

Janus head: the dual role of HLA-G in CNS immunity

Yu-Hwa Huang · Laura Airas · Nicholas Schwab ·
Heinz Wiendl

Received: 21 October 2010 / Accepted: 22 October 2010 / Published online: 18 November 2010
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Abstract The central nervous system (CNS) is considered an immune-privileged organ that maintains an adaptable immune surveillance system. Dysregulated immune function within the CNS contributes to the development of brain tumor growth, and robust immune activation results in excessive inflammation. Human lymphocyte antigen-G (HLA-G) proteins with tolerogenic immunoreactivity have been implicated in various pathophysiological processes including immune surveillance, governing homeostasis and immune regulation. In this review, we describe the wealth of evidence for the involvement of HLA-G in the CNS under physiological and pathological conditions. Further, we review regulatory functions that may be applicable as beneficial strategies in the therapeutic manipulation of immune-mediated CNS immune responses. Additionally, we try to understand how this molecule cooperates with other CNS-resident cells to maintain normal immune homeostasis, while still facilitating the development of the appropriate immune responses.

Keywords Multiple sclerosis · Central nervous system · Human lymphocyte antigen-G · Immune surveillance · Blood–brain barrier · Immune tolerance

Introduction

The human lymphocyte antigen-G (HLA-G) is a nonclassical HLA class Ib molecule with the *HLA-G* gene located within the HLA at 6p21.3, one of the most polymorphic regions in the human genome [1, 2]. In spite of its close proximity within the chromosome, HLA-G has been called “nonclassical” because of its limited polymorphism [3, 4]. The gene structure of the HLA-G primary transcript generates seven alternative mRNAs that encode membrane-bound (HLA-G1, G2, G3, G4) and soluble (HLA-G5, G6, G7) protein isoforms [2]. HLA-G has a stop codon in exon 7, that results in a truncated cytoplasmic tail [5]. This premature termination leads to the retention of HLA-G in the endoplasmic reticulum until a high-affinity peptide is bound, as well as to an extended surface half-life [6].

Immune response in the CNS

The CNS is generally considered as an immune-privileged site; however, immune responses within the CNS are not uncommon [7, 8]. Based on experimental evidence, under normal conditions extremely low levels of immune-competent cells are able to regularly enter the neural parenchyma without causing subsequent pathology [7]. Moreover, the locally acting antigen-presenting cells (e.g. dendritic cells) can exert important immunological properties [9]. These findings indicate a direct impact of immune responses on intraparenchymal processes under physiological and pathological conditions. Therefore, immune elements play an important role in CNS immune surveillance [9]. Immune competent cells are normally prevented from penetrating the parenchyma by the blood–brain barrier (BBB), a well-developed blood-tissue barrier that protects the brain from

Y.-H. Huang · N. Schwab · H. Wiendl (✉)
Department of Neurology, Inflammatory Disorders
of the Nervous System and Neurooncology,
University of Münster, Domagkstr. 13,
48149 Münster, Germany
e-mail: heinz.wiendl@ukmuenster.de

L. Airas
Department of Neurology, MediCity Research Laboratory,
Turku University Hospital, Turku, Finland

changes in the levels of ions, amino acids, peptides, and other macromolecules (reviewed in reference [10]). However, under pathological conditions, a rapid activation of the immune system can occur within the CNS parenchyma, for example, influx of activated lymphocytes, monocytes, and immunoglobulin [11–13]. The trafficking of immunocompetent cells such as T lymphocytes across the BBB can be facilitated by the expression of distinct sets of adhesion molecules, and chemokines and their receptors [14], whereas CNS-resident cells, in response to inflammation, are considered to be the main producers of proinflammatory mediators [15, 16]. There are four major cell groups within the CNS: astrocytes, neurons, oligodendrocytes, and microglia.

Astrocytes are one of the CNS-resident cell types that are able to mediate immune responses [17]. In addition to their important role in establishing and maintaining the BBB [18], astrocytes are the major glial cells within the CNS that are known to be able to carry out phagocytosis, to express major histocompatibility complex (MHC) class II molecules, and to process and present CNS antigens [17]. The prevailing view on astrocytes is that they mainly exert negative immune regulatory functions, thus contributing to the immune privileged status of the CNS [19].

