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Review

Impact of Toll-Like Receptors (TLRs) and TLR Signaling Proteins in Trigeminal Ganglia Impairing Herpes Simplex Virus 1 (HSV-1) Progression to Encephalitis: Insights from Mouse Models

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Abstract

Herpes simplex virus 1 (HSV-1) or *simplexvirus humanalpha 1* is a neurotropic virus that is responsible for orofacial infections in humans. More than 70% of the world's population may have seropositivity for HSV-1, and this virus is a leading cause of sporadic lethal encephalitis in humans. The role of toll-like receptors (TLRs) in defending against HSV-1 infection has been explored, including the consequences of lacking these receptors or other proteins in the TLR pathway. Cell and mouse models have been used to study the importance of these receptors in combating HSV-1, how they relate to the innate immune response, and how they participate in the orchestration of the adaptive immune response. Myeloid differentiation factor 88 (MyD88) is a protein involved in the downstream activation of TLRs and plays a crucial role in this signaling. Mice with functional MyD88 or TLR2 and TLR9 can survive HSV-1 infection. However, they can develop encephalitis and face a 100% mortality rate in a dose-dependent manner when MyD88 or TLR2 plus TLR9 proteins are non-functional. In TLR2/9 knockout mice, an increase in chemokines and decreases in nitric oxide (NO), interferon (IFN) gamma, and interleukin 1 (IL-1) levels in the trigeminal ganglia (TG) have been correlated with mortality.

Keywords: HSV-1; TLR-induced immune response; encephalitis; defective TLR; innate immunity

1. Introduction

Herpes simplex virus 1 (HSV-1) or *simplexvirus humanalpha 1* is one of the most prevalent human neurotropic viruses. Infection with this virus begins in the oral and epithelial mucosa (blue arrows, Fig. 1) with local viral replication, and it subsequently targets the trigeminal ganglia (TG), where latency is established [1]. Patients may develop more severe diseases, such as herpetic stromal keratitis, which can cause blindness or latency after disease resolution. HSV-1 latency depends on the equilibrium of the virus and host immune response, which usually does not permit virus replication [2,3]. Eventually, reactivation may occur, and the virus is carried by anterograde transport to epithelial cells (green arrows, Fig. 1). A productive infection occurs, and common cold sores can spread the virus to a new host.

In some patients, the virus targets the central nervous system (CNS), enters the brain, and may or may not cause encephalitis and other severe HSV-1-related diseases (red arrows, Fig. 1). Encephalitis can occur after primary infection or reactivation [2]. The latency of HSV-1 depends on several factors [2]. For example, it depends directly or indirectly on innate immunity because the host's antiviral response is initiated with the activation of innate immunity before the adaptive immune response [4]. The fatality rate of patients with untreated encephalitis is approximately 70% [5,6], and long-term neurological sequelae have been reported in children [7,8].

2. Toll-Like receptors (TLRs) and How They are Activated in HSV-1 Infection

TLRs were an exciting discovery in the field of the innate immune response and are the most studied pattern recognition receptors (PRRs) involved in innate immunity [9]. TLRs sense the presence of microorganisms (viral products in the case of this review) outside and inside cells, recognizing pathogen-associated molecular patterns (PAMPs) [9,10]. At least 12 different functional TLRs have been described in mice, and 10 have been described in humans (TLR1 to TLR9, TLR11 to TLR13, or TLR1 to TLR10), which recognize different PAMPs agonists. TLR10 in mice is not functional [9]. Prokaryotic cells and viruses have different characteristics from their counterparts in eukaryotic cells. As a general example, TLR9 recognizes unmethylated CpG dinucleotides, which are abundant in prokaryotic and viral DNA but are rare in eukaryotic DNA [11].

