IMMUNE RESPONSE TO NONIDET P-40 EXTRACTED INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS ANTIGEN IN RABBITS

by

BOONDEE ATIKIJ

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Approved by:

Major Professor

DEDICATION

To all rabbits who have sacrificed and contributed so much during the course of this investigation.

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INTRODUCTION

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Infectious bovine rhinotracheitis (IBR), herpes virus infection in cattle, causes an economic loss of over 200 million dollars every year to cattle industry in the United States. The consequence of IBR virus infection ranges from mild inapparent to adverse clinical manifestations known primarily as respiratory and reproductive forms of the disease (Wilson, 1974; Owen et al., 1964; Madin, 1956). The virus may occur as latent infection in animals and may be reactivated due to physiological and environmental stresses (Kahrs, 1977; Dennett et al., 1976; Sheffy and Rodman, 1973; McKercher, 1968). The ease of transmission, latent nature and ill-defined immune response of this virus make it to be widely distributed among most cattle populations and difficult to be controlled (Lomba et al., 1975; Brunner, 1973; Timoney, 1971, Grieg, 1965).

Both humoral and cell mediated immune (CMI) responses are required for resistance and recovery of IBR virus infection (Johnson and Muscoplat, 1977; Rouse and Babiuk, 1975, 1974). However, recent evidence indicates that cell mediated immune response plays a prominent role in the recovery process (Rouse and Babiuk, 1977, 1975, 1974; Notkins, 1974). Corticosteroids which have been shown to suppress cell mediated immune response (Muscoplat and Shope, 1975; Davies and Carmichael, 1972) and enhance the IBR virus replication (Hall

and Minocha, 1977) are capable of reactivation and recrudescence of the virus (Davies and Duncan, 1973). Interferon, released from cells exposed to the virus has been suggested to be one of the important factors in controlling IBR virus infection (Fulton, 1978; Babiuk and Rouse, 1976; Todd, 1972).

The control of IBR virus infection is primarily based on hygiene, management and vaccination. Adequate immune response with minimum undesirable post vaccinal effects is an important criteria for evaluation of a vaccine. Modified live vaccines (MLV), though providing protection against the disease (Saunders et al., 1972; McKercher, 1971), may produce abortion (Kelling, 1973) and latent infection in vaccinated animals (Casselberry, 1972; Searl, 1971). Administrations of intranasal live vaccines have been reported to provide sufficient protection in cattle without causing abortion (Todd, 1976, 1975, 1972, 1971); however, the problems regarding the latent nature of the virus, post vaccinal reactions and the difficulty of vaccine administration still exist (Kahrs, 1977).

The inactivated vaccines, on the other hand, overcome the concern about post vaccinal undesirable effects and latent infection. However, the efficacy of the inactivated vaccines is controversial, although investigators have reported adequate protection against IBR virus infection in cattle (Haralambiev, 1976; Karadjov, 1976; Judhasz, 1972; Zuscheck and Chow, 1961).

The presence of cell mediated immune response and interferon in vaccinated animals are important criteria for evaluating the efficacy of IBR virus vaccine. However, no serious attempt has yet been made in this regard. The objectives of this investigation are to (1) compare the humoral, cell mediated immune responses and lymphocyte interferon production in rabbits injected with either live or Nonidet P-40 (NP-40) inactivated IBR vaccines and (2) characterize and study the properties of lymphocyte interferon.

REVIEW OF LITERATURE

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History

Infectious bovine rhinotracheitis (IBR) was first recognized in Colorado feedlot in 1950 under the synonyms of "red nose", "dust pneumonia", or "necrotic rhinotracheitis" (Miller, 1955). The identification of specific antibody to virus in serum samples of blood collected in 1941, indicated that the disease, although previously unrecognized, existed earlier (Gillespie et al., 1957). In 1955, it was designated infectious bovine rhinotracheitis virus (McKercher et al., 1955). Shortly afterwards the virus was isolated and cultured on bovine embryonic kidney cells (Armstrong et al., 1961; York et al., 1957; Madin et al., 1956). The virus has been reported in most countries over the world (Gibbs, 1977) and has wide distribution in the U.S.A. Border Nation Surveys indicated that 10-30% of cattle in the U.S. had become exposed to the disease (York, 1968) which has become of major economic importance in North America (Kahrs, 1974; Sheffy and Krinsky, 1973; Rosner, 1968; Pierson and Vain, 1965).

Classification

The virus was isolated from cattle by Madin et al. (1956) and classified as a herpes virus by comparing in vitro growth and morphology with herpes simplex virus (HSV), (Armstrong, et al., 1961). The virus genome contained

double-stranded DNA with M.W. of 54 x 10^6 dultons and buoyant density in cesium chloride of 1.730-1.732 gm/cm^3 (Graham, et al., 1972; Gibbs et al., 1970; Plummer et al., 1969; Bowling et al., 1969; Russell and Crawford, 1964). The virus capsid was constructed of 162 capsomeres in icosahedral symmetry and an envelope surrounded the nucleocapsid (Bocciarellie et al., 1966; Watrach and Baheman, 1966; Cruickshank and Berry 1965). The virus was ether sensitive, acid labile and inactivated at 56° C for 21 minutes (Snowdon, 1964; Griffin et al., 1958); the nucleocapsid developed within the nucleus of the cell and acquired the envelope on budding from the nuclear membrane (Silva et al., 1973; Zee and Talers, 1972; Jasty and Chang, 1971). The virus produced crowdy type A intranuclear inclusions in the infected cells (Armstrong et al., 1961; Cheatham and Crandell, 1957) and most of its physico-chemical characteristics conformed to those of the herpes virus group as reviewed by Gibbs and Rweyemanu (1977), Goodheart and Plummer (1975), Gentry and Randall (1973), Ludwing (1972) and Roizman (1971, 1969).

The virus was named "Bovid Herpes Virus" (BHV_1) according to International Committee on the Taxonomy of Virus (Smith, 1976) and consisted of 9 different strains. Plummer (1964) could not detect any cross neutralization between IBR (Oxford) and 8 different strains of HSV, equine herpes virus and

porcine herpes virus. Other investigators have found no relationship between IBR virus and feline herpes virus 1 (Johnson and Thomas, 1966), canine herpes virus (Poste, 1972), Bovid Herpes Virus 2 (Martin et al., 1966; Pepper et al., 1966) and Bovid Herpes Virus 3 (Bartha et al., 1966). Carmicheal and Barnes (1961) reported that IBR virus (Cooper) and equine herpes virus 1 showed antigenic cross-reactivity by complement fixation and gel diffusion tests. Evans et al. (1972) reported that IBR, herpes virus of Marek's disease and Burkitt's lymphoma showed at least 1 common antigenic component when examined by gel diffusion immunoelectrophoresis and indirect fluorescent antibody technique (FA). Blue and Plummer (1973) detected a common viral antigen by fluorescent antibody in cells infected with IBR (Los Angeles), HSV_1, squirrel monkey herpes virus and equine herpes virus 1. IBR virus and caprine herpes virus 1 has also been reported to share a common antigen (Berrios and McKercher, 1975).

Early serological comparison of different strains of IBR virus did not indicate any antigenic variation (Mohanty and Lillie, 1970; Gillespie et al., 1959; McKercher and Wada, 1964). However, Buening and Gratzek (1967) were able to demonstrate minor difference between BHV_1 (enteritis) and 3 different strains of IBR (Cooper, Los Angeles, Iowa State University 2) by neutralization kinetics. More recent studies supported that minor antigenic differences did exist among

IBR virus strains (Potgieter and Mare, 1974; Crandell, 1973; Bagus, 1972; House, 1972) and it did not correlate with the affinity of virus for particular organ (House, 1972). Further studies on antigenic variation may be important for selecting the strains used for vaccination since different virus strains had varying immunogenecities. House (1972) reported that IBR (Cooper) evoked the greatest immune response in cattle. Studies on ferrets infected with virulent or avirulent strains of IBR virus indicated that only virulent strain produced immunity that protected the animals against the challenging virus (NADC, 1977).

Cultivation and Life Cycle

IBR virus can multiply in a wide variety of cell cultures such as bovine (McKercher, 1959; Madin et al., 1956), ovine, caprine, equine, rabbit (McKercher, 1959), canine (House, 1972), mouse, monkey (Plummer et al., 1969) and human lung WI-38 (Michalski et al., 1976). Infectious bovine rhinotracheitis virus causes typical herpes virus cytopathic effect (CPE), "Syncytial formation" (Roizman, 1962) and induces the formation of large intranuclear inclusion bodies in infected cells (Cheatham and Crandell, 1957). Plaque formation by IBR virus has been reported in various cell cultures (Bagust, 1972; Sabina and Parker, 1963; Rouhandeh and Werder, 1963). The life cycle and sequential development of the IBR virus are similar to other herpes

viruses (Valicek and Smid, 1976; Watson, 1973; Silva, et al., 1973; Roizman and Spear, 1973; Zee and Talen, 1972, 1971; Jasty and Chang, 1971, 1972; Schwartz and Roizman, 1969).

Clinical Diseases Associated With IBR Viruses

The virus has been reported to cause mainly respiratory and reproductive forms of diseases (Schultz et al., 1977). It is also associated with occular, central nervous system, enteric, neonatal, and dermal infections in cattle (Gibbs, 1977; Kahrs, 1977). The diseases produced by the virus are IBR (Madin, et al., 1956), conjunctivitis (Timoney and O'corner, 1971; St. George, 1965; Hughes et al., 1964), infectious pustular vulvovaginitis (Kendrick et al., 1958) previously recognized as "Blaschenausschlag", encephalitis (Beck, 1975; Lomba et al., 1973), mastitis (Gourlay, 1974; Roberts et al., 1974), enteritis (Wellemans et al., 1974), dermatitis (Bwangamoi and Kaminjolo, 1971) and abortion (Wilson, 1974; Lomba, 1973; Kirkbride, 1973; McKercher and Wada, 1964).

Host Heterogeneity

Besides cattle, IBR virus has been isolated and/or causes infection in other animals such as goat (Mohanty et al., 1972), horse (Jubb and Kennedy, 1970), water buffalo (St. George and Philpott, 1972), swine (Nelson et al., 1972), wild beast (Karstad et al., 1974), mustelids (Porter et al., 1975), mule deer (Chow and Davis, 1964) and rabbit (Armstrong, 1961).

Armstrong (1961) produced a mild erythrematous lesion in rabbits inoculated intradermally with IBR virus. Persechino et al. (1965) confirmed that this virus could infect rabbit and produce dermatitis, corneal keratitis and meningocephalitis. Further studies by Bwangamoi and Kaminjolo (1973) showed that virus could produce skin lesion in rabbits inoculated intradermally and cause epididymitis upon intratesticular inoculation. Recent studies by Kelly (1977), and Lupton and Reed (1979) indicated that Cooper and Los Angeles strains of IBR virus could produce similar clinical symptoms in rabbits as in cattle and cause fatal infection in neonatal rabbits. Serological studies in rabbits experimentally infected with IBR virus revealed that humoral immune response was elicited as detected by complement fixation, neutralization and gel diffusion tests (Faye, et al., 1975).

Latent Infections

The unique problem of IBR virus is its capability to establish latent infection as a sequel to a primary infection (Schultz, 1977). The early observation of the epidemiology of IBR virus suggested that it produced a latent infection (Bottcher, 1970; Saxegaard, 1966; Snowdon, 1965). Recrudescence of clinical disease with excretion of viruses may occur as a reaction to stress (Gibbs, 1977).

Smith (1978), Gibbs et al. (1975), Dennet et al. (1976, 1973), Bitsch (1973) and Darcel & Dorward (1972) found that corticosteroid can reactivate IBR virus in naturally infected animals. Viruses were excreted from the nasal secretion and clinical manifestations were occasionally observed. Studies on experimentally IBR infected cattle recovered from primary infection, indicated that the virus could be reactivated after intravenous inoculation with corticosteroid (Davies and Duncan, 1974; Sheffy and Rodman, 1973; Davies and Carmichael, 1973). Sheffy and Rodman (1973) reported that vaccination of cattle with modified live vaccine (MLV) resulted in latent infection since the virus was reactivated with corticosteroid treatment. However, there is yet no evidence to confirm that the excreted virus was the vaccine strain that was inoculated.