Neurons are generally considered rather to be targets than to be initiators of immune reactions. However, bidirectional interactions between immune components and neurons under inflammatory conditions have been reported. Neumann et al. [20] observed that neuronal cells *in vitro* are capable of upregulating MHC class I molecules in response to viral infection; another study showed that myelin-reactive T cells not only produce effector molecules and mediate tissue destruction, but can also produce neurotrophic factors and provide trophic support to aid neuronal survival [21]. Hypothetically, due to the occurrence of immune responses within the CNS, neurons of the CNS have the ability to regulate the immune system and suppress inflammatory conditions in the CNS [22], highlighting the advantages of neurons for CNS inflammation studies.

Oligodendrocytes are able to express MHC molecules under CNS inflammatory conditions [23]. This increases the vulnerability of these cells to MHC class I-restricted CD8 T cell-mediated cytotoxicity, and they therefore act as targets for antigen-specific immune responses [23–25]. Actively induced death of myelin-forming oligodendrocytes in the form of apoptosis or necrosis by immune-mediated processes at the site of CNS inflammation might explain demyelination in the CNS, one of the hallmarks of multiple sclerosis (MS) [26].

Microglia, phagocytic cells of myeloid origin, are considered to be brain-resident macrophages and involved in the innate immune response within the CNS. The

cellular consequences of particular receptors on microglia depend on the environmental context in which they are expressed. With regard to immune responses, special regard has to be given to Toll-like receptor-4 [27], scavenger receptors [28], and Fc receptors [29]. However, even though microglia are considered to be the closest link between the immune system and the CNS, it is still a matter of great debate how potent these cells are in amplifying or initiating specific immune responses within the CNS environment.

Very little HLA-G can be found in the CNS parenchyma under normal conditions, but it is expressed abundantly in CNS autoimmune diseases, for example in MS lesions and periplaque white matter [30], as well as in other CNS diseases (meningitis and Alzheimer's disease) [30]. Within the CNS, HLA-G is expressed by macrophages, microglia and endothelial cells, but not by astrocytes, oligodendroglial cells, or neurons. Specifically in MS, HLA-G has been found to be upregulated in monocytes of the cerebrospinal fluid (CSF) relative to the peripheral blood, and MS patients exhibit significantly higher CSF levels of soluble HLA-G (sHLA-G) than patients with other neurological diseases, emphasizing the immunobiological relevance of this pathway for neuroinflammation during CNS immunoregulation [30]. In accordance with this, sHLA-G has also been found to be present in MS patients and suggested to play a protective role during MS as an antiinflammatory molecule downregulating MS inflammatory responses [30–32]. These previous observations are in good agreement with new findings on the functional significance of HLA-G in CNS immune regulation [33, 34].

Immune paralysis in the CNS

Glioblastomas are highly malignant brain tumors with a poor prognosis, despite intensive research and clinical effort [35]. Regarding the biology of malignant transformations in the CNS, the particular requirements and preconditions for the immune responses against cerebral tumors must be reconsidered. It is known that tumors have a remarkable ability to mold their stromal environment to their own advantage by via powerful mechanisms for proliferation, migration, invasion, matrix degradation, angiogenesis, survival, chemo-/radiotherapy insensitivity and immune evasion (for review in see reference [36]).

Consistent with its antiproliferative ability, HLA-G expression has repeatedly been shown to be upregulated in various cancers [37–42]. Moreover, forced expression of HLA-G in glioma cells enhances their tumorigenic capacity [43, 44]. This association is consistent with the various immunosuppressive activities of HLA-G that have been described in several *in vitro* experimental settings. For

example, constitutive HLA-G expression by glioma cells (U373MG and T98G cell lines) protects them from targeted killing by natural killer cells in a dose-dependent manner [43]. Similarly, glioma-mediated immune suppression by HLA-G expression effectively inhibits the activation of cytotoxic T cells [43, 44].

