and is found on chromosome U3 in cattle and on 12 in humans. Unlike other IFN of other classes, IFN-ϖ is not stable at a pH of 2 at 4°C nor in the presence of SDS.

Finally, there are types of IFN described that do not fit into the accepted scheme of IFN classification. These include acid labile IFN-α (Bielefeldt-Ohmann and Babiuk, 1987), unusual IFN species from Sendai-virus-infected amniotic membranes (Duc-Goiran et al, 1983), and human IFN-delta

(Wilkinson and Morris, 1986).

Production and Purification

Most cell types can be induced to produce at least some IFN by treatment with one of the many inducing substances (Edy, 1984). Since the first report of IFN production by bovine cells (Tyrrell, 1959), various cell lines, primary cell cultures, and organ cultures of bovine origin have been induced to produce IFN (Bielefeldt-Ohmann and Babiuk, 1987; Fulton and Rosenquist, 1976). A phenomenon known as priming, in which exposure of cells to small amounts of IFN results in enhanced IFN production following subsequent stimulation (Isaacs and Burke, 1958), also occurs in bovine cells (Jacobsen et al, 1988).

IFN induction is caused by a variety of substances which have been classified according to differing schemes (Ho, 1984; Torrence and DeClercq, 1981). One classification scheme is: viruses, polyanions (including naturally occurring and synthetic nucleic acids), amines of low molecular weight, antibiotics, bacterial products, agents that stimulate lymphocytes, and microorganisms other than viruses (Torrence and DeClercq, 1981). Additionally, there are lymphoblastoid cell lines that spontaneously produce IFN (Adolf et al, 1982).

Production of IFN suffers from several limitations (Billiau, 1984). First, cells produce only small quantities

of IFN. Secondly, the crude IFN obtained is usually very dilute and contaminated with other proteins. Finally, purification and concentration of the preparations is accompanied by large losses of the biologically active material. Thus, advances in the production and purification of IFN have been arduous and parallelled advances in laboratory techniques and understanding of IFN molecular biology.

Early production systems utilized chicken (Lampson et al, 1963) or murine tissues (Finter, 1964) and suffered from the limitations discussed above. In the early sixties, studies on IFN production by human leukocytes collected from blood donations were conducted in various laboratories. Kari Cantell and his collaborators continued to perfect this method (Strander et al, 1975). The messenger RNA for mouse IFN was isolated from induced cells in 1972 (DeMaeyer-Guignard et al, 1972). and several years later the expression of human IFN in Escherichia coli was accomplished (Taniguchi et al, 1980; Nagata et al, 1980).

Bovine IFN has been induced using both live and killed viruses (Fulton and Rosenquist, 1976). Bovine IFNs have also been produced using recombinant DNA techniques (Capon et al, 1985). Additionally, the development and refinement of other methods of IFN production have continued in cattle (Allen et al, 1988; Jacobsen et al, 1988).

Assay

There exist numerous methods to assay for IFN. As the

indicator of IFN activity, most assays utilize the ability of IFN to render cells resistant to viral infection. In a typical IFN bioassay a series of dilutions of IFN are made, added to an appropriate tissue culture, incubated, challenged with a virus, and after an appropriate time the extent of virus growth is determined. An inverse relation is made between the amount of virus growth and the amount of IFN present (Finter, 1981). Some of the more commonly used bioassays include cytopathic effect inhibition (Ho and Enders, 1959), plaque reduction (Wagner, 1961), and yield reduction of infectious virus, viral hemagglutinin, viral enzymes, or viral nucleic acid (Grossberg and Sedmak, 1984). A number of permutations of test cell, challenge virus, and the method used to measure virus growth have been described. Also, indicators of the cellular biochemical effects of IFN have been used as quantitative indicators of IFN activity (Short and Fulton, 1987).

Less used assay methods include hemadsorption inhibition, immunofluorescent cell counting, reduction of DNA synthesis, agar diffusion, and various others (Grossberg and Sedmak, 1984). Radioimmunoassays (Shiozawa et al, 1988; Scott et al, 1985) and ELISAs (Overall et al, 1989) for IFN have also been described.

The World Health Organization has designated International Reference Preparations for use as standards in IFN assays to permit the calibration of bioassays in international units (IU) from one laboratory to another. The

International Reference Preparations are from human, mouse, rabbit, and chicken cells. Similar reference standards are not available for bovine IFN.

Receptors

IFNs have no biologic activity within the cells in which they are formed but rather must first be secreted and then readsorbed by cells (Vengris et al, 1975). Although the exact mechanism by which IFN exerts its biologic effects is not known, the initial event in their activity is binding to their respective cell surface receptors (Zoon et al, 1982). This receptor is also responsible for the species specificity of IFN (Slate et al, 1978). Type I IFN (α and β) share a common receptor, however Type II IFN (γ) has a distinct receptor (Branca and Baglioni, 1981). Although its exact location is imprecisely known, it appears that the receptor binding region of IFN-α is in the N-terminal half of the molecule (Siemers et al, 1988). ARG_{23}, a highly conserved amino acid among IFNs (Langer and Pestka, 1985), appears to be at least one of the critical amino acids in receptor binding (Siemers et al, 1988). Cell surface gangliosides may also play a role in IFN binding, possibly as early, nonspecific, low affinity receptors (Chany, 1984).

Biological effects: antiviral properties

The myriad of effects that have been attributed to IFN since its discovery have not lessened the significance of

its antiviral activity. IFN appears early in the course of viral infection, before humoral immunity (Baron et al, 1982). Treatment of animals with IFN neutralizing antibodies results in more severe disease (Fauconnier, 1982) and exogenous IFN has been used to treat or prevent viral infections in man (Scott and Tyrrell, 1984). Thus, it is apparent that IFN forms an important first line of defense against viral infections.

However, the mechanisms by which IFN inhibits viral replication are not completely understood. IFN is not the protein that actually inhibits virus multiplication. Rather, IFN is an inducer that causes cells to undergo biochemical modifications, including the synthesis of new proteins, whereby the cells are rendered unable to proceed through the various steps required for the synthesis and assembly of virions. Several mechanisms have been examined as the potential mediators of the antiviral state (Content, 1984). Many of them involve interference with the ability of parental or early viral messenger RNA molecules to be translated.

The two most studied potential mediators of the antiviral effect are the 2',5'-oligo(A) synthetase and the protein kinase systems. The 2',5'-oligo(A) system is discussed in detail in a later section. IFN also induces a protein kinase that phosphorylates several proteins in infected cells, in particular a protein variously referred to as the P_1, 67K, or 72K protein, and the small (α) subunit of eucaryotic protein synthesis initiation factor eIF2

(LeBleu et al, 1976). The phosphorylation eIF2-α results in its inactivation and prevention of mRNA translation (Levin et al, 1976; Kaempfer et al, 1979).

Other less well understood potential mediators of the antiviral effect have been suggested. These include: inhibition of viral penetration (Whitaker-Dowling et al, 1983), inhibition of the primary transcription of the viral genome (Metz et al, 1976; Marcus and Selellick, 1978), methylation of mRNA (DeFerra and Baglioni, 1981), tRNA deficiency or inactivation (Content, 1984), and inhibition of the late stages of virus replication (budding, assembly and glycosylation) (Jay et al, 1983; Maheshwari et al, 1980; Naso et al, 1982).

The IFN-treated cell differs from the untreated cell in many aspects and the induction of the antiviral state is probably extremely complex. The current explanations likely only reveal a limited number of the mechanisms the cells have evolved to protect themselves against viral infection and their importance in an in vivo viral challenge is only speculative.

The species specificity of the antiviral, and other, effects of IFN vary greatly. As an example chick and duck IFN, as well as mouse and rat IFN, exhibit little if any cross protective activity (Joklik, 1985), while human IFN protects bovine and feline cells, but bovine IFN does not protect feline cells (Perino LJ, unpublished data). The antiviral effects of IFN in humans and laboratory animal

models have been reviewed (Content, 1984; Scott and Tyrrell, 1984; Hilfenhaus and Polastri, 1984).

Various bovine viruses, predominantly those with respiratory tropisms, have also shown in vitro sensitivity to different IFNs. Using bluetongue virus serotype 10 induced IFN in Georgia bovine kidney (GBK) cells or bovine nasal secretion IFN, in vitro viral yield was reduced for bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV), parainfluenza-3 virus (PI-3V), goat respiratory syncytial virus (RSV), bovine RSV, bovine adenovirus (BAV) type 7, and vesicular stomatitis virus (VSV) compared to control cultures (Fulton et al, 1984). Similar in vitro results were seen with various recombinant bovine IFNs including IFN-$\alpha_1$1, IFN-β2, and IFN-γ against BHV-1, PI-3V, BVDV, bovine RSV, VSV, and pseudorabies virus (Gillespie et al, 1985; Fulton et al, 1986; Czarniecki et al, 1986; Bielefeldt-Ohmann and Babiuk, 1988). Additionally, recombinant human IFN-α A or IFN-α A/D have efficacy against bovine viruses grown in cells of bovine origin (Fulton et al, 1986).

Biological effects: immune system

IFN can either enhance or depress the humoral and/or cellular immune response depending on the antigen, type and stage of immune response, as well as the dose, route, and type of IFN given. In the case of the immune response to a viral antigen, IFN has immunomodulatory potential from two sources. First, IFN could have direct effects on the immune

system, and second, IFN could exert indirect effects by modulating the amount of viral antigen presented to the immune system through its antiviral or immunomodulatory properties.

B Lymphocytes.

Reports that a 26 KDalton human protein known both as B-cell differentiation factor and interleukin-6 is IFN-β2, suggest that IFNs are mediators in B-cell mediated immune responses (Tosato et al, 1988). Early in vivo studies of antibody formation using IFN-treated mice showed enhanced, suppressed, or unchanged responses, depending upon the system examined and the dose, timing, type, and route of IFN administration (Sonnenfeld, 1984; Booth and Marbrook, 1981). In vitro systems with cells of human or murine origin displayed similar behavior (Sonnenfeld, 1984).

More recently, the advent of IFN clinical trials in humans has allowed some indicators of humoral immunity to be studied. Compared to untreated controls, patients with chronic type B hepatitis that were treated daily or every-other-day with a total of 35 million Units (U) of recombinant IFN-α2 per week showed a decrease in pokeweed mitogen-induced immunoglobulin production after two weeks of therapy (Peters et al, 1986b). The decrease appeared to be due to inhibition of the late stages of B cell differentiation into immunoglobulin producing and secreting cells. Pokeweed mitogen-induced lymphoproliferation was not affected.

Another report compares multiple sclerosis patients injected subcutaneously with 5 million U of human lymphoblastoid IFN every day for 6 months to a placebo treated group of multiple sclerosis patients. After 1 week the pokeweed mitogen-induced secretion of IgG was reduced in the IFN-treated group and remained lower than in controls for the 6 month treatment period (O'Gorman et al, 1987). Experiments using lymphocyte subset mixing suggested the changes in immunoglobulin production were a direct IFN effect on the B lymphocyte subset. These reports suggest that repeated large doses of IFN suppress the immunoglobulin production response to pokeweed mitogen.

Additionally, in vitro studies of the effect of IFN on human B lymphocytes have continued. In an in vitro study of human lymphocytes treated with recombinant human IFN-α2, low concentrations (1 to 100 IU/ml) of IFN enhanced, while high concentrations (10^5 IU/ml) suppressed, pokeweed mitogen stimulated immunoglobulin production (Peters et al, 1986a). Mitogen-induced proliferation was not affected at any concentration (Peters et al, 1986a). The modulation of immunoglobulin production appeared to be a direct effect on B cells. In another report, in vitro treatment of human lymphocytes with recombinant human IFN-α2 (10^5 IU/ml) had a suppressive effect on pokeweed mitogen-induced immunoglobulin production. However, at 10^4 U/ml the suppressive effect on pokeweed mitogen-induced immunoglobulin production was minor. There was a dose related enhancement of a hapten

specific primary antibody response (Evans and Ozer, 1987). These authors further examined this effect, concluding that the IFN modulation was via a radiosensitive helper cell function that may involve binding of IFN to specific cellular receptors expressed on T lymphocytes.

In immunoglobulin M-positive B cells isolated using fluorescence activated cell sorting, all three types of recombinant human IFN (1 to 10^4 U/ml of α, β, or γ) had a dose responsive enhancing effect on the proliferative response induced by mitogenic anti-μ monoclonal antibody (Morikawa et al, 1987). An enhancement of proliferative response was also noted in normal B lymphocyte enriched and some B cell-type chronic lymphocytic leukemia cell preparations treated with 10^3 U/ml recombinant human IFN-α and stimulated with anti-μ antibody (Karray et al, 1988). These authors noted that IFN-α had no effect on B cell proliferation when used alone. Using B cells from healthy individuals, it was shown that recombinant human IFN-α, although devoid of effect when used alone, selectively enhances the interleukin-2 dependent B cell responses to 2,4,6-trinitrophenol when used at 10^3 U/ml and had no suppressive effect even when used at a hundredfold higher concentration (Delfarissy et al, 1988). Thus, <u>in vitro</u>, it appears that lower doses of IFN are able to augment some B lymphocytes responses.

In mice administered a mixture of a malaria vaccine and 100 to 5000 U of IFN-γ, enhancement of antibody production,

T cell helper, and delayed hypersensitivity was noted (Playfair and DeSouza, 1987). In immunocompetent mice inoculated with 10 LD_{50} of street rabies virus, treatment with 10^5 U of IFN-α resulted in a significant increase in production of immunoglobulin against rabies virus despite a 10-fold reduction in rabies virus production in the brain (Marcovistz et al, 1987). In the same experiment, mice immunosuppressed by cyclophosphamide treatment had almost undetectable levels of anti-rabies immunoglobulin, while similarly treated mice that received IFN-α still produced a significant amount of immunoglobulin against rabies virus (Marcovistz et al, 1987). The in vivo effect of lower doses of IFN administered for shorter durations on antigen specific immunoglobulin production has yet to be explored in humans.

In cattle there have been studies on the effects of IFN in viral challenge systems (Babiuk et al, 1985; Roney et al, 1985; Cummins and Hutcheson, 1986; Gillespie et al, 1986; Bielefeldt-Ohmann and Babiuk, 1986c; Bielefeldt-Ohmann and Babiuk, 1985a); however, there have been few published studies on the effect of IFN on the response to a viral vaccine (Cummins and Hutcheson, 1986) or on the in vitro or in vivo effects of IFN on bovine B lymphocytes. BHV-1 seronegative calves were treated intranasally or intramuscularly, daily for one week with 50 x 10^6 U of human recombinant leukocyte IFN-A and challenged intranasally with 3 x 10^6 or 10^4 $TCID_{50}$ of BHV-1 Cooper strain on the first day of

IFN treatment. BHV-1 serum antibody titers were slower to increase and were not maintained as long as those of virus control calves (Roney et al, 1985). The authors attributed this to the inhibition of viral infection in the IFN-treated group.

In another study, BHV-1 seronegative calves were treated orally for three days with a placebo or 0.05, 0.5 or 5 IU/lb body weight of Sendai virus-induced human leukocyte IFN. On the second day of treatment calves were inoculated with 10^3 $TCID_{50}$ of BHV-1 in each nostril. The geometric mean serum antibody titer to BHV-1 of the IFN treatment groups was two to four times greater than the control group at 14 days postinfection. However, by 25 days after inoculation all calves had seroconverted and there was no difference between treatment and control groups. The geometric mean titers of plaque forming units of nasally excreted BHV-1 virus was greater in the IFN-treated groups on days 3, 7 and 10 and in the control group on day 14 after inoculation (Cummins and Hutcheson, 1986). The same authors also reported that calves concurrently given an intramuscular BHV-1/BVDV/PI-3V modified-live virus vaccine and 1 U/lb body weight of Sendai virus-induced human leukocyte IFN orally had enhanced seroconversion to PI-3V. From these two findings the authors concluded that IFN treatment stimulated antibody development and reduced nasal shedding of BHV-1 virus at 14 days postinoculation.

In a study of calves inoculated with $10^{6.5}$ $TCID_{50}$ of the

Holmes strain of BVDV and treated with six daily intramuscular doses of 10^4 or 10^3 U/kg body weight of recombinant bovine IFN-$\alpha_1$1 commencing with the day of infection, neutralizing antibodies to BVDV appeared in the serum at the same time as in the virus controls. Both treated and control calves were completely protected when challenged with BVDV 40 days after the first inoculation of BVDV. The authors concluded that IFN treatment did not interfere with the immune response to the virus infection (Gillespie et al, 1986).

Finally, compounds that induce IFN such as avridine (N,N-dioctadecyl-N,N-bis(2-hydroxyethyl)-propanediamine), DDA (dimethyl dioctadecyl ammonium bromide), tilorone (2,7-bis(diethylaminoethoxy)fluoren-9-one) and others have also been shown to possess adjuvant effects. Whether IFN plays a role in this has not been examined (Jensen, 1986). In summary, in vivo IFN administration to cattle has been shown to either depress, augment, or not affect the bovine humoral immune response to viral antigens.

Cell-mediated immunity and cytotoxic effector cells.

Delayed-type hypersensitivity represents one of the multiple expressions of cell-mediated immunity (CMI) with the tuberculin reaction as the best known and first recognized form (Klein, 1982). In murine studies of the effects of IFN-α and -β on delayed-type hypersensitivity, either immunoenhancement or immunosuppression was observed, depend-

ing on the timing of IFN administration, antigen dosage, and host genotype (DeMaeyer-Guignard, 1984). Assessment of the effects of IFN on delayed-type hypersensitivity responses in cattle has not been reported.

Lymphocyte proliferation in response to T lymphocyte mitogens or antigens is used to assess CMI. Two of the commonly used mitogenic lectins are phytohemagglutinin (PHA), which binds N-acetyl-galactosamine containing oligosaccharides, and concanavalin A (ConA), which binds saccharides containing a terminal D-mannose. Both activate T lymphocytes in a variety of mammalian species (Ashman, 1984). Treatment of mitogen stimulated lymphocyte cultures with any of the three species of IFN results in decreased lymphoproliferative responses in cattle (Jacobsen and Rockwood, 1988).

In a study of some leukocyte functions, including indicators of CMI, calves were given recombinant bovine IFN-α or IFN-ɤ, either intravenously or intramuscularly, at 10^6 or 10^4 U/kg of body weight. High doses (10^6 U/kg) of both IFNs, given either intravenously or intramuscularly, caused a decrease in PHA- and ConA-stimulated lymphocyte proliferation 24 hours after injection. However, this effect could be blocked by addition of interleukin-2 (10 U/ml) to the cultures. At 48 and 96 hours after IFN treatment, an enhanced lymphocyte proliferation response was detected. An enhanced response was also noted for the low dose (10^4 U/kg) of either recombinant bovine IFN-α or IFN-ɤ.

IFN treatment had no consistent effect on the ability of blood lymphocytes to produce interleukin-2 in response to ConA stimulation (Bielefeldt-Ohmann and Babiuk, 1986c). In another study, calves treated intramuscularly with 2 x 10^4 U/kg recombinant bovine IFN-α_I1 had marked reduction in the amplitude of their ConA-induced lymphoproliferative responses from 4 to 24 hours post administration (Griebel et al, 1989). Other data presented in the above report suggested that the decrease in response was due to a responder cell deficit rather than the induction of suppressor cell activity or IFN antiproliferative effects.

In calves challenged with an aerosol of BHV-1, followed four days later by an aerosol of Pasteurella hemolytica, intranasal treatment with 10 mg of recombinant bovine IFN-α 1 (1 x 10^6 U/kg body weight, estimated from materials and methods) 48 hours prior to virus challenge resulted in much higher ConA-stimulated interleukin-2 production at seven days postinfection (Babiuk et al, 1985). In an in vitro study of the ConA-induced proliferative response of bovine intraepithelial leukocytes, the addition of either recombinant bovine IFN-α or -τ caused inhibition (Nagi and Babiuk, 1988). A Cantell-type bovine leukocyte IFN was shown to decrease both lymphocyte response to PHA or ConA and lymphocyte allogenic reactions at high concentrations and no effects were reported at lower concentrations (Jacobsen et al, 1986).

Administration of avridine, a lipoidal amine compound

that has activity as an IFN inducer, as well as a humoral and cell-mediated immune adjuvant, to cattle resulted in higher mean lymphocyte blastogenic responses to mitogens (Roth and Kaeberle, 1985).

Antigen specific lymphoproliferative response to BHV-1 was studied in bovine nonadherent cells treated *in vitro* with 100 U/ml of IFN-α or -r (Eskra et al, 1985). Increased proliferation to BHV-1 was observed in cultures treated with IFN-r but not IFN-α.