Humoral and Cell Mediated Immune Responses to Infectious Bovine Rhinotracheitis Virus

Current evidence indicates that cell mediated immunity may play an important role in controlling herpes virus infection (Shimizu et al., 1976; Russell 1975, 1974; Notkins, 1974; Lodmell et al., 1973). Since the herpes virus can spread by intracellular route (Babiuk et al., 1975; Lodmel et al., 1973), it may not be exposed to extracellular neutralizing antibody (Notkins, 1974; Christian, 1971). Herpes virus infections are also more severe in individuals with

deficiency in CMI response (Allison, 1972; Merigan and Steven, 1971). Furthermore, the appearance of antibody does not correlate with the time of recovery from infection (Rouse et al., 1975; Rosenberg, 1974, 1972). In IBR infected cattle, the clinical recovery and cessation of virus excretion occur at 1 week before anti-IBR antibody can be detected (Rouse and Babiuk, 1974). Bottcher and Mahler (1970) reported the shedding of virus in naturally IBR infected cattle, occurred in spite of presence of circulating IBR antibody.

Humoral immunity, though considered less important in primary infection, has been reported to play important role in facilitating the destruction of herpes virus infected cells by the mechanism of antibody-complement lysis (Rawls and Tompkins, 1975), antibody-complement mediated inflammatory response (Osler, 1976) and antibody dependent cellular cytotoxicity (ADCC) as reported by Rouse et al. (1976) and Bloom (1975). Rouse et al. (1976) and Babiuk et al. (1975) have shown that antibody-complement lysis is an important mechanism in limiting IBR virus dissemination in vitro. Furthermore, the cooperation of ADCC which results in the destruction of IBR infected cells demonstrates its importance in preventing virus spread (Rouse et al., 1976, a, b; Shore et al., 1974). By combining these two mechanisms of ADCC and complement lysis marked reduction of virus dissemination

is observed (Rouse et al., 1977, a, b). The macrophages and lymphocytes are able to act as effector cells (Rouse et al., 1976, a, b, Rouse and Babiuk, 1975). The ADCC mechanism is reported to occur at about the time when virus is disseminated to adjacent cells (Grewal, 1977; Rouse et al., 1976 b). The binding of antibody, effector cells and virus-infected cells with or without complement, results in lysis of infected cells, measured by chromium^{51} (Cr^{51}) released (Rouse et al., 1977 b) or inhibition of viral formation (Rouse et al., 1977 a). Rouse and Babiuk (1978) suggested that the humoral immunity could play a role against the herpes virus infection by antibody-complement lysis and ADCC mechanisms, and it was more important in recovery from recrudescence than in primary infection.

In general, humoral immune response is always used as an epidemiological tool. Since the neutralizing antibody to IBR virus can be detected in the serum of infected cattle at about 10-14 days after infection (Davies and Carmichael, 1973; Rouse and Babiuk, 1974) and persists for up to 5.5 years after infection (Chow, 1972). The techniques used to detect antibody in serum include neutralization test (House and Baker, 1971; Greig, 1969; Mohanty and Lillie, 1965; McKercher and Saito, 1965; Snowdon, 1961; Dawson et al., 1962), passive haemagglutination test (Kirby et al., 1974; Zyambo et al., 1973 a, b; Shimizu et al., 1972; Vengris and

Mare, 1971) gel diffusion (Charton, 1975; Kaminjolo and Gicho, 1972), complement fixation (Wellemans and Leunen, 1973) and fluorescent antibody technique (Assaf et al., 1975). The constant virus varying serum, although has been widely used, is less sensitive than the constant serum varying virus test (House and Babur, 1971) and the passive haemagglutination test (Kirby et al., 1974). The sensitivity of the test is improved either by reducing the concentration of challenge virus (Huck and Woods, 1972; Bitsch, 1970) or adding complement (Potgieter, 1975) or increasing the virus-serum incubation period (Bitsch, 1978). Some investigators have not been able to demonstrate the neutralization antibody in serum of infected cattle (Jan, 1978; Collings et al., 1972; Saxegaard, 1968).

Current evidence also indicates that the CMI response is related more than serum neutralizing antibody to immune status against IBR virus (Rouse and Babiuk, 1978). Therefore the neutralizing antibody in serum of cattle may not be accurate indicator for immunity. Recently, the delayed hypersensitivity test has been developed for the epidemiological survey of IBR virus infection in cattle (Aguilar-Setein, 1979, 1978 a, b); Correa et al., 1977). However, field trial and accuracy of this test must be further evaluated.

The important role of CMI response in IBR infection is supported by the observation that cattle vaccinated with some vaccines cannot withstand the challenge of virulent strain of IBR virus despite the presence of high serum antibody (Schultz, 1977; Sheffy and Rodman, 1973). Most of the studies for quantitation of CMI responses are performed *in vitro* and have included lymphocyte blastogenic response, direct cytotoxicity and lymphokines (interferon) production.

The measurement of thymic-derived lymphocyte (T lymphocyte) function by evaluating lymphocyte blastogenesis *in vitro* in response to phytohaemagglutinin (PHA), a mitogen with specificity to T-lymphocyte, was first reported by Nowell (1960). Sorensen *et al.* (1969) developed quantitation of lymphocyte blastogenesis by measuring the incorporation of ^{3}H-thymidine (^{3}HTdR) into PHA stimulated lymphocytes. Since then, the standard techniques for quantitation of lymphocyte proliferation have been established for many animals including bovine (Lazary *et al.*, 1974; Muscoplat *et al.*, 1974) and rabbits (Mansfield and Wallace, 1973). The lymphocyte response to PHA has been shown to reflect the CMI status in animals (Al-Sarraf, 1972; Cendrowski, 1970).

Lymphocyte stimulation assays for IBR virus have been described by Davies and Carmichael (1973) and Rouse and Babiuk (1974). By using UV-irradiated IBR virus, incorporation of ^{3}HTdR into lymphocytes from IBR infected cattle occurs at about 5 days after infection, peaks at 8-10 days and subsequently declines. The ability of anti-IBR serum to inhibit the lymphocyte blastogenesis in response to UV-IBR antigen together with the inability of other herpes viruses to stimulate the IBR-sensitized lymphocytes indicate specific antigen stimulation. Blastogenesis, however, only gives a measure of antigen recognition by immune lymphocyte and does not give any information whether or not the lymphocytes can act as the effector cells. Sensitized lymphocytes were able to prevent viral plaque formation and markedly decrease virus yield on cell monolayer infected with IBR virus (Rouse and Babiuk, 1975). The inhibition is shown to be immune specific and involves a process of suppression of viral replication rather than destruction of free virus or virus infected cells. Although the nature of inhibition has not been elucidated, the subsequent observation by Babiuk and Rouse (1976) on the production of interferon of peripheral blood lymphocytes (PBL) of IBR infected animals suggests that the plaque inhibition is produced by interferon. The immune interferon appears to be synthesized by T lymphocytes with the cooperation of macrophage in response to IBR antigen.

By using ^{51}Cr release technique, Rouse and Babiuk (1977 c) demonstrated that direct cytotoxicity against IBR infected cells was mediated by T lymphocytes from IBR infected cattle and it was not restricted by genetic incompatability of lymphocytes and target cells.

Interferons are a family of inducible proteins produced by either lymphoid or nonlymphoid cells in response to viral infection (Colby, 1977; Ho and Armstrong, 1973) or other stimuli such as bacteria (Youngner and Steinberg, 1964), endotoxin (Ho and Kono, 1965) protozoa (Remington and Merigan, 1968), rickettsiae (Hahon and Kozkowski, 1968), natural and synthetic nucleic acid (DeClercq, 1974), mitogen (Wheelock, 1965) and antibiotics (Merigan, 1973). Besides the antiviral effect, interferon also affects expression of surface antigen (Lindahl et al., 1973), immune response (Epstein, 1977) and proliferation of both normal and tumor cells (Ho, 1975).

Youngner and Salvin (1973) have established a subclassification of interferon based on mode of induction and stability at pH 2. Generally, "interferon type I" or "Classical interferon" is defined as acid-stable interferon produced by both lymphoid or nonlymphoid cells in response to virus or other stimuli and type II or "immune interferon" (Falcoff, 1972) which is acid-labile, is produced by lymphocytes in response to specific antigens or mitogens.

Subsequent study on comparison of type I and type II interferon indicates that they are different in immunological, biological and physico-chemical properties (Epstein, 1977). Valle et al. (1975) have shown that immune interferon from sensitized human lymphocytes is produced in response to HSV antigen stimulation with the cooperation of macrophages. The immune interferon is labile at pH 2 and at 56° C and has different antigenic properties from other human interferons. Youngner and Salvin (1973) have also described the difference of mouse type I and type II interferons appearing in circulation of mice with delayed hypersensitivity reaction to BCG and challenged with tuberculin extracts. Mouse immune interferon is found to be labile at pH 2 but stable at 56° C and is not neutralized by type I mouse interferon antibody. Comparison of bovine type I and type II interferon is also reported by Rouse and Babiuk (1978).

Compared to type I interferon, type II has been reported to be 100 to 1,000 times more immunopotent than type I and can be immunopotentiating or immunosuppressive both in vivo and in vitro (Youngner, 1977). Furthermore, type II interferon also contains tumor growth inhibiting factor (Salvin et al., 1975) and bactericidal activity (Salvin, 1974). Whether type I or type II interferon will be produced by lymphocytes in response to a given stimuli is not always predictable and can be influenced by the method of preparation

of the cell cultures (Jordan and Merigan, 1974) and the immune status of the donor (Haahr et al., 1976). It has been suggested that primary role of type II interferon is immunoregulation rather than viral inhibition (Youngner, 1977).

Herpes viruses have been reported to be both poor interferon inducers and insensitive to interferon effect (Trueblood and Manjara, 1972; Ho, 1973). However, interferon is detected in serum of calves inoculated with IBR virus (Zygraich et al., 1974; Todd et al., 1971, 1972; Rosenquist and Loan, 1969 a, b) and in IBR infected organs and cell cultures (Fulton, 1976; Smorodintsev, 1968). Rosenquist and Loan (1969) reported that the interferon was detected in serum as early as 6 hours post inoculation, reached peak at 1-2 days and subsequently declined, the later event being closely correlates with the presence of viremia. The correlation between the suppression of nasal viral titres and maximum level of interferon in nasal secretion was also reported by Todd et al. (1971, 1972). Vaccinated calves with high level of nasal interferon were refractory to challenge with virulent IBR virus. This supported the protective role of interferon in IBR virus infection. Furthermore, Theil et al. (1971) observed that cattle injected with an interferon inducer (poly I:poly C) 3 hours prior to infection developed a milder clinical disease than did the untreated cattle. The antiviral effect of bovine

macrophage interferon against IBR virus in interferon-treated bovine tracheal organ cultures reported by Fulton and Root (1978) indicates that interferon may function to protect the respiratory tract against viral infection in cattle.

Further supportive evidence for the important role of antiviral effect of interferon against IBR virus was demonstrated by Rouse and Babiuk (1976). Using macrophage-lymphocyte culture and IBR antigen, they reported that immune interferon was produced by T-lymphocytes within 24 hours after antigen stimulation. The production was augmented by physical contact between autologous macrophages and lymphocytes. The antigen-antibody complex of irradiated IBR infected cells was better at stimulating interferon than free antigens. Furthermore, they also demonstrated, for the first time that IBR virus was inhibited by immune interferon produced from sensitized lymphocytes in response to IBR virus antigen stimulation. Subsequent study on the properties of bovine immune interferon (Babiuk and Rouse, 1978) indicated that it had a wide range of lymphokine activities such as enhancing the level and speed of ADCC mediated by bovine effector cells. Phagocytic activity of macrophage and direct T-cell mediated lymphocytotoxicity were also increased. In addition, bovine immune interferon had greater effect on heterologous cells than type I interferon (Babiuk and Rouse,

1977). The role of bovine immune interferon was suggested to be concerned with limiting spread of IBR virus, thus resulting in recovery from the disease (Babiuk and Rouse, 1976).

Infectious Bovine Rhinotracheitis Virus Vaccines

I. Modified Live Vaccines

(a) Intramuscular Vaccines: The first IBR vaccine developed was a modified live vaccine (MLV) for intramuscular (IM) inoculation (Schwarz et al., 1957) and it was widely used in combination with other vaccines (Schell et al., 1972). The duration of humoral immunity persisted for 3-6 years after vaccination (Chow, 1972; Rosner, 1968). The vaccine elicited both systemic CMI and humoral immune responses in cattle (Gerber et al., 1978). The efficacy of the vaccines was diminished by the presence of maternal antibody in calves less than 6 months of age (Kahrs, 1977). Occasionally, it caused abortion in pregnant cattle (Wilson, 1974; Mitchell, 1974; Kelling et al., 1973; McFeeley, 1968) and produced post vaccinal reactions (Frank et al., 1977; Roberts and Carter, 1974; Reed et al., 1973; Mackey, 1971). Furthermore, many investigators have found that IBR-vaccinated calves shed IBR viruses into nasal secretions, urine, semen and milk which serve as sources of infection to susceptible animals (Straub, 1976; Sheffy and Rodman, 1973; Kelling et al., 1973; McKercher, 1971). Despite the controversy regarding the

efficiency of the intramuscularly administered IBR vaccine, they are generally accepted and widely used (House, 1977; Sheffy, 1974).