These studies suggest that the aberrant expression of HLA-G during proliferation of a cell that has lost the ability to control its cell cycle would generate a tumor that resists immune cell-mediated killing. This hypothesis would fit with the aggressive nature of tumors that over-express HLA-G. In agreement with this, the detrimental role of HLA-G in neuroblastoma, a type of tumor originating from the sympathetic nerve system and the most common extracranial malignancy in childhood, has been revealed [45]. In this study, HLA-G serum levels were not only increased in neuroblastoma patients but also correlated with relapse. In contrast to glioblastoma, the immune escape mediated by HLA-G in neuroblastoma stems from active monocytes instead of the tumor itself [45]. It seems very likely that there are still undefined detrimental effects of HLA-G on immune surveillance and tumor clearance, which may have therapeutic implications in cerebral tumor-specific immunity.

Immunological defense mechanisms clear most viral infections after a few days. This enforced homeostasis implies constantly operating defense mechanisms against both external and internal pathogens. However, some viruses develop strategies for subverting host defenses, facilitating their spread or the establishment of latency. In this respect, another supportive role of the induced expression of HLA-G to react to the intrusion of the CNS is indicated by the study of Lafon et al. [46], in which neural cells were found to upregulate and express HLA-G upon infection with a neurotropic virus (rabies). This HLA-G expression on astrocytes has been claimed to be responsible for a negative immune regulatory function, thus contributing to the virus invasion [46].

Equivocal roles of the nervous system and the immune system in MS

MS affects approximately one million people worldwide, mostly in North America and northern Europe. Women are affected two times more frequently than men. The disease often begins in young adulthood with recurrent inflammatory attacks against the white matter of the brain, involving neurological impairment, e.g. blindness, loss of sensation, lack of coordination, bowel and bladder incontinence, and difficulty in walking [47–49].

The pathological/histological hallmark of MS is the plaque, which is an area of myelin that has been damaged

by inflammation and by non-neural CNS cells, including bone marrow-derived microglia and brain-derived astroglia cells [47–49]. The originating cause of MS is uncertain [47–49]. Both the wound itself and the process of its repair evoke a cascade of immunological activities. However, it is generally believed that the disease is initiated by the immune attack against the white matter that results in degeneration of axons and myelin [47–49]. However, the possibility that the immune response itself is a reaction to a primary neurodegenerative process must also be appreciated [50]. Overall, MS is considered a complex genetic disease associated with environmental factors, together with dysregulated immune responses in the CNS white matter, where the inflammatory cell profile of active lesions has been extensively characterized [47–50].

Considering the biology of inflammation in the CNS, it is clear that although inflammation can cause and exacerbate damage, it can paradoxically also enhance the repair of damage. In other words, although the influx of inflammatory immune cells into the CNS is conventionally regarded as deleterious with pathological consequences, cells whose activation is associated with inflammation, both infiltrating cells and CNS-resident cells, may at the same time contribute to the repair and regeneration, particularly by establishing an immunotolerogenic environment.

The regulation of the inflammatory response is very complex with many cellular players, and for many years immunologists have considered an immune response to be a direct consequence of antigenic stimulation against autoantigens by mechanisms related to “loss of tolerance”. However, emerging evidence suggests that within the CNS, the immune and nervous systems utilize/exploit overlapping mechanisms and share mediators. Among these candidates are brain-derived neurotrophic factors, which promote crosstalk between the two systems [51, 52]. For example, B cell-activating factor, a member of the tumor necrosis factor family required for peripheral B-cell survival and homeostasis [53], is produced and acts in the immune system; however, this molecule was recently shown also to be locally produced by astrocytes to support the long-term survival of B cells in the CNS of MS patients, suggesting cooperative crosstalk between the immune and nervous systems during CNS inflammation [54, 55].

Coincident induction of cerebral tumors in MS: failure in CNS immune tolerance?