The ability of IFN to augment the reactivity of cytotoxic effector cells, including cytotoxic T cells, NK cells, and cells mediating antibody dependent cellular cytotoxicity (ADCC), has been well documented in both murine and human systems, and this may be an important mechanism for the *in vivo* immune effects of IFN (Herberman, 1984; Inghirmai et al, 1985). Similar phenomenon have been reported in bovine systems. The effects of various doses of recombinant bovine IFN-r on natural cell-mediated cytotoxicity (NC) in healthy and BHV-1 infected calves were examined. In healthy calves a single intramuscular dose of 10^3, 10^4 or 10^5 U/kg body weight, or a single intravenous dose of 10^3 or 10^4 U/kg body weight, of IFN-r caused elevation of NC 48 and 96 hours post-administration (Bielefeldt-Ohmann and Babiuk, 1985a). The authors noted that the changes were not statistically significant because of the small number of animals in each treatment group. In BHV-1-infected calves, the decline in NC activity usually seen following infection was completely

prevented by three intranasal doses of 10 mg of recombinant bovine IFN-$\alpha_1$1 (1 x 10^6 U/kg body weight, estimated from the materials and methods) commencing 36 hours prior to virus challenge. In a subsequent experiment with BHV-1-infected calves, a single dose of recombinant bovine IFN-$\alpha_1$1, given 48 hours prior to virus challenge, partially prevented the decrease in NC activity seen in controls and calves treated 24 or 72 hours prior to virus challenge. In a third experiment with BHV-1-infected calves, a single intramuscular dose of 10 mg of recombinant bovine IFN-$\alpha_1$1 (1 x 10^6 U/kg body weight, estimated from the materials and methods) or 2.5 mg of recombinant bovine IFN-τ (1 x 10^5 U/kg body weight, estimated from the materials and methods) resulted in less pronounced or complete prevention of the decline in NC activity seen in infected controls (Bielefeldt-Ohmann and Babiuk, 1985a).

However, another group reported that <u>in vitro</u> treatment of bovine PBML cells with 100 or 1000 U/ml of IFN-α or -τ had no effect on NK-like activity against BHV-1 infected A549 human tumor cells (Eskra et al, 1985).

Administration of avridine, a lipoidal amine compound that has activity as an IFN inducer, as well as a humoral and cell-mediated immune adjuvant, to cattle resulted in an enhanced ability of polymorphonuclear leukocytes (PMN) to mediate ADCC (Roth and Kaeberle, 1985).

Phagocytes.

The fact that IFN-τ is one, if not the main, form of the lymphokines released by activated T cells known as macrophage activating factor, suggests that IFNs have an important role in modulating macrophage function (Schultz and Kleinschmidt, 1983; Male et al, 1987). Macrophages carry out a number of immune functions that could be modulated by IFN, including: ingestion and killing of pathogens, ingestion and destruction of foreign substances, antigen presentation, and the production of various soluble immunoregulatory factors. Studies on murine and human macrophages have shown that IFN can alter macrophage morphology, surface markers and receptors, phagocytic functions, enzymatic activity, arachidonate metabolism, intracellular killing, antigen presentation, monokine production, tumoristatic or tumoricidal activity, and migration (Russell and Pace, 1987; Vogel and Friedman, 1984)

There have been few studies of the effects of homologous or heterologous IFN on bovine macrophage function. Recombinant bovine IFN-$\alpha_1$1 has been shown to decrease both random (chemokinetic) and directed (chemotactic) migration of monocytes and to increase the phagocytosis of opsonized E. coli and Staphylococcus aureus (Bielefeldt-Ohmann and Babiuk, 1984). Bovine alveolar macrophages (BAM) retrieved by lung lavage from calves that had been treated by the intranasal, intramuscular, or intrapulmonary routes with

recombinant bovine IFN-$\alpha_1$1 or -τ were assayed for a variety of indicators of antimicrobial and immunoregulatory potential. They generally showed increased activity levels following IFN treatment (Bielefeldt-Ohmann and Babiuk, 1986a). The same report described inconsistencies between results obtained using alveolar macrophages from IFN-treated calves and those obtained using cells from untreated calves that were subsequently exposed to recombinant bovine IFN *in vitro*. In another study, *in vitro* exposure of alveolar macrophages from normal calves to recombinant bovine IFN-$\alpha_1$1 resulted in reduced replication of PI-3V and VSV upon challenge of the macrophages (Holland, 1988). In an *in vitro* study of bovine macrophages obtained by culture of bone marrow-derived stem cells IFN-α and -τ had differing effects on the expression of Fc receptors (Pontzer and Russell, 1987).

IFN has also been reported to affect PMN phagocytic function. *In vitro* incubation of PMNs with recombinant bovine IFN-α, -τ, or human IFN-τ has been shown to impair their ability to migrate under agarose and increased ADCC and antibody-independent cell-mediated cytotoxicity (Steinbeck et al, 1986; Steinbeck et al, 1984; Steinbeck et al, 1985; Bielefeldt-Ohmann and Babiuk, 1986c). However, there were no effects on *Staph. aureus* ingestion, the myeloperoxidase-hydrogen peroxide-halide antimicrobial system, or generation of oxygen radicals.

The *in vivo* administration of recombinant bovine IFN-α-

I1, by the intranasal or intramuscular routes, increased PMN functions as measured by migration/chemotaxis, the myeloperoxidase-hydrogen peroxide-halide antimicrobial system, and generation of reactive oxygen species (Lawman et al, 1987; Bielefeldt-Ohmann and Babiuk, 1986c). Likewise, treatment of calves with 0.5 mg of recombinant bovine IFN-τ suppressed random migration and augmented iodination responses (Canning, 1987). Additionally in this study, the ability of opsonized Brucella abortus to survive in the presence of PMNs from animals treated with recombinant bovine IFN-τ was decreased (Canning, 1987).

PMN function was also examined in calves treated intranasally with 10 mg of recombinant bovine IFN-$\alpha_1$1 (1×10^6 U/kg body weight) 48 hours prior to challenge with an aerosol of BHV-1, followed four days later by an aerosol of P. hemolytica (Babiuk et al, 1985). Four days after viral infection PMN chemotaxis was reduced in both IFN-treated and untreated calves, however, the PMNs from IFN-treated animals were almost twice as active as those isolated from untreated controls. Additionally, superoxide anion generation of PMNs was increased by 4 days after viral challenge in both IFN-treated and untreated calves, but by the 7th day post-infection this activity was nearly normal in IFN-treated calves while superoxide anion generation continued to increase in untreated calves.

In addition to its possible role in modulating PMN-mediated ADCC mentioned above, administration of the IFN

inducer, avridine, to cattle resulted in an enhanced ability of PMN to ingest Staph. aureus, but had no effect on PMN random migration under agarose or nitroblue tetrazolium reduction (Roth and Kaeberle, 1985).

Major histocompatibility antigen expression.

Changes in cell surface antigen expression have been noted in a variety of different cell types following treatment with Type I or II IFN and this may mediate at least some of the immunological changes associated with IFN treatment (Paulnock and Borden, 1985). Among the best defined alterations in cell surface antigens following IFN treatment are modulation of the expression of Class I and Class II major histocompatibility (MHC) antigens (Gresser, 1984).

IFN-α and IFN-β increase the level of Class I (H-2 and HLA) antigen expression on the surface of many cell types, including lymphocytes (Lonai and Steinman, 1977; Vignaux and Gresser, 1977; Dolei et al, 1983; Zuckermann and Head, 1986). Also, expression of the Class I associated protein, β2-microglobulin, is concomitantly increased in treated cells (Heron et al, 1978; Fellous et al, 1981). Studies of the molecular mechanism of induction have shown that the appearance of new cell surface proteins is the result of an increase in mRNA levels for these proteins (Wallach et al, 1982; Sugita et al, 1986; Lampson and George, 1986; Fertsch et al, 1987). IFN-γ not only increases the level of expression of Class I MHC antigens, but also Class II (Dolei et

al, 1983; Wong et al, 1983; Kely et al, 1984). As in Class I antigens, this induction of cell surface antigen expression is temporally preceded by increases in mRNA for the component polypeptides of these MHC-encoded proteins (Fertsch et al, 1987). Some data have suggested that IFN-ϒ preferentially modulates Class II antigen expression (Wallach et al, 1982). However, studies have shown that IFN-α and -β can also affect expression of these antigens, although considerably higher concentrations of IFN are required to initiate similar increases (Wallach et al, 1982; Dolei et al, 1983).

In cattle, leukocytes isolated from within the epithelium or lamina propria of the small intestine that were pretreated for 18 hours with recombinant bovine IFN-αI1 or -ϒ had moderate enhancement of expression of both class I and class II MHC antigens although there was considerable variability between animals (Nagi and Babiuk, 1988). In another in vitro study, bovine PBML were treated with recombinant bovine IFN-α or -ϒ. Enhancement of expression of class II MHC antigens was observed in cells treated with IFN-ϒ, but not with IFN-α (Eskra et al, 1985).

Leukocyte circulation.

The administration of IFN-α results in changes in the numbers of circulating leukocytes, lymphocyte subset ratios, and in lymphocyte trafficking. These effects tend to be dose related. Less information is available for IFN-β and -

r, but their effects appear to be less marked than IFN-α (Bottomley and Toy, 1984).

Hematologic studies in humans have shown drops in total number of white blood cells, neutrophilic granulocytes, and lymphocytes following IFN treatment (Bottomley and Toy, 1984). In mice treated with murine IFN-α/β or poly(I):poly(C) (an IFN inducer) the expected lymphopenia was noted, as well as a significant decrease in the number of T cells in the thymus and spleen along with a significant increase in the number of non-T cells in the splenic white pulp. Also, the ratio of Lyt1+/Lyt2+ (helper/suppressor-cytotoxic) cells was elevated (Kawasaki et al, 1986). Previous work had shown that injections of murine IFN caused a lymphadenopathy and an enlargement of the spleen of mice (Gresser et al, 1981). These authors speculated that the trapping of circulating lymphocytes in the peripheral lymph nodes was enhanced by IFN, although they did not perform analysis of lymphocyte subsets.

In sheep that were injected with 2×10^7 U of human recombinant IFN-α-2a, the output of recirculating lymphocytes from cannulated popliteal lymph nodes fell to below 1% of pretreatment levels and remained depressed for up to 35 hours (Hein and Supersaxo, 1988). After the period of depressed lymphocyte output, a compensatory surge of cell traffic occurred for 2 to 3 days, during which there was a relative increase in the proportion of CD4+ (helper) T cells in lymph. These phenomena could be associated with high

levels of IFN, but there was no correlation with plasma cortisol concentrations.

When 10^6 U/kg recombinant bovine IFN-α_I1 was administered intravenously, subcutaneously, or intramuscularly to calves, leukopenia, neutropenia, and lymphocytopenia were observed (Gillespie et al, 1986). In calves given hydrocortisone and BHV-1 there was a significant negative correlation between the numbers of circulating lymphocytes and serum IFN titers (Cummins and Rosenquist, 1979). Calves given recombinant bovine IFN-α or IFN-τ, either intravenously or intramuscularly, at 10^6 or 10^4 U/kg of body weight showed a transient, mild leukopenia 12-48 hours post-injection and a lymphopenia that normalized by 24 hours post-injection (Bielefeldt-Ohmann and Babiuk, 1986c). All calves in this study, including PBS-treated controls, showed an increase in PMN numbers 4, 8 and 12 hours post-treatment. In calves challenged with an aerosol of BHV-1, followed four days later by an aerosol of P. hemolytica, intranasal treatment with 10 mg of recombinant bovine IFN-α_I1 (1 x 10^6 U/kg body weight) 48 hours prior to virus challenge resulted in no significant differences compared to untreated controls (Babiuk et al, 1985). Finally, syngeneic twin calves injected intramuscularly with 2 x 10^5 U/kg of recombinant bovine IFN-α_I1 had a lymphopenia at 24 hours post-injection characterized by decreases in both circulating T and non-T/non-B lymphocytes (Griebel et al, 1989). Additional characterization of circulating lymphocytes revealed an

increased CD4+/CD8+ (helper/suppressor-cytotoxic) ratio indicating a relatively greater depletion of the CD8+ subpopulation of T lymphocytes.

Heterologous IFN appears to have similar effects in cattle. Doses of 5 x 10^5 U/kg of recombinant human IFN-α A or A/D, administered to calves intramuscularly, resulted in a leukopenia, lymphopenia, and reduction of the post-handling neutrophilia seen in controls (Fulton RW, unpublished data).

Thus, it appears that although IFN-α causes a lymphopenia in the peripheral circulation, the alteration of lymphocyte trafficking such as augmentation of lymphocytes in lymph nodes and the increased helper/suppressor-cytotoxic T lymphocytes ratio may be immune enhancing effects. Also, some of the depression in T cell mitogen-induced lymphoproliferation noted following in vivo IFN treatment may be partially due to a depletion of responder cells.

Biologic effects: cell growth and differentiation

The pleiotropic effects of IFN were mentioned in the introduction. Within five years of the first description of IFN there were reports of effects on cell growth distinct from the antiviral action (Paucker et al, 1962). Since then, the modulation of cell growth, physiology, and differentiation by IFN has been studied. Reviews and discussion of this IFN effect, are not within the scope of this thesis, but have been published (Taylor-Papadimitriou, 1984;

Sreevalsan, 1984; Grossberg and Taoylor, 1984). These studies have and will continue to contribute to the understanding of the molecular mechanisms of IFN action and cellular function.

Pharmacokinetics

The behavior of exogenous IFN depends on a number of variables including route of administration, as well as type and source of IFN administered. The three types of IFN differ somewhat in their distribution and metabolism, although, in general, they behave like any other small circulating plasma proteins or glycoproteins. Reviews on the pharmacokinetics of IFN have been published, but most of the information concerns the human and murine systems (Bocci, 1981; Bocci, 1982; Bocci, 1984). There is more information on the pharmacokinetics of IFN-α than IFN-β or -γ.

Understanding the role of the kidney in the catabolism of small circulating proteins, including IFN, has led to the realization that the kidneys are responsible for eliminating the bulk of IFN from the plasma pool. Proteins with molecular weights less than 50,000 pass through the glomerular filter to different degrees depending upon factors such as the shape and charge of each protein. These proteins present in the ultrafiltrate are mostly reabsorbed by cells of the proximal tubule and are degraded. IFN molecules fit the criteria for filterable proteins, and, depending on the type, subtype, and degree of glycosylation, are filtered at

a rate proportional to their concentration in the plasma and degraded by cells of the proximal tubule. Other possible sites of IFN catabolism that are less important include liver, cell membranes, muscles, and lungs (Bocci, 1984).

In humans, IFN is rapidly cleared from the circulation, with half-life ($t_{½}$) values for recombinant human IFN-α ranging from 0.75 to 9.8 hours (Wills et al, 1984b; Bornemann et al, 1985; Wills and Spiegel, 1985). When human IFN-α is administered intramuscularly the maximum serum concentrations are seen between 1 and 6 hours after injection, they remain fairly steady for 6-12 hours, then progressively decline and by 18 to 36 hours are undetectable (Bocci, 1984; Wills et al, 1984b; Bornemann et al, 1985). The circulating IFN levels seen after intramuscular administration are markedly lower than those obtained after an intravenous injection (Bocci, 1984; Wills et al, 1984b; Bornemann et al, 1985). The reported $t_{½}$ in various species of animals (dog, rabbit, and monkey) ranges from 1.8 to 9.5 hours (Gibson et al, 1985; Satoh et al, 1984; Wills et al, 1984a).

In dairy calves given a daily dose of 10^6 U/kg of recombinant bovine IFN-α_I1 intramuscularly or subcutaneously for 3 or 5 days, peak serum IFN levels occurred 2 to 6 hours post administration (Gillespie et al, 1986). The peak levels of serum IFN following intramuscular or subcutaneous doses were 10-fold less than the same dose given intravenously. Using the same type of IFN and the same or similar

doses, other authors have reported peak serum IFN values that were less that 50% of the titers after intravenous injection occurring 1 to 2 hours post administration (Bielefeldt-Ohmann and Babiuk, 1986c; Griebel et al, 1989).

None of the above studies reported $t_{½}$ values for IFN in cattle. However, from data presented, a calculated $t_{½}$ for IFN of 8.6 hours was derived (Bielefeldt-Ohmann and Babiuk, 1986c). Intramuscular doses of 5×10^5 U/kg of recombinant human IFN-α-A given to cattle had a $t_{½}$ of 3.1 hours, compared to a $t_{½}$ of 12.6 hours for recombinant human IFN-αA/D (Fulton RW, unpublished data).

Other routes of IFN administration have been studied in humans and laboratory animals including intraperitoneal, oral, intranasal, and rectal (Bocci, 1984). Reports on the presence or absence of detectable serum IFN and/or clinical effects following dosage by these various routes are sometimes conflicting.

Another consideration in the pharmacokinetics of IFN is the induction of IFN-neutralizing antibodies. In a report of 28 calves given repeated intranasal or intramuscular doses of IFN at the rate of 10^6 U IFN/kg, only one developed IFN-neutralizing antibodies (Roney et al, 1985). In contrast, 31 of 51 humans treated with recombinant human IFN α-2a developed anti-IFN antibodies. Sera of 16 of the 31 neutralized the antiviral effects of recombinant human IFN α-2a <u>in vitro</u> (Steis et al, 1988). The possibility that these anti-IFN antibodies have clinical relevance in resis-

tance to effects of IFN has been suggested (Inglada et al, 1987; Quesada et al, 1987; von Wussow et al, 1987). The route of administration, duration of treatment, number and size of doses, and immunogenicity of the IFN are all likely to influence the likelihood of development of anti-IFN antibodies. In addition, time of sampling and assay method are likely to influence the detection of anti-IFN antibodies.

2',5'-oligoadenylate synthetase

Investigations into the molecular mechanisms of the array of effects ascribed to IFN have revealed several potential mediators of different IFN-associated phenomena. One of the best characterized is the 2',5'-oligo(A) synthetase/RNase system first described by Kerr and Brown (Kerr and Brown, 1978). Not only is this system of interest because of its importance as a mediator of the antiviral effect of IFN, but also because of its potential importance as an intracellular regulator of cell metabolism through its role as a fundamental metabolic symbol (Silverman, 1984; Luxembourg, 1988).

The 2',5'-oligo(A) system has been reviewed (Silverman, 1984; Luxembourg, 1988; Johnston and Torrence, 1984; Joklik, 1985; Williams and Silverman, 1985) and may be briefly summarized as follows. Synthesis of the enzyme 2',5'-oligo(A) synthetase can be induced by a variety of stimuli, including IFN, steroid hormones, growth factors, drugs, and various physiological or pathological stimuli. Once the

2',5'-oligo(A) synthetase is activated by double-stranded RNA it catalyzes the linkage of adenosine triphosphate (ATP) into oligomers of adenylate linked by a unique 2',5'-phosphodiester bond. The oligomers range in size from 2-15 adenylates, with 3 being the predominant homolog. The 2',5'-oligo(A) synthetase is also capable of catalyzing the synthesis of a great many structures by adding AMP and other nucleoside monophosphates in 2',5' linkage to the 2'-OH side of primer nucleotide structures which contain a 2',3' or 2',5'-terminal adenylate. This 2',5'-nucleotidyl transferase activity is terminal for nucleotides other than ATP. The 2',5'-oligo(A) oligomers, except the dimers, activate a latent cellular endoribonuclease (2',5'-oligo(A) dependent RNase, RNase L or F). The activated endoribonuclease degrades mRNA, thus inhibiting translation. 2',5'-oligo(A) is rapidly degraded by an intracellular 2',5'-phosphodiesterase. The activities of phosphatases that can transform 2',5'-oligo(A) from the active tri- or diphosphorylated oligomers to inactive mono- or dephosphorylated oligomers may also play a role in 2',5'-oligo(A) catabolism.

Several methods for determination of 2',5'-oligo(A) synthetase have been described, all of which consist of 3 steps: 1) preparation of 2',5'-oligo(A) synthetase from cells, 2) <u>in vitro</u> 2',5'-oligo(A) synthesis, and 3) quantification of 2',5'-oligo(A) (Peska, 1981; Luxembourg, 1988; Johnston and Torrence, 1984; Bruchelt et al, 1987). Different techniques have been employed to effect these steps.

Three basic methods for 2',5'-oligo(A) enzymatic synthesis are used: 1) direct synthesis using soluble enzyme activated by soluble poly(I):poly(C), 2) synthesis by enzyme adsorbed to poly(I):poly(C) linked to agarose, sepharose or cellulose, and 3) synthesis by enzyme adsorbed to 2',5'-diphosphate linked to sepharose or agarose and activated by soluble poly(I):poly(C). Likewise, there are four basic methods for assaying for 2',5'-oligo(A): 1) synthesis of oligomers from radiolabeled ATP followed by chromatographic separation and isotope quantification, 2) determination of biologic activity through nuclease L inhibition of translation or degradation of RNA, 3) competition of binding to nuclease L, and 4) radioimmunoassay or enzyme-linked-immunosorbent assay using 2',5'-oligo(A) specific antibodies. Additionally, a spectrophotometric method for quantification of 2',5'-oligo(A) has been described recently (Short et al, 1987).

The use of IFN assay to monitor IFN therapy suffers from the fact that IFN serum $t_{½}$ is short. However, the kinetics of 2',5'-oligo(A) synthetase elevation are very different from that of the inducing IFN. In humans administered varying doses of human lymphoblastoid IFN-α, 2',5'-oligo(A) synthetase activity increased in a dose dependent fashion and was a more sensitive indicator of IFN administration than was measurement of the level of circulating IFN (Merritt et al, 1986). Injection of doses of recombinant human IFN-α_{2a} that ranged from 0.3 to 18 x 10^6 U also caused

increases in 2',5'-oligo(A) synthetase activity in a dose dependent fashion and the increases were detectable for a longer time than was serum IFN (Witter et al, 1987; Barouki et al, 1987). Additionally, a parallel time course between induction of 2',5'-oligo(A) synthetase activity and development of the antiviral state in PBML was demonstrated.

Similarly, the use of IFN as a viral diagnostic test is limited because of the transient IFN production coupled with the short $t_{½}$ (Skidmore and Jarlow, 1987). The use of changes in levels of 2',5'-oligo(A) synthetase activity in the diagnosis and prognosis of human diseases has been examined (Read et al, 1985; Furuta et al, 1987; Fujii et al, 1987; Ferbus et al, 1988; Kennedy and Tilles, 1988). Additionally, modulation of serum IFN levels and 2',5'-oligo(A) synthetase activity has been monitored in humans receiving viral vaccines (Tilles et al, 1987; Nakayama et al, 1988).

