IMMUNITY IN LATENT HERPES SIMPLEX VIRUS INFECTION

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Summary. – Immune responses against productive Herpes simplex virus 1 (HSV-1) and/or Herpes simplex virus 2 (HSV-2) (HSV) infection together with associated immune escape mechanisms are to a great degree understood. Due to a limited RNA expression and lacking a convincing evidence for production of virus proteins during latency, HSV in latently infected neurons had been for a long period considered invisible to immune system. This review analyzes currently accumulating data indicating an important role of immune response to HSV-1 latency and/or to early steps of HSV-1 reactivation process. Although this review focuses on HSV-1, it is likely that general concepts apply to both HSV-1 and HSV-2, since they share a great deal of similarity in their biological features including a high degree of sequence similarity at the nucleic acid level.

Key words: Herpes simplex virus 1; Herpes simplex virus 2; latency; latency-associated transcripts, latency-associated proteins, CD8+T cell

Contents:

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Abbreviations: HCMV = Human cytomegalovirus; GFP = green fluorescence protein; HBV = Hepatitis B virus; HSV = HSV-1 and/or HSV-2; HSV-1 = Herpes simplex virus 1; HSV-2 = Herpes simplex virus 2; IF = immunofluorescence; LAT = latency-associated transcript(s); ICP = infected cell protein; MHC = major histocompatibility complex; MHC-I = MHC class I; TAP = transporter associated with presentation of antigens

1. Latency

Following initial productive infection HSV-1 rapidly enters the sensory ganglia innervating the infection site and passes by retrograde transport to the ganglion**.** After short period of productive infection, viral DNA circularizes, forms an episome in cell nucleus and a latent infection is established inside neurons of the sensory ganglion (typically TG). It is not clear how neurons in ganglia survive the primary infection. During

latency no infectious virus that would represent the progeny from infecting HSV is produced. Neurons are non-dividing, terminally differentiated cells, and so once the latency is established, viral genome needs not replicate to persist for the life-time of the neuron. For that reason sensory neurons can serve as a reservoir of the virus.

The sensory neurons are extensively interconnected through synapses and can thus operate as excellent conduits for transport of virus particles to and from mucosal surfaces allowing not only establishment of the latency but also reactivation of latent virus. A recurrent herpetic disease results from the reactivation of latent virus and its anterograde axonal transport to the periphery but not from exogenous re-infection (Roizman, 1996, Daheshia *et al*., 1998; Deshpande *et al*., 2000; Favoreel *et al*., 2000; Nash *et al*., 2000; Jones 1998, 2003; Bloom, 2004; Becker, 2002).

2. Experimental models

The reproductive cycle of HSV-1 is readily studied in cell culture and experimental animals. However, latency can only be established with difficulty in primary neuronal cultures. Much of the work on latency therefore relies on experimental infection of animals. Primary animal models are the mouse and rabbit for HSV-1 and guinea pig for HSV-2. HSV-1 is usually inoculated by the ocular route, allowing the virus to replicate within epithelial tissues and enter local nerve termini to ensure fast axonal transport to neuronal nuclei. Data suggest that repression of viral gene expression in the latency is under neuronal control because latency may be preferentially established in certain phenotypic classes of sensory neurons. HSV-1 in infected human individuals and in rabbits (ocular rabbit model) periodically reactivates from latency in sensory neurons, is transported by an anterograde axonal transport, and is shed at peripheral sites, leading to recurrent disease. Spontaneous HSV-1 reactivation is rarely observed in mice (Stanberry, 1994; Wagner and Bloom, 1997).

