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P.P. PASTORET, E. THIRY, B. BROCHIER and G. DERBOVEN

Department of Virology, Faculty of Veterinary Medicine, University of Liège, 45, rue des Vétérinaires, B-1070 Brussels, Belgium

General plan

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1. Brief history of the disease associated with *Bovid herpesvirus 1* infection of cattle

Infectious Bovine Rhinotracheitis (IBR) is a respiratory disease of cattle, first described in the United States twenty-eight years ago (Schroeder and Moys, 1954 ; McKercher *et al.,* 1954 ; Jensen *et al.,* 1955 ; Miller, 1955).

Infectious bovine Pustular Vulvovaginitis (IPV) is a venereal disease of cattle known for a long time in Europe, especially in Germany, where it was described for the first time in the middle of the nineteenth century by Büchner (cited by Kokles, 1967) ; in 1894. Trommsdorf gave it its definite name : *Bläschenausschlag.* The same venereal disease was described in the United States for the first time in 1895 (Steddom, 1895, cited by Kendrick *et al.,* 1958) which means that infectious bovine pustular vulvovaginitis existed before infectious bovine rhinotracheitis both in Europe and in the United States.

Virus isolation was first reported in 1956 from cases of infectious rhinotracheitis which occurred in cattle gathered into feedlots in the United States (Madin *et al.,* 1956 ; York *et al.,* 1957). This isolate was shown to be serologically identical to other isolates originating from cases of infectious pustular vulvovaginitis (Gillespie *et al.,* 1959 ; Wagner and Gillespie, 1959 ; McKercher *et al.,* 1959). Both of these clinical entities seemed thus to be caused by the same agent, at the moment known as *Bovid herpesvirus 1* (BHV1) (Roizman *et al.,* 1973 ; Roizman and Furlong, 1974).

Experiments were designed to compare the pathogenicity of both types of isolates (Gillespie *et al.,* 1959), either from the respiratory (IBR) or the genital (IPV) tract, and it was shown that both types of isolates give the same results in the genital tract, whereas IPV strains given intranasally only produce a very mild respiratory disease and one hundred times less virus is excreted by the animal.

In fact, infectious rhinotracheitis and infectious pustular vulvovaginitis very seldom occur at the same time, in the same herd, notwithstanding some ethological habits of cattle (Kahrs and Smith, 1965 ; Kahrs, 1977). So IBR and IPV viruses, although serologically identical and very difficult to differentiate by conventional serological or virological means, differ by some clinical or biological properties, and most people agree that infectious bovine rhinotracheitis virus emerged from infectious pustular vulvovaginitis virus in the United States or, later, in Europe, as the result of certain major changes in the rearing of cattle, such as the gathering of numerous animals into feedlots (Gilbert and Saurat, 1970 ; Pastoret, 1979).

In African wildlife, *Bovid herpesvirus 1* has only been isolated from cases of pustular vulvovaginitis in wildebeest *(Connochaetes taurinus)* although many other *bovidae* may harbour the virus (Karstad *et al.,* 1974 ; Straub, 1978b ; Pastoret *et al.,* 1982a, submitted for publication).

2. Introduction

Thus infectious bovine rhinotracheitis (IBR) is at present a well-known respiratory disease of cattle (Straub, 1978a ; Dannacher *et al.,* 1980) and infectious pustular vulvovaginitis (IPV) a well-known genital disease, both caused by *Bovid herpesvirus 1* (BHV 1). BHV 1 is also responsible for numerous other clinical manifestations (Wellemans, 1975 ; Kahrs, 1977 ; Gibbs and Rweyemamu, 1977 ; Dhennin *et al.,* 1979).

One of the most striking features of BHV 1 infection in cattle is latency (Pastoret *et al.,* 1978a). Latency can be defined as the masked persistence of the virus in the host, so that it cannot be detected by conventional virological means. Certain endogenous or exogenous stimuli may unmask the virus from time to time. The virus may thus be later reactivated and sometimes reexcreted. Latency is a phenomenon observed with many other herpesviruses *(Herpesviridae),* but it is particularly interesting to study in the infection of cattle with BHV 1 for two main reasons. First of all, BHV 1 can be experimentally reactivated by the use of glucocorticoids (dexamethasone) and it shares this peculiarity with *Feline herpesvirus 1* (Gaskell and Povey, 1977 ; Povey, 1979). Then, the phenomenon can be studied in the proper species where it appears and in a naturally fixed situation.

