Pathogenesis, latency and reactivation of infections by herpesviruses

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Summary: After a brief review of the pathogenesis of herpesvirus infections, the essential aspects of latency are presented: the sites, the state and control of latency. The stimuli of reactivation are reviewed and the putative mechanisms leading to reactivation are discussed. Re-excretion, which is the direct consequence of reactivation, is also detailed. Latency has a great epizootiological significance, mainly because of re-excretion. Re-excretion of virus by latently infected carriers, without any clinical sign, ensures the silent transmission of virus to susceptible in-contact animals. New prophylactic measures must consider not only the prevention of the clinical effects of infection with a virulent virus, but also the prevention of re-excretion.


INTRODUCTION

Herpesvirus infections of domestic animals are of great importance in livestock and poultry production, especially because herpesviruses have developed sophisticated strategies to persist in susceptible populations of restricted size. Latency is the property shared by all herpesviruses which allows them to persist indefinitely in their host after a primary or secondary infection (45). The mechanisms of establishment and maintenance of latency are not yet fully elucidated, but molecular studies are in progress for several herpesvirus models and recent data suggest that latency is under the control of viral latency genes. New hypotheses are available concerning the coding regions involved in the establishment and maintenance of latency and for the level of genome expression during latency.

Nevertheless, these mechanisms may differ according to the herpesvirus studied and the term “latency” has certainly not the same meaning for each one. Differences between herpesviruses are indeed observed in the site of latency, the state of the latent viral genome, the ability to furnish in vitro models of latency, the frequency of reactivation and the stimuli able to induce it. Moreover, virus-host relationships must be taken into account: the behaviour of the same herpesvirus may differ depending on the animal species or the cells infected by the virus.

This paper deals with general and comparative aspects of pathogenesis, latency, reactivation and re-excretion of animal herpesviruses. Emphasis will be given to mechanisms which allow herpesviruses to escape the measures, mainly vaccination and eradication, used until now to control their propagation (40).

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PATHOGENESIS OF HERPESVIRUS INFECTIONS

The range of diseases caused by herpesvirus infections is quite large. Moreover, the same virus can provoke different diseases, depending on the viral strain, the age of the animal or the route of infection. Bovine herpesvirus 1 (BHV-1) and Suid herpesvirus 1 (SHV-1) are good examples: BHV-1 infection of cattle causes rhinotracheitis, vulvovaginitis, conjunctivitis, abortion in pregnant cows, encephalitis in young calves, metritis; BHV-1 is also associated with enteritis and mastitis (44). SHV-1 infection of pigs is followed by abortion in pregnant sows, by septicaemia in neonates, by encephalomyelitis in piglets or by respiratory diseases in young pigs and adults (3).

In domestic animals, clinical disease is usually produced following a primary infection; recrudescent disease, i.e. caused by a reactivated virus, seems to be rare. The situation differs in humans: labial and genital recrudescent lesions are commonly observed in humans infected with herpes simplex virus type 1 and 2 (HSV 1 and 2), even in the presence of a high level of specific immunity (34). Shingles is a painful expression of reactivation of varicella-zoster virus, another human herpesvirus.

Virulence is a genetically controlled property and regions of the viral genome carrying virulence markers are beginning to be identified, especially for HSV and SHV-1. Thymidine kinase (TK) gene was the first gene of virulence unambiguously demonstrated. TK-negative mutants are shown to be avirulent (4, 22, 23, 31), but markers of virulence are also found in other parts of the genome (5, 27, 50, 64). Proteins coded by these regions remain to be studied. The knowledge of the genomic regions bearing virulence markers will lead, in the near future, to building specifically avirulent deletion mutants designed as artificially attenuated vaccine strains.

LATENCY

Latency is defined as the silent persistence of the virus in the body, not detectable by conventional virological procedures (44). Infectious virus is only recovered from latently infected organs by prolonged culture of organ cells or co-culture of these cells with susceptible cells (30, 47). Therefore, no infectious virus is reisolated in cell cultures inoculated with a triturated organ latently infected.

Establishment of latency

Expression of some viral genes is required for the establishment of the virus in a latent state. The TK gene was the first gene of latency recognized, but this point is conflicting: even low TK-producing mutants of HSV-1 remain latent (14). TK activity is not absolutely required for the multiplication of HSV in the trigeminal ganglion (10). Moreover, Kit et al. have recently reported the successful establishment of TK-negative mutants of BHV-1 in a latent state (23).