After recognizing PAMPs via TLRs located in plasma or on the endosome membrane, a signal is transmitted to the cytoplasm of defense cells through myeloid differen-

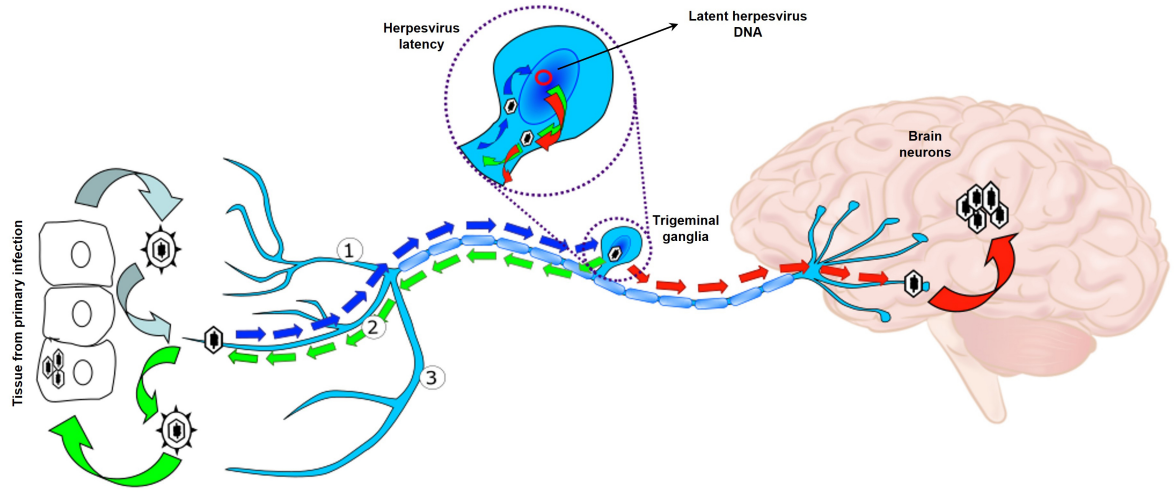

Fig. 1. HSV-1 infection. The primary site of infection occurs in epithelial cells, with productive infection through one of the three branches of the trigeminal nerve: the ophthalmic branch ①, the maxillary branch ②, or the mandibular branch ③. Particles are carried by retrograde transport through axons (blue arrows) to sensory TG, where latency occurs. Eventually, reactivation may occur, the virus is carried by anterograde transport (green arrows) to the epithelial cells, and a productive infection occurs again. Reactivation and targeting of the virus in the brain can occur in cases of low host immunity, causing encephalitis (red arrows). HSV-1, *Herpes simplex virus 1*; TG, trigeminal ganglia.

tiation factor 88 (MyD88) for all TLRs except for TLR3 [4,9,12]. This occasionally occurs in conjunction with TIRAP (TIR domain-containing adaptor protein). CD14 serves as an adaptor molecule for various TLRs, including TLR4 (triggered by lipopolysaccharide (LPS) or other molecules), TLR2 (activated by peptidoglycan or other molecules), TLR9 (unmethylated DNA from microorganisms or from mitochondria), and TLR7 (activated by single strand RNA). CD14 enhances the activation of some TLRs [13].

MyD88 recruits interleukin 1 (IL-1) receptor-associated kinase (IRAK), which initiates a phosphorylation cascade. Subsequently, IκB is phosphorylated and degraded by the proteasome, freeing nuclear factor kappa B (NF-κB) with its nuclear localization signal in the cytoplasm. NF-κB then moves to the nucleus, binds to specific regulatory sites on the DNA of defense immune genes, and functions as a transcription factor for pro-inflammatory genes, like genes for cytokines or other substances [9,13]. When HSV-1 infects a host (Fig. 2), defense cells encounter the virus, triggering an immune response via TLR2, which forms dimers with TLR6 or TLR1 [13–15]. This process recruits the adaptor proteins MyD88 and TIRAP [16]. Like in other microorganisms, this leads to a phosphorylation cascade of various proteins, as described previously [14].