In fact, neutralizing antibodies against BHV 1 are mainly found in species belonging to the family *Bovidae* and related families (Pastoret *et al.,* 1982a submitted for publication). The virus remains latent and can be reactivated in at least two of these species, domestic cattle *(Bos taurus)* and wildebeest *(Connochaetes taurinus)* (Karstad *et al.,* 1974), also in English ferrets *(Mustela putorius furo L.)* (Smith, 1978) and rabbit *(Oryctolagus cuniculus)* (Rock and Reed, 1982).

Thus, studying latency of BHV 1 in cattle allows errors arising from experimental contrivance to be avoided, because it is well known, in

veterinary medicine, that the same herpesvirus infection may give quite different results according to the animal species involved (Vindevogel *et al.*, 1980a and b ; Vindevogel and Pastoret, 1981). This paper will try to describe the main characteristics of BHV 1 pathogenesis, latency, and their consequences ; it will also try to answer two questions yet unsolved : where is the latent virus in the body, in which form does it exist (Roizman, 1965) ? It will also deal with the mechanisms implied in the maintenance of the latent state, those involved in the reactivation and those that control reexcretion.

3. Main characteristics of the virus (*Bovid herpesvirus 1*, BHV 1)

Infectious Bovine Rhinotracheitis virus belongs to the family *Herpesviridae* (Armstrong *et al.*, 1961 ; Knock and Liess, 1961 ; Straub, 1981) and shares the same morphological features as other members of the family (Pastoret *et al.*, 1978d). Its genome consists of a double-stranded DNA molecule (MW 88×10^6 daltons) (Hahnefeld and Hahnefeld, 1964 ; Skare *et al.*, 1975) covalently linked to some ribonucleotides (Babiuk and Rouse, 1976). The buoyant density of the DNA molecule is approximately 1.730 g/ml (Russell and Crawford, 1964 ; Geder *et al.*, 1978) ; which corresponds to a high percentage (72 %) of Guanine + Cytosine (Plummer *et al.*, 1969 ; Graham *et al.*, 1972 ; Ludwig, 1972a and b ; Black and Slack, 1972). DNA molecule represents more than 6 % of the viral particle (Pastoret, 1979) and is inserted into the core (Bocciarelli *et al.*, 1966 ; Langenberg and Sharpee, 1978).

Restriction patterns of the DNA molecule have recently been obtained (Skare *et al.*, 1975 ; Geder *et al.*, 1978 ; Pastoret *et al.*, 1980b ; Engels *et al.*, 1981) and further information has been obtained on the genomic structure (Farley *et al.*, 1981). This DNA molecule codes for both non-structural (such as virus-induced DNA-polymerase) and structural proteins (Misra *et al.*, 1981 ; Schwers *et al.*, 1980a and b ; Weinmaster *et al.*, 1982). The viral particle contains at least twenty-one structural polypeptides, ten of which are glycosylated (Pastoret *et al.*, 1980c). Strains from respiratory and genital origins differ slightly in this respect. In fact, it is often very difficult to distinguish between strains of different origins (Pastoret *et al.*, 1980c), the best technique being restriction cleavage of DNA molecule (Ludwig, 1981 ; Taylor *et al.*, 1982). The temperature-sensitive attenuated vaccine strain can also be easily distinguished from the others since its *ts* character is genetically stable even after reactivation (Pastoret *et al.*, 1980b ; Zygraich, 1981).

The antigenic structure of BHV 1 is not yet well known (Darcel *et al.*, 1978 ; Darcel and Jericho, 1981) ; it belongs to the first neutroseron as defined by Honess and Watson (1977).

BHV 1 seems to have oncogenic potential (Michalski and Hsiung, 1975 ; Geder *et al.*, 1978), but does not provoke tumour in cattle.

