T cell immunity in HSV-1- and VZV-infected neural ganglia

T cell immunity in HSV-1- and VZV-infected neural ganglia

Marlies Boeren[1,2,3], Pieter Meysman[4,5,6], Kris Laukens[4,5,6], Peter Ponsaerts[2], Benson Ogunjimi[3,4,7,8], Peter Delputte[1,9].

[1] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium

[2] Laboratory of Experimental Hematology (LEH), Vaccine and Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium

[3] Antwerp Center for Translational Immunology and Virology (ACTIV), Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

[4] Antwerp Unit for Data Analysis and Computation in Immunology and Sequencing (AUDACIS), Antwerp, Belgium

[5] Adrem Data Lab, Department of Computer Science, University of Antwerp, Antwerp, Belgium

[6] Biomedical Informatics Research Network Antwerp (biomina), University of Antwerp, Antwerp, Belgium

[7] Centre for Health Economics Research & Modeling Infectious Diseases (CHERMID), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium

[8] Department of Paediatrics, Antwerp University Hospital, Antwerp, Belgium

[9] Infla-med, University of Antwerp, Antwerp, Belgium

*Correspondence: peter.delputte@uantwerp.be (P. Delputte)

Abstract

Herpesviruses hijack the major histocompatibility complex class I (MHC I) and class II (MHC II) antigen presentation pathways to manipulate immune recognition by T cells. First, we illustrate herpes simplex virus-1 (HSV-1) and varicella-zoster virus (VZV) MHC immune evasion strategies. Next, we describe MHC - T cell interactions in HSV-1- and VZV- infected neural ganglia. Although studies on the topic are scarce and use different models, most reports indicate that neuronal HSV-1 infection is mainly controlled by CD8+ T cells through non-cytolytic mechanisms, whereas VZV seems to be largely controlled through CD4+ T cell-specific immune responses. Autologous human stem cell-derived *in vitro* models could

substantially aid in elucidating these neuro-immune interactions and are fit for studies on both herpesviruses.

Keywords: T cell, MHC, nervous system, varicella-zoster virus, herpes simplex virus-1, immune evasion.

Highlights

- Herpesviruses exert a multitude of MHC I and MHC II immune evasion strategies to establish persistent infections. A newly described mechanism involves epitope evasion through depletion of high-affinity peptides that fit into the MHC I binding cleft.
- MHC I and MHC II molecules are also expressed in the 'immune-privileged' nervous system.
- T cell immunity plays an active but not fully understood role in the nervous system to prevent HSV-1 and VZV reactivation.
- CD8+ T cells can control viral infection without causing neuronal cell death via (i) non-lytic cytokines such as IFN-y and/or (ii) via lytic granules such as granzyme B that degrade specific viral proteins.
- Neuronal HSV-1 infection seems to be mainly controlled by CD8+ T cells through non-cytolytic mechanisms, while VZV seems to be largely controlled through CD4+ T cell-specific immune responses.

MHC class I and class II antigen presentation pathway and herpesvirus immune evasion strategies

Herpesviruses have co-evolved with humans and can persist in humans by harnessing various strategies to circumvent both the innate and adaptive immune system. General herpes simplex virus-1 (HSV-1) and varicella-zoster virus (VZV) immune evasion strategies are reviewed in Amin et al. 2019 [1] and Abendroth et al. 2010 [2], respectively. We here focus on modulation of the major histocompatibility class I (MHC I) and class II (MHC II) antigen presentation pathway as an immune evasion strategy. In general, endogenous epitopes are presented by MHC class I molecules and exogenous epitopes are presented by MHC class II molecules.

Cytoplasmic proteins, including viral proteins, are processed by the **MHC class I antigen presentation pathway** (Figure 1).

Hijacking the transporter associated with antigen presentation (TAP) to avoid recognition by the adaptive immune system is frequently done by viruses, especially herpesviruses [3, 4]. For instance, HSV ICP47 cytoplasmic protein binds to TAP and prevents the transfer of viral peptides into the endoplasmic reticulum (ER), and as such also the loading onto MHC I (Figure 1) [4-7]. Human cytomegalovirus (HCMV) US6 impairs TAP function by interfering with ATP binding to the transporter (Figure 1) [4, 8]. Although VZV UL49.5 protein interacts with TAP, it does not block its function and has no effect on antigen recognition by human leukocyte antigen (HLA)-A1 and HLA-A2 restricted cytotoxic T lymphocyte (CTL) clones [9]. This is in agreement with the observation that MHC I is retained in the Golgi apparatus of VZV-infected cells and not in the ER where TAP plays its active role [10]. Interestingly, VZV ORF66 protein kinase activity is, at least partially, responsible for the accumulation of MHC I in the Golgi apparatus [11]. It is plausible that the VZV ORF66 protein interacts with another yet-to-be-identified protein at the stage of vesicle egress from the Golgi apparatus, or with the VZV UL49.5 protein (Figure 1). Furthermore, HCMV US2 and US11 can downregulate MHC I expression by enhancing the degradation of ER-localized MHC I heavy α-chain (Figure 1) [4]. In addition, y-herpesviruses genome maintenance proteins (GMPs) inhibit their own presentation on MHC I molecules during latency, a strategy called *cis*-acting immune evasion [12]. GMPs target many different steps in the MHC I antigen peptide presentation pathway all in the early stages of MHC I maturation (Figure 1) [12]. Finally, recent data showed that herpesviruses have evolved yet another MHC I immune evasion strategy: epitope evasion through depletion of high-affinity peptides that fit into the MHC I binding cleft (Figure 1) [13]. Computational enrichment analyses to determine proteins that are depleted across the 30 most common human HLA-I genes showed that protein products of VZV ORFs 4, 9, 32, 61, 62, and 63 are significantly depleted in the number of high-affinity peptides across many alleles, independently of the VZV strain used [13]. Interestingly, four of these proteins: ORFs 4, 61, 62, and 63 are transcriptional regulators, important in the early stages of infection and reactivation [14-19]. Indeed, by delaying detection to later stages of infection, the virus prevents early immune-induced destruction of a host cell and increases the probability of budding [13]. To illustrate clinical relevance: HLA-A molecules with poor VZV IE62 presentation capabilities were found to be more common in a cohort with a herpes zoster history as compared to a nationwide control group, and this tendency was most pronounced for herpes zoster cases at a young age where other risk factors are less prevalent [20]. In addition, post-herpetic neuralgia, the most common complication of herpes zoster, is also associated with certain HLA alleles [21-23]. Of note, in simian varicella virus (SVV), the closest but non-human VZV

analog, ORFs 9, 61, 62, and 63 were found to be depleted too [13]. Furthermore, the homolog of VZV ORF62, HSV1 protein RS1 also showed depletion [13].