TLR3 is activated by double strand RNAs (dsRNAs) (Fig. 2), a byproduct of HSV-1 replication [12,15], and employs the Toll/interleukin-1 receptor (TIR) domain-containing adapter-inducing interferon-β (TRIF) adapter. This activation initiates a phosphorylation cascade through IRF-3 and IRF-7, resulting in the induction of type I interferon (type I IFN) [14,16–18]. TLR4 can also use the TRIF adapter in some cases [14]. TLR9 is activated after agonists (HSV-1 and its DNA in the present study [19]) enter the cell and recruit MyD88. This triggers a phosphorylation cascade in two possible directions: through IRAK, leading to NF-κB moving to the nucleus and activating pro-inflammatory genes [9] or via IFN regulator factors (IRF) IRF-3 and IRF-7 (Fig. 2), resulting in type I IFN activation [16–18,20,21].

After infection by microorganisms, mitochondria are stimulated (Fig. 2), producing excess ATP [22]. The ATP exits and re-enters the cell and triggers the NRLP3 inflammasome complex [22]. This leads to the conversion of pro-IL-1 beta into IL-1 beta by caspase-1 [9,19,20,22]. IL-1 beta then exits the cells and initiates inflammation [19,20,22]. Concurrently, IFN type I acts against virus replication [19]. IFN gamma is released from cells (Fig. 2) and enhances the specific activity of macrophages, dendritic cells (DCs), neutrophils, and lymphocytes [19]. Various models, including animal (mouse, rabbit) models, have been employed to study these pathways.

3. TLRs and TLR Signaling Proteins in TG Impairs HSV-1 Progression to Encephalitis

The murine model of HSV-1 infection is excellent for studying TLRs and TLR signaling pathways and their role in initiating acquired immunity. Various studies have utilized murine models to examine the immune response against HSV-1, demonstrating its affinity tropism for the TG and the brain [17,22–24]. In both a murine intranasal in-