3. Latency-associated transcripts (LAT)

During latency, the viral genome is present as a circular episome and is associated with cellular histones. For the duration of latency the only abundantly expressed viral RNAs are latency-associated transcripts (LAT), although a limited transcription of some immediate/early genes (notably ICP4) and early genes (e.g. thymidine kinase) has been reported. It seems likely that LAT may have an important function in establishment of latency, maintenance of latency and/or reactivation from latency. Analysis of LAT mutants in animal models demonstrated that the LAT were not unconditionally required for establishment or maintenance of latency. HSV-1 LAT mutants, however, exhibit impaired reactivation in explanted mouse trigeminal ganglia (TG) and in the rabbit eye model (Wagner *et al*., 1988; Cook *et al*., 1991; Spivack *et al*., 1991; Thompson and Sawtell, 1997; Preston, 2000; Jones, 2003; Kent *et al*., 2003).

To establish latency in ganglia neurons must survive the primary infection. Importantly, the LAT gene has been demonstrated to encode an antiapoptotic function both *in vitro* and *in vivo*, which may contribute to the survival of infected neurons and hence may affect both establishment of latency and subsequent reactivation. The ability of LAT to interfere with apoptosis correlates with their ability to promote spontaneous reactivation, suggesting that the antiapoptotic activity of LATs is important during the latency-reactivation cycle (Jerome *et al*., 1998; Hill and Masucci, 1998; Jones 2003; Kent *et al*., 2003; Bloom, 2004). LAT are capable of preventing neuronal cell death by inhibiting both the receptor-mediated (caspase-8) and mitochondrial-mediated (caspase-9) pathway. Elucidation of how the LATs ability to block apoptosis influences the HSV life style is difficult due to HSV-1 genetic complexity and the fact that it contains at least five other apoptosis-modulating genes. Immediate early (ICP27), early (E, US2 and US5) and late gene products (gD and gJ) have been shown to block apoptosis primarily during the acute/productive infection. The LATs are encoded by a region located in the internal long repeat (IR_L) and partially overlapping the 3' terminus of the ICP0 gene; they are transcribed from the DNA strand complementary to that encoding ICP0 mRNA (Fig. 1). The 8.3 kb polyadenylated primary LAT gives rise to a family of LATs restricted to the nucleus, including a very stable 2 kb LAT (Cook *et al*., 1991; Preston, 2000; Jones, 1998, 2003; Thompson *et al*., 2003; Bloom, 2004). The LAT may operate via RNA (e.g. an antisense regulation of ICP0 and/or ICP34.5 expression). Even though the 3' termini of the LATs overlap with the 3' end of ICP0 mRNA, the antisense regulation of ICP0 by LATs has been ruled out. Replication of LATs mutants in tissue culture or *in vivo* is not affected, ICP0 expression is normal, and latent infection is established. Reactivation of latent LATs mutants is rather impaired, not stimulated as would be predicted in the absence of the antisense inhibition of ICP0. Furthermore, a region within the first 1.5 kb of the 8.3 kb primary transcript has been shown to be essential for spontaneous reactivation in a rabbit model. This region cannot function via the antisense mechanism, because it does not overlap with any other known HSV-1 gene (Everett, 2000).

4. Latency-associated proteins

4.1 LAT ORFs do not encode a protein essential for efficient spontaneous reactivation

LAT-encoded protein(s) may play an important role in spontaneous reactivation and in immune response to HSV-1 latency. The LAT-encoded protein, essential for efficient