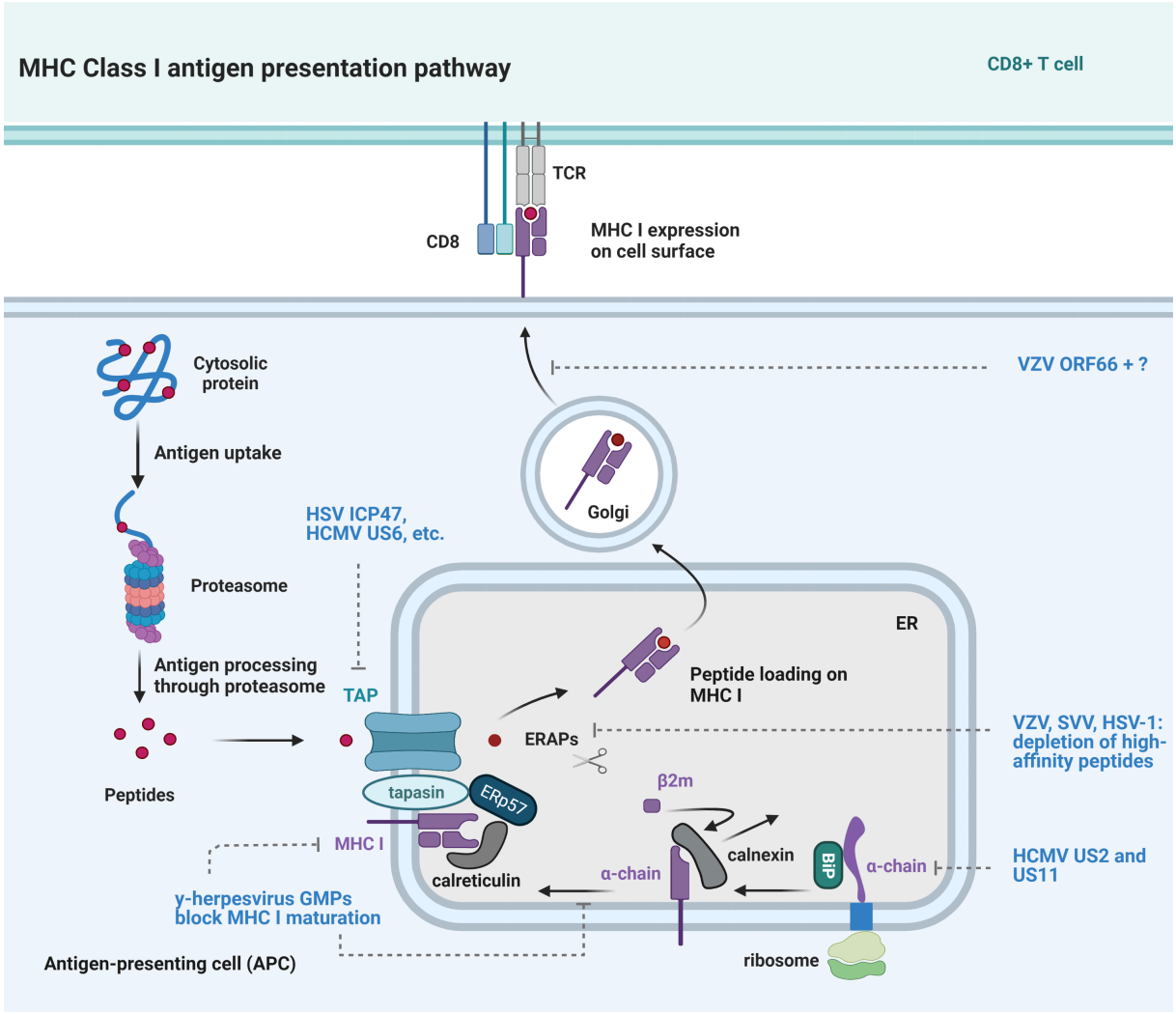
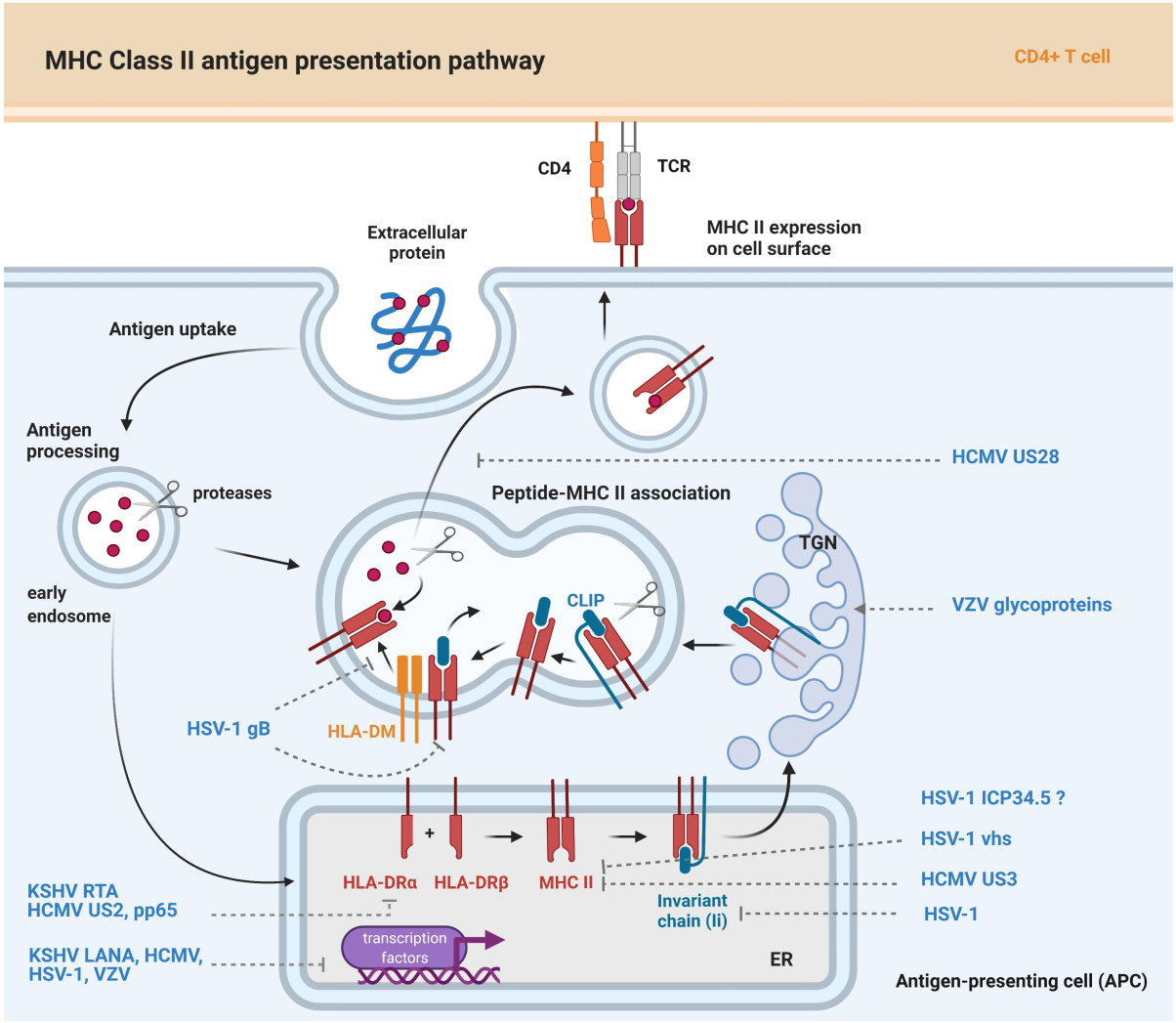


Figure 1: Herpesvirus strategies to modulate MHC class I antigen presentation Cytoplasmic proteins are processed by the proteasome. The resulting peptides are transported into the ER lumen by TAP and can be further trimmed by endoplasmic reticulum aminopeptidases (ERAPs). MHC I heavy α-chain associates with binding immunoglobulin protein (BiP) and next with calnexin. β2-microglobulin (β2m) assembles with the heavy α-chain to form the MHC I dimer. Calnexin is being exchanged for calreticulin. The MHC I-calreticulin complex associates with TAP/Tapasin–ERp57. The peptide is loaded onto the MHC I complex with concurrent dissociation of the TAP/Tapasin-ERp57. Finally, the MHC I – peptide complex is transported to the cell surface through the Golgi apparatus where it can be recognized by CD8+ T cells [24, 25]. Dashed lines indicate the stages where herpesviruses interfere with the MHC class I antigen presentation pathway.