4. Clinical features

Contrary to infectious pustular vulvovaginitis, infectious rhinotracheitis can be a very severe disease, particularly when helped by certain bacteria (Asso, 1976 ; Yates, 1982).

Clinical lesions are usually restricted to the anterior respiratory tract, with nasal exsudation and tracheitis, but may also extend to the posterior respiratory tract, with bronchitis and pneumonia. Viral multiplication produces lysis of epithelial cells and therefore the destruction of the epithelium. During viral multiplication, transient intranuclear inclusion bodies are formed (Straub, 1978a). Local clinical symptoms are preceded and accompanied by an intense hyperthermia (Shroyer and Easterday, 1968).

Experimental infection with IBR isolated by the intranasal route produces a disease that mimics the natural one, being acute but generally less severe (Pastoret *et al.*, 1978c).

Infectious bovine rhinotracheitis infection is often associated with numerous other symptoms, such as conjunctivitis, abortion, metritis after cesarean section, encephalitis in young calves, rare cases of enteritis (Wellemans and Leunen, 1974 ; Lomba *et al.*, 1976 ; Straub, 1978a ; Pastoret, 1979).

The great variety of symptoms stems from the pathogenesis of the disease and from the privileged relationships that exist between virus and organism.

Both the foetus and the newborn animal are very susceptible to BHV 1 infection. If a foetus is infected, a generalized acute disease follows, leading to death and expulsion, that is to say its abortion (Owen *et al.*, 1964 ; Molello *et al.*, 1966 ; Kennedy and Richards, 1964 ; Stubbings and Cameron, 1981).

Inoculation of the foetus with a wild strain of the virus, within the uterus, is nearly always fatal (Ludwig and Storz, 1973 ; Kendrick, 1973).

Infection of pregnant cows by special strains provokes abortions, which can be experimentally reproduced, either by parenteral or even by intranasal inoculation. Prevalence of abortion in a herd depends on several factors, the most important being the immune status of the mother (Owen *et al.,* 1968).

The newborn calf is also very susceptible to IBR virus, as has been shown by experimental inoculation (Baker *et al.,* 1960 ; Kendrick and Straub, 1967). If it is not protected by colostral antibodies, the infection usually produces a generalized, highly fatal disease (Moretti *et al.,* 1964 ; Espinasse *et al.,* 1974).

Encephalitis is also observed in very young animals, but the encephalitic strains may differ from the others (French, 1962 ; Barenfus *et al.,* 1963 ; Hall *et al.,* 1966 ; Bagust, 1972 ; Jetteur *et al.,* 1979).

Reasons why young animals seem to be more susceptible are still being discussed (Rossi and Kiesel, 1977 ; Babiuk and Rouse, 1979).

5. Pathogenesis

The genesis of the local form of the disease after primary infection, such as rhinotracheitis or conjunctivitis, can be easily explained ; the other forms of the disease follow the generalization of the local infection. The role of the level of infection on clinical signs (Gaskell and Povey, 1979) and on the establishment of latency is not well known.

Generalization of the infection is generated by three different ways :

a) viraemia ;

b) neural spread ;

c) cell-to-cell transmission of the virus through intercellular bridges, even in the presence of specific antibodies.

a) *viraemia*

After primary infection a very transient viraemia can take place before the appearance of specific antibodies in the serum of the animal. If the animal is pregnant, the foetus can be contaminated by this way, leading to an acute infection and to abortion. Some attenuated vaccine strains given intramuscularly can cause abortion because of the artificially-produced viraemia (McKercher and Wada, 1964 ; McFeely *et al.,* 1968). Therefore it seems better to use attenuated strains given intranasally in a dairy herd, especially strains like the temperature-sensitive one, which is truly apathogenic for the foetus, and to vaccinate heifers before their first pregnancy, for the foetus is then protected by the immunity of its mother (Saunders *et al.,* 1972 ; Kahrs *et al.,* 1973 ; Durham, 1974).

The pathogenesis of abortion explains why it can follow any kind of local form of the disease such as rhinotracheitis or conjunctivitis. Viraemia also explains the genesis of most of the rare cases of enteritis that can be observed (Gratzek *et al.,* 1966a and b).