Increases in 2',5'-oligo(A) synthetase in PBML have been associated with IFN treatment, modified live viral vaccine administration, and viral infection in cattle. In calves treated with doses of recombinant bovine IFN-α_I1 ranging from 10^4 to 10^7 U/kg increases in 2',5'-oligo(A) synthetase activity in PBML were IFN dose related and tended to remain elevated longer than serum IFN levels (Perino et al, 1987).

Daily intramuscular administration of 10^6 U/kg body weight of recombinant human IFN-α2 for seven days induced 2',5'-oligo(A) synthetase in bovine PBML. When these calves

were challenged with vaccinia virus there was good correlation between antiviral activity and 2',5'-oligo(A) synthetase activity (Vanden Broecke et al, 1985). In the same study, cattle infected with BHV-1 and treated intramuscularly with 10^6 U/kg body weight human IFN-α2Arg for six days starting the day before infection were not protected against primary infection or from the establishment of viral latency. Calves that were challenged and treated with IFN had higher 2',5'-oligo(A) synthetase activity than did calves treated with IFN, and enzyme levels in both groups were higher than in controls. BHV-1 primary infection alone did not cause a very marked increase in 2',5'-oligo(A) synthetase, on the average. Three months after primary infection calves were treated with five consecutive daily intravenous injections of dexamethasone. Upon reactivation of the virus there was a significant increase in 2',5'-oligo(A) synthetase even if no IFN could be detected in the circulation. Dexamethasone treatment in the control animal had no effect on levels of 2',5'-oligo(A) synthetase.

In another study, calves injected once, intramuscularly, with 10^6 U/kg body weight of recombinant bovine IFN-α_I1 had increased levels of 2',5'-oligo(A) synthetase compared to controls (Short and Fulton, 1987). These authors also report that calves injected intravenously with a commercial BHV-1/BVDV/PI-3 MLV vaccine had elevated 2',5'-oligo(A) synthetase levels compared to controls. <u>In vivo</u> treatment of calves with recombinant bovine IFN-α_I1 increased the

levels of 2',5'-oligo(A) synthetase activity in both alveolar macrophages and PBML (Holland, 1988). In a different study using the same IFN, levels of 2',5'-oligo(A) activity increased 8 hours following IFN administration and returned to baseline within 72 hours (Griebel et al, 1989). These authors reported the level of enzyme activity in counts per minute, so comparison with data in cattle and other species is not possible.

Calves that were naive to BHV-1 showed increased levels of 2',5'-oligo(A) synthetase in PBML following aerosol inoculation with BHV-1 (Bielefeldt-Ohmann et al, 1989). The peak 2',5'-oligo(A) synthetase activity occurred 4 to 5 days post-inoculation which corresponded temporally with the period of highest susceptibility to secondary bacterial infection. Additionally, a relationship between 2',5'-oligo(A) synthetase levels on day 5 post-infection and clinical outcome of a dual infection with BHV-1 and P. hemolytica was noted. Aerosol challenge with P. hemolytica did not induce 2',5'-oligo(A) synthetase nor alter the kinetics of virus-induced enzyme.

Clinical applications

Reviews have been published on the use of IFN in humans to treat various infections and tumors (Merigan, 1988; Goldstein and Laszlo, 1986; Billiau, 1985; Finter, 1985). The use of exogenous IFN in cattle to prevent or reduce viral infection has had mixed success. Calves treated daily

for 1 week with 50 million U of human recombinant IFN-αA, intranasally and/or intamuscularly, and inoculated with BHV-1 on the first day of treatment had less severe respiratory tract disease and delayed onset of infection, viral shedding, and appearance of viral neutralizing antibodies compared with controls (Roney et al, 1985).

Intramuscular doses of 10^6 U/kg recombinant human IFN-α2 given to calves daily for 7 days prior to infection provided partial or complete protection from intradermal infection with vaccinia virus (Vanden Brocke et al, 1985). However, in the same report, calves treated daily with the same dose for 6 days, starting the day before BHV-1 challenge, had identical clinical symptoms as controls both during primary infection and dexamethasone induced reactivation.

Treatment of calves intranasally with 10 mg of recombinant bovine IFN-α_1 (1 x 10^6 U/kg body weight, estimated from material and methods) 48 hours prior to challenge with BHV-1 increased the ability of the animals to withstand an aerosol challenge with P. hemolytica four days later (Babiuk et al, 1985). In another report by same authors, intranasal or intramuscular treatment of calves with 10 mg of recombinant bovine IFN-α_1 48 hours prior to challenge with BHV-1 and P. hemolytica reduced clinical signs, number of sick days, lung lesions, and weight loss (Babiuk et al, 1987). Based on in vivo viral replication in the upper respiratory tracts of calves in these two trials the authors concluded

that the reduction in viral-bacterial synergy observed following IFN treatment did not appear to be due to a direct effect of the IFN on virus replication and postulated it originated from the immunomodulatory effects of IFN.

Treatment of calves with 5 mg of recombinant bovine IFN-$\alpha_1$1 (approximately 4 x 10^6 U/kg body weight) prior to shipment, after shipment, or at both times resulted in slightly reduced number of days sick, clinical scores, and morbidity compared to untreated controls (Perino LJ, Smith RA. Unpublished data, 1986).

The effects of IFN on BVDV infection has also been examined. Intramuscular treatment of dairy calves with 10^4 or 10^5 U/kg of recombinant bovine IFN-$\alpha_1$1 for six consecutive days commencing the day of challenge with the Holmes strain of bovine virus diarrhea virus resulted in delayed onset of clinical signs and slightly reduced disease severity (Gillespie et al, 1986a).

Bovine Herpesvirus-1

Infection with BHV-1 is associated with a spectrum of clinical diseases in cattle including respiratory and genital disease as well as conjunctivitis, abortion, encephalitis, enteritis, and a generalized disease of newborn calves (Fenner et al, 1987). The colloquial names infectious bovine rhinotracheitis (IBR), red nose, and necrotic rhinitis refer to the acute, contagious upper respiratory tract disease described during the early 1950s in Colorado feed-

lots and California dairies (Jensen and Mackey, 1979). IBR may occur as a subclinical, mild, or severe disease with morbidities as high as 100%. High mortalities may also be seen if secondary bacterial infection occurs subsequent to viral debilitation of pulmonic defenses.

Various etiologic mechanisms for the predisposing effects of BHV-1 to secondary bacterial infection have been explored. Some studies have examined the effects of BHV-1 on airway function (Conlon et al, 1987) but most have explored various indicators of pulmonic and systemic immune function.

BHV-1 has been shown to have detrimental effects on the bacterial defense mechanisms of the lung. Aerosol exposure to BHV-1 will facilitate infection of the lung by a usually noninfectious dose of P. hemolytica resulting in fibrinous pneumonia (Yates et al, 1983). In vitro and in vivo experiments have shown cytopathic effects (CPE) on respiratory epithelium that results in compromise of the mucociliary defense mechanisms (Rossi and Kiesel, 1977; Allen and Msolla, 1980).

Altered BAM functions have also been attributed to BHV-1. BHV-1 has been reported to have caused CPE on BAM (Rossi and Kiesel, 1977; Toth and Hesse, 1983),even though it appears to replicate in BAM at very low levels (Toth and Hesse, 1983; Forman et al, 1982; Forman and Babiuk, 1982) or not at all (Rossi and Kiesel, 1977). Other BHV-1 mediated effects noted in these in vitro BAM studies include reduc-

tion of Fc-mediated receptor activity and phagocytosis, complement receptor activity, and ADCC (Forman and Babiuk, 1982).

In vivo BHV-1 infection also affects BAM recovered by lung lavage. One study reported that only a small proportion of BAM appeared to become infected in aerosol or intranasal challenged calves (Forman et al, 1982). In this study, even though clinical signs of viral infection were noted, the BAM from these calves showed unaltered Fc and complement receptor activities, phagocytic activity, and ability to mediate ADCC. In another study there was a reduction in the number of BAM with Fc and complement receptors and in the ability of BAM to phagocytize and kill Staph. epidermidis (Brown and Ananaba, 1988). Macrophage activity as measured by chemiluminescence is also impaired in BHV-1 infected calves (Conlon and Eyre, 1985). In other BHV-1 challenged calves, lavage recovered BAM showed increases in the percentage of cells expressing a MHC class II antigen, increased Fc-mediated phagocytosis, increased β-glucuronidase, and increased production of prostaglandin E_2 (Bielefeldt-Ohmann and Babiuk, 1986a). Selective suppression of BAM-mediated cellular cytotoxicity and interleukin-1 generation was also observed in this study.

Macrophage/neutrophil interaction, as well as neutrophil function, may also be impaired in calves exposed to an aerosol of BHV-1 followed five days later by an aerosol of P. hemolytica (McGuire and Babiuk, 1983). In this study,

analysis of sequential lavage fluid suggested that neutrophil infiltration into the lung in response to the presence of the bacteria was delayed. In vitro studies of these cells showed that neutrophils from BHV-1 infected animals displayed little random migration and did not respond to a chemotactic stimulus. Also, macrophages were not able to produce neutrophil chemotactic factors. In another BHV-1 aerosol challenge study, significant depression of peripheral blood neutrophil chemotactic response was again noted, but the antibacterial activity of the neutrophils was not significantly affected (Filion et al, 1983). A later study in calves challenged with BHV-1 or BHV-1 and P. hemolytica showed decreased neutrophil chemotaxis and enhanced generation of superoxide anions (Bielefeldt-Ohmann and Babiuk, 1985b). In vivo aerosol exposure of calves to BHV-1 resulted in decreased random migration of peripheral blood neutrophils and enhanced ingestion of Staph. aureus (Briggs et al, 1988). Preincubation of bovine peripheral blood neutrophils with BHV-1 in vitro did not impair their ability to ingest and kill P. hemolytica nor their random or directed migration, suggesting the BHV-1 induced dysfunction is not a direct effect (Noel et al, 1988).

The importance of CMI in BHV-1 infection was suggested by the appearance of antigen-specific lymphocyte transformation responses that corresponded temporally with the time of recovery from both primary and recurrent BHV-1 infections in cattle (Davies and Carmichael, 1973) and that CMI responses

occurred only in immunized or infected animals (Rouse and Babiuk, 1974).

In a BHV-1 aerosol challenge study, significant depression occurred in the lymphocyte blastogenic response to PHA, P. hemolytica, and P. multocida (Filion et al, 1983). Another report of calves exposed to BHV-1 confirms the diminished proliferative response to mitogens following virus infection and also describes a significant leukopenia and a transient depression of NC expressed against a xenogeneic cell line (Bielefeldt-Ohmann and Babiuk, 1985b). Intravenous inoculation of BHV-1 decreased the number of circulating lymphocytes by more than 50% (Cummins and Rosenquist, 1979). In vitro infection of bovine lymphocytes with BHV-1 or exposure to virus-free culture supernatants inhibited blastogenic response of bovine peripheral blood mononuclear cells to ConA while exposure to ultraviolet-inactivated BHV-1 had no effect (Pollard et al, 1985).

Vaccines have been developed in an attempt to prevent or reduce the severity of BHV-1 infection. Both intranasal and intramuscular vaccines appear efficacious in experimental challenge systems and induce circulating antibody; however, there are conflicting reports on the induction of nasal antibody and questions as to its relevance to protection (McKercher and Crenshaw, 1971; Frank et al, 1977; Gerber et al, 1978). Induction of both acid-labile and acid-resistant IFN following BHV-1 challenge of immunized calves has been shown (Gerber et al, 1978).

CHAPTER III

INTERFERON AND 2',5'-OLIGOADENYLATE SYNTHETASE IN SERUM AND PERIPHERAL BLOOD MONONUCLEAR LEUKOCYTES OF CATTLE FOLLOWING INJECTION OF BOVINE INTERFERON-$\alpha_1$1

Summary

Cell extracts prepared from peripheral blood mononuclear leukocytes from sixty-six samples obtained from six clinically healthy calves contained an average 2',5'-oligoadenylate (2',5'-oligo(A)) synthetase activity sufficient to synthesize 186±82 pmoles 2',5'-oligo(A)/h/10^6 cells. These calves showed no measurable serum interferon (IFN) activity. Five calves were given intramuscular injections of 10^4, 10^5, 5 x 10^5, 10^6 and 10^7 units of Bovine IFN-$\alpha_1$1 per kg of body weight at two-week intervals. Five different dose sequences were used employing a 5 x 5 Latin square design such that each animal received each dose once. Activity of 2',5'-oligo(A) synthetase increased at 24 hours in response to all dosages of IFN and then declined following first order kinetics and with an apparent $t_{½}$ of 2.1±0.5 days. The area under the concentration-time curve (AUC) for 2',5'-oligo(A) synthetase increased with dose of IFN more rapidly than did

peak response. Serum IFN measured at one-day intervals following administration of IFN was consistently measurable only at dosages above 10^6 units (U) IFN per kg body weight. Half-life for circulating IFN was 12.4±1.0 hours. Over all dosages, increases in 2',5'-oligo(A) synthetase activity were measurable for 3.5 days longer than were increases in IFN following intramuscular injection of IFN. No animals developed detectable anti-IFN antibodies. This information on the pharmacokinetics of both the 2',5'-oligo(A) synthetase response and the serum IFN response to treatment with IFN should be of value in studies on the prophylactic or therapeutic use of IFN.

Introduction

Interferons (IFNs) are cellular proteins produced in response to viral infection as well as other stimuli (Torrence and DeClercq, 1981). In addition to their role in defense against viral infection, they inhibit viral replication, inhibit cell division, and modulate immune responses (Stewart, 1979). The usefulness of serum IFN measurements for assessing the presence of viral disease and evaluating appropriate dosages of IFN is limited because the assay procedures are lengthy and laborious and because endogenous serum IFN levels increase at varying times after virus infection and endure for only a few days (Treuner et al, 1980; Vilcek and Kohase, 1977). Similarly, injected IFN disappears rapidly from the serum (Gibson et al, 1985; Satoh

et al, 1984; Sugino et al, 1986; Witter et al, 1987). Thus patients with viral infection or those administered IFN may have measurable levels of IFN in their sera only briefly if at all. Recently a rapid method for measuring serum IFN has been reported (Skidmore and Jarlow, 1987). The problem remains, however, that IFNs can be found for only a short time, if at all, following viral infection or IFN injection. Moreover, in the case of prophylactic or therapeutic use of IFN, biological response is a more important measure than is the concentration of IFN itself in serum.

IFNs-α, β, and γ share the property of being able to induce the intracellular enzyme 2',5'-oligoadenylate (2',5'-oligo(A)) synthetase. This enzyme catalyzes synthesis of 2',5'-oligo(A) which activates a latent endoribonuclease. The RNase digests single-stranded RNA thereby inhibiting protein synthesis (Floyd-Smith et al, 1981). 2',5'-oligo(A) synthetase is present in extracts of various cells, including peripheral blood mononuclear leukocytes (PBML). In many cases its level can be raised by exposure to IFN (Sugino et al, 1986; Baglioni et al, 1979; Ball, 1979; Kimchi et al, 1979). Compared to serum IFN, 2',5'-oligo(A) synthetase in PBML appears earlier after viral infection (Schattner et al, 1981a), reaches more readily measurable levels sooner and more reliably after viral infection (Schattner et al, 1981a), and persists at elevated levels longer (Schattner et al, 1981a; Barouki et al, 1987; Lodemann et al, 1984; Merritt et al, 1986). For these reasons 2',5'-oligo(A)

synthetase has been used as an index of biologic responsiveness of cells to and a marker for IFN (Barouki et al, 1987; Merritt et al, 1986; Buffet-Janvresse and Hovanessian, 1984; Chousterman et al, 1983; Merlin et al, 1981; Schattner et al, 1982).

We are interested in the 2',5'-oligo(A) system in cattle not only because a number of economically important diseases in cattle are caused by viruses, but also because it should be useful in assessing the response of cattle to immunizing agents, to IFN inducers, and to natural and recombinant IFNs. Bannai has recently shown that two human IFNs, one natural and one recombinant, have different 2',5'-oligo(A) inducing capacities (Bannai, 1986). Similar variations are likely to occur in cattle. The work reported herein was undertaken to gain information about normal concentrations of 2',5'-oligo(A) synthetase in PBML, the relationship between amount of IFN given and 2',5'-oligo(A) synthetase levels in PBML, and the duration of elevated 2',5'-oligo(A) synthetase after administration of IFN.

Materials and Methods

Materials--Agarose-poly(I)·poly(C) and poly(I)· poly(C) were purchased from Pharmacia P-L Biochemicals, Piscataway, N.J. [8-014C]was purchased from ICN Pharmaceuticals, Inc., Irvine, Calif. Bovine IFN-$\alpha_1$1, synthesized in Escherichia coli, was obtained from CIBA-GEIGY Corp., Greensboro, N.C. Bacterial alkaline phosphatase (AP) and phosphocreatine

kinase were obtained from Sigma Chemical Co., St. Louis, MO. Dowex 1x2, 100 to 200 mesh, was washed sequentially with 20 bed volumes of 1 N NaOH, water, 1 N HCl and water; this wash sequence was repeated five times ending with HCl and water washes to leave the resin in the Cl^- form (Mirua and Hayashi, 1967).

Animals--Calves of mixed breeding, weighing 325 to 400 kg each, were obtained from commercial sources. They were maintained on grain supplement and free-choice hay.

Cell extracts--Bovine PBML were collected from 10 ml of heparinized blood by dilution of the buffy coat with an equal volume of phosphate buffered saline (PBS) and centrifugation on a Ficoll-Hypaque cushion at 1000 x g for 20 min. Cells collected at the interphase were washed twice with 5 ml portions of PBS and suspended in 400 μl PBS. Two samples of cells (10 μl each combined with 25 μl 0.1 percent crystal violet in 0.1 M acetic acid) were counted on a hemocytometer. If the counts differed from each other by less than two times the square root of the mean of the counts, the mean was taken as the cell count. If the counts differed from each other by more than two times the square root of the mean of the counts, additional paired counts were done until this criterion was met. Then either i) all counts were averaged or ii) one count was rejected before the remaining counts were averaged. One count was rejected if its deviation from the mean of the counts was more than four times the average deviation of the remaining counts. After

counting, PBML were sedimented at 1500 x g for 1 min and the supernatant solution was removed. Sufficient lysis buffer (Schattner et al, 1981a) was added to the cell pellet to give a concentration of 1.3 x 10^8 cells/ml. After 30 min at 4°C, cell debris was removed by centrifugation at 12,000 x g for 6 minutes. The resulting cell extract was used immediately for assay or stored at -70°C.

Activity of 2',5'-oligo(A) synthetase--Assays were conducted as previously described (Schattner et al, 1981a; Short and Fulton, 1987). Changes in 2',5'-oligo(A) synthetase were taken as the measured activity (pmoles 2',5'-oligo(A)/h/10^6 PBML) minus the mean control value.

Measurement of serum IFN--IFN assays were performed by a plaque reduction method in 24-well tissue culture plates containing confluent Madin-Darby bovine kidney (MDBK) monolayers with vesicular stomatitis virus (VSV) as the challenge virus (Fulton and Pearson, 1980; Fulton et al, 1986). Four-fold dilutions of the initial dilution (1:50) were made. IFN titers (in units per ml) were expressed as the reciprocal of the dilution that reduced the number of VSV plaques by fifty percent (Fulton and Pearson, 1980; Fulton et al, 1986). Changes in serum IFN were taken as measured U/ml minus 50 (the reciprocal of the initial dilution, at which all controls were negative).

Assay for neutralizing antibodies to IFN--Serum antibodies were measured by a modification of the IFN assay described above (Fulton and Pearson, 1980; Fulton et al,

1986). A 1:5 dilution of serum was mixed with 200 U of bovine IFN-$\alpha_1$1/ml and incubated at 37°C for 1 hour. The resulting final dilution (1:10 serum with 100 U of IFN/ml) was added to confluent MDBK monolayers and assayed for antiviral activity as described above.

Assay for viral antibodies--A microtitration virus neutralization test in cell culture was utilized as previously described (Fulton et al, 1982). Viruses included BHV-1, BVDV, PI-3V, and bovine RSV. Two-fold dilutions of serum were made beginning with a 1:2 initial dilution (which resulted in a 1:4 final dilution after addition of virus). Acute sera were from the day of the initial injection of IFN (day 0) and the convalescent sera were from the collection seven days after the fifth and final injection of IFN (day 63).

Study design--2',5'-oligo(A) synthetase was measured in the PBML of six normal calves for 14 days prior to any injections of IFN. During this equilibration period, two calves, randomly selected, were given intramuscular injections of IFN carrier medium. Subsequently these six calves served as experimental and sentinel subjects in a study designed to assess the effect of dose of IFN on 2',5'-oligo(A) synthetase activity in PBML. Five animals were given intramuscular injections of 10^4, 10^5, 5 x 10^5, 10^6 and 10^7 units of IFN per kg of body weight at two-week intervals using five different predetermined and randomly assigned dose sequences employing a 5 x 5 Latin square design (Fisher

and Yates, 1963) such that each animal received each dose once during the 10-week period (Table 1, page 65). One additional animal was maintained with and treated the same as the five experimental subjects except that it received no injections of IFN.

