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The role of varicella zoster virus immediate-early proteins in latency and their potential use as components of vaccines

Sadzot-Delvaux C, Rentier B

Department of Microbiology, Fundamental Virology, Liege University, Sart Tilman-Liege, Belgium

Summary. Varicella zoster virus immediate-early (IE) proteins are intracellular regulators of viral gene expression. Some of them (IE62 and IE63) are found in large amounts in infected cells but are also components of the virion tegument. Several IE and early genes are transcribed during latency, while late genes are not. Recently, we demonstrated the presence of protein IE 63 in dorsal root ganglia of persistently infected rats as well as in normal human ganglia; other IE proteins have been found since in human ganglia. Cell-mediated immunity (CMI) to IE 62 has been evidenced. We found both humoral immunity and CMI to IE 63 in immune adults. In elderly zoster-free individuals, CMI to IE 63 remained high. The differences in the CMI to IE 63 among young adults, elderly people and immunocompromized patients have to be analyzed according to their status relative to zoster, to determine whether the decrease in CMI, particularly to IE proteins, could be responsible for viral reactivation and for the onset of shingles. Hopefully, the waning of the CMI to VZV IE 63 and perhaps to other IE proteins could become a predictive marker for herpes zoster and reimmunization, not only with the vaccine strain, but also with purified IE proteins could help prevent zoster at old age.

Introduction

Varicella zoster virus (VZV) has long been considered as a virus whose functioning could be deduced from that of herpes simplex virus (HSV), the prototype of Alphaherpesviruses, which is easy to produce in vitro and for which experimental models are available. Indeed VZV shares many morphological and biological properties with HSV and all other alphaherpesviruses. The most interesting characteristic certainly is the fact that, following primary infection, the virus can reach sensory ganglia and remain latent in the peripheral nervous system for many years before being reactivated and producing a rash, usually restricted to a single der-matoma.
However, it is now obvious that the mechanisms involved in VZV latency and reactivation are not identical to and perhaps not even similar to those described for HSV. HSV-1 remains latent in neurons only, and its latency is characterized by the accumulation of antisense latency-associated transcripts (LATs) in the nucleus of infected cells. Although these transcripts are thought to contain at least one open reading frame, no corresponding protein has been detected so far and it is still not clear whether the LATs play a role in the induction or in the maintenance of latency, while they could be involved in viral reactivation [44]. These characteristics are shared by the other alphaherpesviruses such as pseudorabies virus, equine herpesvirus-1 or bovine herpesvirus-1 but not by VZV, whose genome lacks any homolog of the HSV LAT-encoding sequence.
In situ hybridization and Northern blot analysis have provided evidence for a restricted transcription of the VZV genome during latency: both α (ORFs 4, 62 and 63) and putative β genes (ORFs 29 and 21) are transcribed during latency while no γ gene is [9-11, 31, 43]. The viral proteins corresponding to these transcripts have now been detected in rat [12] and in latently infected human ganglia [28, 30]. Three IE proteins (IE 4, 62 and 63) and two E proteins (ORF21p and ORF29p) accumulate in latently infected cells while no late protein seems to be expressed.

Key roles of the immediate-early proteins in productive infection

The VZV replication cycle can be subdivided into three phases: (1) virus adsorption and entry, (2) viral gene transcription and translation and (3) viral assembly and egress. Once the virus has penetrated the cell by fusion of its envelope with the plasma membrane, involving interactions between viral glycoproteins and cell receptors, the nucleocapsid reaches a nuclear pore through which the viral genome is released into the nucleoplasm. Viral genes are transcribed in the nucleus and transcripts are translated in the cytoplasm in three successive phases. A first wave leads to the very early expression

of ORFs 4, 61, 62 and 63, in the absence of de novo protein synthesis. The proteins produced, called immediate-early proteins (IE), migrate back into the nucleus and induce the expression of a second class of proteins called early (E), mainly the enzymes involved in viral DNA replication. The third and last wave of protein expression occurs after DNA replication and results in the synthesis of late (L) proteins that will constitute the viral particle. The capsids are assembled in the nucleus, wrapped around the newly replicated DNA before acquiring an envelope and being released.
In this highly controlled process, the expression of the IE proteins is certainly the most critical step since these proteins play a key role in the regulation of expression of most other viral genes. Moreover, except for ORF6 lp, these proteins are found associated with purified virus particles [23, 24] and could constitute important targets for the immune system.