In adult cattle, viraemia is, in fact, very seldomly followed by a secondary localization of the disease. This is not the case for the newborn calf, however, which suffers from acute generalized disease provoked by viraemia. This pathogenesis can explain why passive immunization of the newborn conferred by colostral antibodies is effective and protects it from the worse effects of the infection (Rosner, 1968 ; House and Baker, 1968).

b) *neural spread*

The virus, that multiplies intensively at the local site of infection, contaminates the peripheric nerves and, by that route, reaches the central nervous system where some strains may cause encephalitis (Johnson *et al.,* 1964 ; Straub and Bohm, 1965 ; Hall *et al.,* 1966 ; Bagust and Clark, 1972).

Both intranasal and intravaginal experimental inoculation of the virus produce generalization of the infection by the nervous pathway. In both cases, the distribution of the virus is quite similar, despite differences in the localization within the nervous system : the virus can be found both in the brain and in the spinal chord when the animals are inoculated by intravaginal route, but it remains confined to the brain when they are inoculated by the intranasal route. This should be the consequence of the distribution of the sensory nerves to the peripheric organs which are affected with an intense viral multiplication.

The major part of the macroscopic or microscopic lesions are observed at the initial site of contamination and the extension to the nervous system produces no important damages, except within the Gasserian ganglions (Narita *et al.,* 1976 ; 1978a and b).

c) *spread through intercellular bridges*

Bovid herpesvirus 1, like other *Herpesviridae,* can use intercellular bridges to propagate itself

from cell to cell, avoiding therefore the extracellular fluid. This explains why the virus can be disseminated to other cells in the presence of high titres of specific antibodies. This particular mode of propagation also explains why the virus can produce plaques in cell culture in the presence of specific antibodies in the supernatant (Stevens and Groman, 1964 ; Zuffa *et al.,* 1976 ; Aguilar-Setién *et al.,* 1980). This kind of viral spread may be important for viral propagation after reactivation.

6. Latency : definition and introduction

After the invading period that follows the primary infection, the virus maintains itself in the organism at the latent stage. Latency is one of the main biological properties of *Bovid herpesvirus 1,* shared with many other *Herpesviridae* (Honess and Watson, 1977).

Latency can be defined as the persistence of the virus in the recovered host in a hidden form, undetectable by conventional virological means, with subsequent intermittent episodes of reexcretion. The persistence of *Bovid herpesvirus 1* in the infected animal after recovery has been investigated very early, since Storch, in Germany, suggested it already in 1910 for infectious pustular vulvovaginitis.

Several questions arise : where is the hidden virus, in which state, by which mechanism is it reactivated (Roizman, 1965) ?

The actual site of BHV 1 latency is not yet known, but it is believed that, like other herpesviruses, it can remain latent in nervous cells, lymphoid cells, as well as epithelial cells (Pastoret *et al.,* 1980b). It is well known that the latent virus remains localized near the site of its first multiplication and will be reexcreted in the primary infected organ (Davies and Carmichael, 1973 ; Davies and Duncan, 1974 ; Narita *et al.,* 1978c ; Pastoret *et al.,* 1980a).

Local lesions observed during reexcretion periods are either due to a viral reactivation in the epithelial cells themselves or to a reinfection of those cells occurring after viral reactivation in the sensitive nervous system innerving those tissues ; it has also been suggested that independent viral recrudescences take place at both sites (Davies and Duncan, 1974).

BHV 1 can be isolated from trigeminal ganglia of clinically normal cattle (Homan and Easterday, 1980), viral DNA can be detected in the same organ (Ackermann *et al.,* 1981 and 1982) during the latent period, and trigeminal ganglionitis can be observed during recrudescence (Narita *et al.,* 1981), but the discovery that a vaccine thermosensitive strain which is unable to invade the nervous system (Zygraich *et al.,* 1974a, b and c) can be reactivated by dexamethasone treatment of cattle (Pastoret *et al.,* 1980b) seems to indicate that the virus remains latent in the epithelial cells as well.

Another bovine herpesvirus has already been reactivated *in vitro* from bovine epithelial testicular cells (Thiry *et al.,* 1981a and b).