Endosomal and lysosomal viral proteins, typically ingested by antigen-presenting cells (APC), are processed by the **MHC class II antigen presentation pathway** (Figure 2). However, viral proteins normally found in the cytoplasm and exocytic compartments can also be efficiently presented by MHC II proteins [26, 27]. For instance, MHC II presentation of cytosolic antigens HCMV immediate early protein 1 (IE1) [28] and Epstein-Barr nuclear antigen 1 (EBNA1) [29, 30] was reported. Moreover, the autophagy pathway can serve as an additional entry route for presentation by MHC II, as illustrated by EBNA1 [31].

MHC class II immunoevasins have been detected for all members of the herpesvirus family. They are presumed to be primarily expressed during the lytic cycle when virus particles can be transmitted to other hosts, rather than during latency when protein expression is already tightly contained [27, 32]. The MHC class II transactivator (CIITA) is the master regulator of MHC class II expression and is regulated by IFN-y signaling through the JAK/STAT pathway [26, 27]. Several herpesviruses target **transcription factors upstream of CIITA.** For instance, Kaposi Sarcoma Herpesvirus (KSHV)-encoded latency-associated nuclear antigen (LANA) can downregulate CIITA transcription by reducing the transcriptional activity of CIITA promoters PIII and PIV (Figure 2) [33]. LANA can also bind RFX proteins which prevents the association of CIITA with the MHC II promotors, leading to an even greater MHC II downregulation [34]. Furthermore, HCMV can induce enhanced proteasomal degradation of JAK1, leading to MHC II downregulation (Figure 2) [35]. Similarly, HSV-1 can target STAT1 and STAT2 [27], and VZV can target STAT1α and JAK2, thereby blocking downstream transcription of IFN regulatory factor 1 (IRF1) and CIITA, resulting in downregulation of MHC II expression (Figure 2) [36]. Moreover, VZV can inhibit IFN-y induction of MHC II cell surface expression, which may transiently protect cells from CD4+ T cell immune surveillance [36]. HCMV, HSV-1 and VZV genes involved in targeting the transcriptional factors in the JAK/STAT pathway remain to be elucidated [27]. Herpesviruses also interfere with MHC II expression post-translationally at different stages of **MHC II assembly and trafficking to the cell surface**. For instance, KSHV replication and transcription activator (RTA) protein, an important regulator of the viral life cycle, can downregulate MHC II expression directly through redirection of HLA-DRα to a proteasomal degradation pathway and indirectly by enhancing the expression of MARCH8 which results in downregulation of its substrate HLA-DRα (Figure 2) [37]. Furthermore, three HCMV-encoded proteins, HCMV US2, US3, and pp65, downregulate MHC II expression during lytic infection and act at different stages of assembly and trafficking. HCMV US2 can induce rapid proteasomal degradation of MHC II heavy chains. HCMV US3 can bind MHC II αβ complexes leading to mislocalization, and HCMV pp65 can traffic MHC

II complexes into perinuclear lysosomes where the HLA-DRα chain is degraded (Figure 2) [27, 38, 39]. During HCMV latency, MHC II expression is downregulated via retention of HLA-DR within cytoplasmic vesicles that also contain HLA-DM [40]. This mechanism is attributed to the HMCV US28 protein (Figure 2) [41]. Alpha-herpesviruses also interfere post-translationally with MHC II expression. For instance, HSV-1 infection strongly reduces the expression of the invariant chain (Ii) in B lymphoblastoid cells, impairing the formation of stable MHC II-peptide complexes (Figure 2) [42]. In addition, HSV-1 viral envelope glycoprotein B (gB) can bind to HLA-DR which leads to competition with viral peptides for binding to Ii (Figure 2) [43]. HSV-1 gB can also associate with HLA-DM molecules (Figure 2). These three mechanisms ultimately serve to inhibit MHC II cell surface expression and antigen presentation [42]. Moreover, the HSV-1 host shutoff *(vhs)* protein, encoded by the U_L41 gene, and the infected cell protein 34.5 (ICP34.5), encoded by the $\gamma_1 34.5$ gene, can block antigen capturing and transport of MHC II proteins to the cell surface leading to decreased MHC II expression on glioblastoma cells [44]. The *vhs* protein acts early in infection to block the *de novo* synthesis of MHC II proteins, whilst the ICP34.5 protein acts later in the infection cycle through a yet-to-be-elucidated mechanism (Figure 2) [44]. It is unlikely that VZV interferes at the stage of MHC II transport to the cell surface since experimental MHC II upregulation on fibroblasts, through IFN-γ stimulation, is not reversed by VZV infection. For VZV to be able to persist latently in the host, transient downregulation of MHC II cell surface expression is indeed favored over a prolonged blocking through multiple mechanisms which would completely circumvent CD4+ T cell immune surveillance and lead to a productive infection [36]. Remarkably, herpesviruses' final replication stages occur in endosomal compartments containing proteins for virion assembly. VZV acquires its viral glycoproteins through vesicles derived from the TGN. These proteins can directly be processed for presentation by MHC II molecules on APC instead of being used for viral assembly [26, 45]. Whether this is a viral strategy to limit viral spread in favor of the establishment of latency remains to be elucidated.



168

169 **Figure 2: Herpesvirus strategies to modulate MHC class II antigen presentation**
170 Endosomal or lysosomal viral proteins are first degraded into peptides within their acidic
171 vesicles. Meanwhile, in the ER newly synthesized class II α and β chains associate with the
172 invariant chain (Ii). These complexes are directed to the trans-Golgi network (TGN) and
173 subsequently to the vesicles containing the peptides to be presented. The class II invariant light
174 chain peptide (CLIP) that occupies the peptide-binding cleft is exchanged for the antigen, a
175 process facilitated by an important accessory protein HLA-DM. Finally, the MHC class II –
176 peptide complex is transported to the cell surface for antigen presentation to CD4+ T cells [27,
177 46]. Dashed lines (blockade) and arrows (entry route) indicate the stages where herpesviruses
178 interfere with the MHC class II antigen presentation pathway.

MHC class I - CD8+ T cell interactions in HSV-1- and VZV-infected ganglia

Although MHC class I molecules are expressed at the surface of almost all nucleated cells, their expression levels may strongly vary depending on cell type and conditions [47]. Initially, it was generally accepted that neural cells, in an immune-privileged site, do not express MHC I at their surface. This concept has been increasingly challenged as MHC I expression may be upregulated on neurons during neuronal HSV-1 and/or VZV infection. Yet, this is not without risk as MHC I expression would make infected neurons susceptible to CD8+ T cell killing and as such - as neurons are non-renewable - would potentiate loss of local neuronal network integrity. Nevertheless, besides **cytolytic mechanisms**, CD8+ T cells can also exert **non-cytolytic mechanisms** to clear virus-infected cells (Text Box 1).

Text Box 1: **CD8+ T cell mechanisms to clear virus-infected cells**

CD8+ T cell cytolytic mechanisms:

(i) Release of **perforin** alone or together with **granzymes**.
Perforin alone can lead to immediate necrosis of the target cell through the formation of large pores, causing swelling and rupture of the cell membrane. In addition, perforin can mediate the trafficking of granzymes into the target cell leading to apoptosis [48].