(b) Intranasal Vaccines: The first intranasal (IN) vaccine used containing parainfluenza 3 and IBR virus was introduced by Todd et al. (1971). This vaccine has gained widespread acceptance since it may overcome the problem of causing abortion when injected into pregnant calves (Smith, 1978; Kahrs et al., 1973; Todd et al., 1976, 1972, 1971). The IN vaccine provided protection within 72-96 hours post vaccination against virulent IBR virus challenge (Straub, 1976; Todd, 1974, 1972). The resistance to reinfection was attributed to the production of local antibody and interferon detected as early as 40 hours post inoculation. McKercher (1971) reported no distinct advantage of IN vaccine over IM vaccine for systemic antibody production. However, Todd (1975) reported that only IN vaccines had the capability of eliciting the local antibody which was important defense mechanism against viruses with respiratory tract as a primary route of infection (Notkins, 1974; Todd, 1972; Ogra, 1970). Another advantage of IN vaccine is its efficacy for vaccinating calves with colostral antibodies (Todd, 1976). It was also reported that the IBR vaccine strain was stable and did not infect the fetus (Todd, 1976; Gibson, 1973). However, the vaccine was difficult to administer and may produce post vaccinal reactions (Kahrs, 1977).

Recently, temperature sensitive (TS) strain of IBR virus has been developed for intranasal inoculation (Zygraich et al., 1976; 1974 a, b). The vaccine strain is chemically treated to induce a temperature sensitive characteristic, thus it cannot replicate at the body temperature of cattle. The growth of the virus is restricted to the nasal mucosa so that it is incapable of systemic replication or fatal infection. Kucera et al. (1978) reported that TS vaccine elicited neutralizing antibody in cattle, protected the vaccinated animals against challenge with virulent virus and was safe for vaccination in pregnant cattle. In addition, both local and systemic humoral immune response and CMI response were elicited in vaccinated cattle (Gerber et al., 1978).

II. Inactivated Vaccine

The inactivated vaccine has been developed to overcome the problems of latency and reactivation of live virus vaccines together with post vaccinal reactions in animals, particularly in pregnant cattle (Roberts, 1974; Wilson, 1974; Kelling et al., 1973; McKercher, 1971). There is, however, a controversy regarding the efficacy of the vaccine as some investigators cannot detect any neutralizing antibody in vaccinated cattle (Msolla, 1979; Schipper and Kelling, 1975; Hamdy and Trapp, 1964). By using adjuvant with inactivated vaccines, many investigators have demonstrated serum

neutralizing antibody in cattle (Kendrick and Osburn, 1973; Kolar et al., 1972). Judhasz et al. (1972) prepared formalin inactivated IBR adjuvated with aluminum hydroxide gel absorbant. Subcutaneous (SC) administration of this vaccine resulted in high titers of serum antibody. Matsuoka (1972) demonstrated that formalin inactivated IBR vaccine without adjuvant protected cattle against challenge with IBR viruses. In addition, shedding of viruses and post vaccinal reactions were not observed in vaccinated cattle. Haralambiev (1976) demonstrated that IBR viruses inactivated by treatment with ethanol and saponin were capable of eliciting neutralizing antibody in cattle by IN or SC routes. Antibody in nasal secretion was also observed and the animals were resistant to challenge with virulent virus. Kristov and Karadzhov (1976) confirmed that IBR virus, inactivated with formaldehyde and saponin, administered with oil adjuvant could elicit neutralizing antibody in cattle after second injection. Other supportive evidence for inactivated IBR vaccine with adjuvant was also reported by Karadjov (1976). Inoue et al. (1977), by using complement requiring serum neutralizing antibody as an indicator, reported that cattle injected with inactivated vaccine did not show serum antibody after first injection; however, serum antibody titers markedly increased after second injection. The adverse effects of inactivated vaccines including fatal hypersensitivity and other nonfatal allergic syndromes have been discussed (Kahrs, 1977).

Although there is a good evidence of humoral immune response, CMI response has not been evaluated in animals injected with inactivated vaccines.

MATERIALS AND METHODS

MATERIALS AND METHODS

Animals and Cell Cultures

Eight New Zealand rabbits, 4 to 6 months of age, were used in these studies. All rabbits were tested for the absence of antibodies to IBR virus.

Primary and secondary rabbit lung and kidney cultures were prepared from one to two week old albino rabbits. The animals were asphyxiated with carbon dioxide (CO_2), lungs and kidneys were aseptically removed, minced and trypsinized in 0.25% trypsin (1:250) in phosphate buffered saline, 0.01M (PBS) and growin into monolayers in Falcon tissue culture flasks in CO_2 incubator at 37° C. The cells were maintained in minimal essential medium (MEM), Eagles (GIBCO, N. Y.) supplemented with 10% heat inactivated fetal calf serum (FCS, K C Biological, Inc., Lenexa, KS), 0.1% sodium bicarbonate ($NaHCO_3$) and antibiotic mixture of penicillin (100 units/ml), streptomycin (10 ug/ml), kanamycin (5 ug/ml) and amphotericin B (fungizone, 0.5 ug/ml). Primary cultures were passed once to get secondary cultures which were used in the experiments.

Chick embryos, 12 days old, were removed from eggs, head and limbs were cut off, the tissues were minced and processed as previously described for rabbit tissues.

Bovine kidney (BK) cells were obtained from Dr. R. M. Phillips (Diagnostic Laboratory, College of Veterinary

Medicine, K.S.U.) and grown into monolayer in 250 ml capacity tissue culture plastic flasks. The cells were maintained in MEM plus 10% FCS. Madin-Darby bovine kidney (MDBK) cells were obtained from American Type Culture Collection (ATCC) laboratory (Rockville, MD), grown and maintained in MEM plus 10% horse serum (HS).

Propagation and Assay of Virus Stock

Infectious bovine rhinotracheitis virus (Cooper strain) was obtained from Dr. David Reed (Iowa State University, Ames, Iowa). Vesicular stomatitis virus (VSV) was provided by Dr. T. C. Johnson (Division of Biology, K.S.U.). Both viruses were passed for several passages on rabbit kidney cultures.

Monolayer of secondary rabbit kidney or lung cultures were inoculated with either IBR or VSV at a multiplicity of 0.1 plaque-forming unit (PFU) per cell, incubated for 1 hour and maintained in 1 x MEM plus 5% FCS (anti-IBR antibody free) at 37° C in CO_2 incubator. The infected cultures were harvested when most of the cells showed cytopathic effect (CPE), approximately 48-72 hours post inoculation. The suspension was frozen and thawed twice, then centrifuged at 1,000 g for 15 minutes at 4° C. The stock viruses were stored at -70° C and infectivity titers were determined by PFU assay (Dulbecco, 1952) or tissue culture infectious doses 50 ($TCID_{50}$).

Serial log dilutions of viruses in MEM plus 5% FCS (anti-IBR antibody free) were adsorbed for 1 hour on rabbit lung monolayers in 60 x 15 mm dishes (0.2 ml per dish). Eight ml of mixture of 2 x purified agar in deionized distilled water and 2 x MEM supplemented with 5% FCS was overlaid on cultures that were incubated in CO_2 incubator (3 days for IBR virus and 2 days for VSV). Plaques were observed 12-24 hours after the addition of neutral red (0.01%) in media-agar mixture on plates.

Determination of $TCID_{50}$ was performed on 96 wells-microtiter plates (Falcon, Microtest II). Monolayer of secondary rabbit lung cultures, grown in microtrays, were infected with 0.2 ml of serial log dilutions of stock virus (4 wells per dilution). The cultures were observed for CPE under the microscope 48-72 hours post infection. One unit of $TCID_{50}$ was determined as the dilution that showed CPE in 50% of cultures.

Bovine viral diarrhea virus (BVD, NADL strain) was obtained from ATCC laboratory. The virus was propagated and assayed essentially as described for IBR except that MDBK cultures and horse serum were used.

Bovine Parainfluenza-3 (PI-3), Shope Rabbit Fibroma virus (SFV), Bovine Adeno Virus 3 (Adeno 3) and Bovine Adeno Virus 5 (Adeno 5) were kindly provided by Dr. R. M. Phillips (College of Veterinary Medicine, K.S.U.). The viruses were

propagated on rabbit lung cultures and $TCID_{50}$ were determined as described previously.

Preparation of IBR Virus Antigen

Monolayer of secondary rabbit kidney cultures were inoculated with IBR virus at a multiplicity of 0.1 per cell and maintained in MEM plus 5% FCS (anti-IBR antibody free) at 37° C in CO_2 incubator. When maximum cells showed CPE (72-96 hours) the cultures were harvested and centrifuged at 4° C. The pellet was washed 2 x with PBS, frozen, thawed twice and extracted with 2% NP-40 in Tris buffer, pH 7.0 (1×10^7 cells per ml of NP-40). The suspension was sonicated for 15-30 seconds in ice-alcohol bath, stirred for 1 hour at 4° C, then centrifuged at 1,200 g for 15 minutes and the supernate was stored at -70° C. Prior to injection the supernate was emulsified with equal amount of Freund's incomplete adjuvant (Grand Island Biological Co., N.Y.) in a multi-churn apparatus (Mulsi Jet, Inc., Elmhurst, Ill.). The inactivation of the viruses was confirmed by the lack of plaque formation on monolayer cultures. Uninfected rabbit kidney cells were extracted with NP-40 for the preparation of normal cell antigen.

Inoculation of Rabbits

The following groups of 2 rabbits each were inoculated intramuscularly with 1 ml of antigen at 2 different sites:

1) NP-40 extracted IBR infected rabbit kidney cells plus adjuvant.

2) NP-40 extracted uninfected rabbit kidney cells plus adjuvant.

3) Live IBR virus (3×10^3 PFU).

4) Normal saline.

All rabbits were bled from ear arteries at 12 hours, 1, 2, 4 and 6 weeks post inoculation. Serum were collected and stored at -70° C.

Isolation of Rabbit Lymphocytes

Blood was collected into syringe containing heparin (10 units per 1 ml of blood) and was diluted with equal volume of PBS. Four ml of diluted blood was gently layered on 4 ml of Ficoll-Hypaque mixture (density = 1.077 at 25° C). The lymphocytes were collected from the interface after centrifugation at 400 g for 30 minutes, diluted in PBS and layered on second Ficoll-Hypaque solution and centrifuged to remove the residual red blood cells. Lymphocytes were collected, washed 2 x with PBS and resuspended in Rosville Park Memorial Institute (RPMI) medium 1640 with 10% FCS to contain 1×10^6 cells per ml of media.

Preparation of UV-Inactivated IBR Virus

Stock IBR or BVD viruses in 1 ml amounts were UV irradiated for 5 minutes in 60 x 15 mm plastic petri dishes (uncovered) at a distance of 10 cm from a UV germicidal

sterile lamp (630 T_8, 30 watt, Westinghouse Electric Co., Bloomfield, N. J.). Inactivation of virus was confirmed by plaque assay method.

Serum Neutralization Test

Serum samples were inactivated at 56° C for 30 minutes. Two fold dilutions of inactivated serum were prepared in MEM with 5% FCS and incubated with 100 $TCID_{50}$ of IBR virus for 1 hour. Serum-virus mixtures (0.2 ml) were added on monolayer of secondary rabbit lung cultures in microtrays (4 wells per dilution). The titers of serum antibody were expressed as the reciprocal of the serum dilution that protected 50% rabbit lung cultures and were calculated by the method of Karber (1931).

Lymphocytes Stimulation Studies

Each lymphocyte culture (2 ml) in RPMI 1640 plus 10% dialyzed FCS received either 5 ug of PHA (Sigma Chemical Co., MO) or UV-inactivated IBR or BVD viruses (3×10^6 PFU) and incubated for 72 hours in CO_2 incubator at 37° C. Twenty-four hours before termination of cultures, 1 ml of lymphocytes suspension from each tube was transferred into another tube to which 1 uCi of ^{3}HTdR (specific activity = 6 Ci/M mole, Schwarzman) was added. The cultures were harvested, centrifuged at 1,000 g for 15 minutes at 4° C. The supernate was aspirated and the cultures were washed 2 times with 5 ml of cold PBS, then precipitated with cold 5%

trichloroacetic acid (TCA) and kept in ice bath for 10 minutes. TCA insoluble fractions were collected onto glass fiber filter papers (GF/C, 2.4 cm., Whatman, England) using a millipore manifold (Model 3025, Belford, Mass.). The tubes were washed 2 X with 5 ml of 5% cold TCA. Filters were placed in scintillation vials containing 10 mls of scintillation fluid (Research Production International Corp., IL). Samples were counted in scintillation spectrometer (Packard, Tri-Carb, model 3002, Downers Grove, IL) for 10 minutes. The data presented were the average of duplicate cultures.