IE 62

ORF62p, a 175 kDa protein encoded by ORF 62 and also by ORF 71, is present in large amounts in the viral tegument [24] and thus brought in by the incoming virus. It is expressed as a nuclear IE phosphoprotein, thus it is called IE 62 and it exerts an important regulatory function in VZV replication, as evidenced by transient expression experiments demonstrating that it is capable of transactivating genes of all classes and increasing viral DNA infectivity [21, 38, 41]. Moreover, IE 62 regulates its own promoter [5, 16]. However, these regulatory properties appear to be dose-dependent because IE 62 can positively or negatively regulate the expression of other genes, depending on its concentration.
However, IE 62 does probably not act on its own, but in synergy with other IE proteins or even with cell proteins. A direct interaction with IE 4 specifically modifies the intracellular localization of the latter by transporting it to the nucleus [14]. Based on these data, it seems reasonable to propose that IE 62, brought in at a low concentration by the incoming virus, initiates the IE phase by transacti-vating the expression of α genes. In a second step, IE 62 could participate in the activation of β genes and later of 7 genes. Due to its autoactivating properties, its intracellular concentration increases progressively and its expression is thereby down-regulated.

IE 4

ORF4p, a 55 kDa protein, is also present in large amounts in the viral tegument [23] and produced very early on in infected cells [13]. Its localization is mostly cytoplasmic but thanks to a direct interaction with IE 62, IE 4 can be transported to the nucleus [13]. IE 4 transactivates promoters of all three classes of genes, either on its own or in synergy with IE 62 [15], but it has no demonstrable transrepressing activity [37]. Its transactivating properties could require the presence of other functional cellular proteins.

IE 63

ORF63p is a 45 kDa phosphoprotein encoded by genes 63 and 72. It is a component of the viral tegument, shown to be a true IE protein and it is strongly expressed during lytic infection in cell culture as well as in skin lesions [12, 23, 34]. Its localization is mostly nuclear, but it can also be detected in the cytoplasm of infected cells [12]. Even though IE 63 shows a slight repression of IE 4 transac-tivating properties on the IE 62 promoter [12], it lacks significant transactivating properties and its role at the very beginning of the replicative cycle has not yet been elucidated.

ORF61p

The phosphoprotein encoded by ORF 61 (ORF61p, 62-65 kDa) has been considered as an IE protein only on the basis on its homology with HSV-1 ICP-0, but an experimental demonstration of its IE nature has not yet been provided. Contrary to the three other IE proteins, ORF61p does not belong to the viral tegument [23]. It enhances infectivity of VZV and HSV-1 DNA as shown by transient expression experiments [32] and, depending on its cellular concentration and on host cell lines, it can either repress or transactivate the functions of IE 4 and IE 62 on other VZV gene promoters [8, 32, 33].

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Latency

Besides their functional properties as regulatory proteins in productive infection, proteins IE 4, 62 and 63 appear to be of great interest because they are also expressed in large amounts during latency. An expression of viral proteins has never been described during alphaherpesvirus latency, but it has been demonstrated for IE 63, first in an animal model [12, 39] then in humans [30]. Later, other IE or E proteins have been detected in latently infected human cells [28]. Mostly intranuclear during productive infection according to their functions, these proteins are detected predominantly in the cytoplasm of latently infected cells but they become detectable in nuclei when the virus reactivates. The reasons for this modified distribution during latency are still not understood. It is also not clear whether cytoplasmic sequestration is the result of a failed process that normally carries them to the nucleus where they perform their regulatory tasks or whether their accumulation by itself inhibits replication. Cytoplasmic overloading of these proteins during latency suggests new hypotheses for the establishment and maintenance of VZV latency [27]: the virus enters the cell as in productive infection and the cycle initiates by the expression of some of the regulatory IE proteins (IE 4, 62 and 63) which probably migrate to the nucleus to exert their regulatory functions, but they are in such low quantities that they remain undetectable.
Even a low amount of IE proteins migrating to the nucleus allows the expression of E proteins encoded by ORFs 21 and 29 [28], which also accumulate in large amounts in the cytoplasm. It is not known whether other E proteins are expressed and whether viral DNA replication occurs at all, but so far, no L protein has been detected during latency.
The mechanisms involved in this process have yet to be identified, but it is tempting to conclude that cells in which the virus becomes latent lack the necessary elements to process IE proteins and to give them the conformation by which they become functional. Phosphorylation or other post-translational modifications are candidate impaired process mechanisms. It is also possible that some cellular components interact with viral proteins or with viral promoters that can interfere with the replication cycle and lead to replicative arrest. For instance, Patel and collaborators have demonstrated that the isoforms of the cellular transcription factor Oct-2 expressed specifically in neuronal cells can inhibit basal activity of the VZV IE 62 promoter in neuronal cells but not in other cell types, suggesting a cell type-specificity [35]. This mechanism could be of particular importance for the onset of VZV latency in sensory ganglia.
However, such explanations still need experimental confirmation and must take into account the observation that inhibition is reversible in yet undetermined conditions when reactivation occurs.
Another important difference between VZV and HSV latency is the nature of the cells in which the virus remains latent. HSV clearly resides in neurons only whereas the precise localization of persistent VZV is still debated: using *in situ* hybridization or *in situ* PCR, evidence of latency in neuronal cells [17, 20], in non neuronal cells [11, 31] or in both cell types [26, 29] has been shown. Animal models did not allow elucidation of this issue because in mice and rats the viral genome was detected in both cell types [39, 45].