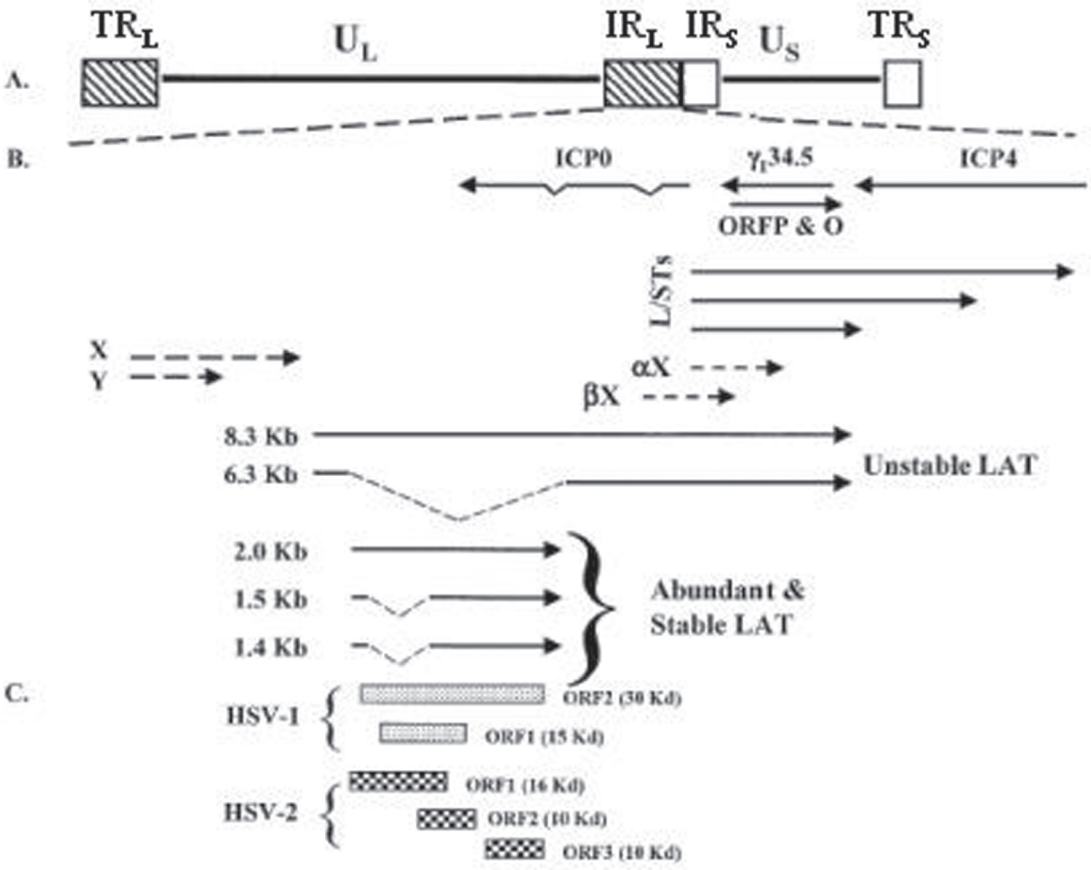

Fig. 1

Location of genes within the HSV-1 long internal repeat (IR_L) (Acording to Jones, 2003)

(A). U_L and U_S denote long and short unique sequences, respectively; IR_L and IR_S denote long and short internal repeats, respectively; TR_L and TR_S denote long and short terminal repeats, respectively.

(B). Transcription map of IR_L. The location of LAT, ICP, γ134.5, ORF and L/STs are indicated by solid lines. Partially mapped transcripts αC and βX are denoted by dashed arrows.

(C). Positions of potential ORFs within the abundant and stable LAT of HSV-1 strain 17 syn+ and HSV-2 strain 333. The approximate size of the representative ORF is given in parentheses.

spontaneous reactivation, must map within the first functional 1.5 kb LAT. The absence of a well-conserved ORF in this region among all HSV-1 strains capable of high reactivation suggests that it is very unlikely that LAT encode any protein essential for efficient spontaneous reactivation (Kent *et al*., 2003; Drolet *et al*., 1998). There are, however, other ORFs located in other regions of primary LAT, and interestingly, LAT are also bound to polyribosomes in cultured neuronal cells and mouse TG during latent infection (Nicosia *et al*., 1994; Goldenberg *et al*., 1998).

The presence of the 2.0 kb LAT in the cytoplasm of infected cells and its association with polysomes has led to the proposal that the 2.0 kb LAT is translated. Accordingly, the LAT-encoded viral proteins, which are uniquely or predominantly expressed during viral latency, might exist. All HSV strains so far sequenced contain putative ORFs of significant size within the stable 2.0 kb LAT. The largest of HSV-1 ORFs encoding a protein of ~274 aa is conserved at amino acid level among HSV-1 strains suggesting an important function. Interestingly, the ORF in the 2 kb HSV-2 LAT (encoding ~419 aa) is not similar to that in HSV-1. No protein can be directly translated from the 2.0 kb LAT, because is not capped or polyadenylated and is thought to be a stable intron. Yet, an alternative splicing of the primary 8.3 kb LAT resulting in non-nuclear polyadenylated molecules containing ORF has not been ruled out, and such molecules may function as LAT mRNAs.