Statistical methods--All statistical analyses were performed using SAS (SAS Institute Inc, Cary, North Carolina). Sensitivity of measurement of changes in 2',5'-oligo(A) synthetase activity was compared to sensitivity of measurement of changes in serum IFN levels with a paired t test. The assumption of homogeneity of variances as determined by Levine's test could not be made for the serum IFN levels or peak 2',5'-oligo(A) synthetase; thus the analysis of variance ANOVA was performed after rank transformation of the data.

Results

Baseline period and experimental controls--During the fourteen days prior to initiation of IFN treatment, ten blood samples were obtained from each calf for measurement of 2',5'-oligoadenylate synthetase activity. Figure 1 (page 67) shows a frequency distribution of enzyme activity values during the baseline period. The average 2',5'-oligo(A) synthetase activity was 186±82 pmoles 2',5'-oligo(A) synthesized/h/10^6 PBML, making the 95% confidence interval 22 to 350 pmoles 2',5'-oligo(A) synthesized/h/10^6 PBML. Sera tested during this baseline period had no detectable IFN

(<50 U/ml). Levels of 2',5'-oligo(A) synthetase activity in PBML and serum IFN of calves were unaffected by administration of IFN carrier.

The calf that received no injections of IFN had no detectable levels of serum IFN during the experiment. All calves appeared clinically normal throughout the experiment and no signs of toxicity were noted at any IFN dose. Serum antibody titers to BHV-1, PI-3V, BVDV, and bovine RSV did not change significantly during the experiment (Table 2, page 66).

Prior to administration of the final dose of IFN, the calf that received no injections of IFN and 2 of the 5 experimental calves had unexplained elevations in 2',5'-oligo(A) synthetase levels. The remaining calves were unaffected. During the period when the untreated calf showed aberrant 2',5'-oligo(A) synthetase levels, data from all calves were omitted from the analysis.

Effects of dose of IFN on 2',5'-oligo(A) synthetase levels in PBML and serum IFN levels--Time course of 2',5'-oligo(A) synthetase response to IFN: Figures 2a and 2b (pages 69 and 71) show the time course of 2',5'-oligo(A) synthetase induction in PBML and serum IFN levels, respectively. Both 2',5'-oligo(A) synthetase and serum IFN generally tended to peak 24 hours after IFN administration for all doses of IFN. The response of both 2',5'-oligo(A) synthetase and serum IFN increased in both magnitude and duration with increasing dosages of IFN. The measured

increases in 2',5'-oligo(A) synthetase were greater in magnitude and longer in duration than the respective increases in serum IFN at all doses of IFN. Based on combined data display in from Figure 2a (page 69), the apparent $t_{½}$ of 2',5'-oligo(A) synthetase is 2.1±0.5 days. The correlation coefficient for a first order decay process was 0.96 to 0.98 for doses of IFN above 5 x 10^5 U/kg. In contrast the data from Figure 2b (page 71) for the 10^6 and 10^7 U/kg doses give an estimated $t_{½}$ for IFN of 12.4±1.0 hours.

Sensitivity of detection of changes in 2',5'-oligo(A) synthetase activity in PBML and serum IFN--The duration of the 2',5'-oligo(A) synthetase response was compared to the duration of increased serum IFN by examining the mean number of days a positive enzyme response (>350 pmoles/h/10^6 PBML) was detected, compared to the number of days serum IFN was increased over 50 U/ml following a single injection of IFN (Figure 3, page 73). At a dose of 10^4 U IFN/kg, 2',5'-oligo(A) synthetase activity was positive for 3.5 days while serum IFN was detected in only one animal on one day. Even at dose levels where serum IFN was readily detectable, the enzyme activity was positive longer. Over all doses, increases in 2',5'-oligo(A) synthetase were detectable an average of 3.5 days longer than were increases in IFN ($P<0.0005$).

Cumulative responses--The area under the concentration-time curve (AUC) of 2',5'-oligo(A) synthetase activity was compared to the AUC of serum IFN for varying doses of IFN

(Figure 4a and 4b, page 75 and 77). The average AUC for 2',5'-oligo(A) synthetase activity were 1411, 1606, 2504, 2726, and 4349 pmoles, respectively for doses of IFN of 10^4, 10^5, 5 x 10^5, 10^6 and 10^7 U IFN/kg. The corresponding AUC values of serum IFN were 2, 29, 97, 487 and 2381 respectively.

ANOVA of rank transformed AUC values of 2',5'-oligo(A) synthetase indicated that the effect of dose of IFN was highly significant (P=0.0002), the effect of animal was less significant (P=0.01), and the effect of dose order (week) was not significant (P=0.26). ANOVA of the rank transformed AUC values for IFN also indicated that the effect of dose was highly significant (P<0.0001). Effect of animal was not significant (P=0.36) nor was time period (P=0.06).

Peak responses--Changes in 2',5'-oligo(A) synthetase activity were compared to changes in serum IFN levels at varying doses of IFN by comparing peak responses following administration of IFN (Figure 5a and 5b, page 79 and 81). The average peak levels of 2',5'-oligo(A) synthetase activity were 466, 704, 904, 935, and 1154 pmoles, respectively at 10^4, 10^5, 5 x 10^5, 10^6, 10^7 U IFN/kg dosages. The average peak values for serum IFN at the corresponding dosages were 2, 29, 76, 310, and 1625, respectively. ANOVA of the rank transformed 2',5'-oligo(A) synthetase peak values indicated that dose effects were highly significant (P=0.0022). Effect of animal was not significant (P=0.15) but time period was significant (P=0.007). ANOVA of the rank trans-

formed IFN peak values indicated that dose effects were highly significant ($P<0.0001$). Effect of animal was not significant ($P=0.53$) nor was time period ($P=0.15$).

Serum antibodies to IFN in calves receiving IFN--Sera tested were taken on the initial day of treatment with IFN and seven days after the calves had received all five different doses of IFN (day 63). No neutralization of IFN activity was detected in either acute or convalescent sera (1:10 final serum dilution against 100 U IFN) of IFN-treated or control calves.

Discussion

Activity of 2',5'-oligo(A) synthetase in PBML of cattle reported in this work, 186±82 pmoles/h/10^6 PBML, is somewhat higher than values previously reported by this laboratory 88±41 pmoles/h/10^6 cells (Short and Fulton, 1987). Such an increase is likely due to changes in procedures for counting PBML and for transferring oligonucleotide product to chromatography columns. Values reported for humans are 810 (95% confidence interval 190-3520) pmoles/h/10^6 PBML at 30°C (Barouki et al, 1987), 114±56 pmoles/h/10^6 PBML (Buffet-Janvresse and Hovanessian, 1984), 1860±780 pmoles/h/10^6 PBML at 37°C (Chousterman et al, 1983), 70 pmoles/h/10^6 PBML at 37°C (95 percent confidence interval 7 to 669 pmoles/h/10^6 PBML (Merritt et al, 1985), and 1300±390 pmoles/h/10^6 cells 30°C (Schattner et al, 1981a). From one laboratory to another the average measured activity per 10^6 PBML varies

more than ten-fold but standard deviations seem to be about the same, 30-40 percent. Our results, using the assay developed by Schattner et al. (Schattner et al, 1982) modified to measure incorporation of all nucleoside residues, show that 2',5'-oligo(A) synthetase of PBML of cattle are comparable to those for man.

Cattle showed, in general, incrementally increasing levels of 2',5'-oligo(A) synthetase in PBML to increasing doses of IFN from 10^4 to 10^7 U/kg. Peak 2',5'-oligo(A) synthetase activity of PBML at 24 hours after administration of IFN increased in proportion to the log of the dose of IFN as shown in Figure 5a (page 79). Larger doses of IFN increased not only the activity of 2',5'-oligo(A) synthetase in PBML of cattle but also the number of days during which 2',5'-oligo(A) synthetase activity was significantly above normal. Our observation that 2',5'-oligo(A) synthetase levels in PBML cattle remain elevated for two to seven days as a function of dose agrees with the observation that administration of 2.5×10^5 U IFN/kg in man caused an elevation of 2',5'-oligo(A) synthetase that persists for four days (Barouki et al, 1987). The pattern of decline of 2',5'-oligo(A) synthetase following induction by IFN shown by Barouki et al. (Barouki et al, 1987) is strikingly similar to the pattern reported herein. In order to assess both total magnitude of the 2',5'-oligo(A) synthetase response and peak response to IFN we compared peak responses and AUC for each dose level of IFN from 1 to 11 days (Figure 4a and

5a, page 75 and 79). Peak activity of 2',5'-oligo(A) synthetase as an estimate of biologic response to IFN yields useful results, but they are low compared to estimates based on AUC of 2',5'-oligo(A) synthetase activity.

The maximum dose of 10^7 units IFN/kg caused no apparent adverse effects in cattle. Gillespie et al. similarly observed no clinical signs beyond a transitory moderate febrile response after an initial intramuscular dose of 10^6 U IFN/kg in cattle (Gillespie et al, 1986). Furthermore, they noted no clinical signs after intravenous, subcutaneous or intranasal administration of IFN. At doses estimated to be 2.5×10^5 units per kg, IFN caused distinct clinical symptoms in humans (Witter et al, 1987). Even though IFN was given repeatedly to each experimental animal over several weeks in the work reported here, none developed detectable anti-IFN antibodies. Similarly Roney et al. (Roney et al, 1985) reported that of 28 calves given repeated intranasal or intramuscular doses of IFN at the rate of 10^6 U IFN/kg, only one developed IFN neutralizing antibodies. In contrast, 31 of 51 humans treated with recombinant human IFN α-2a developed anti-IFN antibodies. Sera of 16 of the 31 neutralized the antiviral effects of recombinant human IFN α-2a in vitro (Steis et al, 1988). The possibility that these anti-IFN antibodies have clinical relevance in resistance to effects of IFN has been suggested. (Inglada et al, 1987; Quesada et al, 1987; von Wussow et al, 1987). The route of administration, duration of treatment, number and

size of doses, and immunogenicity of the IFN are all likely to influence the likelihood of development of anti-IFN antibodies. In addition, time of sampling and assay method are likely to influence the detection of anti-IFN antibodies.

The experiments reported herein (based on a Latin Square design) demonstrate that the effect of dose of IFN on 2',5'-oligo(A) synthetase was highly significant for peak ($p=0.002$) and AUC ($p=0.0002$). Dose order (or time) and animal also affected the 2',5'-oligo(A) synthetase response to IFN but to a much lesser degree than dosage of IFN. These findings are consistent with those reported in humans by Merritt et al. with respect to the effect of dose of IFN on 2',5'-oligo(A) synthetase (Merritt et al, 1986). We have no basis for comparison of ANOVA on ranks by animal and by dose order. Treatment with IFN should not influence animal response to its subsequent use.

Elevated serum IFN levels, measured 24 hours after injection of IFN, could be demonstrated using grouped data at all doses above 10^4 U/kg but only at and above 5×10^5 u/kg could the change be detected in all animals. ANOVA of rank transformed data show that the effect of dose on peak concentrations of IFN is highly significant ($p=0.0001$). We attribute the extremely low P-value to the fact that changes in serum IFN at one day post-treatment became consistently measurable only at dosages of at least 10^6 U IFN/kg body weight and then increased dramatically from 0 to about 2000

units as shown in Figure 5b (page 81). Elevated serum concentrations of IFN were detectable on the average for 3.5 days less than changes of 2',5'-oligo(A) synthetase. These results support earlier reports that following administration of IFN at 10^6 U/kg body wt or less, changes in 2',5'-oligo(A) synthetase of PBML are more reliable measures of effective concentration of IFN than are changes in serum IFN (Sugino et al, 1986; Schattner et al, 1981a; Merritt et al, 1986). They also confirm that the 2',5'-oligo(A) synthetase produced in response to injecting with IFN persists long after IFN becomes undetectable in the blood (Barouki et al, 1987).

Once induced to high levels 2',5'-oligo(A) synthetase is measurable for several days and for about four times as long as IFN. From the data of Bielefeldt-Ohmann and Babiuk we have calculated a $t_{½}$ for IFN of 8.6±1.0 hours (Bielefeldt-Ohmann and Babiuk, 1986c), somewhat shorter than our reported value. Differences for $t_{½}$ of IFN may be related to the time period of measurement--24 to 96 hours post-injection in our work and 1 to 24 hours post-injection in the work of Bielefeldt-Ohmann and Babiuk. It is possible that during the first 24 hours after injection the apparent rate constant for removal is affected by distribution within the body or sequestration. The reported $t_{½}$ in various species of animals (rabbit, dog monkey and man) is 3.5-5.7 hours (Gibson et al, 1985; Satoh et al, 1984; Wills et al,1984a; Wills et al, 1984b). The difference between the

$t_{½}$ of IFN-α in cattle and in other species examined is probably related to size and metabolic rate.

Clarification of the effect of IFN on 2',5'-oligo(A) synthetase activity of cells of cattle is important for several reasons. If IFN is to be used in prophylaxis or therapy of bovine diseases, it is important to have information on the dose response relationship for IFN. Activity of 2',5'-oligo(A) synthetase in PBML provides a useful means of assessment of pharmacological response to IFN in cattle as it does in humans (Merritt et al, 1986; Schattner et al, 1981a). The duration of elevated 2',5'-oligo(A) synthetase as a function of dose of IFN is likely as important as the peak response. Data presented herein on the time course of elevated 2',5'-oligo(A) synthetase of PBML and corresponding AUC data should provide useful information on the pharmacological response of an animal to a single dose of IFN. Such information should be helpful in establishing when to administer IFN for prophylactic purposes and in determining frequency of repeated administration. Although we did not examine PBML for antiviral activity during period of elevated 2',5'-oligo(A) synthetase activity, Barouki et al. have clearly demonstrated that increased antiviral activity parallels increased 2',5'-oligo(A) synthetase activity (Barouki et al, 1987).

In many cases conditions under which cattle are liable to develop respiratory disease syndrome are predictable. Under such circumstances prophylactic protocols would be

beneficial in ameliorating or preventing disease. Use of IFN as a prophylactic agent is currently being investigated. Information on the dose response relationship of IFN is essential to evaluation of its the prophylactic value. In this work, using 2',5'-oligo(A) synthetase in PBML as an indicator, we have reported the pharmacological response of cattle to a wide range of doses of IFN.

In addition to its potential value in assessing prophylactic and therapeutic responses to IFN, 2',5'-oligo(A) synthetase could be of diagnostic value. Although the 2',5'-oligo(A) system is known to be stimulated by bacteria, some chemical substances and autoimmune diseases, the response to viruses is generally greater than to other agents (Torrence et al, 1981; Sugino et al, 1986; Buffet-Janvresse and Hovanessian, 1984; Chousterman et al, 1983; Schattner et al, 1981b). Once produced 2',5'-oligo(A) synthetase persists at elevated levels in PBML for longer than does the stimulating IFN. Its level can be measured in hours whereas virus isolation takes a week or more; therefore, assays of 2',5'-oligo(A) synthetase levels could provide information to support or oppose a diagnosis of a viral disease and do so more rapidly and reliably than IFN and more rapidly than virus isolation. Use of 2',5'-oligo(A) synthetase activity in evaluating autoimmune and infectious disease states is also feasible in cattle, as it is in humans (Schattner et al, 1981a; Schattner et al, 1981b) but viral diseases are

generally of greater economic concern than autoimmune disorders in cattle.

TABLE 1

LATIN SQUARE DESIGN FOR IFN DOSAGES

	Treatment Week				
Animal	1	2	3	4	5
11	B[a]	A	C	D	E
09	D	B	A	E	C
32	E	C	B	A	D
19	A	E	D	C	B
30	C	D	E	B	A

[a] dosages in U IFN/kg body weight: $E = 10^4$, $A = 10^5$, $D = 5 \times 10^5$, $B = 10^6$ $C = 10^7$.

TABLE 2

SERUM ANTIBODY VIRAL NEUTRALIZATION TITERS ON THE FIRST DAY OF INJECTION OF IFN (DAY 0) AND SEVEN DAYS AFTER THE LAST INJECTION (DAY 63)

	Virus / Day							
	IBR		BVDV		PI-3V		RSV	
Animal	0	63	0	63	0	63	0	63
CONTROL	16[a]	16	64	64	512	256	16	16
1	<4	<4	128	64	256	256	32	32
2	32	32	32	64	256	128	32	32
3	<4	<4	<4	<4	64	64	16	16
4	8	8	32	32	32	64	8	8
5	16	16	64	16	128	128	32	32

[a] The lowest dilution tested was 1:4.

Figure 1. Frequency Distribution of Levels of 2',5'-oligo(A) Synthetase Activity Values in Calves During the Baseline Period.

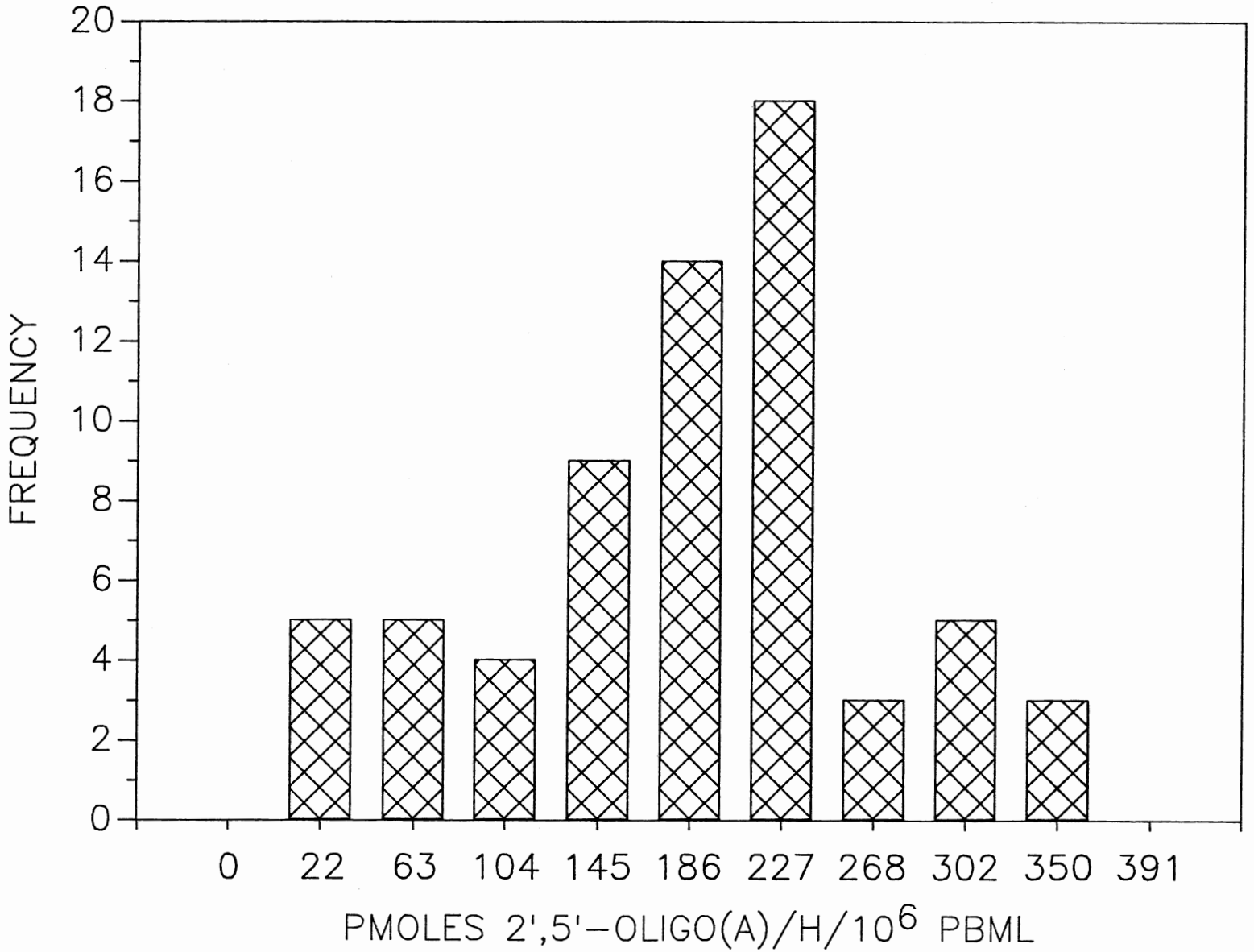

20
18
16
14
12
10
8
6
4
2
0
FREQUENCY
0
22
63
104
145
186
227
268
302
350
391
PMOLES 2',5'-OLIGO(A)/H/10^6 PBML

Figure 2a. Time Course of 2',5'-oligo(A) Synthetase Response in PBML Isolated from Calves Treated with Varying Doses of IFN (10^4 U/kg, ▼ ; 10^5 U/kg, ■ ; 5 x 10^5 U/kg, + ; 10^6 U/kg, • ; 10^7 U/kg, ▲).