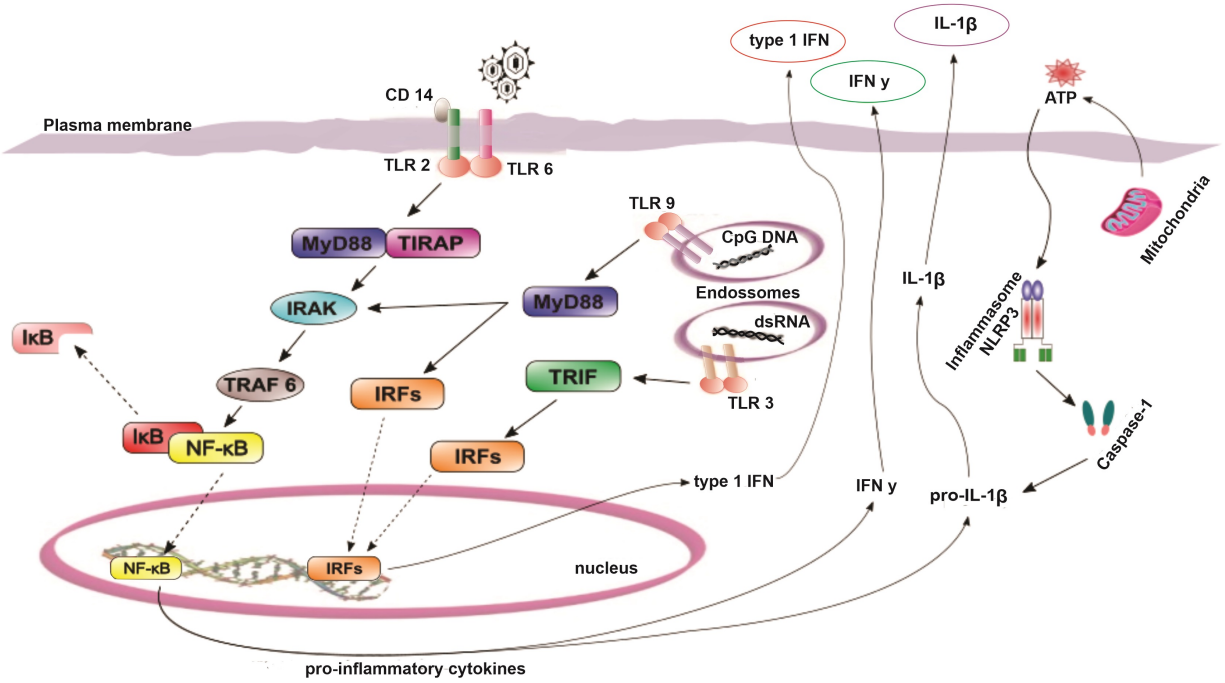

Fig. 2. Pathways involved in the TLR-dependent innate immune response to HSV-1 infection. When HSV-1 infects a host, dendritic cells detect the virus and start an immune response. This involves TLR2 and TLR6 receptors, working with a protein called CD14 to activate a complex chain reaction inside the cell. CD14 lacks transmembrane and cytoplasmic domains and helps enhance this reaction, particularly by activating NF-κB protein. This protein moves to the cell's nucleus and turns on genes that produce inflammatory substances. Additionally, when the virus enters a cell, it activates another receptor, TLR9. This leads to similar chain reactions, producing pro-inflammatory cytokines and type I IFN, which are crucial for fighting the virus. TLR3, another receptor, is activated by viral double-strand RNA during replication. This triggers a response that also leads to the production of type I IFN. After infection, mitochondria in cells increase their activity, generating more ATP. This ATP exits and re-enters cells, activating the inflammasome, and the caspase cuts pro-interleukin (IL)-1 into IL-1, which initiates inflammation. Type I IFN work to prevent the virus from multiplying both inside and outside cells. Outside the cell, IFN gamma enhances the defensive functions of various immune cells. TLR, toll-like receptor; NF-κB, nuclear factor kappa B; Type I IFN, type I interferon; IFN, interferon; dsRNA, double strand RNA.

fection model and a rabbit model, HSV-1 follows the same nerve pathway to target the TG [25,26]. Murine corneal scarification is another method for studying the significance of TLRs [27]. Reinert *et al*. [28] found that microglia sensing of HSV-1 infection in the CNS orchestrates an antiviral program, including type I IFNs and immune-priming of other cell types. Two types of mouse infection, intracranial and cornea scarification, are mentioned here due to their relevance in TLR response against HSV-1. However, cornea scarification can significantly alter host gene transcription in both the cornea and the TG (the site of HSV-1 latency) [14].

Researchers such as Wang *et al*. [29] and Sato *et al*. [30] have used intracerebral mouse inoculum to study the innate immune response in the brain. Wang *et al*. [29] reported that when TLR-2 is triggered by intracerebral inoculation of HSV-1 in mice, it leads to an exacerbated immune response. They also found that TLR9 had no significant impact on HSV-1 defense when inoculated intracerebrally. These studies are essential for understanding TLR functions in the brain, but the brain's immune defense is not efficient. In contrast, the immune response in the TG is optimal against HSV-1, preventing the virus from targeting the brain and thus averting encephalitis [19,25,31,32]. The present review is focused on the intranasal inoculum mouse model, which closely resembles human infection (Fig. 1).

The recognition of HSV-1 by TLRs has been documented in murine models, indicating that HSV-1 activates TLR2 [25,31]. Bansode *et al*. [33] and Cai *et al*. [15] identified the dimerization of TLR2 with TLR1, TLR6, or another TLR2 in response to HSV-1 glycoproteins. Krug *et al*. [23] demonstrated that TLR9 is essential in defending against HSV-1 by activating plasmacytoid DCs to produce type I IFN. Other researchers have found that deficiencies in both TLR2 and TLR9 in mice infected with a low-passage isolate of HSV-1 often lead to encephalitis, frequently with fatal results [19,25,31,32]. The defense against HSV-1 ideally occurs in the TG before the virus reaches the brain [19,31,32].