4.2 Detection of a LAT ORF-encoded protein in latently infected neurons

There has been one report of a LAT-encoded protein in latently infected primary neuron cultures (Doerig *et al*., 1991). In this case, the authors focused on the larger of the two ORFs (ORF 2) from the KOS strain of HSV-1 potentially encoding a 305 aa protein. This ORF is highly conserved among HSV-1 strains. An antiserum was prepared against the potential polypeptide encoded by ORF 2 by immunizing rabbits with a bacterially produced fusion protein containing a part of this polypeptide. Immunochemical analysis showed that all neurons present in a latently infected culture were stained with this antiserum but not with an ICP0 antiserum. No LATs-specific antigen was detected in mock-infected neurons in productively infected Vero cells or in neurons latently infected with a mutant virus carrying a deletion in the LAT gene. Achilles' heel of this report was an 80 K protein recognized by the antiserum in latently infected neurons. The LAT ORF 2 would be expected to yield a protein of about 33 K. This discrepancy was not satisfactorily explained, and the antigen reported in this paper was not later confirmed.

4.3 Recombinant LAT ORF-encoded protein with high HSV-1-specific biological activity

Recently, cell lines were produced in which the 2 kb LAT ORF (encoding ~274 aa), excised from a HSV-1 genomic clone, was expressed in ND7 and BHK cells under the control of a strong Human cytomegalovirus (HCMV) promoter (Thomas *et al*., 1999, 2002). ND7 is a neuronal cell line of low permissiveness for HSV-1 due to presence of transcription factors repressing the expression of IE genes. BHK is a fibroblast cell line of high permissiveness for HSV-1. The virus growth curves obtained showed that both cell liners stably transfected with the LAT ORF gave considerably enhanced permissiveness for HSV-1 compared to control cells transfected with control plasmid. The growth enhancement was considerably more marked (up to 1000 times) in ND7 compared to BHK cells. Viruses were also constructed in which the LAT ORF under HCMV promoter control was inserted into non-essential sites in the HSV-1 genome (US5, UL43). This enhancement of growth was even greater when the LAT ORF expressing viruses were used to infect cells which already had been expressing the LAT ORF. The mechanism by which the LAT ORF is able to enhance virus replication is unclear. Interestingly, the HSV-1 LAT ORF provided a growth enhancement in both ND7 and BHK cells for HSV-1 but none for HSV-2. Although both HSV-1 and HSV-2 genomes are collinear and the vast majority of the protein-coding sequences are very similar, they have generally different sites of latency and reactivation (TG and sacral dorsal root ganglia, respectively). The HSV-2 LAT also contains an ORF potentially encoding a protein of significant size (~419 aa), but they have no sequence similarity with any of HSV-1 LAT ORFs. Differences in HSV-1 and HSV-2 LAT ORFs sequences may be in part responsible for reactivation from different sites. LAT ORF-expressing viruses also show enhanced replication *in vivo*.

To explore the LAT ORF function further an epitope-tagged LAT ORF, LAT*myc*His was synthesized. Generation of such a protein allowed easy intracellular localization of the LAT ORF protein by immunoblot analysis and immunofluorescence (IF) examination using an anti-myc antibody. The IF examination showed that LAT*myc*His formed punctate structures in the cell nucleus reminiscent of the structures formed by ICP0 during the early stages of HSV-1 infection. Nevertheless, a co-localization of LAT*myc*His and ICP0 was not confirmed. Interestingly, deficiencies in IE gene expression, namely those concerning ICP0, an important regulator of viral gene expression, could be overcome *in vitro* by the expression of LATs ORF (Thomas *et al*., 1999, 2002).