The induction of inhibitory effects in the priming phase of CNS-specific immune responses as well as in the effector phase and the interactions between effector lymphocyte subsets and CNS-resident cells might facilitate the

coincidence or the induction of tumor-derived immune suppression. Aarli et al. [56] reported a 63-year-old man who died of a cerebral tumor after the onset of MS. The autopsy revealed a glioblastoma which had developed adjacent to MS plaques, leading to the possibility that the induction of tumor-derived immune suppression might be associated with MS [56]. Subsequent relevant cases supporting this idea have also been reported [57–59].

Given the fact that cerebral tumors and MS relapses can show overlapping clinical and magnetic resonance imaging (MRI) features [56, 59], the appearance of new neurological symptoms and signs in a patient with MS might be indicative of a reactivation of the disease. To better understand the underlying concurrence mechanisms in MS and cerebral tumors, we refer to Frisullo et al. [60], who described a patient with relapsing–remitting MS with recent onset of new neurological signs due to glioblastoma multiforme with disturbances in the transcription factors pSTAT1, T-bet (Th1) and pSTAT3 (Th17) in circulating lymphocytes (e.g. CD4 and CD8 T cells, and monocytes). In other words, the initiation of CNS immune tolerance against cerebral cancer might be linked to an autoimmune attack. We should also consider the possibility that a dys-regulated CNS tolerance might be responsible for the failure of systemic cellular immunity and for the induction of tumor-derived immune suppression in the CNS as well. It is not known whether cerebral cancer pathology might be induced by CNS inflammation or if the same insult may have different effects in different regions of the CNS. Different effects might be due to differences in genetic background and complex tumor immunogenicity. The fact that a beneficial effect against inflammation could be associated with the risk of developing a tumor should not to be overlooked. Understanding the underlying concurrent mechanisms of MS and cerebral tumor might help in developing beneficial disease-modifying therapeutic interventions.

Could HLA-G be a biomarker for CNS-specific alterations of the immune system?

A biological marker is a parameter that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic interventions. For example, sHLA-G has been reported to be a biomarker for embryo quality in human in vitro fertilization; the secretion of sHLA-G was shown to be necessary for implantation [61, 62].

Because of the exquisitely sensitive response of HLA-G to a variety of immune disorders, transplantation, and malignancies [4, 63, 64], the pathological implications of HLA-G expression on clinical correlations, including

overall survival and the risk of developing metastatic diseases, is probably of interest. Fainardi et al. [31, 32] have reported that the level of sHLA-G is significantly increased in MS patients compared to patients with other neurological diseases. Recently, the same group showed that a balance might exist between intrathecally produced molecules that play opposing roles in the balance between inflammation (sHLA class I) and immunomodulation (sHLA-G), which are inversely related to disease activity both clinically and on MRI [31, 32]. Hypothetically, sHLA-G in serum and/or in the CSF of MS patients might be useful as a biomarker for monitoring disease activity and could also be a potential marker associated with clinical outcome.

An important mechanism involved in malignant transformation is that tumors can grow by shedding and releasing exosomes [65, 66]. Exosomes are a normal part of cell-to-cell communication [67], carrying many proteins and RNA molecules and are important for cell proliferation and migration, development of blood vessels, and immune responses [68]. Researchers have recently discovered that glioblastoma-derived exosomes contain proteins that traverse the BBB, and carry cancer genes (e.g. EGFRvIII and miRNA-21) that induce cell proliferation; this could potentially be used as a new biomarker [69]. HLA-G expression has been shown in various cancers [4, 37, 38, 41, 42, 46]. Moreover, Riteau et al. [70] reported that both tumor cells and tumor-derived exosomes express HLA-G, suggesting an essential role of HLA-G in tumor progression and metastasis and pointing towards the potential of applying HLA-G as a biomarker for tumor-associated immune suppression.