Zhang *et al*. [34] revealed that human TLR3 expressed in the CNS is vital for defense against HSV-1. Menasria *et al*. [17] showed that TLR3 orchestrates the innate immune response against HSV-1 through TRIF and through interferon regulatory factors 3 and 7 (IRF-3, IRF-7) in a murine model. Sato *et al*. [30] found that TLR3 is necessary in neurons and astrocytes in the brain for defense against intracerebral inoculation of HSV-1. Reinert *et al*. [28] reported that microglia are the main source of HSV-induced type I IFN expression in CNS cells induced via the TLR3 pathway, but it was insufficient to fully counteract HSV-1.

In immunocompetent mice intranasally infected with HSV-1, the virus targets the TG (Fig. 3) [19,25]. The mice respond by producing chemokines such as IFN gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 alpha (MIP-1 alpha) (Fig. 3A). These chemokines attract macrophages, DCs, natural killer (NKs) cells, and other lymphocytes [19]. Using wild and knockout mice for *tlr2/9*, Lucinda *et al*. [19] demonstrated that DCs and monocytes/macrophages (Mo/Mϕ) are the primary sources of IL-1β and iNOS, respectively, which are crucial for the immune response against HSV-1 and dependent on TLR2/9. They also found that granzyme B produced by T CD8$^+$ and NK lymphocytes is important in the immune response of wild-type mice [19].

TLRs are activated in DCs, leading to the production of IL-1 beta and IL-12 [9,19]. Upon antigen presentation by DCs to naïve T helper lymphocytes, they differentiate into Th1 (T CD4$^+$) cells, which produce IFN gamma. Additionally, activated T CD8$^+$ cells produce IFN gamma, porins, and granzyme. NK cells also produce IFN gamma, while macrophages produce IL-1 and NO [19,31]. IFN gamma further activates macrophages, DCs, NK cells, and other lymphocytes, as well as T CD8$^+$ cells, which respond by producing more IFN gamma and granzyme [19]. These activated macrophages, DCs, NK cells, and other lymphocytes control the infection [19,31,32], which prevents the production of more chemokines, halts inflammation [19,31,32], and keeps the virus latent in the TG [19]. In humans the virus persists for life in the TG, when not reactivated [35].

In immune-deficient mice intranasally infected with HSV-1, the virus penetrates the brain, causing encephalitis. This is observed in mice lacking both TLR2 and TLR9 [19,31] and is depicted in Fig. 3B. In such cases, the virus also targets the TG, and mice respond by producing chemokines, attracting macrophages, DCs, NK cells, and other lymphocytes [19,31,32]. However, these immune cells have no functional TLR2/9, so they cannot be activated, they do not produce IL-1 beta or IL-12, and they cannot present viral antigens to naïve T lymphocytes. Macrophages, DCs, NK cells, and other lymphocytes are not activated, T CD8$^+$ cells cannot produce IFN gamma and granzyme, and the infection cannot be controlled [19]. The chemokines IP-10, MCP-1, and MIP-1 alpha appear not to be dependent on TLR and continue to be produced, perpetually drawing immune cells to the site of infection. Nonetheless, these cells are ineffective against the virus, exacerbating non-specific inflammation [19].

Due to TLR2/9 deficiency, the virus advances to the brain, resulting in encephalitis [19,31,32]. The reasons for the virus migrating to the brain following this heightened non-specific inflammation are not yet understood. Some researchers have demonstrated that TLR3 is critical in mounting a host defense against HSV-1, producing type I IFN in neurons and microglia, or in causing encephalitis when TLR3 is functionally absent [28,30,34].

4. Discussion

Studies of the immune response to HSV-1 can be developed using mice that are susceptible to experimental infection. Mouse models serve as an excellent tool for understanding TLR activation. They are useful not only for studying HSV-1 but also for exploring various methods of activation of several microorganisms [10–13]. Murine models provide valuable insights into the balance between the virus and the host's immune response. While *in vitro* and *in silico* studies enhance our understanding of this topic, they fall short in evaluating the extensive array of alternative pathways available to a vertebrate.