Few facts are known about the state of virus during latency. The actual mechanism of reactivation is still being disputed. There are two main hypotheses.

According to the first one, the virus being maintained in a latent stage by certain immunological mechanisms, if those mechanisms are depressed, for whatever reason, then viral multiplication is allowed to complete itself and the virus is reactivated (Babiuk and Rouse, 1979). For instance, dexamethasone treatment of cattle induces such an immunodepression that the immune control of viral latency breaks off, leading to the reactivation.

The second hypothesis postulates that the virus is reactivated by a direct effect of dexamethasone on the latently infected cells (Pastoret *et al.,* 1978a and d, 1979b and c).

7. Reactivation and reexcretion

Persistence, reactivation and reexcretion of BHV 1 in natural conditions are well supported (Storch, 1910 ; Parsonson, 1964 ; Studdert *et al.,* 1964 ; Saxegaard, 1966 ; Hyland *et al.,* 1975). Snowdon (1964, 1965) reported long-term intermittent excretion of IBR virus. Persistence of BHV 1 in animals was investigated by McKercher *et al.* (1963) and Straub and Bohm (1964) at the same period.

In fact, certain endogenous or exogenous physiological modifications in the animal may provoke reactivation of the virus, which leads to intermittent reexcretion.

Reactivation can be experimentally provoked, particularly by the use of glucocorticoids like dexamethasone (Kubin, 1969 ; Böttcher and Mähler, 1970 ; Sheffy and Davies, 1972 ; Bitsch, 1973 ; Darcel and Dorward, 1975 ; Gibbs *et al.,* 1975), by superinfection stimulus (Mensik *et al.,*

1976) or by other stimuli like 3-methyl-indol (Espinasse *et al.,* 1982, personal communication).

Persistent infection with BHV 1 has also been established in mice, English ferret and rabbit (Smith, 1978 ; Geder *et al.,* 1981 ; Rock and Reed, 1982).

Frequent recurrences of clinical BHV 1 is seen in closed herds (Hyland *et al.,* 1975) and reactivation of BHV 1 can be provoked several times in the same animal by dexamethasone treatment (Kabelik *et al.,* 1976 ; Pastoret *et al.,* 1979a, 1980b ; Narita *et al.,* 1981).

Reexcreted strains do not seem to differ from the original ones from the biochemical point of view (Pastoret *et al.,* 1978b, 1980a and b) but some biological differences have been described (Pastoret *et al.,* 1979b ; Pastoret, 1979, thesis ; Homan and Easterday, 1981) ; that may be related to the fact that reactivated strains are sometimes rather difficult to isolate (Saxegaard, 1966, 1970). However, Castrucci *et al.,* (1980) have shown that BHV 1 does not undergo significant modification in its pathogenicity when reactivated from latently infected animals for the first time.

The rest of this paper will deal with the reactivation of wild virus, temperature-sensitive and non temperature-sensitive BHV 1 attenuated strains given intranasally in cattle, by the use of dexamethasone, and the assessment of various immune parameters following dexamethasone treatment in an attempt to understand the mechanisms whereby the immune response can prevent reexcretion of virus in natural conditions. After which, the consequences of latency, this peculiar feature of the pathogenesis of *Bovid herpesvirus 1,* on epizootiology, diagnosis and prophylactic measures will be described, with particular emphasis on vaccination.

8. Reactivation of wild strains

Two and a half months after primary infection with virulent virus, cattle were intravenously injected with six consecutive daily doses of dexamethasone (0.1 mg/kg body weight). Nasal swabs were collected daily for two weeks and serum collected weekly for three weeks. This procedure was repeated two times later, using double doses of dexamethasone on the last occasion (Pastoret *et al.,* 1978a and d, 1979a).

Infectivity of the swabs was titrated daily and physical particles were counted on electron micrographs after negative staining, according to the pseudoreplication technique.

After the first treatment with dexamethasone, animals excreted high levels of physical particles, one of them as early as 24 hours after the first injection, whereas no physical particles were detected following the second or third course of dexamethasone ; infectious virus was detected at low levels in only one animal after each of the second or third treatments. A significant increase in neutralizing antibodies occurred following the first injection of dexamethasone, but neutralizing titres remained stable following the second or the third treatment.