Immunogenicity of the IE proteins

One of the parameters of obvious importance for the control of virus infection is host immunity. Clinical observations have shown that the frequency and severity of viral reactivations increase in patients whose cell-mediated immunity (CMI) is impaired because of age, pathological disorders or immunosuppressive treatments prior to transplantation [3, 6, 18].
Previous studies have shown that viral tegument proteins, including IE 62 and 63, and the major glycoproteins, are important targets for CMI to VZV. They elicit a long term humoral and cellular immune response after natural VZV infection [1,4, 40]. T lymphocytes from most VZV naturally immune donors proliferate in vitro after stimulation with these proteins and they can lyse autologous target cells that express IE 62, 63, gC, gE, gG or gI.
The critical role of immune control has been suggested by Hope-Simpson as early as 1965 [19]: VZV primary infection appears to be limited by host "resistance" (immune response) that remains high for many years, with a slow decrease over time. This decrease could, however, be partly counterbalanced by frequent viral reactivations or contact with infected individuals, which would contribute to maintaining an efficient immunity until reaching a critical level under which the host resistance would be too low to control viral reactivation. This hypothesis, based only on clinical observations is still valid today even if it appears now that it is mostly the CMI that limits viral reactivation. Indeed, virus

reactivates in spite of high anti-VZV antibody titers and the zoster episode is not correlated with hypogammaglobulinsemia.
It is tempting to think that the expression of viral proteins in latently infected cells constitutes another way to trigger the immune response and could thus contribute to maintain it at a protective level. In this context, it will be of interest to characterize the immune response to viral proteins expressed during latency, in the elderly and in immunocompromised patients who have a high incidence of herpes zoster. It is well known that the CMI is often impaired with age, as documented using in vitro PBMC stimulation by whole VZV antigens [18]. Such a study has not been performed with purified VZV proteins and in particular with proteins expressed in latently infected cells.
However, many questions are raised by this hypothesis of a role for the viral 'latency proteins' in maintaining a specific immune response. The nervous system is indeed protected from immune recognition by anatomical barriers and neurons lack classical MHC molecules at their surfaces . On the contrary, satellite cells surrounding neurons express MHC and they could play an important role in antigen presentation. It is thus critical to define clearly in which cells the virus remains quiescent. So far, during latency, IE 63 has been observed only in neuron cytoplasms. If neurons are the only cells expressing viral antigens, the mechanisms leading to the recognition of viral peptides must be clarified. It is possible that non-classical MHC proteins are being expressed at the cell surface in response to viral infection, as it has been suggested for HSV [36]. However, in HSV-infected cells, peptide presentation by MHC molecules appears to be impaired because of a viral protein that inhibits peptide processing [22, 42]. VZV has been shown to selectively downregulate cell-surface MHC class I expression on human fibroblast cells and to inhibit IFN-7 induction of MHC class II cell surface expression [2].
Enhancing the immune response to IE 63 may become an important strategy for preventing VZV reactivation from latency. If so, IE 63 could be a suitable candidate as an additive for a VZV vaccine to be given to ageing adults in order to boost their immunity and to prevent herpes zoster [7, 25]. Because recognition by the immune system of proteins expressed during latency, proteins such as IE 62 and 63, could play a role in the control of latency, it must be documented using animal models or by studies involving a larger number of donors, particularly donors with a high probability of reactivation of the virus.

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