Potential contribution of HLA-G to pregnancy-associated alleviation of MS

Pregnancy has a stabilizing effect on most Th1-type autoimmune diseases, including MS [71]. Particularly the late stages of pregnancy are clearly associated with a decrease in relapse rate and disease activity on MRI [72–74]. This is likely associated with the general attenuation of the adaptive maternal immune responses taking place during pregnancy [75, 76]. This modulation of the immune system is a biological imperative for the initiation and maintenance of a normal pregnancy. Under “normal” circumstances the mother’s immune system would reject the semiallogenic fetus-graft, but under the special immunological conditions of pregnancy the fetus is protected [76, 77]. Although the majority of the immunosuppression aiming at the protection of the fetus takes place locally at the placenta and starts early in the pregnancy, profound systemic alterations are also evident [76]. During late pregnancy the levels of estrogen

and progesterone are greatly increased, and this likely contributes to the systemic immunomodulation by directly affecting the function of immune cells, which express estrogen receptors such as CD4⁺ and CD8⁺ T cells, natural killer cells and macrophages [73, 76, 78]. Other putative beneficial mechanisms in the control of autoimmune diseases during pregnancy include a shift from a prevailing Th1 response to a Th2 type response [79–81] and an increase in the number of functional regulatory T cells (T_{reg}) [82, 83].

Pregnancy-specific immunoregulatory factors include specific serum proteins and tolerance-promoting signaling molecules such as HLA-G, alpha-fetoprotein, CD200, Fas-ligand, coinhibitory B7 molecules and indoleamine 2,3-dioxygenase [84–87].

In an attempt to identify immunological factors which contribute to the modulation of MS during and after pregnancy, we performed a microarray study in which we compared gene expression in peripheral blood lymphocytes isolated from MS patients during and after pregnancy with similar samples obtained from healthy controls [84]. Out of 5,000 genes, we identified two genes that were differentially regulated in MS patients versus healthy controls (HLA-DR3 and HLA-G): HLA-G was significantly downregulated in the postpartum setting in all MS patients studied, but in none of the controls. When the expression of HLA-G was reanalyzed at the protein level in a larger patient cohort, it became evident that both sHLA-G and HLA-G expressed on peripheral blood lymphocytes are downregulated in the postpartum period in patients exhibiting simultaneous postpartum disease activity, but upregulated in patients with stable postpartum disease [84]. These findings support the hypothesis that HLA-G, via its tolerance-promoting properties, is critically involved in the immune-regulatory mechanisms balancing inflammatory disease activity in MS.

Integrated insights into HLA-G-modulating CNS inflammation: a therapeutic bullet

We have recently identified a new population of regulatory T cells expressing HLA-G. Detection of HLA-G-expression by single positive thymocytes (CD4⁺ or CD8⁺) suggest their thymic origin [88, 89]. HLA-G expressing T_{reg} cells are CD25[−] and FoxP3[−] [88]. They exhibit potent suppressive properties, which are initiated via T-cell receptor activation and critically depend on interleukin-10 (IL-10) production [89]. Importantly, HLA-G-expressing T_{reg} cells can be detected in elevated frequencies at the sites of inflammation during acute neuroinflammation as well as in MS lesions in brain biopsies [34, 88]. Specifically, CCR5⁺ HLA-G-expressing T_{reg} cells, a subpopulation within HLA-G-expressing T_{reg} cells in the periphery, possibly with a potent dynamic capacity to respond to the CNS-specific inflammatory milieu, have been

found to preferentially migrate from the periphery to the CSF and CNS compartments during autoimmune CNS inflammation [34]. This supports their putative role in the outcome of the ongoing disease, and is the first example of beneficial T-cell inflammation in human CNS autoimmunity (also see Fig. 1).

Our group is interested in CNS immune homeostasis, particularly the role of regulatory T cells in regard to self-tolerance and autoimmune diseases. The *in vivo* expression of HLA-G by human T cells and its upregulation under pathological conditions along with the inhibitory activity of HLA-G in T cells indicate that HLA-G has the potential to control T cell activation *in vivo* in the context of immunopathological processes in which autoreactive effector T cells are involved. Thus, the inflammatory immune response, traditionally considered detrimental, might also involve the enhancement of immune-tolerogenic molecules and the triggering of protective processes.