These results suggest that dexamethasone causes production of non-infectious (probably inactivated) virus particles as a first effect and it leads us to think that dexamethasone may exert a direct effect on latently infected cells, causing a recrudescence of herpesviruses.

9. Reactivation of vaccine strains (*ts* and non-*ts*) intranasally administered

Nine month old, healthy, male or female Hereford cattle, were randomly divided into two groups of eight animals each. One group was vaccinated intranasally with *ts*-IBR and the other group was vaccinated with non-*ts* IBR (Con-IBR).

Five and six weeks later, nasal swabs and blood samples were collected from all animals for serum neutralization, antibody-dependent-cell-mediated-cytotoxicity and blastogenesis assays, to obtain a background level of antiviral activity as well as for a haematological profile of each animal (Pastoret *et al.,* 1980b). Animals were then treated for five consecutive days with dexamethasone. Nasal swabs were obtained daily for virus isolation and titration ; blood samples were collected for haematological and immunological studies. Three weeks after the end of the first dexamethasone treatment, the *ts* group was challenged with virulent virus (strain 108) and the non-*ts* group was treated with dexamethasone, for a second time, as described above. Both groups were once again monitored for virus excretion and immunological responses. Four weeks after challenge with virulent (108) virus, the *ts*-IBR group was once again treated with dexamethasone and monitored for virus excretion and immunological responses.

Treatment of animals with dexamethasone resulted in a rapid increase in the total leukocyte counts. This increase in total leukocyte counts

occurred within 24 hours of initiating dexamethasone treatment and was due to a dramatic increase in polymorphonuclear neutrophils and a slight increase in monocytes which overcompensated for the twofold decrease in lymphocytes. In all instances, continued administration of dexamethasone did not further increase or decrease specific cell types.

Following the first dexamethasone treatment, 6 out of 8 non-*ts* and 7 out of 8 *ts*-IBR animals excreted infectious virus. However, all animals were probably latently infected and reexcreted virus since all of them exhibited a rise in specific anti-IBR antibody levels. Furthermore in the non-*ts*-IBR group, animals that did not excrete detectable virus after the first dexamethasone treatment did so after a second treatment with dexamethasone.

Ts-IBR vaccinated animals, on average, excreted more virus (5.25 log vs 3.75 log) over a longer period of time suggesting that *ts*-IBR vaccines produced latency as readily as the non-*ts* one did. In an attempt to prove that the excreted virus was temperature-sensitive, it was tested for its ability to grow at 39 °C. The virus isolated from *ts*-IBR vaccinated animals was indeed temperature-sensitive, since the plaquing efficiency was very low at 39 °C. Furthermore, in all cases, the restriction enzymes cleavage pattern of the *ts*-reactivated virus was similar to that of the original *ts*-vaccine strain and it differed from the non-*ts* attenuated strain (Pastoret *et al.,* 1980b).

When the *ts*-vaccinated animals were infected with the virulent strain 108, they excreted this virus for up to 6 days after infection. When this group was later treated a second time with dexamethasone, no virus excretion could be detected. It was therefore not possible to determine whether recombination between attenuated and virulent field virus occurs or not.

10. Immune control of reexcretion

As already mentioned, several immune parameters were measured in the same animals in order to study how the immune system controls reexcretion.

First of all, reactivation must be differentiated from reexcretion since reactivation may occur in some animals where no excretion of infectious particles can be detected.

In order to study the immune control of reexcretion, blastogenesis index, the level of neutralizing antibodies and the level of those acting in antibody-dependent-cell-mediated-cytotoxicity (ADCC) were measured.

After primary infection with non-*ts* attenuated strain, animals have a normal amount of neutralizing antibodies, but a low amount of antibodies participating in the ADCC reaction and a low blastogenesis index.

After a first treatment with dexamethasone, there is an increase in neutralizing antibodies titre and a steady increase in both ADCC and blastogenesis index together with an important viral reexcretion. When the second treatment with dexamethasone is given, there are less infectious particles reexcreted, no increase in neutralizing antibodies, no increase in ADCC antibodies, but still an increase in blastogenesis index.