4.4 Synthesis of LAT ORF-encoded proteins is inhibited during HSV-1 infection

The weakness of experiments described in the previous paragraph is that a protein with high and specific biological activity was translated from a recombinant gene from which all LAT regulatory elements were removed. The evidence that this 2.0 kb LAT ORF might be expressed in natural context within the LAT gene was missing. Therefore the possibility that ORFs within the 2.0 kb LAT are translated was also investigated by introducing the gene for green fluorescence protein (GFP) into the 2.0 kb LAT sequence. The GFP gene was inserted within the LAT 2.0 kb sequence both into a LAT expression plasmid (LAT-GFP) and HSV-1 (LAT-GFP HSV-1). GFP expression was observed upon transient transfection of cells of both neuronal and non-neuronal origin with LAT-GFP. However, GFP expression could not be detected from the virus (LAT-GFP-HSV-1) in culture or in TG at any stage of the infectious cycle despite the production of alternately spliced LAT-GFP RNAs. Accordingly, the transcripts produced in LAT-GFP-transfected cells should have acted as mRNA for GFP expression, but the transcripts produced in cells infected with LAT-GFP HSV-1 virus should have not. These results suggest that the inhibition of LAT ORF expression during viral infection occurred primarily at the level of translation. (Lock *et al*., 2001). There is a possibility that, in HSV-1 infected cells, the 2.0 kb LAT associated with ribosomes does not function as mRNA but may have another role, possibly related to translation, as indicated by its apparent association with ribosomal complexes. Interestingly, the

expression of β-gal gene from the LAT promoter in the context of HSV-1 differs similarly at transcriptional and translational levels (Lock *et al*., 2001).

In summary, convincing evidence that LAT ORFs are translated in natural context during latency is missing. Nevertheless, there is a possibility that the LAT ORFs are translated at very low levels or in a highly regulated manner during a particular phase of infectious cycle. Such a hypothesis can easily explain why a protein translated from a LAT ORF from recombinant gene has a high HSV-1- or HSV-2-specific biological activity. A novel influenza A virus protein PB1-F2 was discovered only recently, after remaining 10 viral proteins have been know for more than 20 years. Interestingly, the PB1-F2 protein is translated from alternative (+1) ORF of PB1 gene (Chen *et al*., 2001). It would be interesting to examine whether alternative ORFs are also translated from HSV-1 or HSV-2 genome, particularly during latency.

Recently, a protein encoded by an HSV-1 ORF in the LAT promoter region and expressed late in productive infection was identified and designated UOL. The UOL transcript contains 466 nucleotides, is polyadenylated, and has one ORF capable of encoding a 96 aa polypeptide of 11 K. The amino acid sequence of the UOL protein is highly conserved in HSV-1 strains. Importantly, the sera from mice infected with HSV-1 recognized the UOL protein expressed in *Escherichia coli*. It follows from this that the UOL protein is expressed in mice infected with HSV-1. Its function is unknown, it might contribute to HSV-1 virulence (Naito *et al*., 2005).

4.5 Other than LAT ORF-encoded proteins

In the past, a few viral antigens have been detected in latently infected TG *in vivo*, and in latently infected neurons in *ex vivo* TG cultures. For example, it has been reported that an ICP4 antibody reacts with latently infected rabbit TG, and low levels of TK and ICP47 transcripts can be detected in latently infected mouse TG. The low level of expression of viral genes expressed strongly in lytic cycle (productive infection) can be explained in two ways. Either many latently infected neurons express very low levels of viral RNAs other than LAT or rare neurons express high levels of lytic cycle RNAs. It is also possible that many latently infected neurons express very low levels of lytic cycle genes and few rare neurons express strongly the same genes (Green *et al*., 1981; Liu *et al*., 2000; Feldman *et al*., 2002; Sawtell, 2003).