The multiplication of herpesvirus is not absolutely necessary for the establishment of latency. Thermosensitive mutants, which are unable to multiply in the mouse, may persist in a latent form (26). Nevertheless, virus multiplication greatly increases the amount of viruses within the body and therefore the possibility to produce latency.
The sites of latency

Three sites of latency are described for the herpesviruses: nervous, epithelial and lymphoid sites. Epstein-Barr virus (EBV; human herpesvirus 4) persists in a latent state in peripheral B-lymphocytes of infected humans (36). The virus, as well as Marek's disease virus (MDV; Gallid herpesvirus 1) is a member of the Gammaherpesvirinae and lymphoid latency is a property of this sub-family (49). A group of bovine herpesviruses, tentatively classified as bovid herpesvirus 4 by Ludwig (29), which comprises a cluster of respiratory and genital isolates (strain DN599, Movar 33/63, etc.), seems also to establish lymphoid latency in cattle (38) and, in experimentally infected rabbits, monocytes could be a site of latency (39). Other characteristics (growth in cell culture, electron microscopy of infected cells) have led to classify them as bovine cytomegalovirus (60). Murine cytomegalovirus, a true member of the Betaherpesvirinae, is able to establish latency in salivary gland cells and in spleen lymphocytes (48). Alphaherpesvirinae possess a nervous site of latency: the nervous ganglions and especially the trigeminal one (6, 49). Nevertheless, skin is an important site for bovine herpesvirus 2 (BHV-2) latency (32, 57) even if latency in the nervous system has also been described (7). Moreover, HSV establishes latency in the skin of the guinea pig and mouse (19, 58). An epithelial site of latency must therefore be carefully considered for other Alphaherpesvirinae.

The state of latency

Two states of latency are classically described (6): (a) the dynamic state: virus multiplication still occurs in latently infected cells, so that, at any time, the cells contain a reduced amount of infectious virus; (b) the static state: no productive infection occurs in latently infected cells where the genetic information of virus is at least retained, since infectious virus can be recovered by in vitro cultivation of these cells.

The second hypothesis is the most probable (6, 66). Indeed, latency can be established by thermosensitive mutants which are unable to multiply in the mouse (26).

The state of the latent viral genome may vary: integration into cellular DNA of cytomegalovirus genome (11) or presence in an episomic form. The two forms can coexist, as described in vitro for EBV (2, 25) and MDV (55). In several systems, it is demonstrated that certain parts of the genome are transcribed during latency (6; Rock D. L., Mayfield J. and Osorio F. A., personal communication; Rziha H.-J., Ohlinger V., Mettenleiter T.C., Bandtlow I. and Falser N., personal communication). Latency is sometimes defined as a transcriptional block, but it must be partial (37). The regions of the genome transcriptionally active during latency could support the genes which are involved in the maintenance of latency. The level of expression on the viral genome is different depending on the site of latency. When latency occurs in a neuronal site, and this is the case at least for Alphaherpesvirinae, transcription is very reduced. However, if the site of latency is the lymphocyte, viral genome is more expressed and specific viral antigens are present on the surface of persistently infected lymphoblastoid cells (17).

In vitro models of latency have been described for HSV. Inhibition of virus multiplication and incubation at supraoptimal temperature are classically used to establish persistent infection in vitro (54).

Since our knowledge of natural latency is very limited, it is impossible to assess the relevance of such in vitro models.
Control of latency

The control of latency and the mechanisms leading to reactivation are closely related since these mechanisms provide a counter-order to the order of maintaining the latent state.

Virus could be maintained in a latent state either by a control of the specific immune response or by a biological relationship between virus and latently infected cells. A better knowledge of the genes involved in latency will contribute to the study of such relationship.

The first hypothesis, control by the immune response, supposes at least the expression of viral antigens on the membrane of latently infected cells. It seems that Alphaherpesvirinae do not express antigens in latently infected cells. Only an immediate early polypeptide (ICP4) of HSV has been identified in the nucleus of latently infected neurons (15). On the other hand, Gammaherpesvirinae, like EBV, do express viral antigens on the surface of latently infected lymphocytes and these cells are effectively destroyed by immunocytolysis (16); nor does the immune response really control latency in the latter case. The putative involvement of the immune response in the control of latency will also be discussed in the section dealing with stimuli of reactivation.