There are several ways to manipulate CNS immune responses: (1) stimulation with immunogenic vaccines, (2) adoptive stem cell transfer therapy, (3) supply with functional relevant T_{reg} subsets, and (4) modification of the host CNS environment to eliminate antigen-specific autoimmune cells (reviewed in reference [90]). However, in terms of efficiency, specificity, and cost, current success rates are rather low due to the complexity of the imbalanced immune tolerance. Continuing studies characterizing the role of HLA-G-expressing T_{reg} cells in controlling parenchyma inflammation will yield further insights into maintaining the balance between the CNS and immune system; it is very important to point out that the upcoming wave of therapeutic agents for MS will tackle the need for cellular immune therapy [34, 88, 89]. Advances unraveling the modes of action of HLA-G-expressing T_{reg} cells may provide an important pathophysiological example of “beneficial” T-cell inflammation in CNS, interesting from both an immunopathogenic as well as a therapeutic view [34, 89]. However, we are all aware of the fact that the severity, frequency, specific clinical symptoms, and pathology vary greatly in among MS patients. Assuming that HLA-G proves to be an important immune tolerance factor/element in CNS inflammation biology, one could think that high levels of HLA-G reflect a beneficial role during the autoimmune attack; however, this molecule might also paralyze the immune responses. HLA-G might be used beneficially in strategies against inflammatory aggression, but the reduction of immune reactions against pathogens or even the occurrence of cerebral cancer must be taken into consideration. Further studies on HLA-G biology are required to better understand the interplay between the autoimmune attack and the malignant transformation in the CNS as well as the fragile interactions