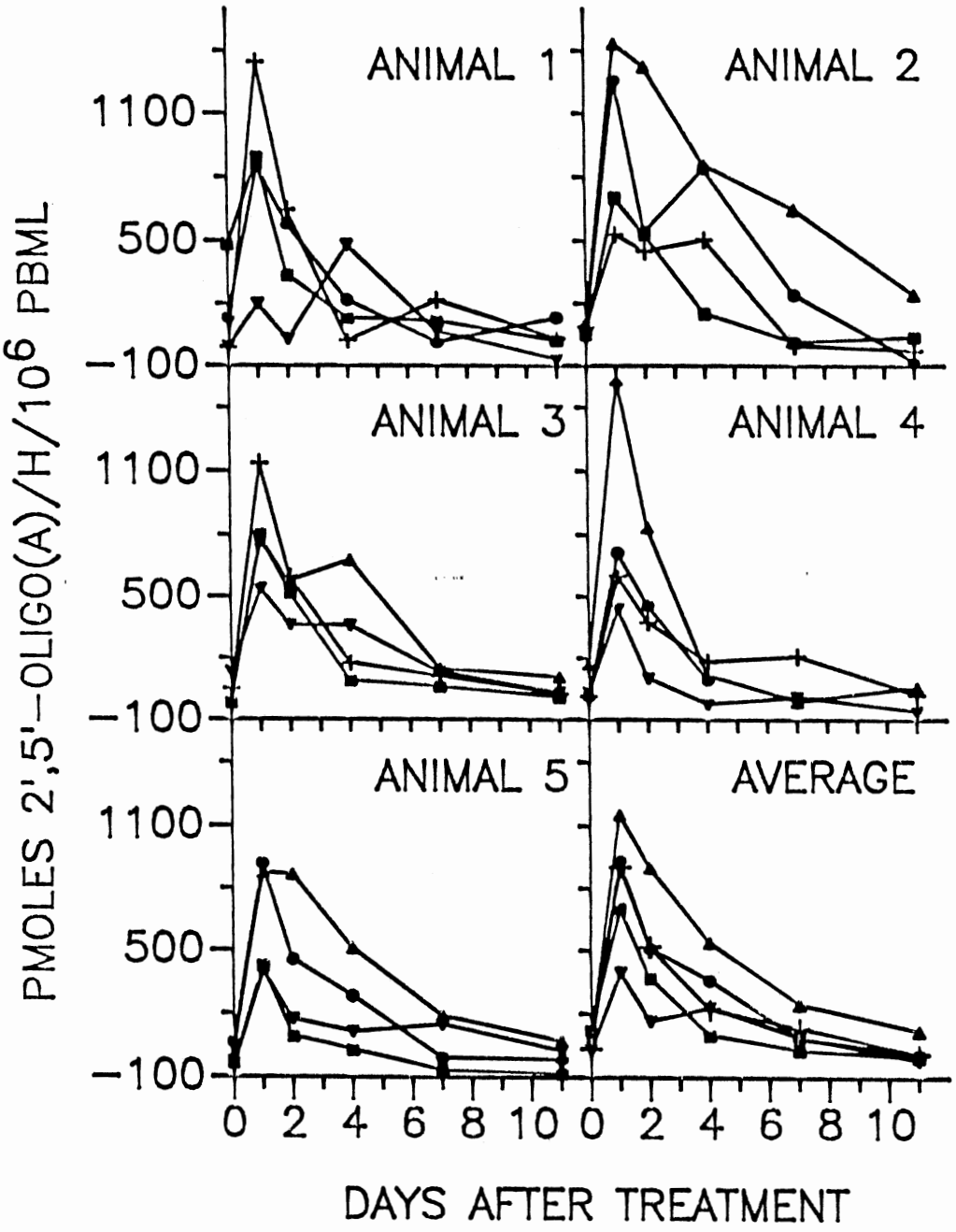

ANIMAL 1
ANIMAL 2
ANIMAL 3
ANIMAL 4
ANIMAL 5
AVERAGE
1100
500
−100
0 2 4 6 8 10
PMOLES 2',5'−OLIGO(A)/H/10^6 PBML
DAYS AFTER TREATMENT

Figure 2b. Time Course of Serum IFN Response in Calves Treated with Varying Doses of IFN (10^4 U/kg, ▼ ; 10^5 U/kg, ■ ; 5 x 10^5 U/kg, + ; 10^6 U/kg, • ; 10^7 U/kg, ▲).

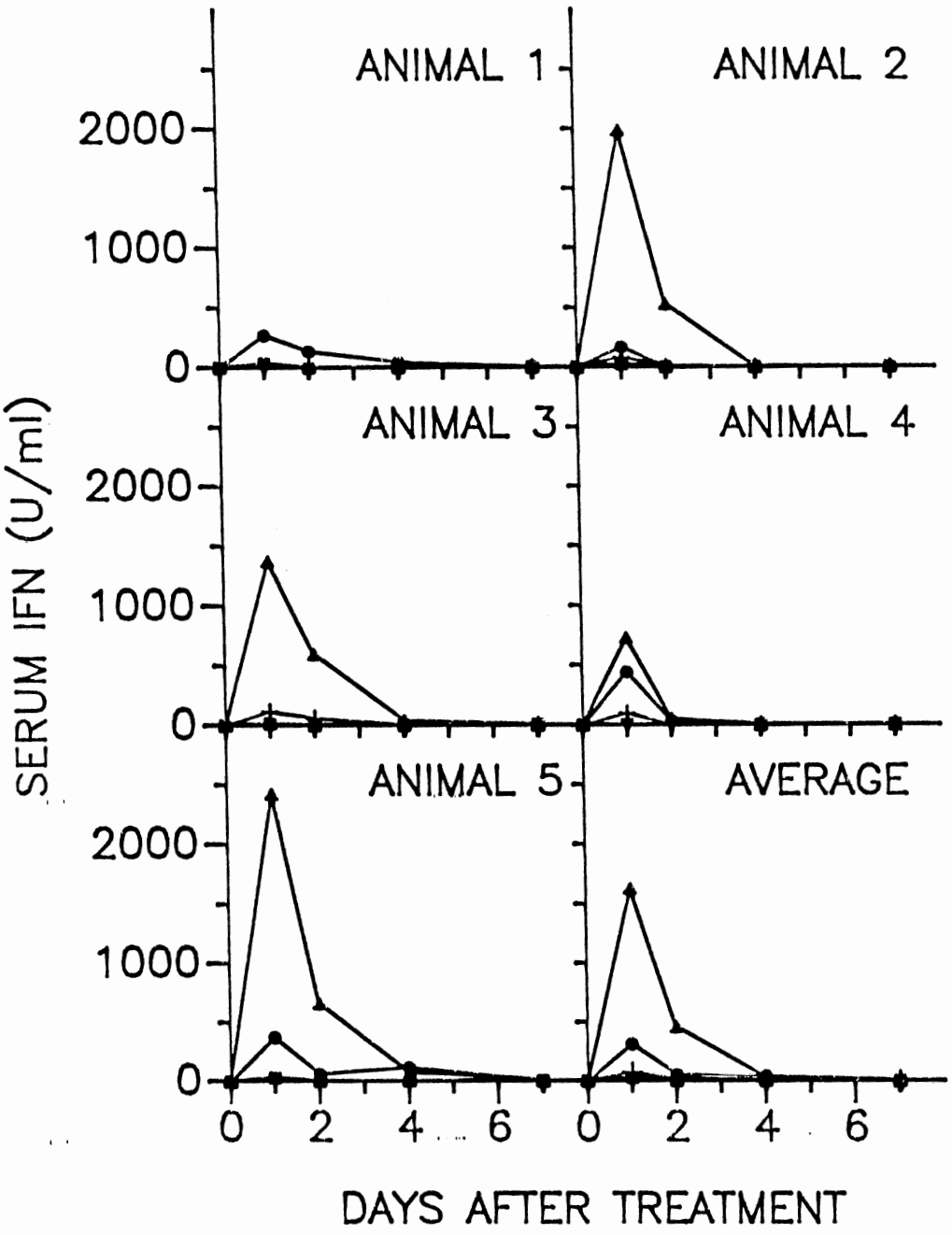

ANIMAL 1
ANIMAL 2
ANIMAL 3
ANIMAL 4
ANIMAL 5
AVERAGE
2000
1000
0
SERUM IFN (U/ml)
0 2 4 6 0 2 4 6
DAYS AFTER TREATMENT

Figure 3. Mean Number of Days Positive for 2',5'-oligo(A) Synthetase (>350 pmoles/h/10^6 PBML, ▨) and Serum IFN (>50 U/ml, ▨) for Each IFN Dose.

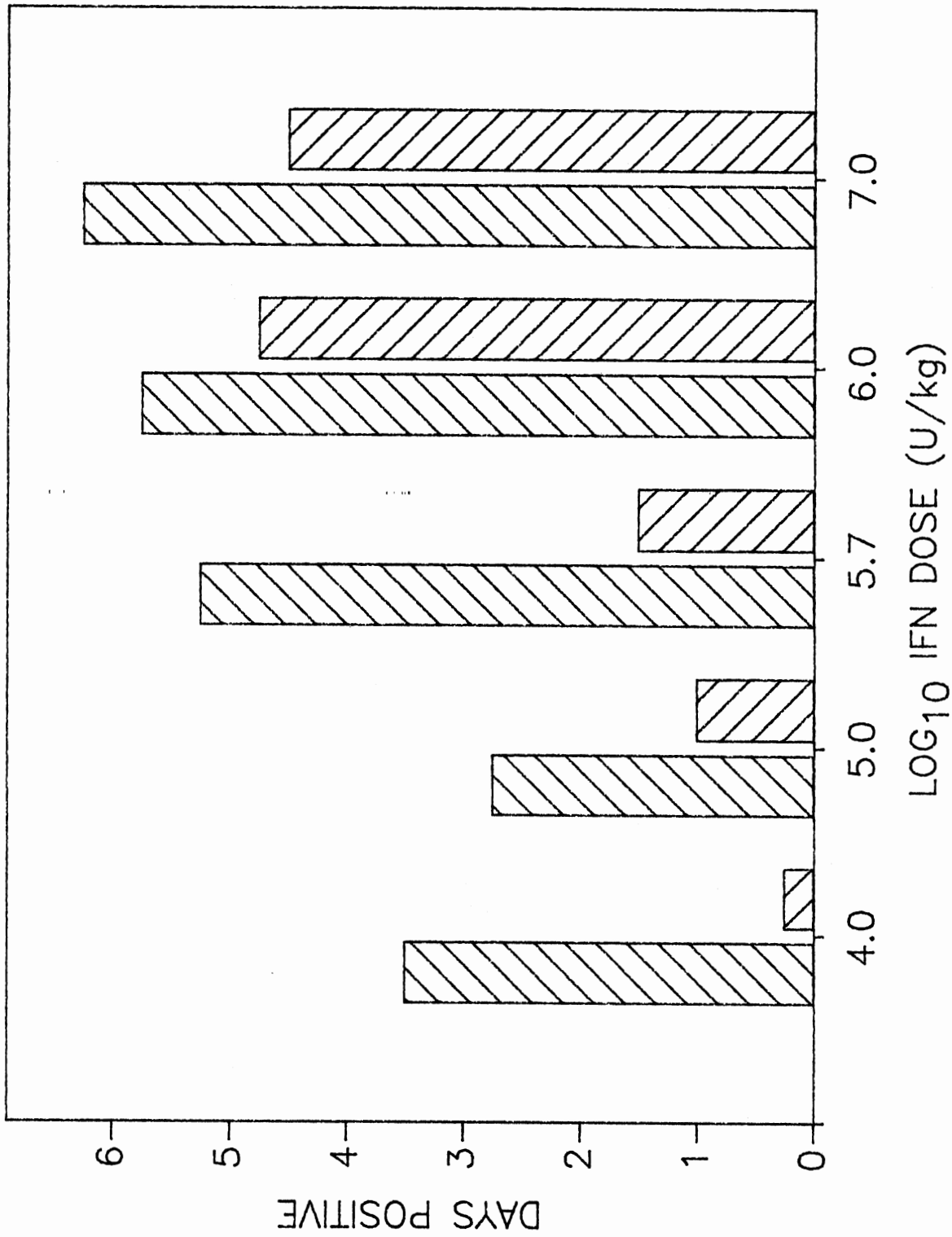

DAYS POSITIVE
0
1
2
3
4
5
6
4.0
5.0
5.7
6.0
7.0
LOG10 IFN DOSE (U/kg)

Figure 4a. AUC Values for 2',5'-oligo(A) Synthesized per Hour by Enzyme from 10^6 PBML for Varying Doses of IFN.

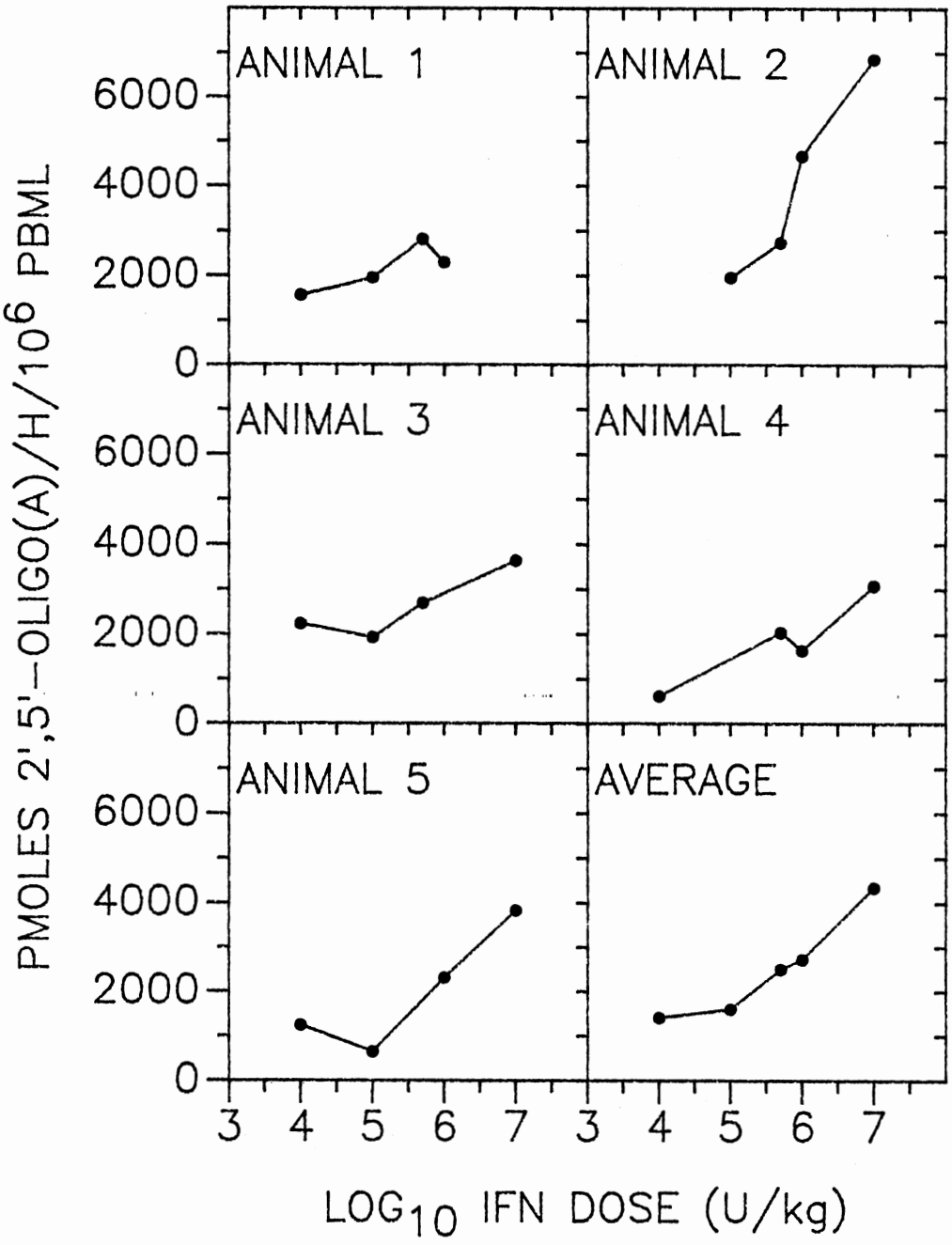

ANIMAL 1
ANIMAL 2
ANIMAL 3
ANIMAL 4
ANIMAL 5
AVERAGE
6000
4000
2000
0
3
4
5
6
7
PMOLES 2',5'-OLIGO(A)/H/10^6 PBML
LOG10 IFN DOSE (U/kg)

Figure 4b. AUC Values for Serum IFN for Varying Doses of IFN.

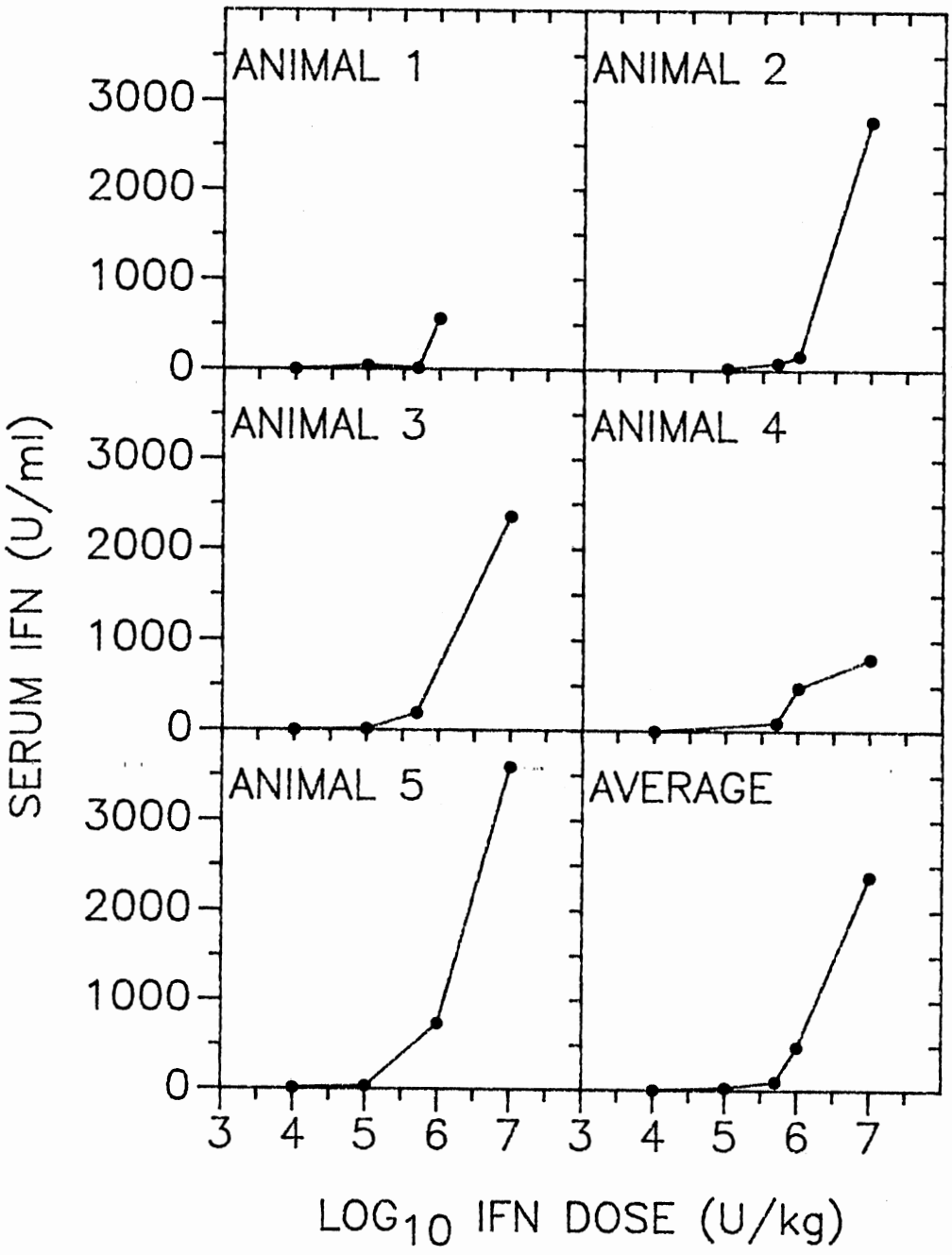

ANIMAL 1
ANIMAL 2
ANIMAL 3
ANIMAL 4
ANIMAL 5
AVERAGE
SERUM IFN (U/ml)
3000
2000
1000
0
3
4
5
6
7
LOG10 IFN DOSE (U/kg)

Figure 5a. Peak Values for 2',5'-oligo(A) Synthesized per Hour by Enzyme from 10^6 PBML for Varying Doses of IFN.