(ii) Ligation of the **Fas-Ligand** (FasL) with the **Fas-receptor** (Fas) activates the caspase cascade and leads to apoptosis [48, 49].

CD8+ T cell non-cytolytic mechanisms:

(i) Virus-infected cells release type I interferons (IFNs) including **IFN-α and -β**, immediately upon infection in response to Toll-like receptor signaling and other RNA sensors. Type I IFNs induce pro-apoptotic molecules but also activate immune cells (NK cells, B cells, T cells, dendritic cells, macrophages) and trigger the activation of non-cytolytic intracellular pathways resulting in the production of interferon-stimulated genes that limit viral spread [50, 51].

(ii) CD8+ T cells (and NK cells) can produce antiviral cytokines, primarily **IFN-y and TNF-α**, in response to IFN-α/β that can potentially result in complete viral clearance without killing the affected cell [50].

(iii) Besides the typical cytolytic activity of **granzymes**, they can also inhibit viral replication and reactivation in cell death–independent manners. They can induce proteolysis of viral or host cell proteins necessary for viral entry, release, or intracellular trafficking, and can amplify the antiviral cytokine response [52].

MHC class I – CD8+ T cell interactions in HSV-1-infected ganglia

MOUSE STUDIES

Pioneering work from Pereira et al. in mice showed transient expression of the murine equivalent of human MHC I (H2 surface antigen) in primary sensory neurons, satellite glial cells (SGCs), and Schwann cells, upon HSV-1 infection. Interestingly, this was only observed during acute infection but not during latency at 64 weeks after infection [53]. CD8+ T cells were observed tightly associated with some neurons from seven days post-inoculation, concurrent with the first detectable expression of H2 surface antigens and termination of infection [53]. At this point, most MHC I-expressing neurons were thus not productively infected [53]. As hypothesized by Medana et al., MHC I-expressing neurons that contain a high viral load may be rapidly killed by CTLs thereby implying the sacrifice of a limited number of non-renewable neurons to protect healthy adjacent neurons [54]. However, termination of productive infection does not necessarily require neuronal cell death. Indeed, there is clear evidence for non-cytolytic CD8+ T cell responses. It was shown that control of HSV-1 latency in mice involves a non-cytolytic CD8+ T cell response via IFN-y signaling, but also via lytic granules such as granzyme B (grB) that do not kill the entire affected neuron. Instead, grB specifically degrades HSV-1 ICP4, which is essential for viral gene expression and reactivation [55, 56]. In another report, CD8+ T cells specific for the immunodominant HSV-1 $gB_{498-505}$ epitope were found to be selectively retained in the ophthalmic branch of the latently infected trigeminal ganglia (TG) in mice. CD8+ T cells also showed the capacity to produce IFN-γ and showed T cell receptor polarization to junctions with neurons, suggesting active surveillance of HSV-1 gene expression during latency [57]. Likewise, Liu et al. showed that CD8+ T cells block HSV-1 reactivation in latently infected mouse sensory ganglia without cytolysis [58].

They hypothesized that early in reactivation a low density of HSV-1 epitopes, derived from HSV IE and E proteins, are expressed on MHC I which may favor the production of antiviral cytokines by CD8+ T cells without cytolysis [58]. However, high-density presentation of an immunodominant HSV-1 CD8+ T cell epitope, gB_{498}, did also not activate cytolytic CD8+ T cell responses and did not affect latency nor virus loads in mouse sensory ganglia [59]. Another proposed control mechanism involves MHC I presentation of HSV-1 structural antigens immediately following entry in the neuron and prior to viral replication. Such a mechanism would allow immune surveillance by CD8+ T cells, thereby limiting viral spread and the establishment of latency without cytolysis [53].

HUMAN GANGLIA

A recent study in latently HSV-1 infected human TG showed clusters of CD4+ and CD8+ T cells surrounding neurons and expression of transcripts and proteins associated with antigen recognition and antiviral functions [60]. Moreover, expression of perforin and grB directly correlated with HSV-1 DNA levels, and grB and TIA-1 protein expression co-localized with CD8+ T cells in the proximity of neurons [60]. MHC I expression, however, was not studied in that report. Interestingly, in an earlier study in which human TG were latently infected, most T cells were CD8+ with only a few CD4+ T cells [61].

Taken together, these data point toward a functional role of CD8+ T cells in controlling HSV-1 infection, without causing neuronal cell death, via non-lytic cytokines and/or via lytic granules such as grB that degrade specific viral proteins.

MHC class I – CD8+ T cells interactions in VZV-infected ganglia

HUMAN GANGLIA

Examination of human sensory ganglia from donors who experienced herpes zoster 1 to 4.5 months prior to death, revealed no MHC I expression on either VZV-infected or non-infected neurons and no CD8+ T cells in proximity of the neurons [62]. Non-cytolytic CD8+ T cells, identified by the absence of TIA-1 or grB expression, were the most abundant immune cell type in the ganglia whereas only very few cytolytic CD8+ T cells were observed [62]. Together, this suggests that CD8+ T cells do not play a major role in controlling VZV reactivation or that they do so in a non-cytolytic manner and without direct contact with infected neurons. Possibly, CD8+ T cells interact with other cell types within the ganglia that do express MHC I, such as SGCs [63]. Alternatively, MHC I expression on neurons could be too low to be detected by

immunofluorescence staining or was downregulated to a steady-state level at the time of investigation. Interestingly, Steain et al. did not detect MHC I in neurons of patients with a reactivated VZV infection at the time of death. In contrast, MHC I expression was detected on all other cell types within the ganglia [63]. Characterization of the infiltrating T cells revealed many CD4+ T cells and cytolytic, grB-expressing, CD8+ T cells of which many were closely associated with neurons within the reactivated ganglia. However, there was little evidence of T cell-induced neuronal apoptosis in either reactivated or non-infected ganglia [63]. As mentioned above, grB can specifically degrade HSV-1 ICP4 instead of affecting the entire ganglion [55]. Similarly, grB may degrade a specific VZV protein without causing neuronal apoptosis. Interestingly, grB can also cleave VZV ORF62, the homolog of HSV-1 ICP4, and HSV ICP27 and its homolog VZV ORF4 [64].

Thus, while there is clear evidence for MHC I expression on neurons and direct interaction with CD8+ T cells upon HSV-1 infection, this is not the case for VZV infection.

MHC class II - CD4+ T cell interactions in HSV-1- and VZV-infected ganglia

As MHC II expression is restricted to professional APC, its expression in the nervous system was thought to be reserved for microglia, the central nervous system counterpart of macrophages [65, 66]. Nonetheless, MHC II expression was described on different neural cell types, including neurons, especially upon induction with IFN-y [27, 67, 68].
In general, CD4+ T cells can recognize peptides presented by MHC class II molecules and act together with CD8+ T cells to clear the invading pathogen (Text Box 2). However, CD4+ T cell functions extend beyond aiding CD8+ T cells (Text Box 2).

Text Box 2: CD4+ T cell mechanisms to clear virus-infected cells

<u>CD4+ T cells' key roles in ensuring a robust and optimal antiviral response</u>:

(i) CD4+ T cells are required for the **generation of cytotoxic and memory CD8+ T cell populations**.
CD4+ T cells promote memory differentiation of CTLs during priming. Help signals enhance IL-15-dependent maintenance of central memory T cells and regulate the size and function of effector memory T cells [69, 70].