4.6 Productive infection in rare neurons

The mouse TG is the most commonly used model for the study of HSV-1 latency. In contrast to humans and rabbits latently infected with HSV-1, spontaneous reactivation is rarely detected in mice. Therefore, it has been expected that latent infection could be preferably studied in this system. In the absence of a detectable infectious virus in TG, a careful examination of TG neurons for viral gene expression in latently infected mice that involved thousands of tissue sections demonstrated that abundant viral transcripts, viral protein, and viral DNA replication occur in rare neurons. A rare neuron means, in this context, approximately one neuron(in) per 10 TG. The viral transcripts examined were those corresponding to ICP4, TK, glycoprotein C, and LAT. The same neurons that were expressing high levels of transcripts were invariably surrounded by foci of infiltrating white blood cells (Feldman, 2002). Productive viral gene expression realized to certain extent during latency is probably due to incomplete virus reactivation or is the result of virus producing neurons that are quickly recognized by the immune system. These results are suggesting that there might exist different subsets of neurons, and it would be interesting to know whether specific populations of neurons are prone to spontaneous virus reactivation. Interestingly, it has been demonstrated that whereas some neuronal populations of the mouse TG are more permissive for establishment of a latent infection with LAT expression, the infection of other neuronal populations most likely results in a productive infection (Yang *et al*., 2000). A quantitative analysis of HSV-1 reactivation in the mouse model (Sawtell, 2003) revealed the presence of (i) viral protein in about 1 of 17 uninduced, latently-infected mouse ganglia, and (ii) only one viral protein expressing neuron in each of these ganglia. Because these reactivating neurons showed evidence of disintegration, it is likely that reactivating neurons do not survive. Fascinatingly, Sawtell (2003) used a whole ganglion immunohistochemistry approach, which allowed him to observe an entire ganglion in one view. Therefore he also clearly demonstrated that only cells morphologically distinguishable as neurons were found to express viral proteins, in agreement with the view that the neuron as the site of latency in the ganglion and that reactivation *in vivo* appears to be restricted to neurons. No protein was detected by a polyclonal antiserum directed against lytic viral proteins in latently infected neurons, which were not undergoing latency (Sawtell and Thompson, 2004).

5. CD8+T cell control of latency

Despite the lack of any convincing evidence that HSV-1 protein(s) are produced during latency, observations from several laboratories suggest that the immune system provides active surveillance of latently HSV-1-infected neurons in mice. Latently infected mouse and human TG are infiltrated with CD8+T cells suggesting an important role for CD8+T

cells in preventing the HSV-1 reactivation or maintaining the virus in latent state (Posavad *et al*., 1996; Khanna *et al*., 2003, 2004a,b; Theil *et al*., 2003).

5.1 MHC-I expression in neurons

Some investigators have argued that CD8+T cells are incapable of providing immune surveillance against HSV-1 reactivation from latency in humans because neurons do not express the MHC class I molecules that are required to present antigenic peptides to CD8+T cells. Nevertheless, mouse neurons do express MHC class I molecules during HSV-1 lytic/productive infection and at the point when latency is being established (Pereira *et al*., 1994; Pereira and Simmons, 1999; Song *et al*., 1999; Abendroth *et al*., 2000). In humans, the HSV ICP47 protein binds efficiently to the transporter of antigenic peptides (TAP) and blocks peptide transport from cytosol into the endoplasmic reticulum for loading on MHC class I molecules (York *et al*., 1994; Hill *et al*., 1995; Hill, 1996). Nevertheless, even in human cells ICP47 is unable to inhibit completely TAP transporting activity, and can be overcome by IFN-γ, which appears to be produced continually in latently infected TG. Moreover, some HSV-1 gene products in neurons could be degraded to peptides and loaded on MHC-I molecules prior to the accumulation of ICP47. Due to extremely high sensitivity of CD8+T cells even very low expression of some viral and MHC-I molecules in latently infected neurons can provide direct surveillance of viral gene expression by CD8+T cells (Sykulev *et al*., 1996).