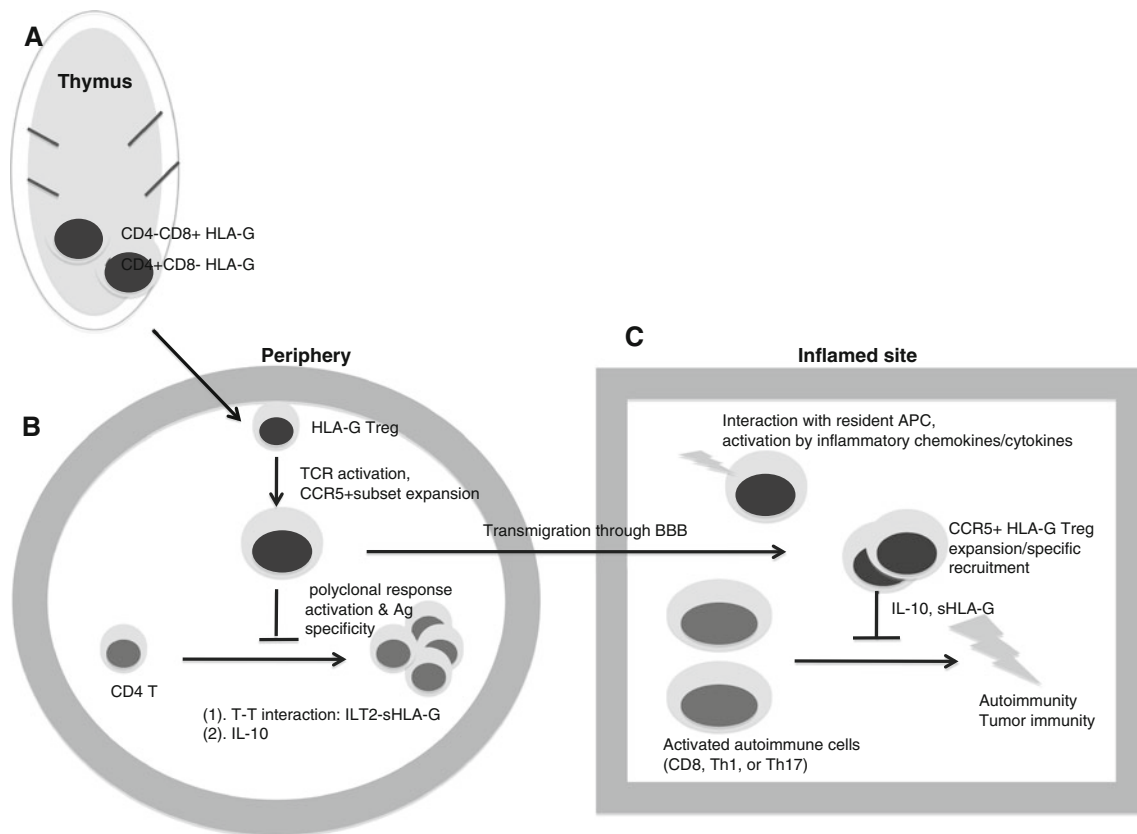


Fig. 1 HLA-G-expressing regulatory T cells: a hypothetical concept of development, mode of action and relevance to CNS inflammation. **a** Development: HLA-G-expressing regulatory T cells seem to originate in the thymus. **b** Mode of action: T cell receptor (*TCR*) engagement on HLA-G-expressing regulatory T cells is crucial for initiation of the immune regulation; this activation then promotes

inhibitory cytokines including the release of IL-10, which is critical for the suppressive capacity. **c** CNS inflammation: upon autoimmune attack in the CNS, e.g. in MS, functional HLA-G-expressing regulatory T cells can be specifically recruited to the site of inflammation; this pathway depends critically on the expression of chemokine receptor 5 (*CCR5*)

between CNS-resident cells (e.g. astrocytes, microglia, oligodendrocytes) and CNS-infiltrating immune cells (e.g. regulatory T cells) [33].

Perspectives

Although HLA-G has been intensively studied in other systems, its role in controlling immune functions within the CNS has just started to be investigated. In this review, we have highlighted data supporting the role of HLA-G in the regulation of CNS autoimmunity and cerebral cancer (Fig. 2). Despite the potential beneficial role that HLA-G might have in the prevention of a predisposed inflammatory CNS, the apparent conflicting role in fostering tumor surveillance has to be taken into consideration. Does HLA-G mediate activation and/or survival of certain types of CNS-resident cells with immunological properties, thereby promoting their detrimental function in individuals susceptible to autoimmunity? Are other subsets of regulatory

cells induced by HLA-G involved in the self-derived suppressive mechanisms? Which cytokines, chemokines and receptors control the function and recruitment of HLA-G-expressing cells to the CNS under physiological and pathological conditions? Continuous effort is needed to unravel the molecular mechanisms of HLA-G expression, differential splicing, subcellular localization, monomeric versus dimeric states, and transcriptional regulation (e.g. demethylation) before the contribution of this captivating molecule to cellular responses can be fully evaluated. This mission is challenging due to the disparate functions attributed to the complex cellular interplay between CNS-resident cells and immune cells. We need to understand how HLA-G cooperates with other activators and inhibitory immune receptors expressed by the CNS to maintain homeostatic immune reactions, while providing tissue-specific immune regulation. Unfortunately, the lack of a rodent HLA-G homologue hinders the possibility of addressing these variables and of combining them with ongoing immune responses. We recently started the