(ii) **T-helper 1 cells** (T_H1) cells produce a large amount of **IFN-y**, which can activate APC to kill ingested microbes.

Naïve CD4+ T cells develop into effector CD4+ T cells upon recognition of an antigen presented by MHC II molecules on activated APC [69] and can differentiate into specific CD4+ effector subtypes depending on the cytokine milieu of the microenvironment [71]. Typically, IL-12 produced by APC, IFN-y produced by NK cells, and type I IFNs drive the differentiation of naïve CD4+ T cells into T_H1 cells. Hence, CD4+ T cells generated in response to viral infection generally have a T_H1 phenotype [46, 69, 71-73].

(iii) Subsets of **CD4+ T cells displaying potent direct antiviral activity** themselves were recently reported in animal models and humans. These untraditional CD4+ T cells are likely to play an important, yet underrated, role in the control of viral replication *in vivo* [27, 74].

(iv) **Follicular helper cells** (Tfh) interact with **B-cells**, leading to B-cell activation with the production of **virus-specific antibodies** [46, 75]

MHC class II – CD4+ T cell interactions in HSV-1-infected ganglia

MOUSE STUDIES

One of the first studies that focused on HSV-1 spread to the central nervous system of mice indicated that MHC II-restricted presentation of viral antigens is required for the control of HSV-1 infections in the nervous system [76]. The morphology of the MHC II-expressing cells in that study suggests that these are most likely microglia or infiltrated macrophages and not neurons [76].

HUMAN GANGLIA

In a later report in which human TG latently harboring HSV-1 were examined, retention of virus-specific CD4+ T cells, next to CD8+ T cells, within TG was observed [60]. SGCs are the most evident non-neuronal cells in the peripheral nervous system presumed to serve as non-professional APC. They have phagocytic capacity related to macrophages and myeloid

dendritic cells, express CD45, co-stimulatory molecules and MHC II, and envelope neuronal cell bodies [60, 77]. Hence, SGCs could support HSV-specific CD4+ T cell responses within latently infected human TG. However, as mentioned earlier, CD8+ T cells are the most dominant cell type during HSV-1 latency [61].

Taken together, little research has been done on MHC II – CD4+ T cell interactions in HSV-1-infected ganglia. Instead, MHC I – CD8+ T cell interactions are more clearly described and generally considered more important in controlling HSV-1 infection and reactivation.

MHC class II – CD4+ T cell interactions in VZV-infected ganglia

AFRICAN GREEN MONKEYS: SIMIAN VARICELLA VIRUS (SVV)

Analyses of ganglia obtained from African green monkeys inoculated with the non-human primate counterpart of VZV, SVV, revealed an influx of $CD8^{bright}$ T cells, equivalent to human CD8+ T cells, and $CD8^{dim}$ T cells, which develop from CD4+ T cells, concurrent with a decline in viral load. Increased MHC II expression on SGCs and a specific influx of $CD8^{dim}$ T cells that recognize peptides presented on MHC II indicate a major role for MHC class II-restricted T cell responses [78]. This is in agreement with the finding that depletion of CD4+ T cells, but not CD8+ T cells or B cells, during primary SVV infection results in sustained lytic viral gene expression in ganglia [79].

HUMAN GANGLIA

For VZV, Steain et al. observed infiltration of cytolytic CD8+ T cells but also CD4+ T cells in the ganglia from patients who were suffering from active herpes zoster at the time of death [63]. They showed that MHC I, but more significantly MHC II expression, was upregulated on SGCs within both reactivated and neighboring ganglia and on some other infiltrating inflammatory cells [63]. Of note, data from Zostavax® and Shingrix® vaccine trials point out an essential role for CD4+ T cells in preventing VZV reactivation [80-83].

All things considered, SGCs are likely to play a role in controlling VZV reactivation: (i) as non-professional APC by taking op VZV antigens, expressing MHC II, and presenting viral peptides to CD4+ T cells which could then kill the virus by the production of IFN-γ or through unconventional direct antiviral mechanisms; (ii) by expressing MHC I and interaction with CD8+ T cells.

An overview of MHC I – CD8+ T cell interactions and MHC II – CD4+ T cell interactions to control HSV-1 and VZV infection in the nervous system is presented in Key Table 1. As described here, these mechanisms differ between HSV-1 and VZV and between latency and reactivation or lytic infection. The exact contribution of each cell population, however, remains to be elucidated and merits further in-depth investigation.

Key Table 1: Site of MHC I and MHC II expression in HSV-1- and VZV-infected neural ganglia and proposed mechanism of control

HSV-1		
MHC I and MHC II expression	**Proposed mechanism of viral control in neural ganglia**	**Main sources**
<u>Mouse sensory ganglia:</u> H2 surface antigen (equivalent to human MHC I): on primary sensory neurons, SGCs, and Schwan cells during acute infection, not during latency. MHC II: on microglia or infiltrating macrophages	<u>Acute infection:</u> Non-cytolytic control via CD8+ T cells that produce antiviral cytokines such as IFN-y and via grB that specifically degrades HSV-1 ICP4. Peptide presentation to CD4+ T cells, presumably by microglia or infiltrating macrophages, is required for the control of an acute HSV-1 infection.	[53, 55, 56, 58, 76]
<u>Human TG:</u> MHC I: not studied MHC II: likely on SGCs	<u>Reactivation:</u> Non-cytolytic control via CD8+ T cells, releasing perforin and grB, in cooperation with CD4+ T cells that interact with MHC II, presumably on SGCs. <u>Latency</u>: CD8+ T cells are much more abundant than CD4+ T cells.	[57, 60, 61]

	Hypothesis: **More prominent role for CD8+ T cells than CD4+ T cells in controlling HSV-1 reactivation.**	
VZV		
MHC I and MHC II expression	**Proposed mechanism of viral control in neural ganglia**	**Main sources**
Human TG: MHC I: on all cell types (infiltrating cells and SGCs) except neurons, during latency and reactivation. MHC II: on SGCs in reactivated and neighboring cells (not studied during latency)	Reactivation: Non-cytolytic control via CD8+ T cells that release grB, in cooperation with CD4+ T cells that interact with MHC II on SGCs. Hypothesis: SGCs are likely to play a role in controlling VZV reactivation: (i) as non-professional APC by taking op VZV antigens and presenting viral peptides on MHC II to CD4+ T cells, which could then kill the ingested virus by the production of IFN-y or through other antiviral mechanisms; (ii) by expressing MHC I and interaction with CD8+ T cells. Latency: Non-cytolytic control via non-cytolytic (grB and TIA-1 negative) CD8+ T cells that do not interact directly with neurons. Hypothesis: **More prominent role for CD4+ T cells than CD8+ T cells in controlling VZV reactivation.**	[62, 63]

319

Human stem cell-derived models to study neuro-immune interactions in the context of HSV-1 and VZV infection