Following HSV-1 infection in immunocompetent rabbits [3,26] and mice [2,15,17,19,23,27,28,31], the virus is known to travel to the TG, and similar phenomenon has been reported in humans [2,34,35]. Kurt-Jones *et al*. [36] reported that TLR2 is activated following HSV-1 infection in mice. Additionally, Krug *et al*. [23] demonstrated the importance of TLR9 in defending against HSV-1 infection in mice by activating type I IFN-producing cells, a key antiviral response [37].

Lucinda *et al*. [19] demonstrated that in mice infected with HSV-1, the chemokines IP-10, MCP-1, and MIP-1 alpha are produced locally in the TG. Interestingly, these chemokines seem not to be dependent on TLR in this case. They attract immune cells such as macrophages, DCs, and lymphocytes to the site. Once there, DCs and macrophages recognize the virus through TLR2 and TLR9, subsequently producing cytokines like IL-12 to guide Th0 to Th1-cell presentation. They also produce pro-IL-1beta, which is then converted to IL-1 beta after inflammasome action, leading to inflammation [9,14,20,21,23].

Following cell presentation, T CD8$^+$ and T CD4$^+$ lymphocytes produce optimal concentrations of IFN gamma. This induces other immune cells to mount an effective response, which is characterized by increased production of NO, IL-1 beta, IFN gamma, and the activation of T CD8$^+$ killer cells, thereby controlling the infection [19]. Moreover, T CD8$^+$ cells are crucial in resolving HSV-1 infections by specifically targeting the gD HSV-1 protein [38]. This combined response prevents the virus from en-