Stimulation indeces (S.I.) were calculated by the following formula:

$$S.I. = \frac{\text{Disintegration per minute (DPM) of } {}^{3}\text{HTdR of stimulated lymphocyte cultures}}{\text{Disintegration per minute of } {}^{3}\text{HTdR of control lymphocyte cultures}}$$

Preparation of Interferon Samples

The remaining one ml of lymphocyte suspension in the experimental procedure described above were incubated further for 24 hours (total 72 hours) then frozen and thawed. The samples were centrifuged at 100,000 g for 1 hour at 4° C in an ultracentrifuge to remove cell debris and virus particles and frozen at -70° C until assayed.

Interferon Assay

Serial 2 fold dilutions of interferon samples were prepared in 1 x MEM plus 10% FCS. Secondary rabbit lung cultures grown to monolayer in microtiter plates were incubated with interferon samples (4 wells per dilution) for 24 hours at 37° C in CO_2 incubator. Monolayers were washed once with PBS and challenged with 100 $TCID_{50}$ of VSV. The cultures were examined under the microscope at 48 hours post challenge for CPE. Virus control cultures were incubated with 0.2 ml of 1 x MEM with 10% FCS for 24 hours before challenge with VSV. The interferon titers were expressed as the reciprocal of interferon dilution that protected 50% of rabbit lung cultures against 100 $TCID_{50}$ of VSV challenge.

Kinetics of Interferon Synthesis

Lymphocyte cultures from rabbits were incubated with 3 x 10^6 PFU of UV-inactivated IBR virus and harvested at 0, 24, 48 and 72 hours post incubation. The cultures were processed and the interferon titers were determined as previously described.

Characterization of Interferon

Rabbit lymphocytes interferon was treated as follows:

1) Heat: Interferon samples were inactivated at 56° C for 1 hour.

2) pH 2: The samples were dialyzed against potassium chloride (KCl) buffer (pH 2) for 24 hours at 4° C and back dialyzed in 1 x MEM (pH 7.4) for 24 hours at 4° C.

3) Trypsin: The samples were incubated with trypsin (final concentration 0.1%) for 2 hours at room temperature. Equal amount of soyabean trypsin inhibitor was added and samples were reincubated for 2 hours more.

4) DNAase and RNAase: 40 ug/ml of either DNAase or RNAase were added to interferon samples. The mixtures were incubated for 1-1.5 hours at room temperature.

All treated interferon samples and control interferon (without treatment) were assayed for interferon titers on secondary rabbit lung cultures as described.

Protection of Different Cell Cultures by Interferon

Secondary rabbit lung and kidney cultures, chick embryo (CE) cultures and bovine kidney cultures grown to almost monolayer in microtiter plates were incubated with serial two fold dilutions of interferon (4 wells per dilution) at 37° C in CO_2 incubator. Twenty-four hours after incubation, the monolayers were washed once with PBS and challenged with 100 $TCID_{50}$ of VSV. The cultures were examined under the microscope at 48 hours post challenge and interferon titers were determined.

Interferon Protection of Rabbit Lung Cultures Challenged With Different Viruses

Secondary rabbit lung cultures grown to monolayer in microtiter plates were incubated with 4 units of interferon contained in 0.2 ml for 24 hours at 37° C in CO_2 incubator. The cultures were washed once with PBS and challenged

separately with 100, 50 and 10 $TCID_{50}$ (4 wells per virus concentration) of VSV, PI_3, Adeno 3, Adeno 5, SFV and IBR virus in 1 x MEM plus 10% FCS (anti-IBR antibody free). The cultures were examined for CPE under the microscope 48-72 hours post challenge.

Inhibition of Lymphocyte Stimulation by Interferon

Lymphocyte cultures from nonimmune rabbits were incubated with 5 ug PHA in the presence or absence of 4 units of interferon for 72 hours. Cultures were exposed to ^{3}HTdR (1 uCi) for 24 hours and then processed. Radioactive incorporation into lymphocytes was determined and stimulation indeces were calculated as described previously.

Effect of Hydrocortisone on Interferon Synthesis and CMI Responses

A stock solution of hydrocortisone (10^{-2} M) was prepared in an acetone-methanol (1:1) mixture and diluted in RPMI 1640 to various concentrations (10^{-6} to 10^{-4} M). Different concentrations of hydrocortisone were added to lymphocyte cultures containing either 5 ug PHA or UV-inactivated IBR virus (3×10^6 PFU). Control lymphocytes cultures were maintained in RPMI 1640 with 10% dialyzed FCS containing 5 ug PHA or UV-inactivated IBR virus without hydrocortisone. Incorporation of ^{3}HTdR and interferon synthesis in lymphocytes were determined as previously described.

Infectious Bovine Rhinotracheitis Virus Growth in Lymphocytes

Lymphocyte cultures were pelleted and incubated with either IBR virus at a multiplicity of 1, or with MEM for 2 hours, then washed 2 x with Hanks Balanced Salt Solution (HBSS) and resuspended in RPMI 1640 plus 10% dialyzed FCS (anti-IBR antibody free) and distributed in tubes, each containing 1 x 10^6 cells per ml of media. One uci of ^{3}HTdR was added into lymphocyte cultures that were harvested at 0, 24, 48 and 72 hours post incubation. One half of each sample (1 ml) was frozen, thawed, centrifuged and plaqued. The other half was washed 2 times with PBS and digested with 1 ml of 0.1 N sodium hydroxide (NaOH) for 1 hour. One ml of 0.1 N hydrochloric acid (HCl) was added to neutralize the samples and 0.1 ml of samples was counted in scintillation spectrometer. The amount of protein was determined by Folin-Phenol method (Lowry, 1951) and the specific activity was determined on the basis of DPM/ug protein.

RESULTS

RESULTS

Appearance of Serum Antibody in Vaccinated Animals

The serum neutralizing antibodies were not detected until after 1 week post inoculation in rabbits injected with either live or NP-40 inactivated virus (Fig. 1). The maximum antibody level (1:24) was observed in rabbits given NP-40 extracted antigen at 2 weeks post inoculation, followed by a gradual decline in titers. In rabbits injected with live virus, the antibody titers reached 1:18 at 2 weeks, remained constant until 4 weeks and declined thereafter until 6 weeks post inoculation. No serum neutralizing antibody was detected in rabbits injected with either NP-40 extracted uninfected rabbit kidney cells or with normal saline.

Cell Mediated Immune Response

Lymphocyte transformation responses as measured by incorporation of ^{3}HTdR into lymphocytes exposed to UV-inactivated IBR virus were first detected at 1 week post inoculation (Fig. 2). Maximum stimulation indeces were observed at 1 week in both NP-40 inactivated IBR and live IBR virus injected rabbits. In rabbits injected with live IBR virus, the S.I. dropped sharply (from 9 to 1.5) at 2 weeks post inoculation but gradually increased thereafter until 6 weeks. Stimulation indeces of lymphocytes from rabbits given NP-40 inactivated IBR virus reached 4 at 2

weeks and gradually declined to 0.5 at 6 weeks post inoculation. Both groups of rabbits, inoculated with either NP-40 extracted uninfected cells or normal saline, demonstrated SI less than 1 throughout the studies.

Kinetics of Interferon Synthesis

Interferon was first detected (titer 1:16) in lymphocyte cultures from rabbits injected with NP-40 inactivated virus at 24 hours (Fig. 3). Maximum interferon synthesis was observed at 48 and 72 hours when the interferon titers increased to 1:125. In rabbits vaccinated with live virus, interferon titers of 1:22 at 24 hours and 1:53 at 48 to 72 hours were observed. No interferon was synthesized in lymphocytes cultures from rabbits injected with normal saline.

Lymphocyte Interferon

Interferon was synthesized in lymphocytes of rabbits, injected with either live or NP-40 inactivated virus (Fig. 4). In rabbits injected with live virus, maximum interferon titers were observed between 1 and 2 weeks, then declined gradually until 6 weeks post inoculation. Interferon titers in rabbits injected with NP-40 inactivated virus reached 1:120 within 1 week, showed a small decrease at 2 weeks, then gradually increased until 6 weeks post inoculation (1:250). Interferon synthesis was not observed in lymphocytes of rabbits injected with normal saline, however a small increase

in titer (1:32) was observed during 2 to 4 weeks in lymphocytes of rabbits injected with NP-40 extracted antigen of uninfected cells.

Specificity of Lymphocytes Stimulation

Lymphocytes from IBR immune rabbits demonstrated S.I. of 1.7 when incubated with UV-inactivated IBR virus. However, S.I. of only 0.4 was observed in cells incubated with UV-inactivated BVD; thus indicating specificity of stimulation of lymphocytes sensitized to IBR virus (Table 1). Likewise, synthesis of interferon was observed only in lymphocytes stimulated with UV-inactivated IBR (titer 1:44).

Characterization of Lymphocyte Interferon

Lymphocyte interferon produced in response to UV-inactivated IBR virus was labile at 56° C for 1 hour and at pH 2 as indicated by % reduction of activity of 98 and 96 respectively (Table 2). The interferon activity was also destroyed by 0.1% trypsin. However, the activity was not affected by DNAase or RNAase treatment, since no reduction of interferon titers was observed.

Effect of Lymphocyte Interferon on Different Cell Types

Table 3 presents results on cell species specificity of lymphocyte interferon. Both rabbit lung and kidney cultures were protected against VSV challenge at interferon titers of 1:89 and 1:64 respectively. However, no interferon protection was observed in chicken embryo and bovine kidney cells with no demonstrable interferon activity (titer 0).

Lymphocyte Interferon Protection in Rabbit Lung Cultures Challenged With Different Viruses

Table 4 presents varying degree of interferon protection on lung cultures when challenged with 10, 50 and 100 $TCID_{50}$ of different viruses. Complete protection of cells was observed when challenged with VSV indicating maximum inhibitory effect of interferon against this virus. The degree of protection of cell cultures was in decreasing order against IBR, SFV, Adeno 3, Adeno 5 virus challenge with no protection being observed against PI-3 virus.

Inhibition of Lymphocyte Stimulation by Interferon

The incorporation of ^{3}HTdR into PHA stimulated lymphocytes was inhibited by interferon. A 9.2 fold increase of label was observed in PHA treated cultures without interferon as demonstrated by the increase in stimulation index (Table 5). Cultures stimulated with PHA in the presence of 4 units of interferon showed much decreased incorporation of ^{3}HTdR (S.I. = 1.7), indicating inhibition of lymphocyte blastogenesis by interferon.

Effect of Hydrocortisone on Cell Mediated Immune Response

Hydrocortisone inhibited CMI response of lymphocytes to UV-inactivated IBR virus or PHA as indicated by decreased incorporation of ^{3}HTdR into lymphocytes (Table 6). Maximum inhibition (71%-81%) occurred with 10^{-4} M. hydrocortisone. Interferon synthesis was also inhibited as demonstrated by 81% decrease in interferon titer. Lower degree of inhibition

of CMI response and interferon synthesis were observed with 10^{-5} M. and 10^{-6} M. hydrocortisone.

Infectious Bovine Rhinotracheitis Virus Growth in Lymphocytes

Figure 5 represents data on the replication of IBR virus in rabbits lymphocytes. Maximum DNA synthesis in infected cultures occurred at 24 hours post infection and was 3 times greater than uninfected lymphocytes. The synthesis of DNA declined at 48 to 72 hours. The increase in PFU titers (10 folds) correlated with DNA synthesis and plateaued thereafter until 72 hours post infection.

DISCUSSION

DISCUSSION

Humoral Immune Response

The results indicated that both live and NP-40 inactivated viruses were capable of eliciting specific neutralizing antibodies in rabbits. Serum antibody was not detected until after 1 week; it reached maximum titer of 1:24 at 2 weeks post inoculation (Fig. 1).