During the last decade, neuronal models derived from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) have been developed to study the infection dynamics of HSV-1 and especially VZV, since mice are refractory to VZV disease [84-86]. hESCs are derived from the inner cell mass of *in vitro*-fertilized human embryos and can differentiate into neurons with a sensory phenotype, which is the site of HSV-1 and VZV latency in the peripheral nervous system [87, 88]. iPSCs can be derived from various cell types such as fibroblasts or peripheral blood mononuclear cells which are reprogrammed, using a group of transcription factors, towards an embryonic stem cell-like stage [89]. Subsequently, the iPSCs can be induced into human neuronal stem cells and further differentiated into neuronal cells with a sensory phenotype, comparable to hESC-derived neurons [84]. In addition, hESC- and hiPSC-derived neurons can be co-cultured with autologous SGCs, macrophages, or other immune cell types. Thus, these models offer a valuable, and even advanced, alternative to studies on (human) cadaver ganglia. Indeed, cadaver ganglia are difficult to obtain, costly, and have the inherent disadvantage of post-mortem changes which influences many factors including gene expression. Moreover, stem cell-derived neuronal models are suitable to study both HSV-1 as well as VZV. These stem cell-derived (co-)culture models clearly hold great potential to gain novel fundamental insights into HSV-1 and VZV (immune)biology. While to date, these models have not yet been exploited to investigate the interaction between HSV-1 or VZV-infected neurons and immune cells, the availability of differentiation protocols for a multitude of cell types makes such studies clearly feasible. It should be noted, however, that differentiation protocols from hESCs or hiPSCs towards T cells still face poor differentiation efficiency and poor scalability [90]. Nonetheless, new differentiation protocols are being developed given the large opportunity for iPSC-derived T cells in the context of immunotherapy [91]. In addition, iPSC-derived neurons can easily be combined with primary T cells from the same donor, thus bypassing the need for differentiation from iPSCs to T cells.

Concluding Remarks and Future Perspectives

HSV-1 and VZV interfere at multiple stages of the MHC class I and class II antigen presentation pathway to modulate MHC I and MHC II cell surface expression. As a consequence,

opportunities for antigen recognition by CD8+ and CD4+ T cells are tightly controlled, especially in neurons. While there is evidence for a more important role of CD8+ T cells over CD4+ T cells in preventing HSV-1 reactivation, the opposite is true for the control of VZV reactivation. In particular, SGCs are likely to play a role in controlling VZV reactivation. In contrast, during HSV-1 reactivation, CD8+ T cells are likely to interact directly with MHC I on neurons. However, the exact mechanisms of control of neuronal HSV-1 and VZV reactivation are still unclear (cf. Outstanding Questions). A limitation to the comparison described in this review, is that most of the HSV-1 data come from studies in mouse models, which cannot be done for VZV as mice are refractory to VZV infection. *Ex vivo* material of (latently) HSV-1 or VZV infected ganglia is valuable but also scarce, costly, and has inherent disadvantages of variability and post-mortem changes. More complex neuro-immune models incorporating autologous neurons, SGCs, and T cells are urgently needed to unravel the mechanisms of viral control in neural ganglia. Human iPSC-based neuronal cell systems are very promising for this type of research. In addition to gaining a fundamental understanding of herpesvirus infection biology, such models can elucidate which mechanisms of immune control are truly relevant, which would be highly valuable in the context of vaccine development.

Outstanding Questions

- Are VZV glycoproteins in TGN-vesicles captured to display viral antigens on MHC II molecules for recognition by CD4+ T cells? Does this contribute to the establishment of latency?
- What is the role of granzyme B in non-lytic control of neuronal VZV infection? Can granzyme B specifically degrade the VZV virion (as shown with HSV-1) instead of killing the entire neuron?
- What is the role of satellite glial cells in maintaining neuronal VZV and HSV-1 latency?
- Are CD4+ T cells and not CD8+ T cells essential in preventing VZV reactivation in human neural ganglia?
- Are CD8+ T cells and not CD4+ T cells essential in preventing HSV-1 reactivation in human neural ganglia?

Acknowledgments

This work was supported by research grants from the Fund for Scientific Research Flanders (FWO-Vlaanderen: G067118N, 1861219N, and G034721N) and the University of Antwerp Industrial Research Fund (POC project 46947).

LMPH is a partner of the Excellence Centre 'Infla-Med'. LEH and CHERMID are partners within VAXINFECTIO that reside under the Excellence Centre ASCID (Antwerp Study Centre for Infectious Diseases).

Figures were created with Biorender.com

Declaration of Interests

The authors declare no competing interests.

References

1. Amin, I., et al., *Herpes Simplex Virus Type 1 and Host Antiviral Immune Responses: An Update.* Viral Immunol, 2019. **32**(10): p. 424-429.
2. Abendroth, A., P.R. Kinchington, and B. Slobedman, *Varicella zoster virus immune evasion strategies.* Current topics in microbiology and immunology, 2010. **342**: p. 155-171.
3. Verweij, M.C., et al., *Structural and functional analysis of the TAP-inhibiting UL49.5 proteins of varicelloviruses.* Mol Immunol, 2011. **48**(15-16): p. 2038-51.
4. Antoniou, A.N. and S.J. Powis, *Pathogen evasion strategies for the major histocompatibility complex class I assembly pathway.* Immunology, 2008. **124**(1): p. 1-12.
5. Hill, A., et al., *Herpes simplex virus turns off the TAP to evade host immunity.* Nature, 1995. **375**(6530): p. 411-5.
6. Orr, M.T., et al., *Inhibition of MHC Class I Is a Virulence Factor in Herpes Simplex Virus Infection of Mice.* PLOS Pathogens, 2005. **1**(1): p. e7.
7. Früh, K., et al., *A viral inhibitor of peptide transporters for antigen presentation.* Nature, 1995. **375**(6530): p. 415-8.
8. Verweij, M.C., et al., *Viral inhibition of the transporter associated with antigen processing (TAP): a striking example of functional convergent evolution.* PLoS pathogens, 2015. **11**(4): p. e1004743-e1004743.
9. Koppers-Lalic, D., et al., *Varicellovirus UL 49.5 proteins differentially affect the function of the transporter associated with antigen processing, TAP.* PLoS Pathog, 2008. **4**(5): p. e1000080.
10. Abendroth, A., et al., *Varicella-zoster virus retains major histocompatibility complex class I proteins in the Golgi compartment of infected cells.* J Virol, 2001. **75**(10): p. 4878-88.
11. Eisfeld, A.J., et al., *Downregulation of class I major histocompatibility complex surface expression by varicella-zoster virus involves open reading frame 66 protein kinase-dependent and -independent mechanisms.* J Virol, 2007. **81**(17): p. 9034-49.
12. Sorel, O. and B.G. Dewals, *The Critical Role of Genome Maintenance Proteins in Immune Evasion During Gammaherpesvirus Latency.* Front Microbiol, 2018. **9**: p. 3315.
13. Meysman, P., et al., *Immunological evasion of immediate-early varicella zoster virus proteins.* Immunogenetics, 2016. **68**(6-7): p. 483-486.