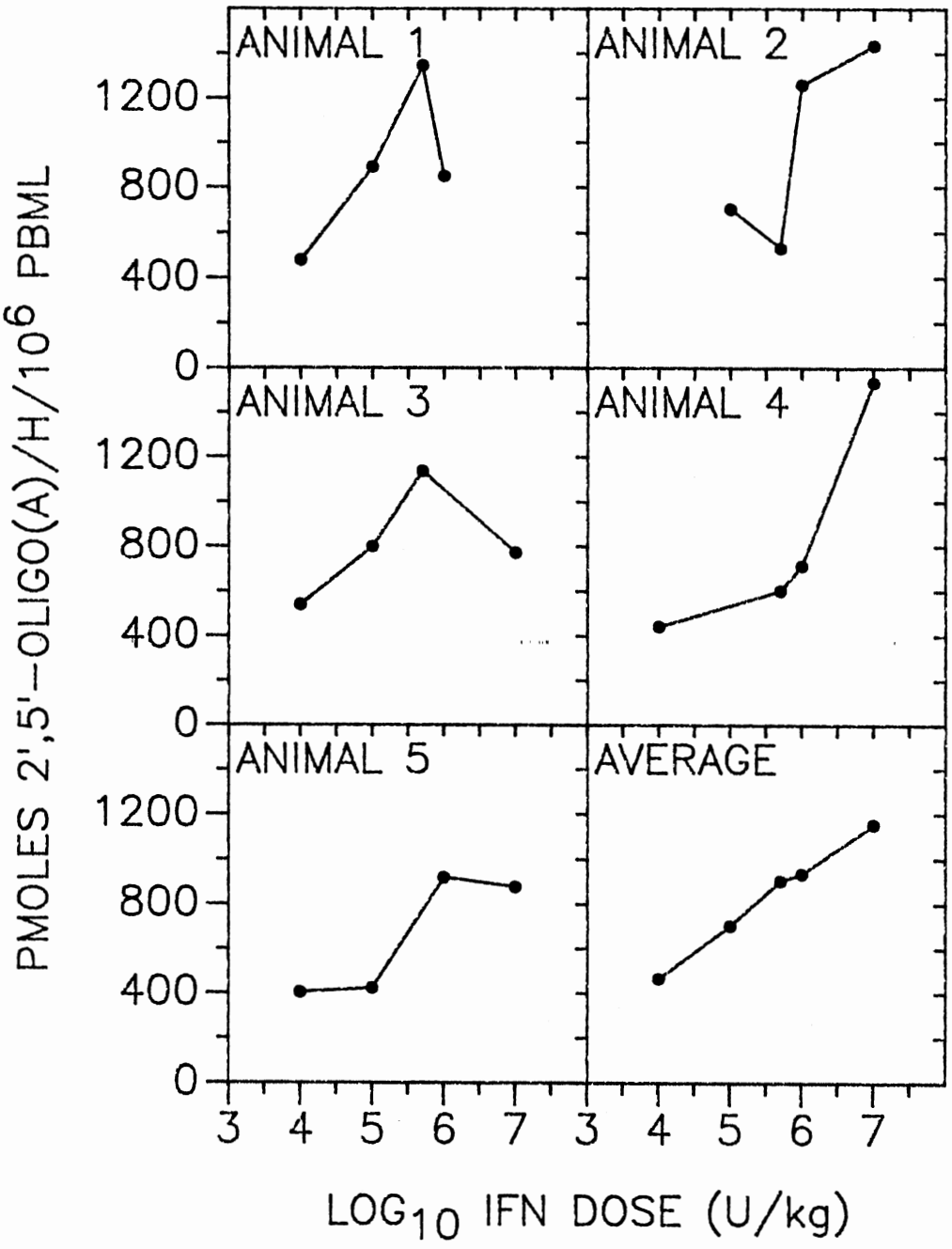
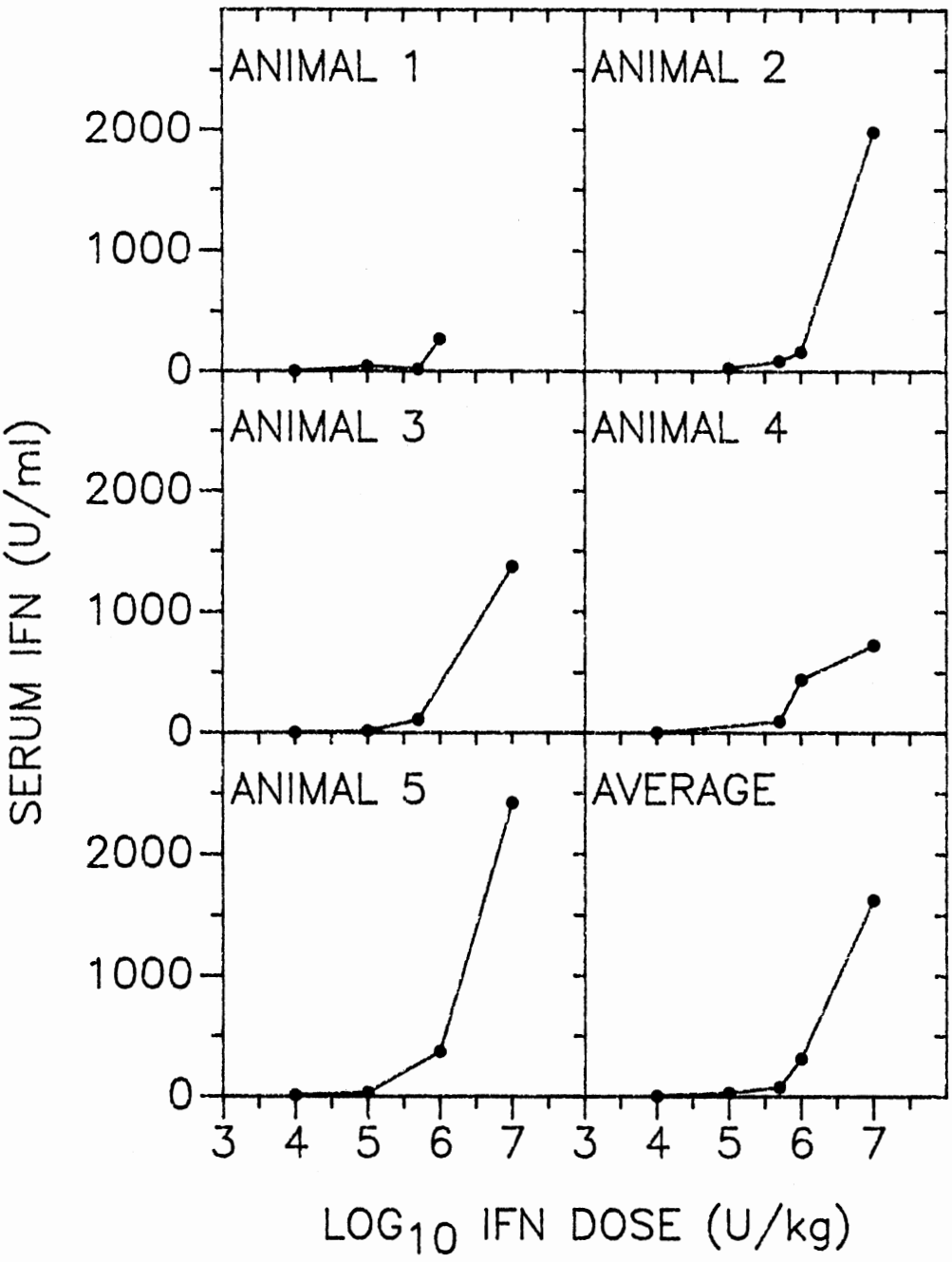

ANIMAL 1
ANIMAL 2
ANIMAL 3
ANIMAL 4
ANIMAL 5
AVERAGE
1200
800
400
0
3
4
5
6
7
PMOLES 2',5'-OLIGO(A)/H/10^6 PBML
LOG10 IFN DOSE (U/kg)

Figure 5b. Peak Serum IFN Values for Varying Doses of IFN.


ANIMAL 1
ANIMAL 2
ANIMAL 3
ANIMAL 4
ANIMAL 5
AVERAGE
2000
1000
0
3
4
5
6
7
SERUM IFN (U/ml)
LOG10 IFN DOSE (U/kg)

CHAPTER IV

SERUM INTERFERON LEVELS AND 2',5'-OLIGO(A) SYNTHETASE ACTIVITY IN PERIPHERAL BLOOD MONONUCLEAR LYMPHOCYTES IN CATTLE AFTER ADMINISTRATION OF RECOMBINANT BOVINE INTERFERON-$\alpha_1$1 AND/OR MODIFIED LIVE VIRUS VACCINE

Summary

In two separate trials, healthy bovine herpesvirus-1 (BHV-1) naive calves were assigned to one of four treatment groups: untreated control, interferon (IFN) only, vaccine-only (modified live BHV-1/PI-3 virus), or concurrent IFN and vaccine. Two weeks later treatments were repeated and calves were monitored for two additional weeks. Both the control group and the group that received only vaccine had undetectable or insignificant levels of serum IFN during the entire 28-day trial period. Administration of IFN or IFN and vaccine resulted in increased concentrations of serum IFN 24 hours after administration following both doses. The high concentrations of serum IFN disappeared rapidly and returned to threshold values two to three days after administration. There was no discernible difference in serum IFN

levels between the IFN-treated group and the group treated concurrently with IFN and vaccine. Following both IFN injections, the calves treated with IFN had large increases in enzyme activity 24 hours after treatment. These responses abated over the subsequent 4 days. In the vaccine-treatment group the level of enzyme activity increased 4 days after the first dose and returned to baseline by day 10, but there was no increase after the second dose of vaccine. The kinetics of enzyme activity in the group of calves that received both IFN and vaccine behaved in a fashion that was a hybrid of the IFN and the vaccine-treated groups.

Introduction

Interferons (IFNs) are cellular proteins produced in response to viral infection or stimuli (Torrence and DeClercq, 1981). In addition to their role in defense against viral infection, they inhibit viral replication, inhibit cellular division, and modulate immune responses (Stewart, 1979). IFNs-α, β and γ induce the intracellular enzyme 2',5'-oligoadenylate (2',5'-oligo(A)) synthetase. This enzyme catalyzes synthesis of 2',5'-oligo(A) which activates a latent endoribonuclease. The RNase digests single-stranded RNA thereby inhibiting protein synthesis (Floyd-Smith et al, 1981).

2',5'-oligo(A) synthetase is present in extracts of various cells, including peripheral blood mononuclear leuko-

cytes (PBML). In many cases its level of activity can be raised by exposure to IFN (Sugino et al, 1986; Baglioni et al, 1979; Ball, 1979; Kimchi et al, 1979). Compared to serum IFN, 2',5'-oligo(A) synthetase in PBML appears earlier after viral infection (Schattner et al, 1981a), reaches more readily measurable levels sooner and more reliably after viral infection (Schattner et al, 1981a), and persists at elevated levels longer (Schattner et al, 1981a; Barouki et al, 1987; Lodemann et al, 1984; Merritt et al, 1986). For these reasons, 2',5'-oligo(A) synthetase has been used as an index of biologic responsiveness of cells to and a marker for IFN (Barouki et al, 1987; Merritt et al, 1986; Buffet-Janvresse and Hovanessian, 1984; Chousterman et al, 1983; Merlin et al, 1981; Schattner et al, 1982) as well as to evaluate clinical disease (Read et al, 1985; Furuta et al, 1987; Fujii et al, 1987; Ferbus et al, 1988; Kennedy and Tilles, 1988). Increases in 2',5'-oligo(A) synthetase in bovine PBML have been associated with IFN treatment, modified live viral vaccine administration, and viral infection (Vanden Broecke et al, 1985; Short and Fulton, 1987; Holland, 1988; Griebel et al, 1989; Bielefeldt-Ohmann et al, 1989).

Bovine herpesvirus-1 (BHV-1) is a clinically important pathogen of the bovine upper respiratory tract. It compromises several specific and nonspecific pulmonary defenses, creating the opportunity for secondary bacterial infection (Rossi and Kiesel, 1977; Forman and Babiuk, 1982; McGuire

and Babiuk, 1983; Bielefeldt-Ohmann and Babiuk, 1985b; Bielefeldt-Ohmann and Babiuk, 1986a; Briggs et al, 1988). Vaccines have been developed in an attempt to prevent or reduce the severity of BHV-1 infection. Both intranasal and intramuscular BHV-1 vaccines appear efficacious in experimental challenge systems and induce circulating BHV-1 specific antibody (McKercher and Crenshaw, 1971; Frank et al, 1977; Gerber et al, 1978).

Like other herpesviruses, BHV-1 is relatively less sensitive than other viruses to the antiviral effects of IFN (Fulton et al, 1984; Gillespie et al, 1985; Fulton et al, 1986; Czarniecki et al, 1986; Griebel et al, 1989; Bielefeldt-Ohmann and Babiuk, 1988). BHV-1 challenge induces IFN and 2',5'-oligo(A) synthetase in cattle (Vanden Broecke et al, 1985; Bielefeldt-Ohmann et al, 1989), and IFN reduces the severity of experimentally induced viral diseases, including respiratory disease involving BHV-1 infection (Babiuk et al, 1985; Roney et al, 1985; Gillespie et al, 1986; Babiuk et al, 1987).

There is limited information on the effects of an antiviral and immunomodulatory protein like IFN on the response to a modified live virus (MLV) vaccine. The purpose of these experiments was to study the effects and interactions of IFN and a MLV BHV-1 vaccine on the enzyme 2',5'-oligo(A) synthetase.

Materials and Methods

Experimental design--This experiment was conducted in two separate trials in the summer (trial A) and in the fall (trial B). Trial A was conducted using 7 male and 7 female calves that weighed an average 226 kg, and trial B was conducted using 14 male calves that weighed an average 210 kg. Prior to weaning, calves were screened for antibodies to BHV-1 to verify that they were seronegative. Calves were transported 230 km to the research facility and allowed at least two weeks to acclimate to their new surroundings. Each calf was randomly allocated to one of four treatment groups and treated accordingly (day 0): control (no injections), IFN only, vaccine-only, or concurrent IFN and vaccine. Two weeks after the initial treatment (day 14), calves received another of their respective treatments and were monitored for an additional two weeks. Calves were maintained on grain supplement and free-choice hay.

Vaccine--A commercial modified live BHV-1/Parainfluenza-3 virus (PI-3V) vaccine was used according to the manufacturer's directions (Resbo serial 210A, Norden Laboratories, Lincoln, NE).

Interferon--The IFN used was a recombinant bovine IFN-$\alpha_I 1$, synthesized in Escherichia coli, obtained from CIBA-GEIGY Corp., Greensboro, NC. Five mg per calf (approximately 3.8×10^6 IFN units/kg) were injected intramuscularly on days 0 and 14.

Assay of 2',5'-oligo(A) synthetase--Assays were conducted as previously described by Short and Fulton, with minor modifications (Short and Fulton, 1987). Briefly, PBML were collected from 8 ml of heparinized blood by adding an equal volume of Sepracell MN (Septratech Corp, Oklahoma City, OK) and centrifugation at 2000 x g for 20 min. Cells collected were washed twice with 8 ml portions of PBS and suspended in 3 ml PBS. Two 10 μl samples of cells were counted on a hemocytometer. If the counts differed from each other by less than two times the square root of the mean of the counts, the mean was taken as the cell count. If the counts differed from each other by more than two times the square root of the mean of the counts, additional paired counts were done until this criterion was met. Then either i) all counts were averaged or ii) one count was rejected before the remaining counts were averaged. One count was rejected if its deviation from the mean of the counts was more than four times the average deviation of the remaining counts. After counting, PBML were adjusted to a final concentration of 5 x 10^6 cells/ml and 1 ml of this solution was placed in a microfuge tube. PBML were sedimented at 1500 x g for 1 min and the supernatant solution was removed. Sufficient lysis buffer was added to the cell pellet to give a concentration of 1 x 10^8 cells/ml. The resulting cell extract was stored at -20°C. The cell extracts were thawed for 30 min at 4°C, cell debris was removed by centrifugation at 12,000 x g for 6 min., and 5 μl

(5 x 10^5 PBM equivalent) of cell extract were removed for assay. Using [8-^{14}C] labeled ATP and scintillation spectroscopy, levels of 2',5'-oligo(A) synthetase activity were determined and expressed as pmoles 2',5'-oligo(A)/h/10^6 PBML. The 2',5'-oligo(A) synthetase reaction was allowed to proceed for 4 hours in trial A and 18 hours in trial B.

Measurement of serum IFN--IFN assays were performed by a plaque reduction method in 24-well tissue culture plates containing confluent Madin-Darby bovine kidney (MDBK) monolayers with vesicular stomatitis virus (VSV) as the challenge virus (Fulton and Pearson, 1980; Fulton et al, 1986). Four-fold dilutions of the initial dilution (1:10) were made. IFN titers (in units per ml) were expressed as the reciprocal of the dilution that reduced the number of VSV plaques by fifty percent (Fulton and Pearson, 1980; Fulton et al, 1986).

Statistical methods--All statistical analyses were performed using SAS (SAS Institute Inc, Cary, North Carolina). One control heifer in trial A that had unexplainable 2 to 4 fold changes in levels of 2',5'-oligo(A) synthetase activity during the acclimation and experimental periods was excluded from the analysis.

Results

Figure 6 (page 96) shows the concentration of serum IFN in the various treatment groups. Both the control group and the group that received only vaccine had undetectable or

insignificant levels of serum IFN during the entire 28-day trial period. The IFN-treated group and the IFN/vaccine-treated group had significant levels ($P<0.05$) of serum IFN 24 hours after administration, following both IFN doses. These high concentrations of serum IFN disappeared within 24 hours and returned to threshold values between two to three days after administration. There was no discernible difference in serum IFN concentration between the IFN only group and the group that received concurrent IFN and vaccine.

Figures 7a (page 98) and 7b (page 100) display the levels of 2',5'-oligo(A) synthetase activity in the four treatment groups for trials A and B, respectively. The control groups for trials A and B averaged 1207 and 571 pmoles 2',5'-oligo(A)/h/10^6 PBML during the experiment, respectively. For each day of the experiment the absolute value of the enzyme activity for trials A and B were different ($P<0.05$). The difference in absolute magnitude of the 2',5'-oligo(A) synthetase activity is likely due to differences in assay conditions.

However, the relative behavior of enzyme activity was quite similar between the two trials. If the 2',5'-oligo(A) synthetase values are expressed as a percent of baseline value (Figure 8, page 102), the percent changes in enzyme activity are not different between trials ($P<0.10$). Calves treated with IFN only had large increases in enzyme activity 24 hours after each IFN treatment that abated over the sub-

sequent 4 days. In the vaccine-only treatment group, the level of enzyme activity increased 4 days following the first dose and returned to baseline by day 10. No increase was seen following the second dose of vaccine. The level of enzyme activity in the group of calves that received both IFN and vaccine concurrently behaved in a fashion that was a hybrid of the behavior of the IFN and the vaccine treated groups.

After both IFN/vaccine treatments there was a sharp increase in enzyme activity at 24 hours. However, after the first dose of IFN/vaccine, the enzyme levels remained elevated for a longer time than did those in the IFN only group. The magnitude of the response during the period of prolonged elevation was similar to the response seen in the vaccine-only group. This additional time of elevation was not noted following the second dose of IFN/vaccine, nor the the second dose of vaccine.

Discussion

The control groups for trials A and B averaged 1207 and 571 pmoles 2',5'-oligo(A)/h/10^6 PBML over the duration of the experiment, respectively. The 2',5'-oligo(A) synthetase activity in PBML of the control cattle in this study is higher than values previously reported by this laboratory (Perino et al, 1989; Short and Fulton, 1987). The increase may be due to changes in procedures such as counting PBML, transferring oligonucleotide product to chromatography

columns, or to differences in calves. The difference in the magnitude of the 2',5'-oligo(A) synthetase activity between the two trials is likely due to differences in assay conditions; specifically, the duration of reaction time. However, the relative behavior of enzyme activity was quite similar between the two trials. Baseline values reported for humans vary greatly, ranging from 70 to 1860 pmoles/h/10^6 PBML with coefficients of variation ranging from 30 to 236% (Barouki et al, 1987; Buffet-Janvresse and Hovanessian, 1984; Chousterman et al, 1983; Merritt et al, 1985; Schattner et al, 1981a). Results in this study, using the assay developed by Schattner et al. (Schattner et al, 1982) modified to measure incorporation of all nucleoside residues, confirm that 2',5'-oligo(A) synthetase activity in PBML of cattle are comparable to those for man.

In the present study the concentration of serum IFN increased dramatically to 258-397 U/ml 24 hours after injection in both of the IFN-treated groups. The rapid return to baseline levels between 48 and 72 hours after treatment is consistent with the rapidly occurring, transient peak and the short $t_{½}$ of IFN in humans (Bocci, 1984; Wills et al, 1984b; Bornemann et al, 1985) and cattle (Gillespie et al, 1986; Bielefeldt-Ohmann and Babiuk, 1986c; Griebel et al, 1989; Fulton RW, unpublished data).

Changes were not seen in serum IFN levels after vaccination. Immunization of human infants with a live measles vaccine resulted in low levels of serum IFN-α (average of 20

U/ml) 7 days after vaccination (Nakayama et al, 1988). Following subcutaneous inoculation of humans with rubella vaccine virus, serum IFN concentrations increased transiently. However, exposure to measles or mumps virus vaccines did not cause detectable changes in serum IFN levels (Tilles et al, 1987). IFN production occurs in the early stages of viral infection and monitoring IFN has been suggested as a viral diagnostic technique (Skidmore and Jarlow, 1987). Given the attenuated nature of the virus used in this study, the pharmacokinetics of IFN, and the low concentrations of IFN induction reported previously with human viral vaccines, failure to detect IFN induction was not unexpected.

Increased levels of 2',5'-oligo(A) synthetase activity in PBML of cattle treated with exogenous IFN (Vanden Broecke et al, 1985; Short and Fulton, 1987; Perino et al, 1987; Griebel et al, 1989), experimentally infected with virus (Vanden Broecke et al, 1985; Bielefeldt-Ohmann et al, 1989), or inoculated intravenously with a MLV vaccine (Short and Fulton, 1987) have been reported previously. In humans inoculated with rubella, measles, or mumps vaccines, elevations in 2',5'-oligo(A) synthetase were seen even in the absence of detectable levels of serum IFN (Tilles et al, 1987).

In the present study, calves treated with IFN showed increased enzyme activity 24 hours after treatment and over the following 4 days. This increase in 2',5'-oligo(A) synthetase agrees with the observation that administration of

2.5×10^5 U IFN/kg in man caused an elevation of 2',5'-oligo(A) synthetase that persisted for four days, and the pattern of decline of 2',5'-oligo(A) synthetase following induction by IFN is similar to the pattern reported herein (Barouki et al, 1987).