Production of antibody response to inactivated IBR vaccines has been controversial. Inoue et al. (1977) reported that no serum antibody was detected in rabbits after first inoculation of commercial inactivated vaccines; however, marked rise of serum antibody was observed after second injection of the vaccine. Matsuoka et al. (1972) reported high level of serum antibody in cattle inoculated with formalin inactivated IBR vaccines. In addition, the vaccine could protect the vaccinated cattle when challenged with virulent IBR virus. Karadjov (1976), Haralambiev (1976), Kristov (1976) and Kendrick (1973) also reported humoral immune response in cattle vaccinated with inactivated IBR vaccines. However, Schipper and Kelling (1975) suggested that no detectable neutralizing antibody was observed in cattle inoculated with inactivated vaccines.

Serum antibody, against herpes virus infection, though may not play a major role, has been reported to be important in preventing infection by the virus either from outside or

through circulation to target organs (Rouse and Babiuk, 1978). Openshaw (1979) reported the possible role of antibody to neutralize herpes simplex virus at the site of peripheral nerves, thus preventing viruses to enter the nerve terminal in immunized mice. The presence of local antibody in respiratory tract has been reported to be related to refractory state of cattle to IBR infection (Todd et al., 1971, 1972). Rouse and Babiuk (1978) suggested the possible role of antibody in preventing the recrudescence of IBR virus by interacting with complement or leukocytes. Thus, the humoral immune status of animals may be important in determining the spread of IBR virus in the body.

In regard to these important findings, NP-40 inactivated IBR virus with adjuvant in the present study produced adequate serum antibody response in rabbits and thus appeared to be a good immunogen.

Cell Mediated Immune Response

The CMI response measured by ^{3}HTdR incorporation into lymphocytes in the presence of UV-inactivated IBR virus was observed before serum antibody was detected (Fig. 1 and Fig. 2). This indicated the possible role of CMI response in protection during early phases of IBR infection. Comparing the results of in vitro studies on CMI response and relating these to the onset of recovery as judged by clinical criteria, Davies and Carmichael (1973) reported that lymphocyte

transformation response occurred at the time of recovery from IBR virus infection. They also reported the appearance of CMI response at 5 days post inoculation, as measured by ^{3}HTdR incorporation into lymphocytes; however, the serum antibody was not detected until 14 days post inoculation. Our results confirmed their investigation and thus indicated the important role of CMI response in early protection and determining the recovery from IBR virus infection.

The marked drop in CMI response (S.I. value of 9 at 1 week to 1.5 at 2 weeks) may be explained in many ways. It has been reported that IBR virus induced viral antigens on the surface of infected cells which can be detected by immunofluorescence technique (Babiuk and Rouse, 1975). Our preliminary experiments indicated that IBR virus could replicate in rabbit lymphocytes as determined by plaque assay and ^{3}HTdR incorporation into IBR virus infected lymphocytes (Fig. 5). It is possible that infected lymphocytes with surface IBR antigens were destroyed by either antibody which was maximum at 2 weeks post inoculation or the sensitized T lymphocytes resulted in the lysis of infected lymphocytes. The other possible explanation was that IBR virus suppressed CMI response in infected animals. Johnson and Muscoplat (1973) reported that BVD virus suppressed T lymphocytes response to mitogen in BVD infected cattle. Cummins and Rosenquist (1978) also reported that IBR infection caused

lymphopenia in infected cattle. It was also possible that IBR virus stimulated the suppressor T lymphocytes; thus resulting in suppression of T cell functions. The recovery of CMI response after 2 weeks was probably due to the recognition and destruction of infected lymphocytes, thus diminishing the chances of spread of IBR virus. Also, the replenishing of T lymphocytes in the body might aid in the recovery process.

Lymphocyte stimulation in response to UV-inactivated IBR virus was specific since exposure to BVD virus did not demonstrate neither lymphocyte blastogenesis or interferon production (Table 1). Rouse and Babiuk (1974) also reported the specificity of blastogenesis of bovine IBR-immune lymphocytes in response to antigen by demonstrating the incapability of herpes simplex virus (HSV) and equine rhinopneumonitis virus to stimulate the proliferation of IBR-immune lymphocytes.

It is possible that part of ^{3}HTdR incorporation into lymphocytes in our studies may have been due to the presence of some bone marrow (B) derived lymphocytes in purified mixed lymphocyte preparation. Although both B and T lymphocytes were present in mixed lymphocyte cultures, most of the peripheral blood lymphocytes (80%) were of thymic origin (Davies et al., 1973). In addition, separate in vitro stimulation of purified B and T lymphocytes by specific

viral antigen demonstrated that the assay was specific for T lymphocytes (Rouse and Babiuk, 1974). Thus, the incorporation of ^{3}HTdR into mixed lymphocyte cultures must represent primarily T lymphocytes stimulation.

There was no relationship observed between CMI response and the amount of interferon produced in rabbits (Fig. 2 and Fig. 4). The degree of CMI response in rabbits injected with live IBR virus was 2 fold higher at 1 week than in rabbits injected with NP-40 inactivated IBR virus (Fig. 2). However, the interferon level was almost identical at that time (Fig. 4). At 6 weeks post inoculation, interferon production was highest, however, CMI response was very low in NP-40 extracted virus inoculated rabbits. In rabbits given live virus, CMI response was highest at 6 weeks when interferon synthesis declined to its lowest level (Fig. 4).

From studies on the kinetics of interferon synthesis the peak interferon activity occurred at 48-72 hours after stimulation (Fig. 3). Mansfield and Wallace (1973) reported that peak of PHA stimulation of rabbit lymphocytes was observed at 72-96 hours. Together, these results supported the idea of Rouse and Babiuk (1978) regarding the different subset of lymphocyte population responsible for proliferation and interferon production. These investigators found that peaks of lymphocyte proliferation measured by ^{3}HTdR incorporation into lymphocytes occurred at 4-5 days post stimulation.

However, peak of interferon production was observed at 24-48 hours. It was possible that low degree of CMI response in rabbits with high interferon was due to immunosuppressive effect of interferon (Brodeur and Merigan, 1975; Wallen, 1975). Our studies on inhibition of ^{3}HTdR incorporation by interferon in PHA stimulated lymphocyte support this hypothesis (Table 5).

Whether or not T or B lymphocytes were responsible for interferon production in our studies is not clear. Rasmussen et al. (1974) and Valle et al. (1975) demonstrated that human immune T lymphocytes were the source of interferon production in response to herpes simplex antigen, since elimination of the T lymphocytes by treatment with anti-T lymphocyte serum plus complement diminished the amount of interferon produced. Babiuk and Rouse (1976) reported that bovine immune interferon was produced by T lymphocytes in response to UV-inactivated IBR antigen, thus indicating that T cells may be the major source of interferon production.

Our results on the inhibitory effect of lymphocyte interferon (Table 4) are in accord with the data reported by Babiuk and Rouse (1976). They demonstrated that IBR-induced lymphocyte interferon could inhibit IBR virus replication, thus suggesting that the virus might be sensitive to type II interferon. It had been previously reported that IBR virus was insensitive to type I interferon (Trueblood, 1973).

Characterization of Interferon

The supernatant of samples from immune lymphocytes, incubated with UV-inactivated IBR virus and centrifuged at 100,000 g, demonstrated the characteristics usually attributed to interferon which is sensitive to heat (56° C, 1 hour) and pH 2 (Table 2). The activity was also destroyed by 0.1% trypsin but not by DNAase or RNAase treatment. The instability to heat and pH 2 of immune interferon produced by rabbit macrophage in response to mitogen was also reported by Smith and Wagner (1967). Immune interferon produced by human lymphocytes in response to herpes simplex antigen stimulation was sensitive to heat and pH 2 (Valle et al., 1975). However, instability to heat and pH 2 were not typical characteristic for all immune interferons studied since Younger (1977) reported that mouse immune interferon was labile at pH 2 but not at 56° C. Rouse and Babiuk (1978) also reported that bovine immune interferon produced by immune lymphocytes in response to UV-IBR virus was sensitive to pH 2 but not to heat. The reduction of interferon activity by trypsin but not by DNAase or RNAase treatment was demonstrable since interferon was glycoprotein molecule which could be digested by trypsin and not by the nucleases.

Rabbit immune lymphocyte interferon showed species specific antiviral activity as it did not protect chick embryo and bovine kidney cultures from VSV challenge (Table 3).

The results did not support the work of Gressor et al. (1974). They reported marked heterologous antiviral activity of human immune interferon on porcine and bovine cell cultures. Rouse and Babiuk (1978) also reported the heterologous antiviral activity of bovine immune interferon on porcine cell cultures. It is probable that the cell cultures used in our studies do not possess receptor sites for rabbit interferon. It may be possible to observe heterologous antiviral activity of rabbit immune interferon on cell cultures other than chick embryo or bovine kidney. At present, there has been no study reported on the heterologous antiviral activity of rabbit immune interferon.

Rabbit immune interferon demonstrated varying degree of antiviral effect against different viruses on rabbit lung cultures (Table 4). Among the viruses tested, VSV was the most sensitive to antiviral effect of rabbit immune interferon followed by IBR, SFV, Adeno 3, Adeno 5 and PI-3 viruses. Trueblood and Manjara (1972), using CPE inhibition test, demonstrated that VSV but not IBR was inhibited by interferon produced by bovine embryo kidney cells or chick embryo fibroblast cells in response to either IBR, PI-3, Newcastle Disease virus (NDV) or influenza viruses. These results suggested that interferon produced in rabbit lymphocytes in response to IBR virus was different from those made in bovine or chick embryo cells. It further pointed out that lymphocyte

interferon might have wide range of antiviral activity against different viruses. This suggests that interferon produced in IBR virus sensitized lymphocytes may be responsible to protect the animal from IBR infection.

Effect of Hydrocortisone on Cell Mediated Immune Response

Both cell mediated immune response and interferon production were inhibited by hydrocortisone (Table 6). Suppression of CMI response was not unexpected since the suppression of immune response by corticosteroid to virus infection in animals had been reported by many investigators (Mendelson and Finland, 1966; Reinicke, 1965; Kass and Finland, 1958; Kilbourne, 1955, 1951). Fulton and Rosenquist (1976) reported the decrease of interferon production induced by IBR virus in bovine fetal spleen and peripheral blood leukocyte cultures after the cells were incubated with hydrocortisone. These parameters may help to explain the effect of stress on decreased host resistance to viral infections in cattle. The viral respiratory disease often occurs in cattle subsequent to exposure to stress such as inclement weather, prolonged transportation, improper handling and vaccinations. It is possible that corticosteroids generated by stressful conditions may influence these viral infections by depressing CMI response resulting in decreased interferon production.

The effect of corticosteroid on interferon production in vivo is controversial. Cummins and Rosenquist (1977, 1978) reported increase of serum interferon in cattle, previously treated with hydrocortisone, when they were injected with IBR virus. They suggested that hydrocortisone resistant lymphocyte population might have been responsible for interferon production during IBR infection.

Further investigation on the role of CMI response and interferon during herpes virus infection should be continued in order to understand the mechanism of resistance and the process of recovery following IBR infection in cattle. In addition, it will help in the development of vaccines that will protect cattle or other animals against herpes virus infection.

TABLES AND GRAPHS

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Figure 1. Appearance of Serum Antibody in Rabbits. Two fold dilutions of heat inactivated serum were incubated with 100 $TCID_{50}$ of IBR virus for 1 hour at room temperature before the mixtures were inoculated in secondary rabbit lung cultures. Cells were examined for cytopathic effects at 48-72 hours post inoculation. Antibody titer as determined by Karber's method was the highest serum dilution that protected 50% lung cultures. Symbols: NP-40 extracted virus (△); live virus (▲); NP-40 extracted uninfected cells (●); normal saline (○).