5.2 Non-lytic mechanisms employed by CD8+T cells

The MHC-I restricted CD8+T cells typically destroy virus-infected cells (Lehner and Creswell, 2004). There is evidence in humans and mice that the HSV-1 infection is associated with partial loss of corneal sensation, suggesting that some rare neurons are indeed killed either by the virus or CD8+T cells. However, upon viral antigen recognition CD8+T cells can also secrete potent antiviral cytokines such as IFN-γ and TNF-α, which can potentially cure viral infections without killing infected cells. Such mechanism has been described for example for Hepatitis B virus (HBV) (Guidotti *et al*., 1996; Guidotti and Chisari 2001). HBV-specific CD8+T cells can abolish HBV gene expression and replication in the liver while killing only a small fraction of hepatocytes. This intracellular inactivation of HBV is mediated by inflammatory cytokines such as IFN-γ and TNF-α. The effects of CD8+T cells include disappearance of cytoplasmic viral nucleocapsid, DNA replicative intermediates, and viral RNA from the liver. Another studies revealed the ability of CD8+T cells to control HIV-1 infection without killing the infected cells. CD8+T cells may be able to halt replication of many other viruses without inducing rapid cell lysis (Levy, 2003).

5.3 CD8+T cells maintain HSV-1 in latency or prevent full reactivation

Recent studies show that CD8+T cells infiltrating TG could block HSV-1 reactivation by a non-lytic mechanism without elimination of latently infected neurons. As neurons cannot be regenerated, such a mechanism is perfectly fitting requirements for the control of HSV-1 latency.

In mice, CD8+T cells invade TG ~7 days after HSV-1 infection, reach maximum ~4 days p.i. and are then retained in a significant number seemingly for the life-time of the animal. Significantly, HSV-1-specific CD8+T cells are persistently stimulated within the latently infected TG. During the early stages of CD8+T cell infiltration into the infected TG, a time when virus replication was subsiding and latency was being established in sensory neurons, a few CD8+T cells expressed the early activation marker CD69. However, the CD69 expression on ganglion-derived CD8+T cells gradually increased as latency was uniformly established in TG and CD69 expression was maintained. The steady expression of IFN-γ and TNF-α in latently infected TG suggests continuous stimulation of the cells producing these cytokines. Expression of these cytokines is tightly regulated and subsides within two hours of withdrawal of the stimulant. Since IFN-γ can block HSV-1 reactivation from latency in sensory neurons, such a CD8+T cell population is ideally suited to the function of long-term protection with minimal tissue destruction (Kodukula *et al*., 1999; Liu *et al*., 2000, 2001; Mueller *et al*., 2003; Stuart *et al*., 2004).

Remarkably, at least 60% of the CD8+T cells in the latently infected TG of C57BL/6 mice were specific for H-2K^b/gB(498–505) complex. Importantly, the *in situ* staining of latently infected TG with the gB(498–505) tetramer revealed many CD8+T cells with polarization to the junction with a neuron, suggestive of a mature immunological synapse. In summary, these findings suggest that HSV-1 gB-specific H-2K^b restricted CD8+T cells are maintained in an activated state through a direct interaction with latently infected neurons that persistently or episodically express the γ1 gene gB. It would be somewhat surprising that a late viral gene product could be targeted by CD8+T cells and that these cells could block HSV-1 reactivation from latency. In contrast to γ2 genes (e.g. gC or gD), the α and γ1 genes (gB) are expressed in the absence of viral DNA synthesis. HSV-1 gB is indeed presented on the surface of infected cells with the kinetics of an early gene product. Thus, it is reasonable to speculate that certain HSV-1 γ1 genes may be expressed in latently infected neurons in the absence of viral DNA synthesis and γ2 expression. This is in agreement with