In the vaccine-only treatment group the level of enzyme activity increased 4 days after the first dose and returned to baseline by day 10. This increase was also seen superimposed upon the exogenously induced 2',5'-oligo(A) synthetase increase in the cattle receiving both IFN and vaccine. The kinetics of this response are consistent with changes in enzyme activity reported for calves experimentally challenged with BHV-1, but the magnitude of the response cannot be compared (Bielefeldt-Ohmann et al, 1989). These enzyme behaviors following exposure to virus or exogenous IFN are consistent with the concept that 2',5'-oligo(A) synthetase activity is a more sensitive indicator of IFN activity than are serum IFN concentrations. Compared to serum IFN, 2',5'-oligo(A) synthetase in PBML appears earlier after viral infection (Schattner et al, 1981a), reaches more readily measurable concentrations sooner and more reliably after viral infection (Schattner et al, 1981a), and persists at elevated concentrations longer (Schattner et al, 1981a; Barouki et al, 1987; Lodemann et al, 1984; Merritt et al, 1986).

Neither of the groups receiving vaccine showed increased 2',5'-oligo(A) synthetase activity following

injection of a second dose on day 14. An explanation for this observation is not readily apparent. A lack of replication of the parenterally administered attenuated virus in calves that had moderate to high concentrations of virus neutralizing serum antibodies is a possibility.

In summary, these data suggest that a MLV vaccine can induce 2',5'-oligo(A) synthetase activity in cattle. The enzyme activity in PBML seems a more sensitive indicator of virus infection and IFN effects than serum IFN concentrations. Finally, concurrent administration of IFN with a MLV vaccine does not appear to affect the kinetics of vaccine induction of 2',5'-oligo(A) synthetase.

Figure 6. Time Course of Serum IFN Levels in Calves Treated with IFN, Vaccine, or Both.

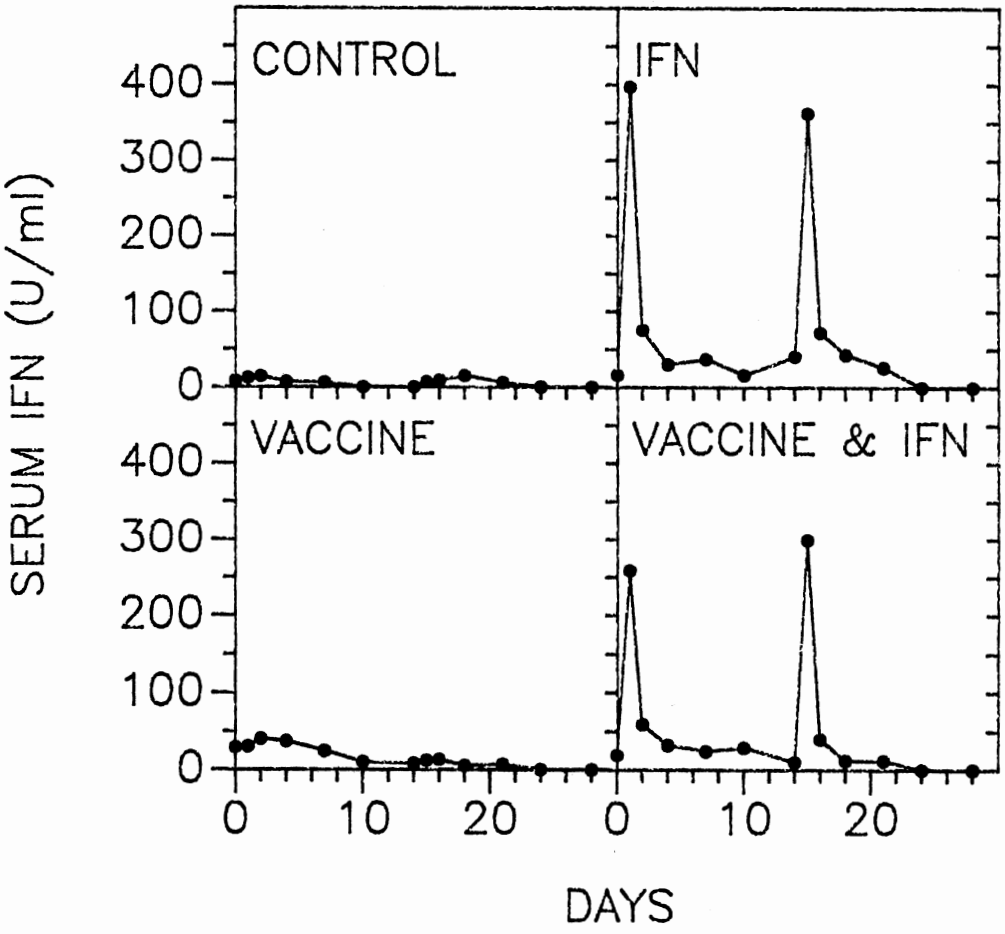

CONTROL
IFN
VACCINE
VACCINE & IFN
SERUM IFN (U/ml)
400
300
200
100
0
0
10
20
DAYS

Figure 7a. Time Course of 2',5'-oligo(A) Synthetase Response in PBML Isolated from Calves Treated with IFN, Vaccine, or Both (Trial A).

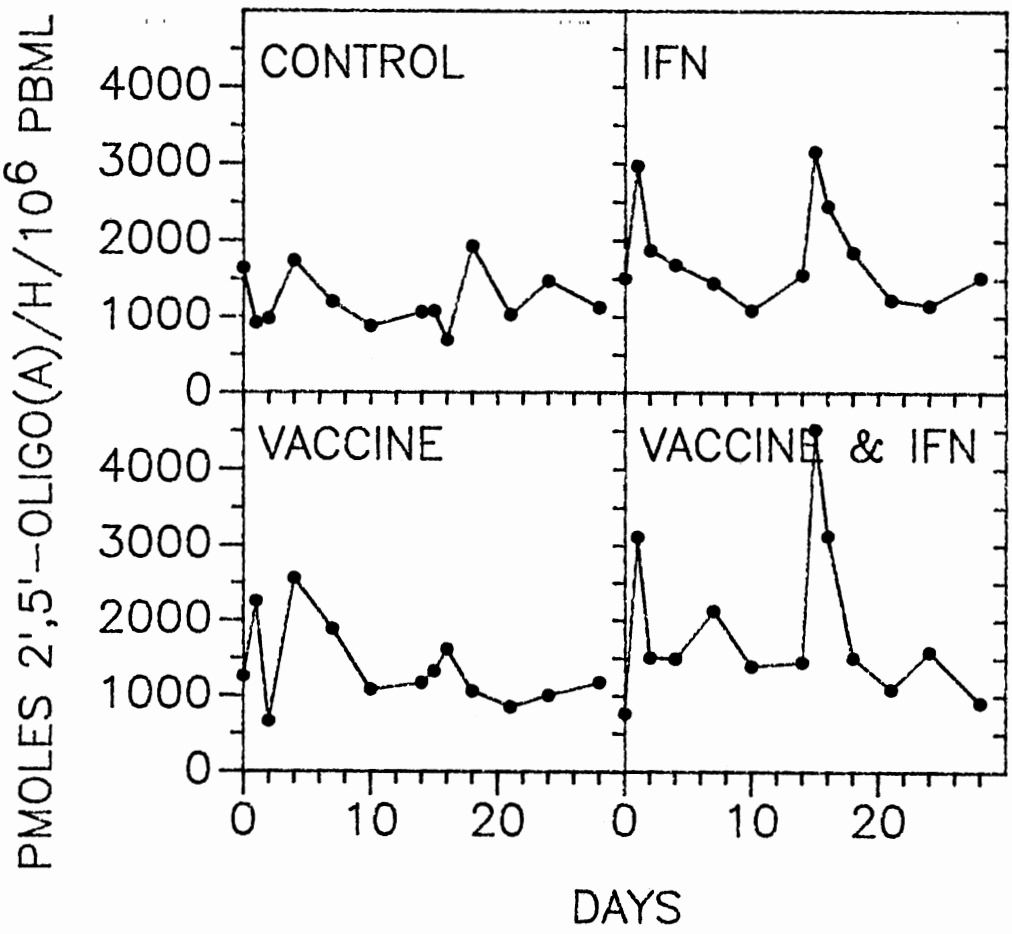

CONTROL
IFN
VACCINE
VACCINE & IFN
PMOLES 2',5'-OLIGO(A)/H/10^6 PBML
4000
3000
2000
1000
0
0
10
20
DAYS

Figure 7b. Time Course of 2',5'-oligo(A) Synthetase Response in PBML Isolated from Calves Treated with IFN, Vaccine, or Both (Trial B).

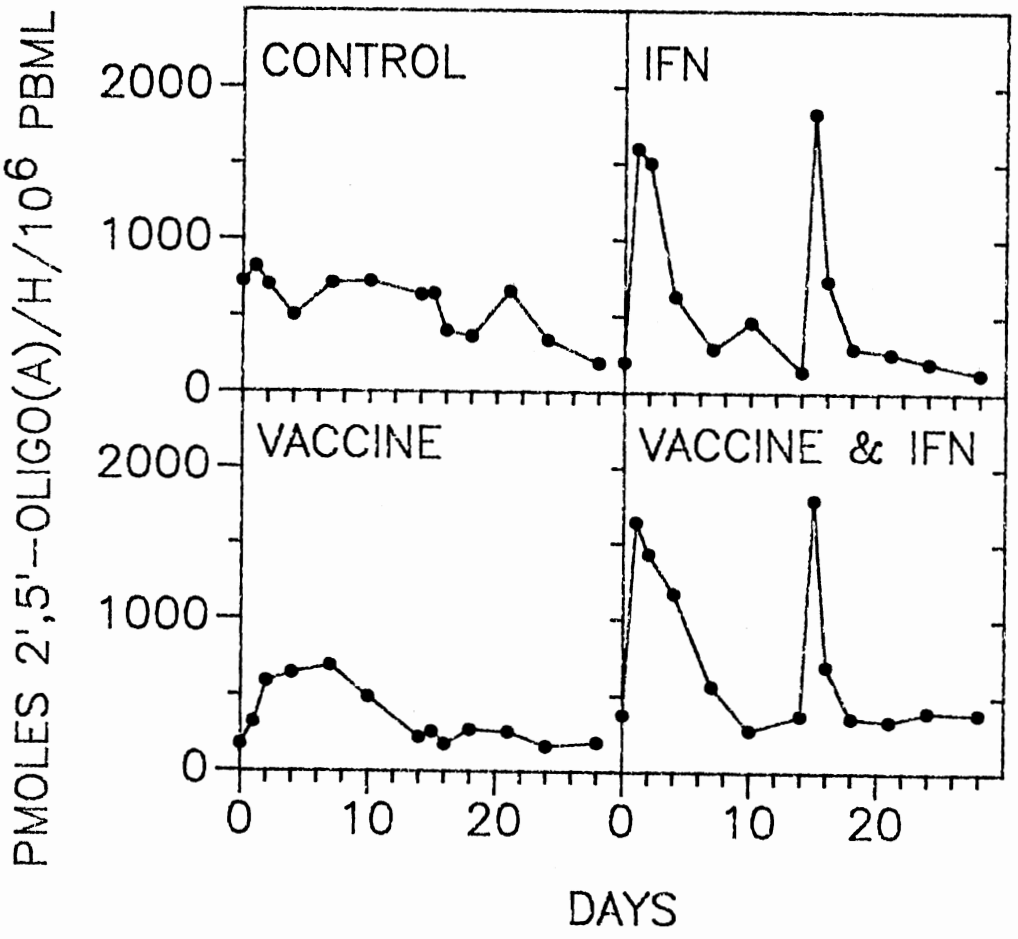
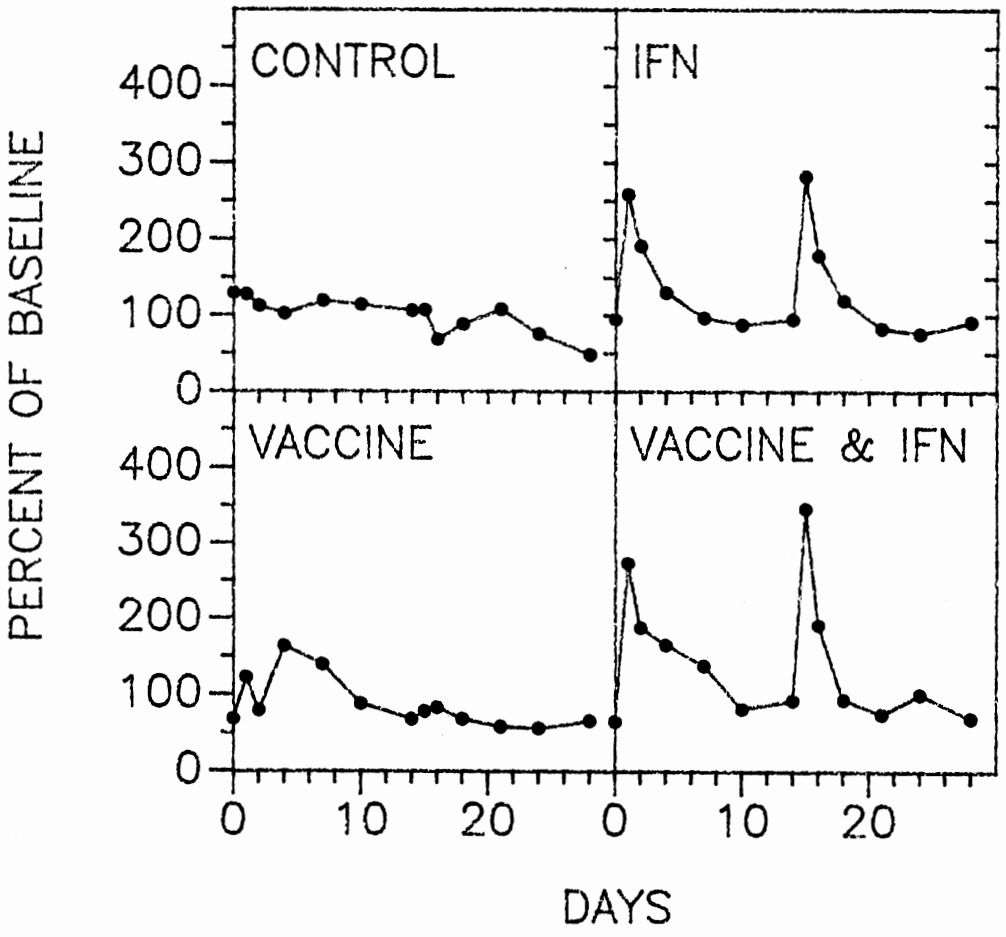

CONTROL
IFN
VACCINE
VACCINE & IFN
2000
1000
0
0
10
20
PMOLES 2',5'--OLIGO(A)/H/10^6 PBML
DAYS

Figure 8. Time Course of 2',5'-oligo(A) Synthetase Response Expressed as Percent Increase over Baseline in PBML Isolated from Calves Treated with IFN, Vaccine, or Both.


CONTROL
IFN
VACCINE
VACCINE & IFN
PERCENT OF BASELINE
400
300
200
100
0
0
10
20
DAYS

CHAPTER V

MODULATION OF THE IMMUNE RESPONSE IN CATTLE TO A MODIFIED LIVE BOVINE HERPESVIRUS-1 VACCINE BY RECOMBINANT BOVINE INTERFERON-αI1

Summary

In two separate trials, healthy bovine herpesvirus-1 (BHV-1) naive calves were assigned to one of four treatment groups: untreated control, interferon (IFN) only (3.8×10^6 U/kg), vaccine-only (MLV IBR/PI-3), or concurrent IFN and vaccine. Two weeks later treatments were repeated and calves were monitored for an additional two weeks. Both the control group and the group that received only IFN had no detectable BHV-1 neutralizing antibodies in their serum during the entire 28-day trial period. However, in both groups injected with vaccine, serum neutralizing antibodies to BHV-1 appeared between day 7-10. There was no difference in the magnitude or time of onset of the antibody response between calves receiving vaccine and those receiving both IFN and vaccine. After the second vaccination calves receiving both IFN and vaccine showed significant increases in BHV-1 antibody titer compared to other groups. Vaccinated

calves also had antigen-specific lymphoproliferative responses beginning between day 4-7. For calves receiving vaccine and those receiving both IFN and vaccine, there was no discernible difference in the magnitude or time of onset of the lymphoproliferative responses after either vaccine dose. Concurrent administration of IFN and a modified live viral vaccine appeared to have no detrimental effect on either the primary humoral or cell-mediated immune response, and may augment the secondary response to a modified live virus vaccine.

Introduction

Interferons (IFNs) are cellular proteins produced in response to viral infection as well as other stimuli (Torrence and DeClercq, 1981). In addition to their role in defense against viral infection, they inhibit viral replication, inhibit cellular division, and modulate immune responses (Stewart, 1979).

The myriad of effects attributed to IFN since its discovery have not lessened the significance of its antiviral activity. IFN production appears early in the course of viral infection, before humoral immunity (Baron et al, 1982); treatment of animals with IFN neutralizing antibodies results in more severe disease (Fauconnier, 1982); and exogenous IFN has been used to treat or prevent viral infections in man (Scott and Tyrrell, 1984). Thus, it is apparent that IFN forms an important first line of defense

against viral infections.

IFN can either enhance or depress the humoral and/or cellular immune response depending on the antigen, type and stage of immune response, as well as the dose, route and type of IFN given. IFN can modulate several immune functions including: MHC antigen expression (Gresser, 1984), lymphocyte proliferation (Bielefeldt-Ohmann and Babiuk, 1986c; Griebel et al, 1989), cytotoxic cell activity (Bielefeldt-Ohmann and Babiuk, 1985a), phagocyte function (Bielefeldt-Ohmann and Babiuk, 1984; Bielefeldt-Ohmann and Babiuk, 1986a; Lawman et al, 1987), lymphocyte trafficking (Hein and Supersaxo, 1988; Griebel et al, 1989), and antibody production (Cummins and Hutcheson, 1986; Playfair and DeSouza, 1987). With respect to the immune response to a viral antigen, IFN has immunomodulatory potential from two sources. First, IFN could have direct effects on the immune system, and second, IFN could exert indirect effects by modulating the amount of viral antigen presented to the immune system through its antiviral or immunomodulatory properties.

Various bovine viruses, predominantly those with respiratory tropisms, are sensitive to different IFNs <u>in vitro</u> (Fulton et al, 1984; Gillespie et al, 1985; Fulton et al, 1986; Czarniecki et al, 1986; Bielefeldt-Ohmann and Babiuk, 1988). Like other herpesviruses, bovine herpesvirus-1 (BHV-1) is relatively less sensitive to the antiviral effects of IFN than other viruses (Fulton et al, 1984; Gillespie et al,

1985; Fulton et al, 1986; Czarniecki et al, 1986; Griebel et al, 1989; Bielefeldt-Ohmann and Babiuk, 1988).

BHV-1 is a clinically important pathogen of the bovine upper respiratory tract. It compromises a variety of the specific and nonspecific pulmonic defenses, creating the opportunity for secondary bacterial infection (Rossi and Kiesel, 1977; Forman and Babiuk, 1982; McGuire and Babiuk, 1983; Bielefeldt-Ohmann and Babiuk, 1985b; Bielefeldt-Ohmann and Babiuk, 1986a; Briggs et al, 1988). Vaccines have been developed in an attempt to prevent or reduce the severity of BHV-1 infection. Both intranasal and intramuscular vaccines appear efficacious in experimental challenge systems and induce virus-specific circulating antibody (McKercher and Crenshaw, 1971; Frank et al, 1977; Gerber et al, 1978).

There is little information on the effects of an antiviral and immunomodulatory protein like IFN on response to a MLV vaccine. The purpose of these experiments was to study the modulatory effects of IFN on the immune response of cattle to a BHV-1/PI-3 MLV vaccine.

Materials and Methods

Experimental design--This experiment was conducted in two separate trials in the summer (trial A) and in the fall (trial B). Trial A was conducted using 7 male and 7 female calves that weighed an average 226 kg, and trial B was conducted using 14 male calves that weighed an average 210 kg. Prior to weaning, calves were screened for antibodies to

BHV-1 to verify that they were seronegative. Calves were transported 230 km to the research facility and allowed at least two weeks to acclimate to their new surroundings. Each calf was randomly allocated to one of four treatment groups and treated accordingly (day 0): control (no injections), IFN only, vaccine-only, or concurrent IFN and vaccine. Two weeks after the initial treatment (day 14), calves received another of their respective treatments and were monitored for an additional two weeks. Calves were maintained on grain supplement and free-choice hay and water.

Vaccine--A commercial modified live BHV-1/parainfluenza-3 virus (PI-3V) vaccine was used according to the manufacturers directions (Resbo serial 210A, Norden Laboratories, Lincoln, NE).

Interferon--The interferon used was a recombinant bovine IFN-$\alpha_I 1$, synthesized in Escherichia coli, obtained from CIBA-GEIGY Corp., Greensboro, NC. Five mg per calf (approximately 3.8×10^6 IFN units/kg) was injected intramuscularly on day 0 and 14.

BHV-1 neutralizing antibody titer--The serum antibody titers were determined by plaque reduction test using Madin-Darby Bovine Kidney (MDBK) cells. Two-fold serial dilutions of serum were mixed with an equal volume (0.25 ml) of BHV-1 virus diluted in medium (Minimal Essential Medium with 2% fetal bovine serum) to contain 25 PFU/ml and incubated at 37°C in a 5% CO_2 atmosphere for 1 hour. Individual wells of

a 24-well plate containing confluent layers of MDBK cells were inoculated with 0.1 ml of the virus-serum mixture, incubated at 37°C for 1 hour, and overlayed with 1% methylcellulose in medium.