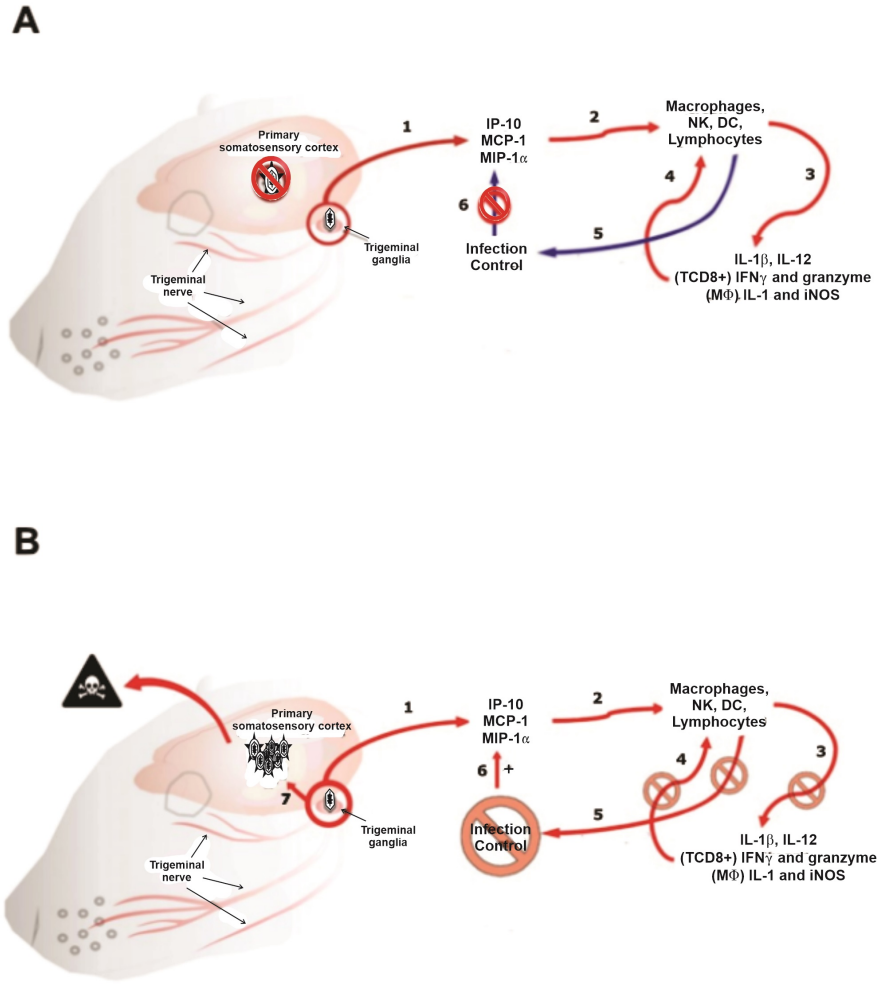

Fig. 3. Cells and cytokines involved in the immune response to HSV-1 infection in immunocompetent (A) and TLR deficient (B) mice. (A) After being intranasally infected with HSV-1, immunocompetent mice exhibit a response where the virus targets the trigeminal ganglia (TG). The mice then produced the chemokines such as IP-10, MCP-1, and MIP-1 alpha (1), which attract macrophages, dendritic cells (DCs), natural killer (NK) cells, and other lymphocytes (2). Upon activation of TLRs in DCs, they produce IL-1 beta and IL-12. Following the presentation of antigens to naïve T lymphocytes, these lymphocytes are polarized into Th1 (T $CD4^+$), which then produces IFN gamma. Additionally, NK cells and T $CD8^+$ also produce IFN gamma. T $CD8^+$ generates granzyme, while macrophages produce IL-1 and NO (3). This IFN gamma further activates macrophages, DCs, NK cells, and other lymphocytes (T $CD8^+$ and T $CD4^+$ cells, leading to an increased production of IFN gamma, particularly by T $CD8^+$cells), as well as granzyme (4). As a result, macrophages, DCs, NK cells, and other lymphocytes become effectively activated to control the infection (5). This activation inhibits the production of additional chemokines, thereby halting the inflammation, and consequently, the virus remains latent in the TG (6). (B) After intranasal infection with HSV-1, TLR-deficient mice exhibit a response where the virus targets the TG. The mice then respond by producing chemokines (1), which attract macrophages, DCs, NK cells, and other lymphocytes (2). In this scenario, the DCs are unable to become activated, failing to produce IL-1 beta or IL-12. Consequently, they cannot present viral antigens to naive T lymphocytes, resulting in the absence of IFN gamma or granzyme production. The macrophages also do not produce IL-1 or NO (3). In this case, there is a lack of activation of macrophages, DCs, NK cells, or other lymphocytes, resulting in the absence of IFN gamma and granzyme production by T $CD8^+$ cells (4). As a result, macrophages, DCs, NK cells, and other lymphocytes does not become capable to control the infection (5). Macrophages, DCs, NK cells, and other lymphocyte chemokines persist in being produced, attracting more immune cells to the infection site. However, these cells and molecules are not immunologically active and perpetuate non-specific inflammation (6). This ongoing non-specific inflammation leads to the virus traveling to the brain, ultimately resulting in encephalitis and death (7). IP-10, IFN gamma-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP-1 alpha, macrophage inflammatory protein-1 alpha; IL-1, interleukin 1; NO, nitric oxide.

tering the brain, halting the overproduction of chemokines, and interrupting the inflammatory process in the TG [19].

Conversely, in mice with double knockout for *tlr2* plus *tlr9*, although the process of chemokine production occurs similarly, the immune cells that arrive in the TG are unable to recognize HSV-1 DNA and other viral molecules due to the absence of functional TLR2/TLR9. Consequently, these cells cannot mount an adequate immune response, leading to uncontrolled infection and continuous production of chemokines, which attract more cells and cause non-specific inflammation. This results in the virus traveling to the brain, where the immune response is also ineffective in TLR2/9-deficient mice, leading to death from encephalitis. This non-specific inflammation may also compromise the blood-brain barrier, facilitating the virus's passage to the brain [19,31].