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SERUM ANTIBODY RESPONSE IN RABBITS

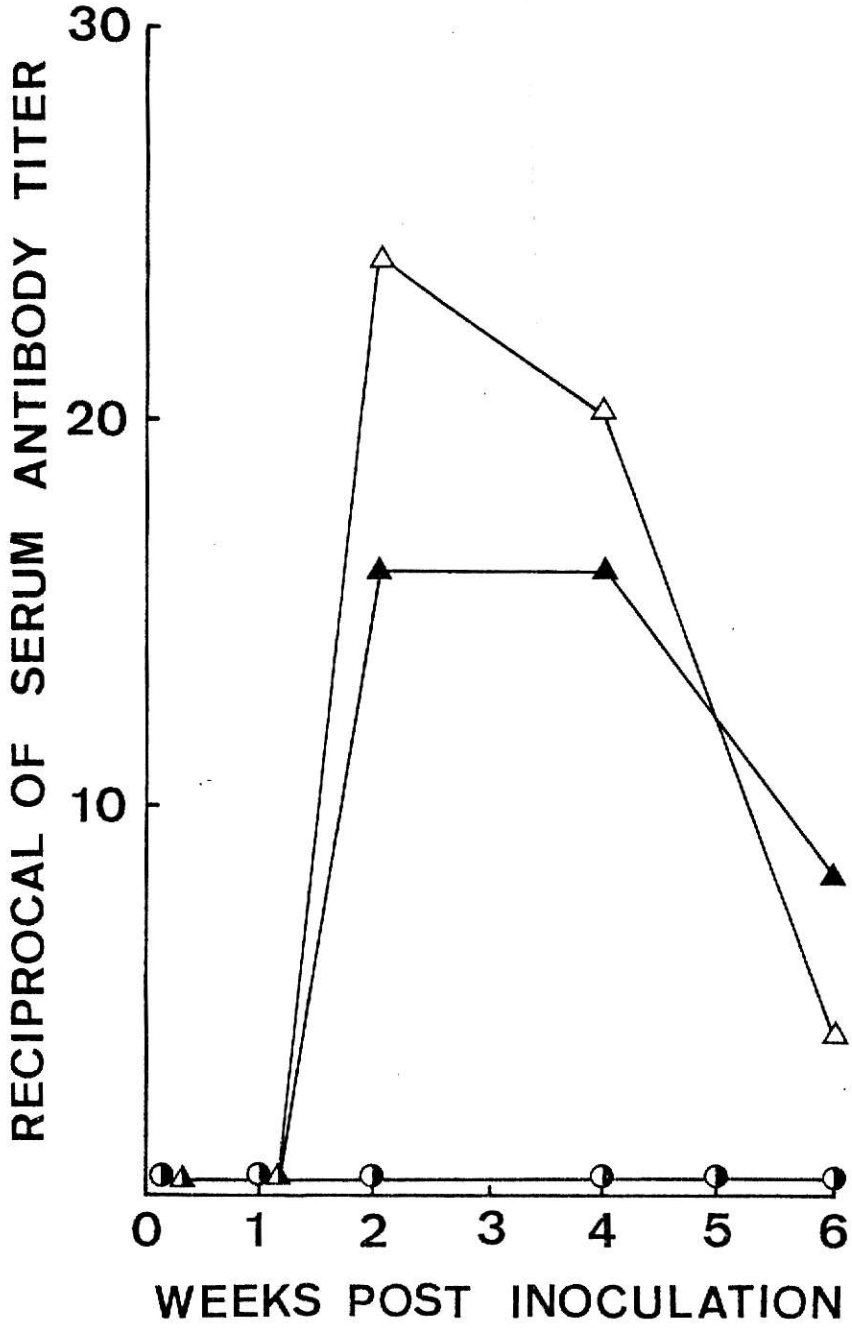


Figure 2. Cell Mediated Immune Response. Lymphocyte cultures in duplicate from each rabbit containing 2×10^6 cells per culture were incubated with 3×10^6 PFU of UV-inactivated IBR virus for 72 hours. Samples were labelled with 1uCi of ^{3}HTdR 24 hours prior to harvest. At termination of cultures, cells were washed, precipitated with TCA, collected on GFC filters and counted in scintillation spectrometer. Stimulation indeces were determined on the basis of ratio of DPM in stimulated lymphocyte cultures over unstimulated cultures. Stimulation indeces of lymphocytes from rabbits injected with NP-40 extracted virus (Δ); live virus (▲); normal saline (O); and NP-40 extracted uninfected cell (●) at different time interval are presented.

CELL MEDIATED IMMUNE RESPONSE IN PERIPHERAL BLOOD LYMPHOCYTES

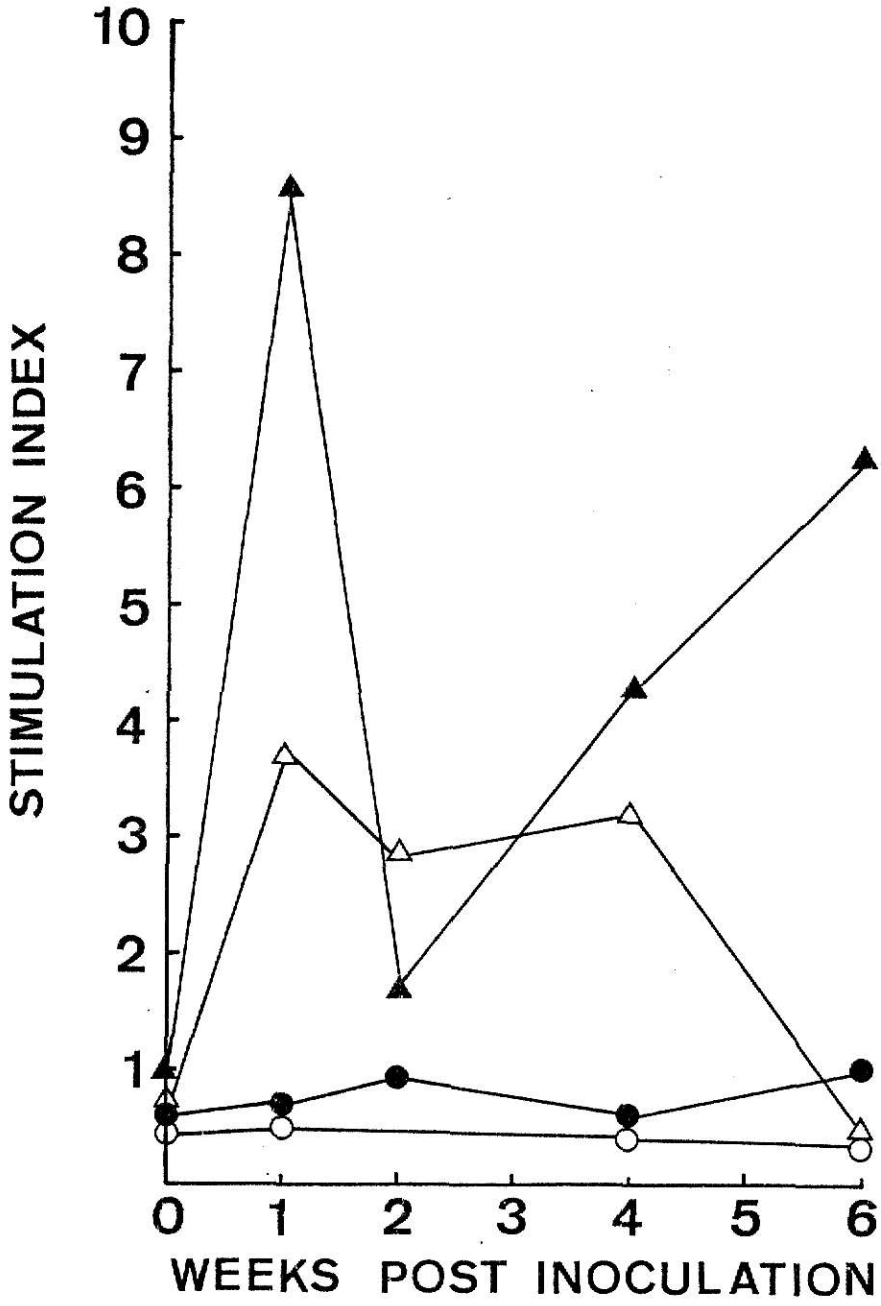


Figure 3. Time Course of Interferon Synthesis in Stimulated Lymphocyte Cultures. Lymphocyte cultures containing 2 x 10^6 cells from immune and nonimmune rabbits were incubated with 3 x 10^6 PFU of UV-inactivated IBR virus. At various time the cultures were harvested, frozen, thawed and ultracentrifuged. The interferon titers in the supernate were determined as the reciprocal of highest dilution of interferon that protected 50% secondary rabbit lung cultures against 100 $TCID_{50}$ of VSV challenge. Time course of interferon synthesis in lymphocytes from rabbits injected with NP_{40}-extracted virus (Δ); live virus (▲); or normal saline (O) are shown.

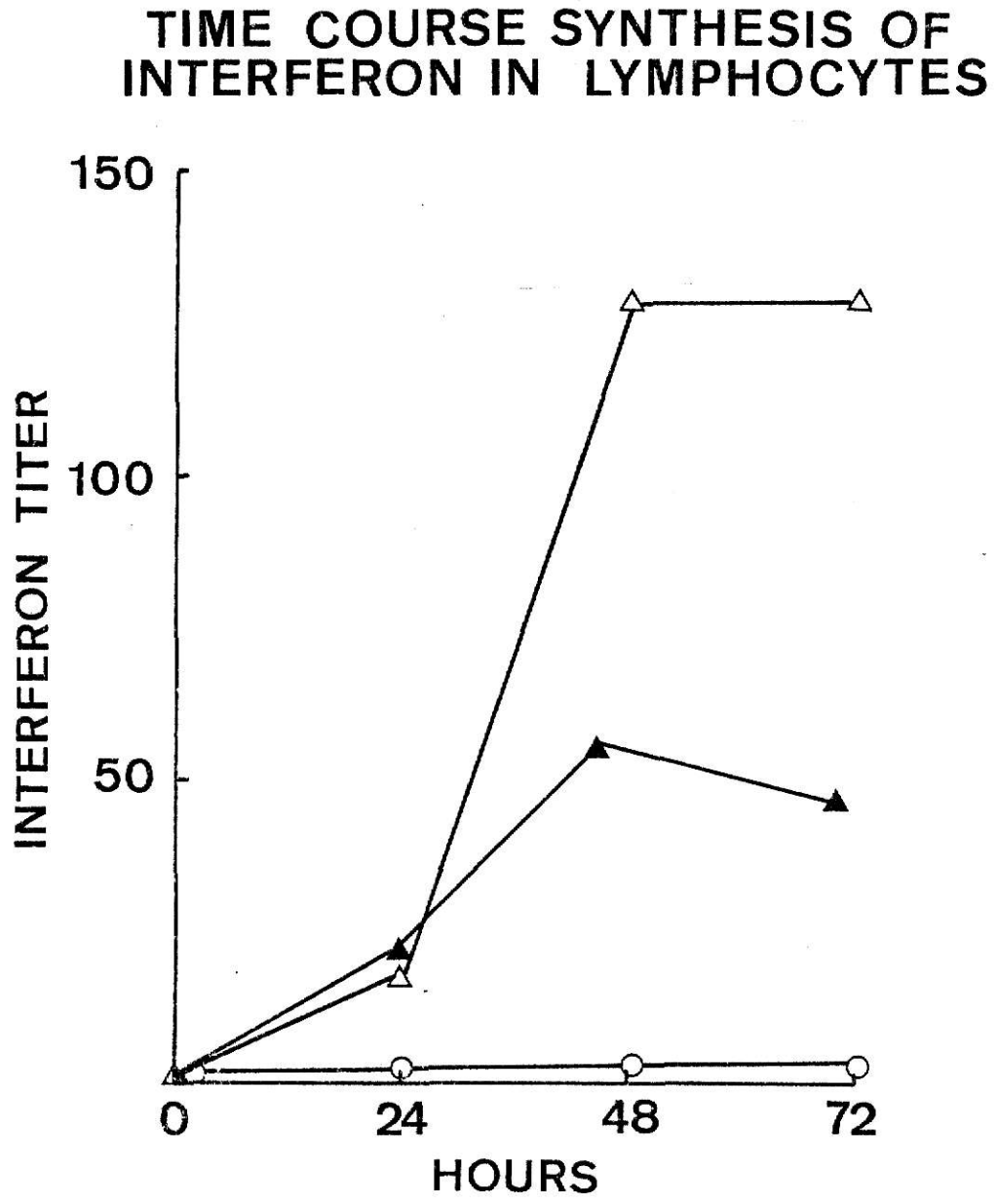

TIME COURSE SYNTHESIS OF
INTERFERON IN LYMPHOCYTES
150
100
50
INTERFERON TITER
0
24
48
72
HOURS

Figure 4. Interferon Production in Peripheral Blood Lymphocytes Stimulated by Specific Virus Antigen. Lymphocytes cultures containing 2 x 10^6 cells from each rabbit were incubated with 3 x 10^6 PFU of UV-inactivated IBR virus for 72 hours; then frozen, thawed and ultracentrifuged. The interferon assay was performed on secondary rabbit lung cultures. The interferon titers were expressed as the reciprocal of the dilution that protected 50% lung cultures challenged with 100 $TCID_{50}$ of VSV. Symbols: NP-40 extracted virus (△); live virus (▲); NP-40 extracted uninfected cells (●); normal saline (○).