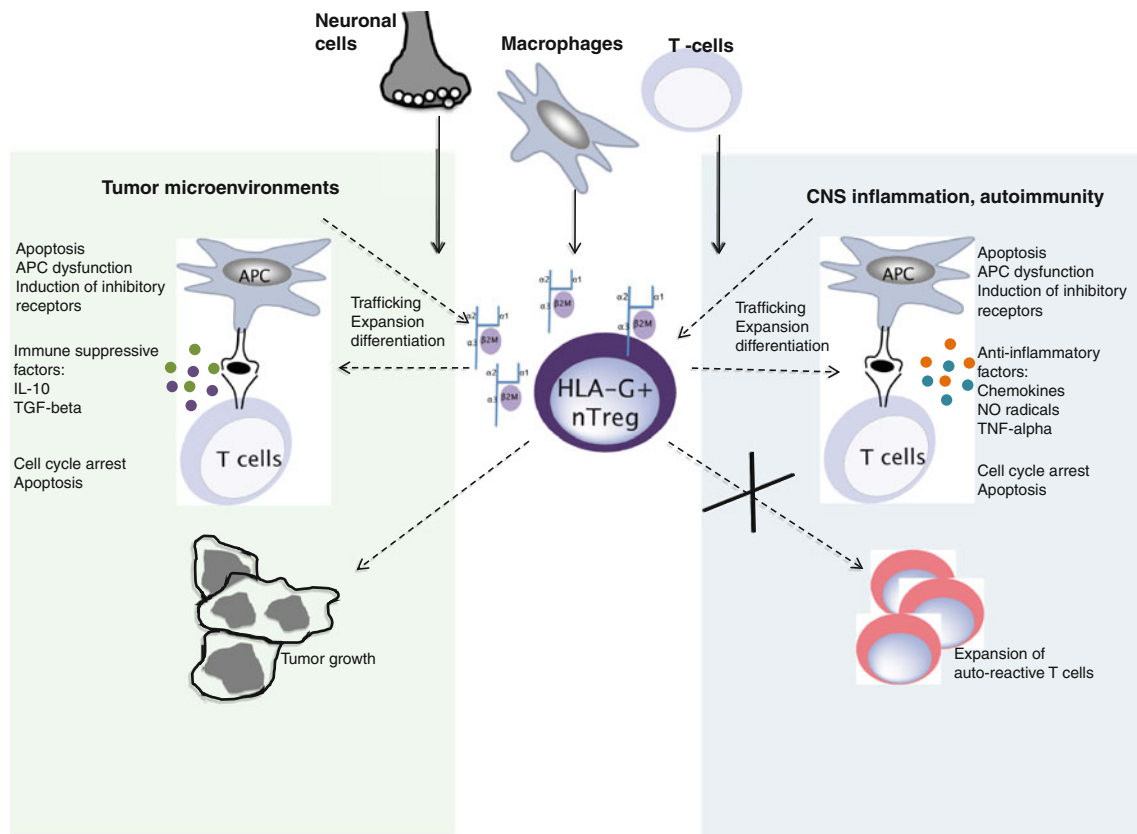


Fig. 2 A hypothetical scenario for the control of CNS immune tolerance by HLA-G secretion and HLA-G-expressing regulatory T cells. Under pathophysiological conditions, HLA-G can be expressed from different sources. HLA-G expression can be expressed by neural cells (nervous source), CNS resident macrophages (nervous source) and T cells (immune source) in response to stimulation (e.g. viral infection and inflammatory stimuli). HLA-G induces immune tolerance towards tumors and inhibits autoimmune responses through different but not mutually exclusive mechanisms. HLA-G is found both in the tumor microenvironment and in inflamed CNS parenchyma. HLA-G induces inhibitory receptor expression by antigen-presenting cells (APC), and in turn these inhibitory receptor-expressing APCs induce

cell-cycle arrest in T cells or they directly kill target cells such as T cells and APCs through perforin- or granzyme B-dependent pathways; they can also function through IL-10 and transforming growth factor beta (*TGF-beta*), chemokines, NO radicals and tumor necrosis factor alpha (*TNF-alpha*) release and can directly inhibit T-cell activation and suppress APC function, thereby inhibiting expression of MHC molecules, CD80, CD86 and IL-12. As a consequence, the host's immune responses towards tumors might be reduced. HLA-G might also be used beneficially in strategies against inflammatory aggression, contributing to the maintenance of an antiinflammatory state and restoring immune tolerance

process of establishing a humanized in vivo mouse model, which could lead to substantial progress in identifying several key mechanisms involved in CNS immune tolerance (regulation).

It is important to identify the factors that are responsible for converting the beneficial “tolerance”-inducing signals in the CNS to detrimental “priming” signals. Common pathways of neuronal damage play an important role in inflammatory neurological diseases. This provides a strong impetus for studying the role of HLA-G in human diseases and emphasizes its relevance for future clinical applications.

Acknowledgments This work was supported by grants from the German Research Foundation (DFG, Wi 1722/6-1 to H.W.) and the German Ministry for Education and Research (BMBF, “German

Competence Network of MS” (KKNMS), UNDERSTANDMS, 01G10907, to H.W.).

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