the finding that the expression of IFN-γ and TNF-α persisted in TG that were latently infected an HSV-1 mutant lacking viral thymidine kinase which was unable to replicate in neurons. CD8+T cells can block the HSV-1 reactivation from latency in *ex vivo* TG cultures derived from syngeneic C57BL/6 mice but not from allogeneic BALB/c mice, which confirms that protective response is MHC-restricted. These findings are consistent with the concept that gB-specific CD8+T cells can directly monitor viral gene activity in latently infected neurons and/or in reactivating neurons at least in this mouse experimental model (Khanna *et al*., 2004a,b). Even when HSV-1 gB from which peptide gB(498–505) is generated cannot be detected in latently infected TG by immunofluorescence, immunoblot analysis, and RIP-SDS-PAGE, its quantity could be sufficient to produce sufficient amounts of MHC-I/peptide complexes for recognition by CD8+T cells. There is evidence that a single peptide-MHC complex on a target cell can elicit a CD8+T cell response (Sykulev *et al*., 1996).

Another possibility is that CD8+T cells infiltrating the sensory ganglion stop reactivation and/or destroy the occasional rogue neuron that decides to support lytic infection. There is very likely a permanent attempt to reactivate a latent virus and CD8+T cells recognize those cells, which are at the very beginning in the reactivation process. Such a conclusion is supported by the observation that IFN-γ can block HSV-1 reactivation only when present early in the reactivation process. The capacity of latently infected neurons to repress viral and MHC gene expression may be frequently perturbed, permitting rapid processing and presentation of some viral gene products to surrounding CD8+T cells. In contrast to latently HSV-1-infected mice, the frequent reactivation of HSV-1 in human TG may reflect a less efficient CD8+T cell recognition. This would be compatible with the fact that HSV-1 ICP47 protein inhibits TAP-mediated transport of antigenic peptides more efficiently in human than in mouse cells. The ICP47 ability to inhibit transport of antigenic peptides, however, does not prevent efficient activation of naive human T cells by cross-priming (Carbone *et al*., 1998; Lehner and Creswell, 2004; Norbury and Sigal, 2003). As ICP47 blocks completely the TAP transport in human neurons, gB or human equivalent epitope is not expressed in MHC-I complex on the cell surface, and such cells cannot be recognized by HSV-1 specific CD8+T cells, even if those are near in a large number (Tomazin *et al*., 1998).

HSV-1 specific CD8+T cells may exert non-lytic or lytic mechanism against latently infected neurons. Epitope density might be a differential signal for CD8+T cell effector response. Viral proteins that are expressed early in reactivation are processed and presented with MHC-I at an initial density on the surface of neurons. The epitope density would increase as reactivation progresses. A recent study has demonstrated that the gB(498–505) epitope is expressed on cells very early (within 2 hours) after lytic infection is initiated, and might also be expressed early during reactivation. Khanna *et al*. (2004a,b) propose that the exposure of gB(498–505)-specific CD8+T cells to neurons early in reactivation, when the gB(498–505) epitope density is presumably low, induces IFN-γ production but not lytic granule release by the CD8+T cells. By contrast, with a neuron late in the reactivation process, when gB(498–505) epitope density is presumably high, the exposure induces the entire functional program of CD8+T cells, including lytic granule release.

CD8+T cells normally block the reactivation in neurons through a non-lytic mechanism, but they can kill those rare neurons that reach a late stage of HSV-1 reactivation and are refractory to non-lytic mechanisms. A therapeutic vaccine containing epitopes that are expressed early in HSV-1 reactivation might boost CD8+T cell protection from reactivation; yet minimize destruction of sensory neurons. An effective vaccine might have to target both reactivation of virus from latency in sensory ganglia and lytic infection at peripheral sites (Koelle and Corey, 2003)

6. Conclusions

Currently accumulating data indicate an important role of CD8+T cells in the control of HSV-1 latency. Although a LAT-encoded protein is not regularly expressed or is so far undetectable in latently infected neurons, the LAT-encoded protein expressed in a recombinant plasmid exhibited a high HSV-1-specific biological activity. Additional studies will be necessary to elucidate how CD8+T cells control the latency and/or reactivation and to determine whether LAT-encoded proteins also take part in this control.

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