REACTIVATION

Reactivation is defined as the appearance of infectious virus at the site of latency. Re-excretion is defined by the presence of infectious or non-infectious virus in peripheral tissues and organs, followed by its excretion outside the body. The terminology usually adopted for HSV infection is:

Recurrence is the appearance of infectious virus in the periphery without any clinical lesion; recrudescence or recrudescent disease consists of clinical lesions in peripheral tissues, produced by a recurrent virus (6).

Reactivation in itself is very difficult to demonstrate. The only way is to directly investigate the site of latency and to check it for the reappearance of virus multiplication. More often, reactivation is studied by its direct consequences: re-excretion and booster immune effect.

Stimuli of reactivation

Several stimuli of reactivation have been recorded and they may differ from one herpesvirus to another. The frequency of reactivation is so high in certain models (e.g. guinea pigs infected with HSV) that it is impossible to identify a stimulus of reactivation because one cannot clearly distinguish between spontaneous and provoked reactivation. On the other hand, the definition of spontaneous reactivation is not obvious: it could be caused by an endogenous condition of the latently infected animal or by a modification of the latent virus-infected cell relationship, at a cellular level (24).

Nevertheless, stimuli of reactivation have been unambiguously identified in most herpesvirus infections. They include stressful conditions, parturition, transport, superinfection by another virus (63), re-housing (13), local irritation of the skin (18) and cyclophosphamide treatment (6, 65). The most important experimental stimulus of reactivation is the injection of glucocorticoids since it reactivates
several herpesviruses of veterinary importance: Bovine herpesvirus 1 (BHV-1) (59), Bovine herpesvirus 2 (BHV-2) (57), Feline herpesvirus-1 (12), SHV-1 (33) and, recently, Equid herpesvirus 1, which has been successfully reactivated and re-excreted by latently infected ponies (9).

The mechanisms of reactivation

The mechanisms of reactivation are related to the factors involved in the control of latency. Three hypotheses must be considered: reactivation of latent herpesvirus could be induced either by immunodepression, or by a direct effect of the stimulus on the latently infected cells or by a combination of these two mechanisms. The two mechanisms can be illustrated by the effect of injection of glucocorticoids. Glucocorticoids could act either by their known immunodepressive properties (51), or by a direct effect on the latently infected cell (41). The second hypothesis is supported by the fact that glucocorticoids enter the cell, are coupled with a vector protein and can selectively induce transcription of certain parts of the genome. In another family of viruses, the Retroviridae, the direct effect of glucocorticoids on the proviral genome has been demonstrated (56).

The mechanism of reactivation is still controversial. For example, reactivation of murine cytomegalovirus is induced by several stimuli; most of them are known to be immunosuppressive. Nevertheless, non-immunological effects accompany these treatments and could represent the true reactivation stimulus (21). Cyclophosphamide is a potent immunosuppressive drug. It induces reactivation of several herpesviruses: HSV (20) and Pigeon herpesvirus 1 (65), but has no effect on BHV-1 latency (42). The dose of cyclophosphamide used also provokes alterations of DNA (6). Therefore, it cannot be concluded that cyclophosphamide effect is due to immunosuppression. Finally, as the genes involved in latency are being identified, the study of the regulation of their expression will certainly contribute to the knowledge of the reactivation phenomenon.

RE-EXCRETION

Re-excretion is effective only when reactivated viruses pass through several barriers. In the case of nervous latency (trigeminal ganglion), viruses migrate through the sensitive branches of trigeminal nerve and finally reach skin or respiratory mucosa. At this stage, virus multiplication can be stopped by cell-mediated cytotoxicity (52). Non-specific immune mechanisms are also involved: monocytes-macrophages, NK cells and interferon also restrict virus multiplication (28). Humoral immunity plays a limited role since herpesviruses pass from cell to cell through intercellular bridges (8). Afterwards, when virus is released in extracellular space, neutralization acts in limiting the re-excretion of infectious virus.