INTERFERON PRODUCTION IN PERIPHERAL BLOOD LYMPHOCYTES IN RABBITS

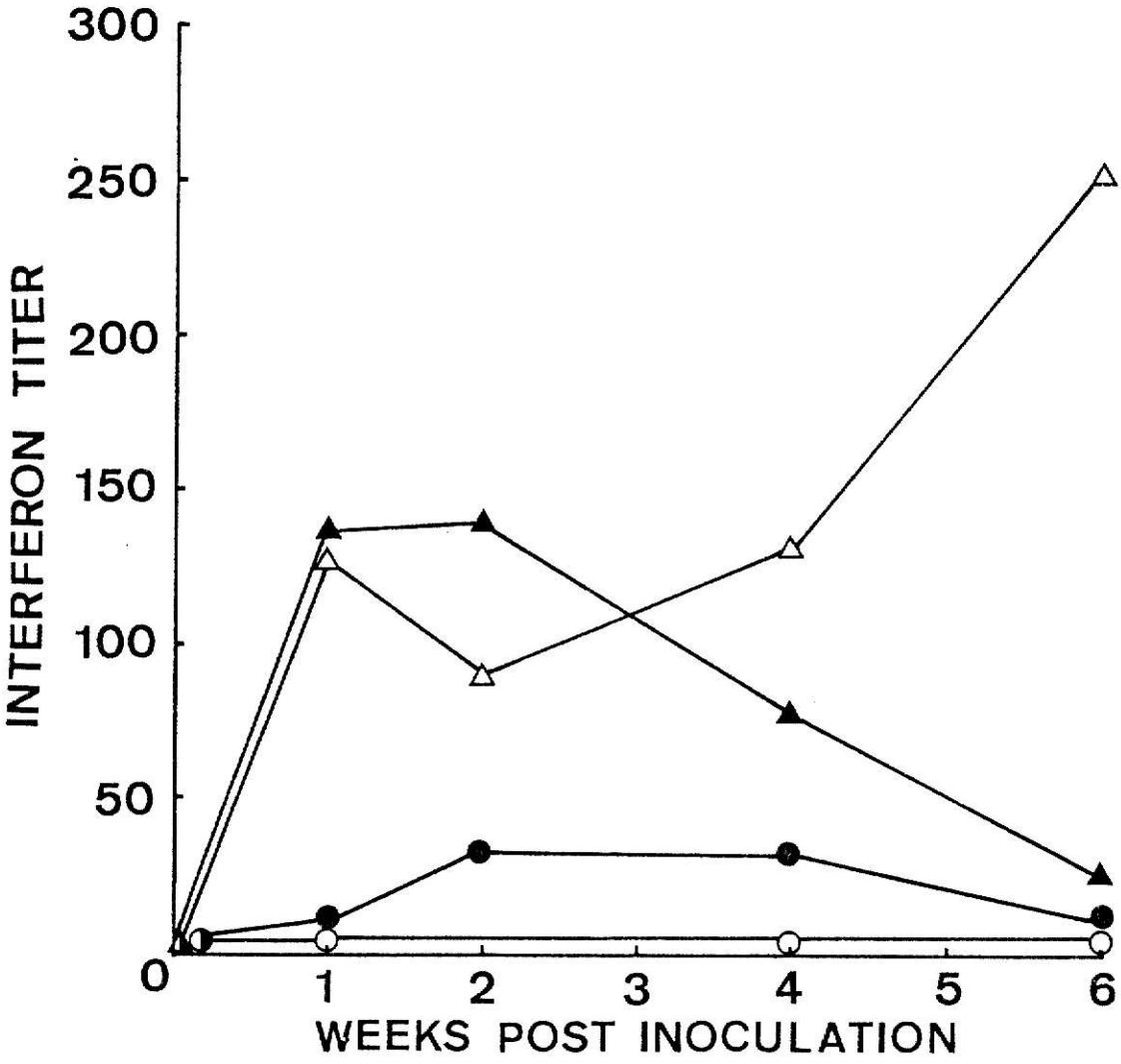
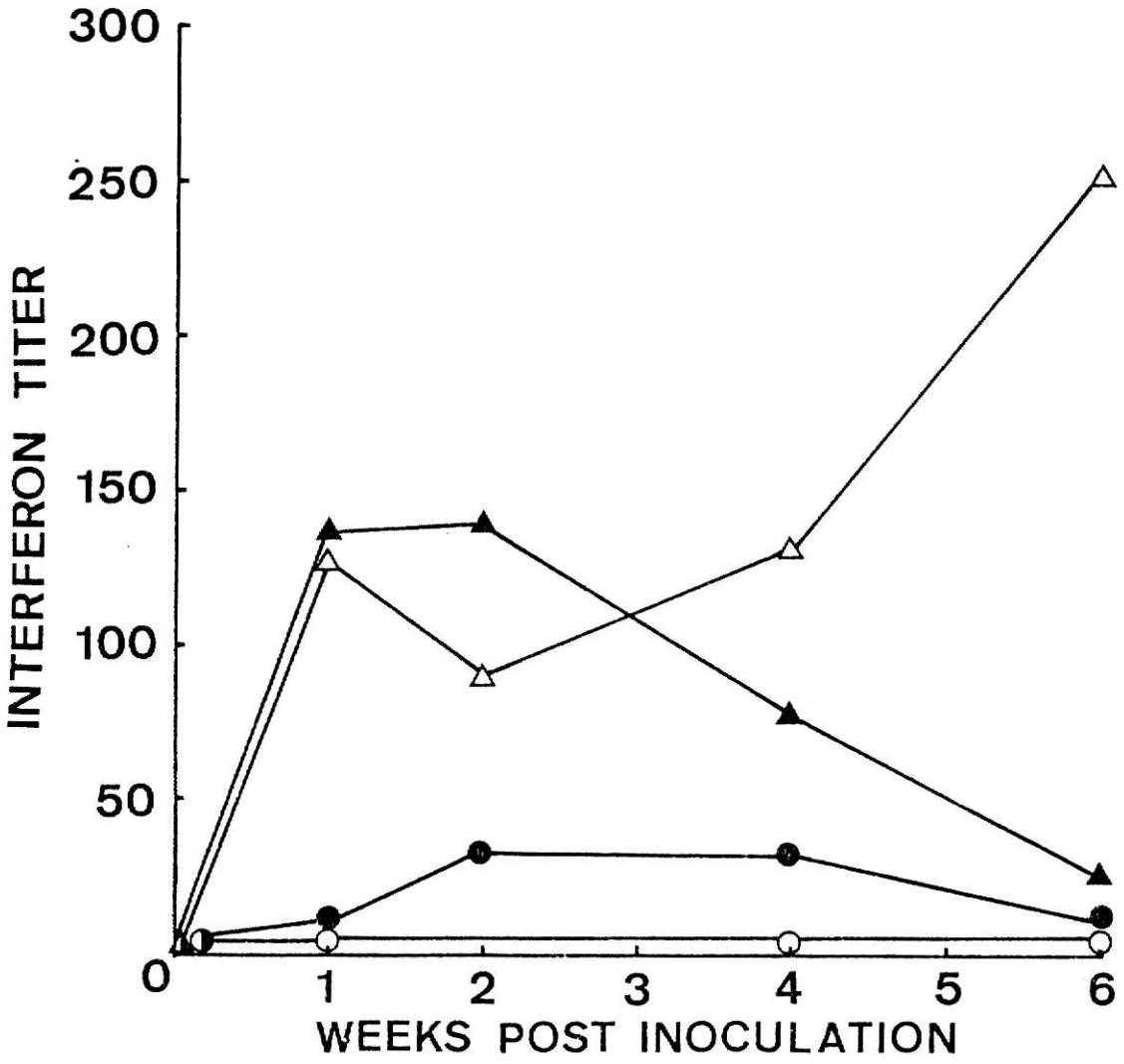


Figure 5. Replication of Infectious Bovine Rhinotracheitis Virus in Rabbit Lymphocytes. Lymphocytes from nonimmune rabbit were incubated MEM or IBR virus at a multiplicity of 1 for 2 hours, washed and resuspended in growth media (0.5 uCi ^{3} HTdR/ml) to contain 1 x 10^{6} cells/ml. Cultures were harvested at 0, 24, 48 and 72 hours post inoculation and the virus infectivity was determined by plaque assay. The amount of protein and ^{3}HTdR incorporation in lymphocyte cultures were determined and specific activities were calculated on the basis of ratio of DPM/ug protein. Symbols: infected lymphocyte cultures (△); uninfected cultures (▲); PFU/ml (●).

INTERFERON PRODUCTION IN PERIPHERAL BLOOD LYMPHOCYTES IN RABBITS



INFECTIOUS BOVINE RHINOTRACHEITIS
VIRUS GROWTH IN RABBIT LYMPHOCYTES

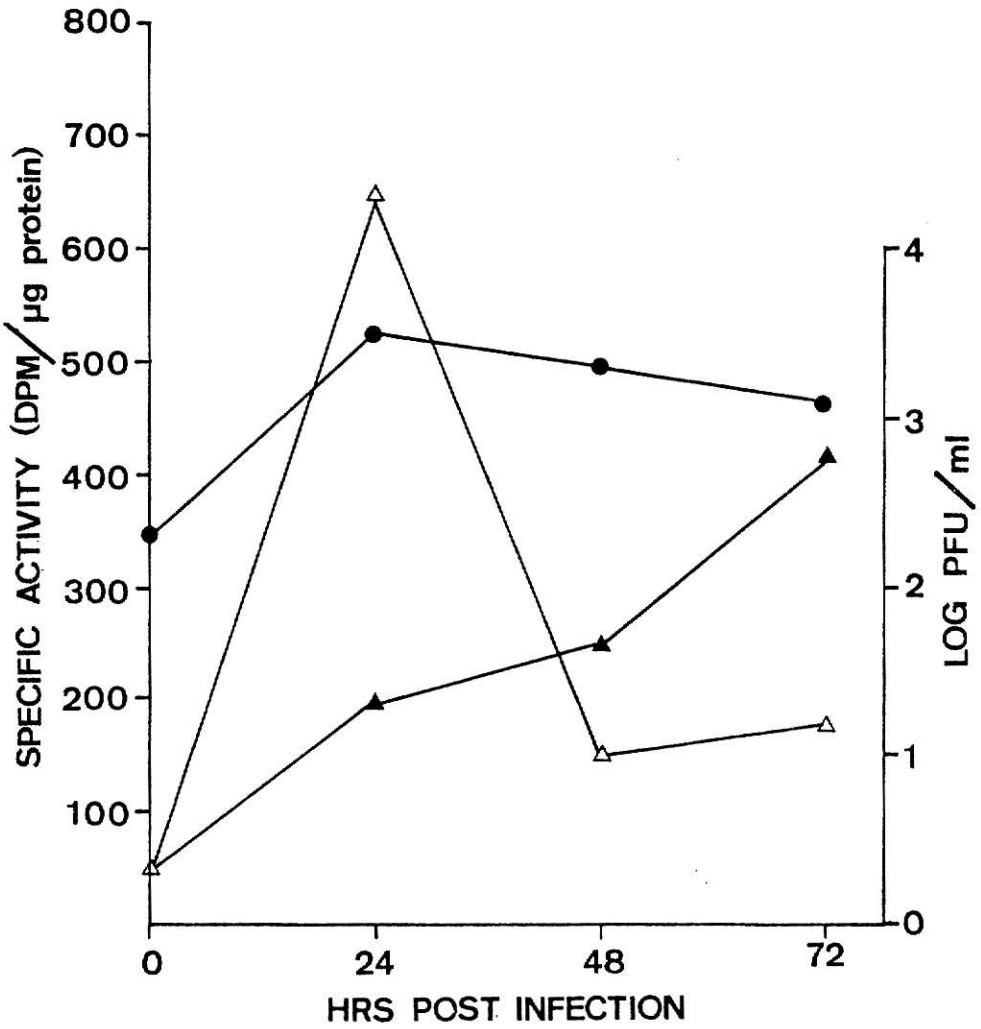


Table 1

SPECIFIC STIMULATION OF IBR VIRUS SENSITIZED LYMPHOCYTES

UV Irradiated Virus	DPM*	Stimulation Index	Interferon** Titer
None	8,688	1	0
IBR	14,926	1.7	44
BVD	3,962	0.4	0

*Incorporation of ^{3}HTdR into stimulated lymphocytes.