If, between the two dexamethasone treatments, some animals are boosted with a virulent strain of IBR virus, the second treatment is unable to provoke reexcretion of infectious particles. There is no change in the amount of neutralizing or ADCC antibodies, but still an increase in blastogenesis.

The sequence of events may be interpreted as follows (Pastoret *et al.,* 1980b) : after a primary infection, the animal has, for instance, a normal amount of neutralizing antibodies, that enable him to prevent the clinical effects of a reinfection with a virulent field virus, but are not sufficient to control an episode of reexcretion. The first viral reexcretion provoked by dexamethasone treatment produces an increase of the immune status and of the efficiency of some immune mechanisms such as ADCC and those which are cellular mediated (Aguilar-Setién *et al.,* 1979b, 1980 ; Pastoret, 1980). These kinds of mechanism can control reactivation better because of the cytotoxic effect exerted on infected target cells, which prevents the production of viral particles before their spread. If the immune mechanisms are reinforced by the booster effect of a virulent strain (for instance), the animal is able to completely control the reactivation induced and no reexcretion occurs.

The reason why the mean level of reexcretion is higher in *ts*-vaccinated animals and why the virus is shed for a longer period, may be that these animals have a lower level of immunity following primary vaccination.

The observation that the animals which excreted the highest level of virus after reactivation are those with the lowest immune response, supports the suggestion that reexcretion is influenced by the immune status of the animal.

11. Mechanism of reactivation

These data, however, do not differentiate whether reactivation is the result of dexamethasone temporarily depressing the immune response of the animal, or whether dexamethasone directly influences the cells harbouring the latent virus, allowing reactivation to occur. Both possibilities may occur and in fact may be required for reexcretion to be detectable.

In order to clarify this last point, the effect of another immunosuppressive drug, cyclophosphamide, on the latency of a virulent strain of BHV 1 has been tested (Pastoret *et al.,* 1980a).

Three months after primary infection with a virulent strain, two cows were intravenously injected with large doses of cyclophosphamide (35 to 40 mg/kg body weight). Two and a half months later, the same animals were injected with 0.1 mg/kg of dexamethasone during five days. Nasal swabs were taken and analysed for the presence of physical or infectious particles of BHV 1. No excretion could be detected after cyclophosphamide treatment, whereas after dexamethasone treatment, both animals reexcreted infectious particles, proving that they were latent carriers.

Cyclophosphamide provoked a large decrease in total leukocyte counts, whereas dexamethasone provoked a steady increase. Lymphocytes were depleted by cyclophosphamide treatment as well as by dexamethasone treatment. It does not seem likely, therefore, that the injection of cyclophosphamide provokes reactivation of BHV 1 in cattle, although it has an immunodepressive effect.

This last observation is more in favour of a specific action of dexamethasone on latently infected cells, for reactivation of BHV 1, than by the means of its immunodepressive properties.

It should also be mentioned that dexamethasone does not depress Fc bearing cells (Pastoret, 1979, Thesis), and that it can reactivate BHV 1 in several unrelated species, whereas it is unable to provoke the reactivation of many other herpesviruses.

12. Consequences of latency on the epizootiology of infectious bovine rhinotracheitis

It is well known that the latency of other herpesviruses ensures the durability of the infection. *Bovid herpesvirus 1* latency plays a prominent role in the epizootiology of the disease :

a) first of all, as already mentioned, one may consider that all animals become latent carriers after a primary infection with a virulent strain ;

b) all the attenuated strains remain latent after vaccination, including those still pathogenic for the fœtus ;

c) vaccination does not prevent the instalment of a virulent strain in a latent stage ;

d) it has not yet been determined whether recombination between attenuated strains and field virus occurs or not in an animal latently infected with both of these strains.

An animal latently infected with a virulent strain or with an attenuated one which is still pathogenic for the fœtus, is a permanent threat for the other animals in its surroundings.