Virus re-excretion is therefore a phenomenon controlled by specific immunity. This is the reason why re-excretion is not always observed after reactivation, although it is a direct consequence of reactivation. Four cases can be considered:

(1) No re-excretion occurs, but viral multiplication is able to boost the immune response and specific immunity may still be stimulated (61);

(2) the specific immunity may no longer be stimulated, reactivation is induced, but it can be demonstrated neither by a booster response nor by an episode of re-excretion;
(3) re-excretion is not accompanied by a booster immune response: reactivation has been provoked in highly immunized animals; some of the reactivated viruses escape the immune defence and are excreted. In this case, the period of re-excretion is short and virus is re-excreted at very low titres (41);

(4) re-excretion is produced and is accompanied by a booster immune response (41).

EPIZOOTIOLOGICAL SIGNIFICANCE OF LATENCY

The epizootiological significance of latency is obviously the persistence of the virus in the population by silent carriers, without evidence of clinical disease. These silent carriers seem healthy and, in certain cases, cannot be detected by the usual serological tests (1).

Prophylactic measures based upon vaccination have been widely used to prevent the clinical manifestations of herpesvirus infections in domestic animals, but they are unable to prevent the installation of a strain in a latent state or to eliminate a latent virus carried at the time of vaccination. Moreover, in such animals, vaccination does not totally prevent the re-excretion of latent virus and the subsequent dissemination of wild-type virus to the surrounding animals. Attenuated live vaccines offer the animal the most complete range of immunogenic components necessary for a well-balanced immune response (53), but attenuated vaccine strains may perfectly well remain in a latent state (43). Cattle vaccinated by an attenuated strain of BHV-1 and subsequently challenged with a virulent strain re-excrete both viruses after reactivation (35). The presence of one virus in a latent state does not exclude latency of another virus: coinfection with two strains is the best procedure to establish latency of two viruses, if the virulence of both strains is the same; superinfection is less efficient, because multiplication of superinfecting virus may be severely restricted by the immune response (62; Yierrel D. L., Blyth W. A. and Hill T. J., personal communication). As recombination occurs easily for HSV and SHV-1, for example, appearance of virulent recombinants must be considered in animals latently infected by two vaccine strains. A recent work has shown that virulent recombinants have been obtained by \textit{in vitro} recombination between attenuated strains of SHV-1 (Lomniczi B., Kaplan A. S. and Ben-Porat T., personal communication).

Newly developed attenuated vaccines must therefore have the following characteristics:

(1) They must be able to prevent re-excretion, since vaccination does not impede the installation of a virulent strain in a latent form;

(2) they have to be deleted in genomic regions involved in latency to prevent their own latency;

(3) they must be deleted in virulence genes and these deletions must be situated in different parts of the genome to prevent the appearance of virulent recombinants;

(4) they must possess markers easily identifiable: biological or biochemical markers, but particularly antigenic markers. This point will be discussed below.

Successful programmes of eradication of herpesvirus infections will take into account the phenomenon of latency. Silent carriers do not show any clinical sign of
the infection and serological analysis fails to identify all these latently infected animals. Moreover, vaccination leads to epidemiological confusion, since it is usually impossible to distinguish by serological testing between animals vaccinated and those infected by a virulent strain. Isolated viruses can sometimes be identified by biological markers (e.g. thermosensitive vaccine strain) or by biochemical markers (e.g. restriction endonuclease fingerprinting). Further research should be devoted to the development of vaccine strains which harbour original antigenic properties, with respect to the parental strain, which do not affect their immunogenicity (Kit S., Sheppard M. and Kit M., personal communication). The recent development of TK-negative vaccine viruses introduces not only a new attenuated property, but also a strain marker (TK-negative) (22, 23, 31). The development of diagnostic methods able to detect all the latent carriers and, selectively, the vaccinated animals will be of great help for eradication programmes.

CONCLUSIONS

Latency increases the complexity of the epizootiology of herpesvirus infections. The consequences of latency are difficult to describe because of the accumulation of data. There is a need to possess a conceptual approach to the interactions between the animal and the latent virus. It would, therefore, be useful to have a model which could analyse any epizootiological situation and suggest an evolution of the system. In this context, logical analysis would be of great help (46).

Molecular biology has provided new tools for the study of herpesvirus latency and pathogenesis: in the near future, the knowledge of the genetic control of latency and virulence will lead to the production of safe vaccines attenuated by specific deletions in the genome. This is a further step to a new rational prophylaxis which should fully prevent the establishment of latent virus and the re-excretion of latently carried virus.

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REFERENCES