**Reciprocal of highest dilution that protected 50% rabbit lung cultures against 100 $TCID_{50}$ VSV challenge.

Table 2

CHARACTERIZATION OF INTERFERON

	Interferon Titer*	% Reduction of Activity
No Treatment	177	0
Heat 56° C, 1 Hr.	4	98
pH 2	6	96
Trypsin 0.1%	26	85
RNAase	> 160	0
DNAase	> 160	0

*Challenged with 100 $TCID_{50}$ of vesicular stomatitis virus.

Table 3

EFFECT OF RABBIT LYMPHOCYTE INTERFERON
ON DIFFERENT CELL TYPES

Cultures	Interferon Titer*	Protection
Rabbit Lung	89	+
Rabbit Kidney	64	+
Chick Embryo	0	-
Bovine Kidney	0	-

*Reciprocal of highest dilution that protected 50% rabbit lung cultures against 100 $TCID_{50}$ VSV challenge.

Table 4

PROTECTION OF RABBIT LUNG CULTURES BY LYMPHOCYTE INTERFERON AGAINST VIRUSES

Virus Challenge	Degree of Protection*		
	10 $TCID_{50}$	50 $TCID_{50}$	100 $TCID_{50}$
Vesicular Stomatitis Virus	++++	++++	++++
Infectious Bovine Rhinotracheitis Virus	++++	++	-
Shope Rabbit Fibroma Virus	+++	+	-
Bovine Adeno 3 Virus	++	-	-
Bovine Adeno 5 Virus	++	-	-
Bovine Parainfluenza-3 Virus	-	-	-

* ++++ = 100%
+++ = 75%
++ = 50%
+ = 25%
- = None

Table 5

INHIBITION OF LYMPHOCYTE STIMULATION BY INTERFERON

Treatment of Lymphocyte Cultures	DPM*	Stimulation Index
None	3,850	1
PHA (2.5 ug/ml)	35,616	9.2
PHA + Interferon (4 units)	6,904	1.7

*Incorporation of ^{3}HTdR into stimulated lymphocytes.

Table 6

EFFECT OF HYDROCORTISONE ON LYMPHOCYTES

Concentration (M) Hydrocortisone	% Inhibition		
	Interferon*	CMI Response**	PHA Response**
0	0	0	0
10^{-6}	38	66	56
10^{-5}	63	66	59
10^{-4}	81	71	81

*Protection of 50% secondary rabbit lung cultures challenged with 100 $TCID_{50}$ of vesicular stomatitis virus.

**Incorporation of ^{3}HTdR into lymphocytes.

DETAILED METHODOLOGY AND REAGENTS

Detailed Methodology

Calculation of Virus, Antibody and Interferon Titers

Karber Formula:

$$\text{Log } TCID_{50} = L\text{-}d\ (S\text{-}0.5) \quad \text{(Karber, 1931)}$$

L = log lower dilution

d = difference between log dilution steps

S = sum of proportions of tissue cultures protected.

Protein Determination

Crystalline bovine albumin was used as a standard protein (1 mg/ml). Standard curves of 10, 20, 30, 40, 50, 75 and 100 ug of bovine albumin were determined. Standard and experimental samples were made to 1.0 ml with distilled water, then 5 ml of solution B was added and mixtures were incubated for 10 minutes at room temperature. As soon as 0.5 ml of folin solution was added, each tube was rapidly mixed, reincubated for 30 minutes at room temperature and read in Klett (#66 filter). The amount of protein in each sample was extrapolated from standard curve.

Reagents

Agar: 2% purified agar is dissolved in deionized distilled water and autoclaved for use. A mixture of 50% agar - 50% 2 X MEM is used for plaquing.

Antibiotics:

Stock solution (100 ml) contains

Penicillin G (1,000,000 units)

Streptomycin (1 g)

Kanamycin (50 mg)

One ml of PSK stock solution is added to give a final concentration of: 100 unit penicillin, 10 ug streptomycin and 5 ug kanamycin per ml media.

DNAase: 2 mg/ml, stock. A final concentration of 40 ug/ml is used.

Hanks Balanced Salt Solutions (HBSS):

10 X HBSS contains:

Solution #1

1) NaCl	100 g	Dissolve in 500 ml
2) KCL	5 g	deionized water.
3) $MgSO_4.7H_2O$	2.5 g	

Solution #2

1) $Na_2HPO_4.7H_2O$	1.4 g	Dissolve in 500 ml
(or $12H_2O$)	1.9 g	deionized water.
2) KH_2PO_4	0.8 g	

Solution #3

$CaCl_2.2H_20$	2.3 g	Dissolve in 125 ml deionized water.

Combine solution #1 and #2, add 250 ml deionized water, shake well and add solution #3. Add 0.25 g of phenol red.

Complete Hanks (1 X):

10X Hanks	100 ml
Antibiotics	10 ml
Fungizone	0.2 ml
Dextrose (10%)	10 ml
Deionized water	1000 ml

$NaHCO_3$ (7.5%) to pH 7.4 or 7.6

Filter to sterilize for use.

Ficoll-Hypaque:

Ficoll 24.3 g is dissolved in 300 ml of deionized water and 50 ml of Isopaque is added to give a density of 1.077 at 25° C. Ficoll-Hypaque is autoclaved for use.

Folin and Ciocalteu's Phenol Reagent:

Dilution of 1 part of folin to 2 parts of distilled water is used.

Fungizone:

50 mg (one vial) plus 20 ml sterile deionized water. Add 0.2 ml/liter of media.

Hydrocortisone:

25 mg, dissolved in 5 ml of acetone alcohol (1:1) mixture to give stock of 5 mg/ml. Diluted in RPMI-1640 to 10^{-2} M. Final concentrations of 10^{-6}, 10^{-5} and 10^{-4}M are used.

Neutral Red:

A solution (0.01%) for plaquing is prepared from powder with deionized water and dissolved in 37° C water bath. Whatman #2 filter is used for filtration. Sterilized by autoclaving and stored at 4° C, protected from light. Add 1 ml of stock/ 100 ml of agar and media.

Nonidet P-40 (NP-40):

2% of NP-40 (v/v) in tris buffer (pH 7.0) is used.

Phytohemagglutinin (PHA):

100 ug/ml in RPMI-1640 stock. A final concentration of 2.5 ug/ml is used.

Potassium Chloride Buffer (0.1 M KCl buffer, pH 2):

74.5 g of KCl is dissolved in 1000 ml of deionized distilled water. Concentrated HCl is used to adjust pH.

Phosphate-Buffered Saline (PBS):

Solution #1

$Na_2HPO_4.7H_2O$ 134 g/l deionized H_2O

Solution #2

$NaH_2PO_4.H_2O$	69.1 g/l deionized H_2O

Buffer Stock:

$Na_2HPO_4.7H_2O$	386 ml - solution #1
$NaH_2PO_4.H_2O$	114 ml - solution #2
Deionized water	500 ml
	pH 7.15

PBS:

80 ml	buffer stock
17 g	NaCl
1920 ml	deionized H_2O

pH 7.2

Autoclave at 15 lbs. for 15 minutes.

RNAase: 2 mg/ml, stock. A final concentration of 40 ug/ml is used.

Solution A:

Na_2CO_3 (2 g) is dissolved in 100 ml of 0.1 N NaOH.

Solution B:

0.5 ml of 1% $CuSO_4.5H_2O$ plus 0.5 ml of 2% NaK tartrate in 50 ml of solution A.

Tris Buffer (Hydroxymethy aminomethane, 0.1 N, pH 7.0):

12.1 g of Tris is dissolved in 1,000 ml of deionized distilled water, HCl is used to adjust pH. Autoclaved for use.

Trypsin: 1 g/400 ml trypsin in PBS made and frozen for use.

Working Solution:

0.25% trypsin	20 ml
PBS	30 ml
10% EDTA	0.5 ml

REAGENTS AND ADDRESSES

Agar	BBL, Cockeysville, MD
Cupric Sulphate ($CuSO_4.5H_20$)	Mallinckrodt Chemical Co., St. Louis, MO
DNAase	Mallinckrodt Chemical Co., St. Louis, MO
Fetal Calf Serum	Kansas City Biological Co., Kansas City, KS
Ficoll	Sigma Chemical Co., St. Louis, MO
Folin and Ciocalteu's Phenol Reagent	Sigma Chemical Co., St. Louis, MO
Fungizone	E. R. Squibb and Sons, NY
Horse Serum	Kansas City Biological Co., Kansas City, KS
Hydrochloric Acid (HCl)	Mallinckrodt Chemical Co., St. Louis, MO
Hydrocortisone	Sigma Chemical Co., St. Louis, MO
Isopaque 440	Winthrop Laboratory, NY
Kanamycin	Bristol Laboratories, Syracuse, NY
Minimal Essential Media (MEM)	Grand Island Biological Co., Grand Island, NY
Neutral Red	Fisher Scientific Co., Fairlawn, NJ
Nonidet P-40	Sigma Chemical Co., St. Louis, MO
Penicillin G	E. R. Squibb and Sons, NY
Phytohemagglutinin	Sigma Chemical Co., St. Louis, MO

Potassium Chloride (KCl)	J. T. Baker Chemical Co., Phillipsburg, NJ
RNAase	Sigma Chemical Co., St. Louis, MO
RPMI-1640	Grand Island Biological Co., Grand Island, NY
Scintillation Fluid	Research Products International Corp., IL
Sodium Carbonate (Na_2CO_3)	Mallinckrodt Chemical Co., St. Louis, MO
Sodium Potassium Tartrate (Nak Tartrate)	Mallinckrodt Chemical Co., St. Louis, MO
Streptomycin	Eli Lily, Indianapolis, IN
Trichloroacetic Acid (TCA)	Mallinckrodt Chemical Co., St. Louis, MO
Tritiated Thymidine	Schwarz/mann Orangeburg, NY
Trypsin	Difco, Detroit, MI

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IMMUNE RESPONSE TO NONIDET P-40 EXTRACTED INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS ANTIGEN IN RABBITS

by

BOONDEE ATIKIJ

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ABSTRACT

Infectious bovine rhinotracheitis (IBR) virus antigen completely free of infectious particles was investigated for the production of immune response and interferon synthesis in rabbits. The antigen was prepared by extracting IBR virus infected secondary rabbit kidney cultures with NP-40 and emulsifying the supernatant with equal volume of Freund's incomplete adjuvant. Each group of 2 rabbits was intramuscularly inoculated with antigen from either virus-infected cells or uninfected cells or live virus.

Humoral immune response measured by serum neutralization was not detected until 2 weeks post inoculation. Maximum serum antibody titers (1:16 to 1:24) were observed in rabbits injected with either live or NP-40 inactivated virus at 2 weeks post inoculation.

Cell mediated immune response in peripheral blood lymphocytes purified over Ficoll-Hypaque as measured by ^{3}H-Thymidine (^{3}HTdR) incorporation was observed within 1 week post inoculation. Stimulation indices (SI) were calculated by the ratio of disintegration per minute (DPM) in lymphocytes cultures stimulated with UV-irradiated IBR virus over unstimulated control cultures. Maximum stimulation indices of 4 and 9 were observed in lymphocytes cultures of rabbits injected with NP-40 inactivated and live IBR virus antigen respectively, at 1 week post inoculation. A sharp drop in

SI of lymphocytes (1.5) from rabbits injected with live IBR was observed at 2 weeks, then gradually increased to 6 in the following 4 weeks. Rabbits injected with either extracted antigen from uninfected cells or normal saline demonstrated SI of <1 during this period.

Lymphocyte interferon synthesis was studied by incubating rabbits lymphocyte cultures with UV-inactivated IBR virus for 72 hours. Cultures were frozen, thawed; ultracentrifuged at 100,000 g and the supernatant was assayed for interferon on secondary rabbit lung cultures in microtiter plates using 100 Tissue Culture Infectious Dose 50 (TCID 50) of Vesicular Stomatitis Virus (VSV) as challenge. Maximum interferon synthesis was observed at 2 weeks (titer = 1:120), then declined gradually until 6 weeks in lymphocytes from rabbits injected with live virus. A titer of 1:120 at 1 week that declined to 1:90 and gradually increased to 1:250 at 6 weeks was observed in interferon preparations of lymphocytes from rabbits given NP-40 extracted antigen from virus infected cultures.

Characterization of lymphocyte interferon demonstrated that it was labile at 56^{o} C for 1 hour and at pH 2, and was inactivated by 0.1% trypsin but not by DNAase and RNAase. Species specific antiviral interferon activity was observed since protection against VSV challenge occurred on rabbit cells only. Virus specific activity was demonstrated in

decreasing order of protection of rabbit lung cultures, challenged with VSV, IBR virus, Rabbit Shope Fibroma virus, Bovine Adeno 3 virus, Bovine Adeno 5 virus and Bovine Parainfluenza-3 virus.