The introduction of a latent, silent carrier in a herd free of the infection, is the best way, nearly the only way to introduce the disease. Uncontrolled therapeutic or zootechnic measures can enhance the risk (Duchatel *et al.,* 1981).

The presence of bulls latently infected with BHV 1 (IPV) is also a major concern for artificial insemination (Schultz *et al.,* 1976 ; Straub, 1978a).

13. Consequences of latency on the diagnosis of infectious bovine rhinotracheitis

First of all, it would sometimes be very useful to possess good diagnostic procedures, not only to ascertain a clinical diagnosis of BHV 1 infection in cattle, but also for the detection of latent carriers of the virus (Aguilar-Setién *et al.,* 1980). A good test should be simple, faithful, specific and not rely on too transient phenomena. Delayed hypersensitivity test is possibly, for several reasons, a good candidate (Aguilar-Setién *et al.,* 1978, 1979a).

Latency also influences the meaning of the laboratory diagnosis of *Bovid herpesvirus 1* infection, even when paired sera are used (Espinasse *et al.,* 1978 ; Asso, 1981). On one hand, if

BHV 1 is reexcreted, cattle may present a steady increase in the titres of neutralizing antibodies without showing any clinical signs and, conversely, BHV 1 can be reexcreted without increase of the titres of neutralizing antibodies. Moreover, cattle may be latent carriers without presenting detectable amounts of neutralizing antibodies (Aguilar-Setién *et al.*, 1979a). It should also be mentioned that neutralization cannot distinguish between an infection due to a respiratory or a genital strain. The isolation of BHV 1 must be cautiously interpreted since the virus can be excreted in the absence of clinical signs and since the attenuated strains remain as latent as the virulent one. Outside the temperature-sensitive mutant, the attenuated strains cannot be distinguished *in vitro* from the virulent ones by other means than by the electrophoresis of DNA fragments obtained after digestion of the nucleic acid by restriction endonucleases. The introduction into the field of attenuated strains has brought about some epidemiological confusions (Asso, 1976).

14. Consequences of latency on vaccination against infectious bovine rhinotracheitis

First of all, vaccination of cattle, either with inactivated vaccines or with attenuated strains does not prevent the further instalment of a virulent strain in a latent stage (Sheffy and Rodman, 1973 ; Nettleton and Sharp, 1980 ; Zuffa and Feketeova, 1980 ; Pastoret *et al.*, 1982b, in press). Conversely, vaccination either with an inactivated vaccine or with an attenuated strain does not prevent the further excretion of a field virus latently carried by the animal before vaccination (Straub, 1979). Moreover it has been known for a long time that at least one attenuated strain, given intramuscularly and still pathogenic for the fœtus, remains latent after vaccination, and the same is true for at least two attenuated strains given intranasally (Darcel le Q. and Dorward, 1975 ; Pastoret *et al.*, 1980b).

As the animal latently infected with a field strain or an inadequately attenuated one is a permanent threat for its surroundings, and since these strains can be disseminated by duly vaccinated animals, vaccination gives a false impression of safety (Nettleton and Sharp, 1980).

The practitioner must know that he cannot really control the dissemination of attenuated strains and the emphasis of the studies in medical prophylaxis of the disease must not only be given on the measures and the mechanisms that help the vaccinated animal to overcome the disease resulting from a contact with a field virulent strain, but also on the measures or the mechanisms that enable the animal to overcome or to control reactivation and reexcretion (Aguilar-Setién *et al.*, 1980).

If animals are sufficiently immunized, they are perfectly able to wholly control the reexcretion and therefore the dissemination of the virus, even if reactivation occurs (Pastoret *et al.*, 1980b). Therefore Straub and Wagner (1977) immunized bulls to avoid contamination of the semen by BHV 1.

Finally, since specific immunity impedes reexcretion, there is a certain lapse of time between the induction of reactivation and the appearance of infectious particles in nasal secretions ; when several animals from different origins are gathered together it is therefore, among other reasons, advisable to vaccinate the animals as soon as possible after their arrival to give them a chance to build up as early as possible a protective immunity (Imray, 1980). It is also advisable to vaccinate heifers before pregnancy (Chow, 1972 ; Saunders *et al.*, 1972).

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