Lymphoproliferative assay--PBML were collected from 8 ml of heparinized blood by adding an equal volume of Sepracell MN (Sepratech Corp., Oklahoma City, OK) and centrifugation at 2000 x g for 20 min. Cells collected were washed twice with 8 ml portions of PBS and suspended in 3 ml PBS. Two 10 µl samples of cells were counted on a hemocytometer. If the counts differed from each other by less than two times the square root of the mean of the counts, the mean was taken as the cell count. If the counts differed from each other by more than two times the square root of the mean of the counts, additional paired counts were done until this criterion was met. Then either i) all counts were averaged or ii) one count was rejected before the remaining counts were averaged. One count was rejected if its deviation from the mean of the counts was more than four times the average deviation of the remaining counts. After counting, PBML were adjusted to a final concentration of 5×10^6 cells/ml of RPMI 1640 medium with 10% fetal bovine serum and 50 µg/ml of gentamicin and 100 µl of this solution was placed in a well of a 96-well plate. To four or six wells for each animal were added 100 µl of the following: RPMI 1640 medium with 10% fetal bovine serum and 50 µg/ml of gentamicin or the same medium containing UV-inactivated BHV-

1 equivalent to 5 x 10^7 plaque forming units/ml of BHV-1. The BHV-1 used was Cooper strain that had been grown on MDBK cells, frozen at -70°C, centrifuged to remove cellular debris, and inactivated by ultraviolet irradiation. Using 100 µl of 5 x 10^7 BHV-1 plaque forming units/ml and 5 x 10^6 PBM/ml resulting in a final multiplicity of infection of 10:1 in each well. Following a 5-day culture at 37°C in a 5% CO_2 atmosphere, 20 µl of RPMI 1640 containing 40 µCi/ml ^{3}H-thymidine was added to each well. Plates were cultured for an additional 18 hours and then frozen at -20°C. After thawing the plate, each well was harvested and lymphoproliferation assessed by determining the ^{3}H-thymidine uptake using liquid scintillation spectroscopy. The relative amount of lymphoproliferation expressed as a stimulation index which was determined by dividing the average counts per minute in the BHV-1 stimulated wells by the average counts per minute in the medium control wells.

Statistical methods--All statistical analyses were performed using SAS (SAS Institute Inc, Cary, North Carolina). One control heifer in trial A that had unexplainable 2 to 4 fold changes in levels of 2',5'-oligo(A) synthetase activity during the acclimation and experimental periods was excluded from the analysis.

Results

Serum levels of BHV-1 neutralizing antibody are shown in Figure 9 (page 118). Both the control group and the

group that received only IFN had no detectable BHV-1 neutralizing antibodies in their serum IFN during the entire 28-day trial period. However, in both groups injected with vaccine, serum neutralizing antibodies to BHV-1 appeared between day 7-10. There was no difference in the magnitude or time of onset of the antibody response between calves receiving vaccine and those receiving both IFN and vaccine. After administration of a second dose of vaccine on day 14, only calves receiving both IFN and vaccine had significant increases in BHV-1 antibody titer ($P<0.05$).

The proliferative responses of PBML to UV-inactivated BHV-1 are shown in Figure 10 (page 120). The calves in the control and IFN-only treatment groups had no positive responses. The calves treated with vaccine or IFN and vaccine had antigen-specific lymphoproliferative responses beginning between days 4-7. There was no discernible difference in the magnitude or time of onset of the lymphoproliferative responses following either dose between calves receiving vaccine and those receiving both IFN and vaccine.

Discussion

In cattle, there have been studies on the effects of IFN in viral challenge systems (Babiuk et al, 1985; Roney et al, 1985; Cummins and Hutcheson, 1986; Gillespie et al, 1986;Bielefeldt-Ohmann and Babiuk, 1986c; Bielefeldt-Ohmann and Babiuk, 1985a); however, there have been few published studies on the effect of IFN on the response to a viral

vaccine (Cummins and Hutcheson, 1986) or on the *in vitro* or *in vivo* effects of IFN on bovine B lymphocytes. A study was done using BHV-1 seronegative calves treated intranasally or intramuscularly, daily for one week with 50 x 10^6 U of human recombinant leukocyte IFN-A and challenged intranasally with 3 x 10^6 or 10^4 $TCID_{50}$ of the Cooper strain of BHV-1 on the first day of IFN treatment. Serum antibody titers to BHV-1 were slower to increase and were not maintained as long as those of control calves (Roney et al, 1985). The authors attributed this delay to the inhibition of viral infection in the IFN-treated group.

In another study, BHV-1 seronegative calves were treated orally for three days with a placebo or with 0.05, 0.5 or 5 IU/lb body weight of Sendai virus-induced human leukocyte IFN (Cummins and Hutcheson, 1986). On the second day of treatment calves were inoculated with 10^3 $TCID_{50}$ of BHV-1 in each nostril. The geometric mean serum titer to BHV-1 of the IFN treatment groups was two to four times greater than the control group at 14 days postinfection. However, by 25 days after inoculation all calves had seroconverted and there was no difference between treatment and control groups. The geometric mean titers of plaque-forming units of nasally excreted BHV-1 virus was greater in the IFN-treated groups on days 3, 7 and 10 and in the control group on day 14 after inoculation. In the same report, calves concurrently given an intramuscular BHV-1/PI-3V/bovine virus diarrhea virus modified-live virus

vaccine and 1 U/lb body weight of Sendai virus-induced human leukocyte IFN orally had enhanced seroconversion to PI-3V. The authors concluded that IFN treatment stimulated antibody development and reduced nasal shedding of BHV-1 virus at 14 days postinoculation.

In a study of calves inoculated with $10^{6.5}$ $TCID_{50}$ of the Holmes strain of BVDV and treated with six daily intramuscular doses of 10^4 or 10^3 U/kg body weight of recombinant bovine IFN-$\alpha_1$1 commencing with the day of infection, neutralizing antibodies to BVDV appeared in the serum at the same time as in the virus controls. Both treated and control calves were completely protected when challenged with BVDV 40 days after the first inoculation of BVDV. The authors concluded that IFN treatment did not interfere with the immune response to the virus infection (Gillespie et al, 1986a).

In immunocompetent mice inoculated with 10 LD_{50} of street rabies virus, treatment with 10^5 U of IFN-α resulted in a significant increase in production of immunoglobulin against rabies virus despite a 10-fold reduction in rabies virus production in the brain (Marcovistz et al, 1987). In the same experiment, mice immunosuppressed by cyclophosphamide treatment had almost undetectable levels of anti-rabies immunoglobulin, while similarly treated mice that received IFN-α still produced a significant amount of immunoglobulin against rabies virus (Marcovistz et al, 1987).

In the present study, serum neutralizing antibodies to

BHV-1 appeared between days 7-10 in both groups injected with vaccine. There was no difference in the magnitude or time of onset of the antibody response between calves receiving vaccine and those receiving both IFN and vaccine, indicating that IFN treatment had no detrimental effect on antibody production. However, differences were noted following administration of a second dose of vaccine. Only calves receiving both IFN and vaccine had significant increases in BHV-1 antibody titer.

The reason for this is not known. Possibly a lack of replication of the parenterally administered attenuated virus in calves that had moderate to high levels of virus neutralizing serum antibodies may have resulted in a minimal secondary response. Also, insufficient time may have elapsed between administration of doses of vaccine. The mechanism for the short-lived, but marked increase in virus neutralizing serum antibodies following the second dose of vaccine and IFN is also open to speculation. IFN is known to have a variety of immunomodulatory effects, including augmenting MHC antigen expression (Nagi and Babiuk, 1988) and altering T-helper and T-suppressor/cytotoxic cell ratios (Griebel et al, 1989). Therefore, the increased serum antibody levels could be due to improved antigen presentation, alteration of lymphocyte subset ratios, or other effects at one of the various steps in immunoglobulin production.

Lymphocyte proliferation in response to T lymphocyte

mitogens or antigens is used to assess CMI. In a study of some leukocyte functions, including indicators of CMI, calves were given recombinant bovine IFN-α or IFN-r, either intravenously or intramuscularly, at 10^6 or 10^4 U/kg of body weight. High doses (10^6 U/kg) of both IFNs, given either intravenously or intramuscularly, caused a decrease in PHA- and ConA-stimulated lymphocyte proliferation 24 hours after injection. However, this effect could be blocked by addition of interleukin-2 (10 U/ml) to the cultures. At 48 and 96 hours after IFN treatment, an enhanced lymphocyte proliferation response was detected. An enhanced response was also noted for the low dose (10^4 U/kg) of either recombinant bovine IFN-α or IFN-r. IFN treatment had no consistent effect on the ability of blood lymphocytes to produce interleukin-2 in response to ConA stimulation (Bielefeldt-Ohmann and Babiuk, 1986c). In another study, calves treated intramuscularly with 2 x 10^4 U/kg recombinant bovine IFN-αI1 had marked reduction in the amplitude of their ConA-induced lymphoproliferative responses from 4 to 24 hours post administration (Griebel et al, 1989). Other data presented in this report suggested that the decrease in response was due to a responder cell deficit rather than to the induction of suppressor cell activity or IFN antiproliferative effects.

In calves challenged with an aerosol of BHV-1, followed four days later by an aerosol of Pasteurella hemolytica, intranasal treatment with 10 mg of recombinant bovine IFN-α 1 (1 x 106 U/kg body weight) 48 hours prior to virus chal-

lenge resulted in much higher ConA-stimulated interleukin-2 production at seven days postinfection (Babiuk et al, 1985). In an in vitro study of the ConA-induced proliferative response of bovine intraepithelial leukocytes, the addition of either recombinant bovine IFN-α or -τ caused inhibition (Nagi and Babiuk, 1988). A Cantell-type bovine leukocyte IFN was shown to decrease both lymphocyte response to PHA or ConA and lymphocyte allogenic reactions at high concentrations and no effects were reported at lower concentrations (Jacobsen et al, 1986). Administration of avridine, a lipoidal amine compound that has activity as an IFN inducer and humoral and cell-mediated immune adjuvant, to cattle resulted in higher mean lymphocyte blastogenic responses to mitogens compared to controls (Roth and Kaeberle, 1985).

Antigen-specific lymphoproliferative response to BHV-1 was studied in bovine nonadherent cells treated in vitro with 100 U/ml of IFN-α or -τ (Eskra et al, 1985). Increased proliferation to BHV-1 was observed in cultures treated with IFN-τ but not IFN-α.

In the present study, the calves had antigen-specific lymphoproliferative responses beginning between days 4-7. This time of onset is in general agreement with previous reports (Babiuk and Misra, 1981; Ghram et al, 1989). There was no discernible difference in the magnitude or time of onset of the lymphoproliferative responses following either dose between calves receiving vaccine and those receiving both IFN and vaccine. It is possible, however, that the

individual variation among responses, described by previous authors and seen in the present trial, masked a treatment effect (Miller-Edge and Splitter, 1986).

In summary, concurrent administration of IFN and a modified live viral vaccine appeared to have no detrimental effect on the humoral or cell-mediated immune response to the vaccine, and IFN may augment the secondary humoral response.

Figure 9. Development of BHV-1 Serum Neutralizing Antibody After *In Vivo* Treatment with IFN, Vaccine, or Both on Days 0 and 14 (Values are Expressed as Geometric Mean Plaque Reducing Antibody Titer).

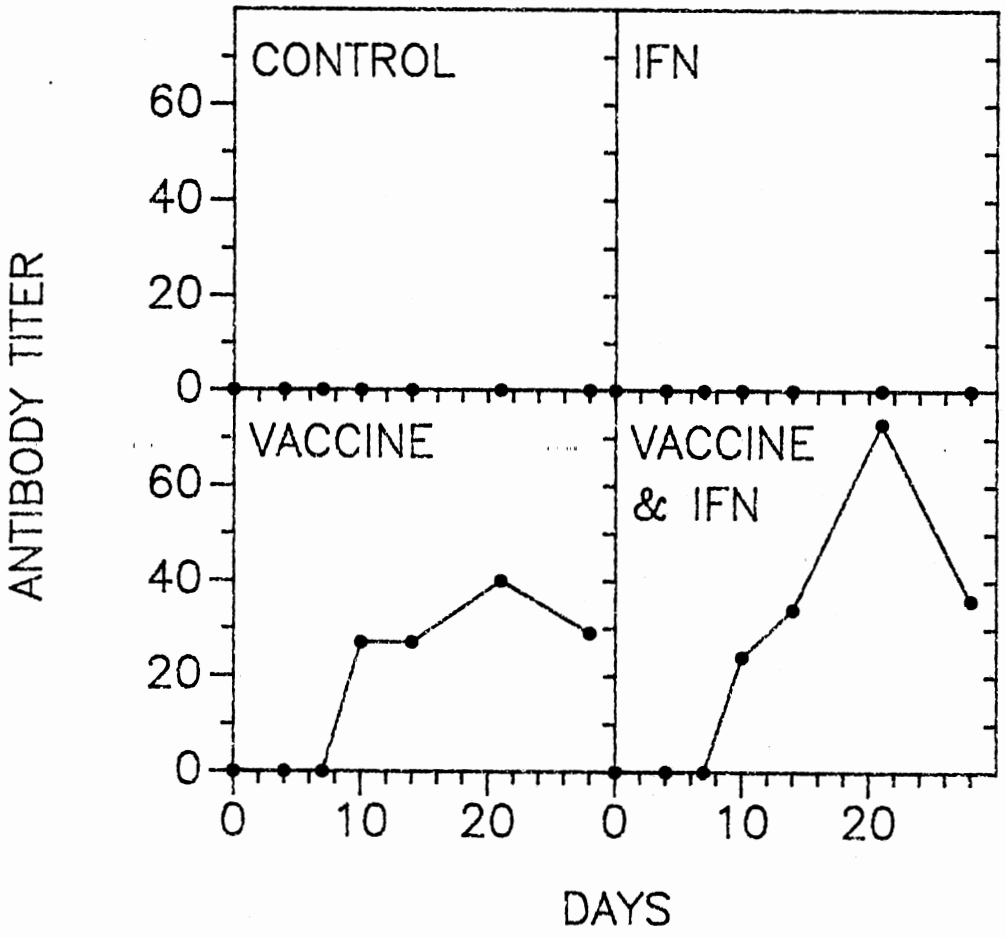
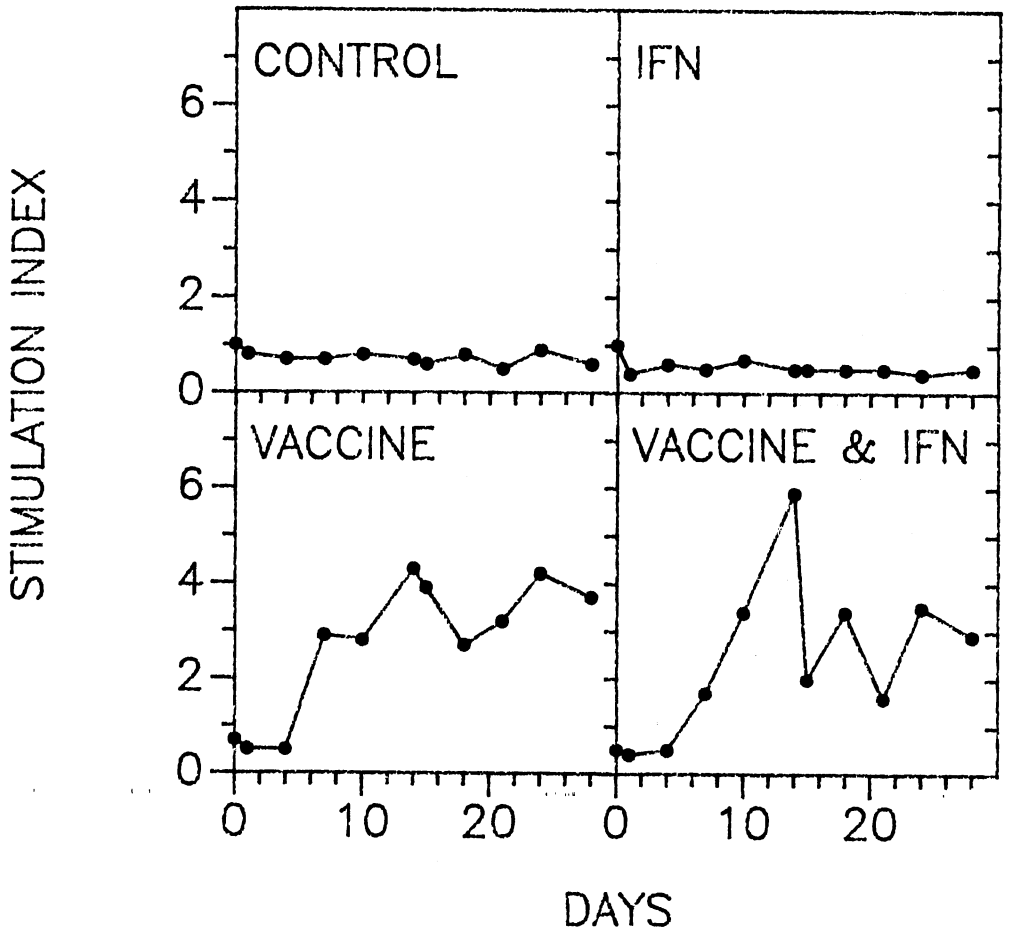

CONTROL
IFN
VACCINE
VACCINE
& IFN
ANTIBODY TITER
60
40
20
0
60
40
20
0
0
10
20
0
10
20
DAYS

Figure 10. Development of PBML Proliferative Response to UV-inactivated BHV-1 After *In Vivo* Treatment with IFN, Vaccine, or Both on Days 0 and 14 (Stimulation Index = Mean CPM in BHV-1 Stimulated Wells / Mean CPM in Medium Control Wells).


CONTROL
IFN
VACCINE
VACCINE & IFN
STIMULATION INDEX
0
2
4
6
0
10
20
DAYS

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of these studies was to determine: 1) the normal concentrations of 2',5'-oligo(A) synthetase in peripheral blood mononuclear leukocytes (PBML) in cattle, the relationship between amount of IFN given and 2',5'-oligo(A) synthetase levels in PBML, and the duration of elevation of 2',5'-oligo(A) synthetase after IFN administration; 2) the effects of a MLV vaccine and/or IFN on 2',5'-oligo(A) synthetase levels in PBML; and 3) the modulatory effects of IFN on the immune response of cattle to a MLV vaccine.

Clarification of the effect of IFN on 2',5'-oligo(A) synthetase activity of cells of cattle is important for several reasons. Study of endogenous or exogenous IFN is hindered by the fact that IFN is present in serum for a very short time. Thus a sensitive indicator of the presence of IFN would be of value. The IFN-induced enzyme 2',5'-oligo(A) synthetase is a candidate for such an indicator in cattle. If IFN is to be used in prophylaxis or therapy of bovine diseases, it is important to have information on the dose response relationship for IFN. Activity of 2',5'-oligo(A) synthetase in PBML provides a useful means of assessment of pharmacological response to IFN in cattle.

Data presented herein on the time course of elevated 2',5'-oligo(A) synthetase of PBML and corresponding AUC data should provide useful information on the pharmacological response of an animal to a single dose of IFN. Such information should be helpful in establishing when to administer IFN for prophylactic purposes and in determining frequency of repeated administration.

The marketing and management practices of the cattle industry in the United States result in tremendous distresses on cattle. This insult, along with the anatomy and physiology of the bovine lung, set the stage for pneumonic disease. In many cases conditions under which cattle are liable to develop respiratory disease syndrome are predictable. Under such circumstances prophylactic protocols would be beneficial in ameliorating or preventing disease. Use of IFN as a prophylactic agent is currently being investigated. Information on the dose response relationship of IFN is essential to evaluation of its the prophylactic value. Work reported herein details the pharmacological response of cattle to a wide range of doses of IFN using 2',5'-oligo(A) synthetase in PBML as an indicator.

In addition to its potential value in assessing prophylactic and therapeutic responses to IFN, 2',5'-oligo(A) synthetase could be of diagnostic value, as well as of value in studying responses to MLV vaccine. Although the 2',5'-oligo(A) system is known to be stimulated by bacteria, some chemical substances and autoimmune diseases, the response to

viruses is generally greater than to other agents. Once produced, 2',5'-oligo(A) synthetase persists at elevated levels in PBML for longer than does the stimulating IFN. The level of enzyme activity can be measured in hours whereas virus isolation takes a week or more; therefore, 2',5'-oligo(A) synthetase could provide information that would or would not support a diagnosis of a viral disease and do so more rapidly and reliably than IFN and more rapidly than virus isolation.

These data suggest that a MLV vaccine is able to induce 2',5'-oligo(A) synthetase in cattle. Enzyme levels seem a more sensitive indicator of virus and IFN effects than serum IFN levels. Also, concurrent administration of IFN with a modified live viral vaccine does not appear to affect the kinetics of vaccine induction of 2',5'-oligo(A) synthetase.

If the administration of exogenous interferon (IFN) proves to be an aid in reducing the impact of the shipping fever complex and the Food and Drug Administration approves IFN for use in cattle, then the logical time to administer it would be when cattle are marketed or during the handling that occurs routinely after arrival at new facilities. It is during marketing and arrival handling that vaccines are also administered, thus concurrent administration of IFN and viral vaccines would be a likelihood. The potential detrimental effects of administering an antiviral protein like IFN concurrently with a MLV vaccine, as well as the potential beneficial effects of the immunomodulatory effects of

concurrently used IFN, are important questions.

From data presented herein, it appears that concurrent administration of IFN and a modified live viral vaccine have no detrimental effect on the humoral or cell-mediated immune response and may augment the secondary response to the vaccine.

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VITA

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