After intranasally inoculating mice with HSV-1, it was observed that the virus was present in the TG in wild-type and *myd88* knockout mice. However, HSV-1 was only detected in the brains of *myd88* knockout mice [25]. Similarly, Lima *et al*. [32] and Zolini *et al*. [31] found comparable results in *tlr2/9* knockout mice. On the other hand, using intracerebral inoculation, Wang *et al*. [29] found that TLR2 activation led to an exacerbated cytokine response, while TLR9 did not significantly affect the survival of mice intracerebrally inoculated with HSV-1. They concluded that TLR9 or TLR2 is not crucial in defending HSV-1 when the virus is inoculated intracranially.

However, in cases of intranasal inoculation, more like to what occurs in humans, wild-type mice respond differently than *tlr2/9* knockout mice, with only the knockout mice exhibiting an ineffective response [19,31,32]. This indicates that TLR2 and TLR9 together are critical for defense against HSV-1, especially when the virus is administered intranasally, suggesting that defense against HSV-1 takes place in the TG before the virus reaches the brain. Notably, Sørensen *et al*. [24] showed that intravaginal inoculation of HSV-2 causes systemic infection in *trl2/9* knockout mice, with the virus targeting the brain, which was like the results of Lima *et al*. [32] and Zolini *et al*. [31] with intranasal HSV-1 inoculation in *tlr2/9* knockouts. Sørensen *et al*. [24] also showed that intravaginal inoculum of HSV-2 in *tlr*-only or *tlr9*-only knockout mice did not cause the virus to target the brain differently from *tlr2/9* knockout mice, concluding that TLR2 together with TLR9 must have a role in joint defense against HSV-2.

Neurotropic HSV-1 is associated with human encephalitis, which Zhang *et al*. [34] highlighted. They reported a higher propensity for patients with a defect in TLR3 to develop encephalitis following HSV-1 infection. Building on this, Sato *et al*. [30] discovered the essential role of TLR3 in mediating innate immune responses to HSV-1 in neurons and astrocytes using an intracerebral infection model in mice. Additionally, Reinert *et al*. [28] found that upon sensing of HSV-1 infection in the CNS, microglia orchestrate an antiviral program, which includes the production of type I IFNs and the immune priming of other cell types, as demonstrated using a cornea scarification mouse model.

From a future research perspective, it is crucial to extend the exploration of human polymorphisms in *tlr* genes beyond *tlr3*, which has already been extensively studied in relation to HSV-1 encephalitis [39]. For instance, Mukherjee *et al*. [39] conducted a review of *tlr* polymorphisms and their impact on the immune response to infectious diseases. Building on these studies, Choudhury *et al*. [40] suggested utilizing *in silico* analysis as a method to further investigate the underlying mechanisms. Investigating polymorphisms in other *tlr* genes could yield deeper insights into the genetic factors that influence susceptibility to HSV-1 encephalitis, which is a severe neurological condition. Additionally, the development and application of TLR agonists could potentially make significant contributions to the treatment and management of HSV-1 encephalitis.

5. Conclusions

There is strong evidence that TLR-dependent immune responses in the TG are crucial for host defense against HSV-1. The responses are mediated by DCs, macrophages/monocytes, NK cells, and T $CD8^+$ lymphocytes. Each of these cells contributes through the production of specific molecules: DCs produce IL-1β, macrophages/monocytes synthesize iNOS, NK cells generate IFN gamma, and T $CD8^+$ lymphocytes release granzyme B and IFN gamma [19]. Additionally, the production of type I IFNs early in infection plays a critical role. Furthermore, TLR2, in conjunction with TLR9 [19], is essential in vertebrates, as demonstrated in the murine model against HSV-1. TLR3 specifically has been proven necessary for protecting humans and mice against HSV-1 induced encephalitis [30,34].

Author Contributions

MAC and EGK conceived, wrote, and revised the manuscript. GPZ drew the figures and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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