14. Braspenning, S.E., et al., *Decoding the Architecture of the Varicella-Zoster Virus Transcriptome.* mBio, 2020. **11**(5).
15. Kost, R.G., H. Kupinsky, and S.E. Straus, *Varicella-zoster virus gene 63: transcript mapping and regulatory activity.* Virology, 1995. **209**.
16. Moriuchi, H., et al., *Varicella-zoster virus (VZV) open reading frame 61 protein transactivates VZV gene promoters and enhances the infectivity of VZV DNA.* J Virol, 1993. **67**(7): p. 4290-5.
17. Perera, L.P., et al., *A major transactivator of varicella-zoster virus, the immediate-early protein IE62, contains a potent N-terminal activation domain.* J Virol, 1993. **67**(8): p. 4474-83.
18. Moriuchi, M., et al., *Varicella-Zoster Virus (VZV) Virion-Associated Transactivator Open Reading Frame 62 Protein Enhances the Infectivity of VZV DNA.* Virology, 1994. **200**(1): p. 297-300.
19. Defechereux, P., et al., *Characterization of the regulatory functions of varicella-zoster virus open reading frame 4 gene product.* J Virol, 1993. **67**(7): p. 4379-85.
20. Meysman, P., et al., *Increased herpes zoster risk associated with poor HLA-A immediate early 62 protein (IE62) affinity.* Immunogenetics, 2018. **70**(6): p. 363-372.
21. Sato, M., et al., *Association of HLA-A*3303-B*4403-DRB1*1302 haplotype, but not of TNFA promoter and NKp30 polymorphism, with postherpetic neuralgia (PHN) in the Japanese population.* Genes Immun, 2002. **3**(8): p. 477-81.
22. Meysman, P., et al., *Varicella-zoster virus-derived major histocompatibility complex class I-restricted peptide affinity is a determining factor in the HLA risk profile for the development of postherpetic neuralgia.* J Virol, 2015. **89**(2): p. 962-9.
23. Sumiyama, D., et al., *HLA alleles are associated with postherpetic neuralgia but not with herpes zoster.* Tokai J Exp Clin Med, 2008. **33**(4): p. 150-3.
24. Hearn, C., et al., *An MHC class I immune evasion gene of Marek's disease virus.* Virology, 2015. **475**: p. 88-95.
25. Hulpke, S. and R. Tampé, *The MHC I loading complex: a multitasking machinery in adaptive immunity.* Trends Biochem Sci, 2013. **38**(8): p. 412-20.
26. Hegde, N.R., M.S. Chevalier, and D.C. Johnson, *Viral inhibition of MHC class II antigen presentation.* Trends Immunol, 2003. **24**(5): p. 278-85.
27. Wiertz, E.J., et al., *Herpesvirus Interference with Major Histocompatibility Complex Class II-Restricted T-Cell Activation.* Journal of Virology, 2007. **81**(9): p. 4389-4396.
28. Le Roy, E., et al., *Infection of APC by human cytomegalovirus controlled through recognition of endogenous nuclear immediate early protein 1 by specific CD4(+) T lymphocytes.* J Immunol, 2002. **169**(3): p. 1293-301.
29. Münz, C., et al., *Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1.* The Journal of experimental medicine, 2000. **191**(10): p. 1649-1660.
30. Voo, K.S., et al., *Identification of HLA-DP3-restricted peptides from EBNA1 recognized by CD4(+) T cells.* Cancer Res, 2002. **62**(24): p. 7195-9.
31. Paludan, C., et al., *Endogenous MHC class II processing of a viral nuclear antigen after autophagy.* Science, 2005. **307**(5709): p. 593-6.
32. Fields, B.N., D.M. Knipe, and P.M. Howley, *Fields virology*. 2007, Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.
33. Cai, Q., et al., *IRF-4-Mediated CIITA Transcription Is Blocked by KSHV Encoded LANA to Inhibit MHC II Presentation.* PLOS Pathogens, 2013. **9**(10): p. e1003751.
34. Thakker, S., et al., *Kaposi's Sarcoma-Associated Herpesvirus Latency-Associated Nuclear Antigen Inhibits Major Histocompatibility Complex Class II Expression by Disrupting Enhanceosome Assembly through Binding with the Regulatory Factor X Complex.* J Virol, 2015. **89**(10): p. 5536-56.
35. Vossen, M.T., et al., *Viral immune evasion: a masterpiece of evolution.* Immunogenetics, 2002. **54**(8): p. 527-42.

36. Abendroth, A., et al., *Modulation of major histocompatibility class II protein expression by varicella-zoster virus.* J Virol, 2000. **74**(4): p. 1900-7.
37. Sun, Z., et al., *Major Histocompatibility Complex Class II HLA-DRalpha Is Downregulated by Kaposi's Sarcoma-Associated Herpesvirus-Encoded Lytic Transactivator RTA and MARCH8.* J Virol, 2016. **90**(18): p. 8047-58.
38. Odeberg, J., et al., *Human cytomegalovirus protein pp65 mediates accumulation of HLA-DR in lysosomes and destruction of the HLA-DR alpha-chain.* Blood, 2003. **101**(12): p. 4870-7.
39. Hegde, N.R., et al., *Inhibition of HLA-DR assembly, transport, and loading by human cytomegalovirus glycoprotein US3: a novel mechanism for evading major histocompatibility complex class II antigen presentation.* J Virol, 2002. **76**(21): p. 10929-41.
40. Slobedman, B., et al., *Latent cytomegalovirus down-regulates major histocompatibility complex class II expression on myeloid progenitors.* Blood, 2002. **100**(8): p. 2867-73.
41. Elder, E.G., et al., *Interferon-Responsive Genes Are Targeted during the Establishment of Human Cytomegalovirus Latency.* mBio, 2019. **10**(6).
42. Neumann, J., A.M. Eis-Hübinger, and N. Koch, *Herpes simplex virus type 1 targets the MHC class II processing pathway for immune evasion.* J Immunol, 2003. **171**(6): p. 3075-83.
43. Sievers, E., et al., *Glycoprotein B from strain 17 of herpes simplex virus type I contains an invariant chain homologous sequence that binds to MHC class II molecules.* Immunology, 2002. **107**(1): p. 129-35.
44. Trgovcich, J., D. Johnson, and B. Roizman, *Cell surface major histocompatibility complex class II proteins are regulated by the products of the gamma(1)34.5 and U(L)41 genes of herpes simplex virus 1.* Journal of virology, 2002. **76**(14): p. 6974-6986.
45. Hambleton, S., M.D. Gershon, and A.A. Gershon, *The role of the trans-Golgi network in varicella zoster virus biology.* Cell Mol Life Sci, 2004. **61**(24): p. 3047-56.
46. Abul Abbas, A.H.L., Shiv Pillai, *Basic Immunology*. Functions and Disorders of the Immune System. Vol. 6th Edition. 2019: Elsevier. 336.
47. Call, M.J., *Chapter 93 - Peptide Loading of MHC*, in *Handbook of Biologically Active Peptides (Second Edition)*, A.J. Kastin, Editor. 2013, Academic Press: Boston. p. 687-696.
48. Waterhouse, N.J., et al., *Cytotoxic T lymphocyte-induced killing in the absence of granzymes A and B is unique and distinct from both apoptosis and perforin-dependent lysis.* J Cell Biol, 2006. **173**(1): p. 133-44.
49. Nagata, S. and P. Golstein, *The Fas death factor.* Science, 1995. **267**(5203): p. 1449-1456.
50. Binder, G.K. and D.E. Griffin, *Immune-mediated clearance of virus from the central nervous system.* Microbes and Infection, 2003. **5**(5): p. 439-448.
51. Swiecki, M. and M. Colonna, *Type I interferons: diversity of sources, production pathways and effects on immune responses.* Curr Opin Virol, 2011. **1**(6): p. 463-75.
52. de Jong, L.C., et al., *Noncytotoxic functions of killer cell granzymes in viral infections.* PLOS Pathogens, 2021. **17**(9): p. e1009818.
53. Pereira, R.A. and A. Simmons, *Cell surface expression of H2 antigens on primary sensory neurons in response to acute but not latent herpes simplex virus infection in vivo.* Journal of virology, 1999. **73**(8): p. 6484-6489.
54. Medana, I.M., et al., *MHC class I-restricted killing of neurons by virus-specific CD8+ T lymphocytes is effected through the Fas/FasL, but not the perforin pathway.* Eur J Immunol, 2000. **30**(12): p. 3623-33.
55. Knickelbein, J.E., et al., *Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1 reactivation from neuronal latency.* Science, 2008. **322**(5899): p. 268-71.
56. Decman, V., et al., *Gamma interferon can block herpes simplex virus type 1 reactivation from latency, even in the presence of late gene expression.* J Virol, 2005. **79**(16): p. 10339-47.
57. Khanna, K.M., et al., *Herpes simplex virus-specific memory CD8+ T cells are selectively activated and retained in latently infected sensory ganglia.* Immunity, 2003. **18**(5): p. 593-603.

58. Liu, T., et al., *CD8(+) T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons.* J Exp Med, 2000. **191**(9): p. 1459-66.
59. Russell, T.A., et al., *Increasing antigen presentation on HSV-1-infected cells increases lesion size but does not alter neural infection or latency.* The Journal of general virology, 2018. **99**(5): p. 682-692.
60. van Velzen, M., et al., *Local CD4 and CD8 T-cell reactivity to HSV-1 antigens documents broad viral protein expression and immune competence in latently infected human trigeminal ganglia.* PLoS pathogens, 2013. **9**(8): p. e1003547-e1003547.
61. Theil, D., et al., *Latent herpesvirus infection in human trigeminal ganglia causes chronic immune response.* Am J Pathol, 2003. **163**(6): p. 2179-84.
62. Gowrishankar, K., et al., *Characterization of the host immune response in human Ganglia after herpes zoster.* Journal of virology, 2010. **84**(17): p. 8861-8870.
63. Steain, M., et al., *Analysis of T cell responses during active varicella-zoster virus reactivation in human ganglia.* J Virol, 2014. **88**(5): p. 2704-16.
64. Gerada, C., et al., *Granzyme B Cleaves Multiple Herpes Simplex Virus 1 and Varicella-Zoster Virus (VZV) Gene Products, and VZV ORF4 Inhibits Natural Killer Cell Cytotoxicity.* J Virol, 2019. **93**(22).
65. Reith, W., S. LeibundGut-Landmann, and J.-M. Waldburger, *Regulation of MHC class II gene expression by the class II transactivator.* Nature Reviews Immunology, 2005. **5**(10): p. 793-806.
66. Hayes, G.M., M.N. Woodroofe, and M.L. Cuzner, *Microglia are the major cell type expressing MHC class II in human white matter.* J Neurol Sci, 1987. **80**(1): p. 25-37.
67. Vagaska, B., et al., *MHC-class-II are expressed in a subpopulation of human neural stem cells in vitro in an IFNγ–independent fashion and during development.* Scientific Reports, 2016. **6**: p. 24251.
68. Neumann, H., et al., *Neuronal control of MHC class II inducibility in rat astrocytes and microglia.* Eur J Neurosci, 1996. **8**(12): p. 2582-90.
69. Swain, S.L., K.K. McKinstry, and T.M. Strutt, *Expanding roles for CD4⁺ T cells in immunity to viruses.* Nature reviews. Immunology, 2012. **12**(2): p. 136-148.
70. Ahrends, T., et al., *CD4(+) T cell help creates memory CD8(+) T cells with innate and help-independent recall capacities.* Nature communications, 2019. **10**(1): p. 5531-5531.
71. Luckheeram, R.V., et al., *CD4⁺T cells: differentiation and functions.* Clin Dev Immunol, 2012. **2012**: p. 925135.
72. Abbas A.K., L.A.H.P.S., *Basic Immunology*. Fourth edition ed. 2014, Philadelphia: ELSEVIER Saunders.
73. Xie, L. and S.-H. Yang, *Interaction of astrocytes and T cells in physiological and pathological conditions.* Brain research, 2015. **1623**: p. 63-73.
74. Soghoian, D.Z. and H. Streeck, *Cytolytic CD4(+) T cells in viral immunity.* Expert review of vaccines, 2010. **9**(12): p. 1453-1463.
75. Crotty, S., *T follicular helper cell differentiation, function, and roles in disease.* Immunity, 2014. **41**(4): p. 529-542.
76. Lewandowski, G.A., D. Lo, and F.E. Bloom, *Interference with major histocompatibility complex class II-restricted antigen presentation in the brain by herpes simplex virus type 1: a possible mechanism of evasion of the immune response.* Proceedings of the National Academy of Sciences of the United States of America, 1993. **90**(5): p. 2005-2009.
77. van Velzen, M., et al., *Neuron-interacting satellite glial cells in human trigeminal ganglia have an APC phenotype.* J Immunol, 2009. **183**(4): p. 2456-61.
78. Ouwendijk, W.J.D., et al., *Characterization of the immune response in ganglia after primary simian varicella virus infection.* Journal of NeuroVirology, 2016. **22**(3): p. 376-388.
79. Meyer, C., et al., *Age and immune status of rhesus macaques impact simian varicella virus gene expression in sensory ganglia.* Journal of virology, 2013. **87**(15): p. 8294-8306.

80. Cunningham, A.L., *The herpes zoster subunit vaccine.* Expert Opin Biol Ther, 2016. **16**(2): p. 265-71.
81. Sei, J.J., et al., *Effector and Central Memory Poly-Functional CD4+ and CD8+ T Cells are Boosted upon ZOSTAVAX® Vaccination.* Frontiers in Immunology, 2015. **6**(553).
82. Weinberg, A., et al., *Varicella-Zoster Virus–Specific Cellular Immune Responses to the Live Attenuated Zoster Vaccine in Young and Older Adults.* The Journal of Immunology, 2017. **199**(2): p. 604-612.
83. Heineman, T.C., A. Cunningham, and M. Levin, *Understanding the immunology of Shingrix, a recombinant glycoprotein E adjuvanted herpes zoster vaccine.* Curr Opin Immunol, 2019. **59**: p. 42-48.
84. Lee, K.S., et al., *Human sensory neurons derived from induced pluripotent stem cells support varicella-zoster virus infection.* PLoS One, 2012. **7**(12): p. e53010.
85. Markus, A., et al., *An in vitro model of latency and reactivation of varicella zoster virus in human stem cell-derived neurons.* PLoS Pathog, 2015. **11**(6): p. e1004885.
86. Pourchet, A., et al., *Modeling HSV-1 Latency in Human Embryonic Stem Cell-Derived Neurons.* Pathogens, 2017. **6**(2).
87. Markus, A., et al., *Varicella-zoster virus (VZV) infection of neurons derived from human embryonic stem cells: direct demonstration of axonal infection, transport of VZV, and productive neuronal infection.* J Virol, 2011. **85**(13): p. 6220-33.
88. Thomson, J.A., et al., *Embryonic Stem Cell Lines Derived from Human Blastocysts.* Science, 1998. **282**(5391): p. 1145-1147.
89. Takahashi, K. and S. Yamanaka, *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.* Cell, 2006. **126**(4): p. 663-76.
90. Nianias, A. and M. Themeli, *Induced Pluripotent Stem Cell (iPSC)-Derived Lymphocytes for Adoptive Cell Immunotherapy: Recent Advances and Challenges.* Curr Hematol Malig Rep, 2019. **14**(4): p. 261-268.
91. Flippe, L., et al., *Rapid and Reproducible Differentiation of Hematopoietic and T Cell Progenitors From Pluripotent Stem Cells.* Frontiers in Cell and Developmental Biology, 2020. **8**.