Journal of Neuroimmunology 337 (2019) 577061

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Review article

The role of immune regulatory molecules in multiple sclerosis

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ARTICLE INFO

Keywords:
Multiple sclerosis
Experimental autoimmune encephalomyelitis
Immune regulatory molecules
Inhibitory molecules

ABSTRACT

Multiple sclerosis (MS) is the most common demyelinating disease which mainly impacts the integrity of central nervous system (CNS). MS etiology is not clearly known but genetic, environmental factors and immune system are the most frequently explored risk factors. Adaptive immune responses have a critical role in MS pathogenesis in which auto-reactive T-cells and autoantibodies are main orchestrators. Immune responses are modulated by inhibitory molecules which regulates adaptive system activation and hemostasis interface. These molecules suppress immune responses through inhibition of cytokine secretion and T cell proliferation and subsequently reducing the inflammation and respective damage. Therefore the critical role of inhibitory molecules in regulating the healthy and safe immune responses make them very attractive target for immunotherapy. In this review paper, the role of inhibitory molecules expressed on the various immune cell types in MS pathogenesis and experimental autoimmune encephalomyelitis (EAE) animal model will be summarized.

1. Introduction

Multiple sclerosis (MS), is a chronic central nervous system (CNS) inflammatory disorder which is classified as an autoimmune disease due to the hyperactivity of adaptive immune response (Goldenberg, 2012; Shabgah et al., 2017). The evidence indicates that more than 2 million people in the world suffer from MS and it is one of the most common neurodegenerative disorder in young adults (Noseworthy et al., 2000). According to the clinical singes, four different types of MS are proposed: primary progressive MS (PPMS), progressive relapsing MS (PRMS), relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS). Since the etiology of MS is unknown, therefore no successful treatment was developed so far. However, it seems that environmental factors, genetic and immune system playing an important role in the triggering and progression of this disease. The adaptive immune response specifically auto-reactive T-cells (CNS antigen-specific CD4+ T-cells), B-cells and antibodies greatly impact on the pathogenesis of MS disease (Loma and Heyman, 2011; Mohammadi et al., 2018; Sospedra and Martin, 2005). Experimental autoimmune encephalomyelitis (EAE) is the experimental model of demyelinating diseases and also an appropriate model for studying and understanding MS mechanisms for developing new therapies (Constantinescu et al., 2011; Fletcher et al., 2010). Regulatory molecules and pathways appear to be immensely disturbed in autoimmune diseases such as MS (Gharibi et al., 2015; Javan et al., 2014; Seyfizadeh et al., 2014, 2015). Nowadays, regulatory molecules and their respective receptors, which play a role in T-cell function, peripheral T-cell tolerance and their activation pathways in MS disease, have been well studied. These molecules suppress immune responses through inhibition of cytokine secretion and T cell proliferation and subsequently reducing the inflammation and respective damage. Therefore the critical role of inhibitory molecules in regulating the healthy and safe immune responses make them very attractive target for immunotherapy. In this review, the role of inhibitory molecules in progression of MS disease and its animal model (EAE) are discussed. In Tables 1 and 2, respectively, the expression of inhibitory molecules in MS and manipulation of coinhibitory molecules

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https://doi.org/10.1016/j.jneuroim.2019.577061
Received 23 May 2019; Received in revised form 4 August 2019; Accepted 4 September 2019

Table 1
Expression of coinhibitory molecules in MS.

Molecule	Expression	Protein/mRNA	Sample	References
CTLA-4	Downregulated	mRNA	PBMC	(Palacios et al., 2008)
Lag-3	Upregulated	protein	CNS of EAE	(Thaker et al., 2018)
PD-1	Upregulated	protein	CNS of EAE	(Salama et al., 2003)
BTLA	Downregulated	protein	B cells	(Piancone et al., 2016)
BTLA	Upregulated	protein	T CD8+	(Brenu et al., 2016)
PD-1	Downregulated	mRNA	PBMC	(Javan et al., 2016)
PD-1	Upregulated	protein	MBP-specific CD4+ and CD8+ T lymphocytes in SMS	(Trabattoni et al., 2009)
PD-1	Downregulated	protein	T cell in lesions	(Machado-Santos et al., 2018)
PD-1	Upregulated	protein	Treg	(Sambucci et al., 2018)
PDL-1	Downregulated	mRNA	PBMC	(Javan et al., 2016)
PDL-1	Upregulated	protein	lesions	(Ortler et al., 2008)
PDL-1	Upregulated	protein	CD19+,CD14+,CD10+, In SMS	(Trabattoni et al., 2009)
TIM-3	Downregulated	protein	T CD4+, TCD4+,CD25+	(Feng and Feng, 2016)
TIM-3	Downregulated	mRNA	CSF	(Koguchi et al., 2006)
TLR-1	Downregulated	mRNA	PBMC	(Singh et al., 2007)
TLR-2	Upregulated	protein	B cell, dendritic cell, Treg, in helminth-infected MS patients	(Suzanne et al., 1986)

Abbreviations: CTLA-4, The cytotoxic T lymphocyte antigen 4; VISTA, V-domain Ig suppressor of T cell activation; Lag-3, Lymphocyte activation gene-3; PD-1, Programmed cell death 1; PD-L1, Programmed death-ligand 1; TIM-3, T cell immunoglobulin and mucin domain 3; BTLA, B and T lymphocyte attenuator; TLR, Toll-like receptors.

Table 2
Manipulation of coinhibitory molecules and their outcome in EAE.

Receptor/Ligand	Manipulation	Outcome in EAE	References
B7H4	B7H4 KO	Exacerbated disease	(Wei et al., 2011)
	Blockade of B7H4	Disease severity	(Prasad et al., 2003
	B7H4-Ig	Treating EAE	(Podojil et al., 2013)
VISTA	VISTA KO	Disease severity	(Wang et al., 2014)
	Blockade of VISTA	Exacerbated EAE	(Wang et al., 2011)
CTLA-4	Blockade of CTLA-4	Exacerbated disease	(Karandikar et al., 1996)
	CTLA-4-Ig	Inhibit EAE induction	(Khoury et al., 1995)
Lag-3	Lag-3 KO	Developed EAE	(Thaker et al., 2018)
	Blockade of Lag-3	Disease severity	(Kadowaki et al., 2016)
PD-1	PD-1 KO	Disease severity	(Wang et al., 2010)
	Blockade of PD-1		(Salama et al., 2003)
PD-L1	PD-L1 KO	Developed EAE	(Latchman et al., 2004) (Carter et al., 2007)
Tim-3	Tim-3 KO—CD8 T cell	-	(Lee and Goverman, 2013
	Blockade of Tim-3	Disease severity	(Monney et al., 2002)
	Galectin9 KO	-	(Zhu et al., 2005)
	Galectin 9	Alleviated EAE	(Zhu et al., 2005)
BTLA	BTLA-HEVEM KO	Exacerbated disease	(Watanabe et al., 2003) (Wang et al., 2005)
	Modified DC with BTLA	Alleviated EAE	(Yuan et al., 2014)

Abbreviations: KO, Knockdown; EAE, Experimental autoimmune encephalomyelitis; DC, Dendritic cell.

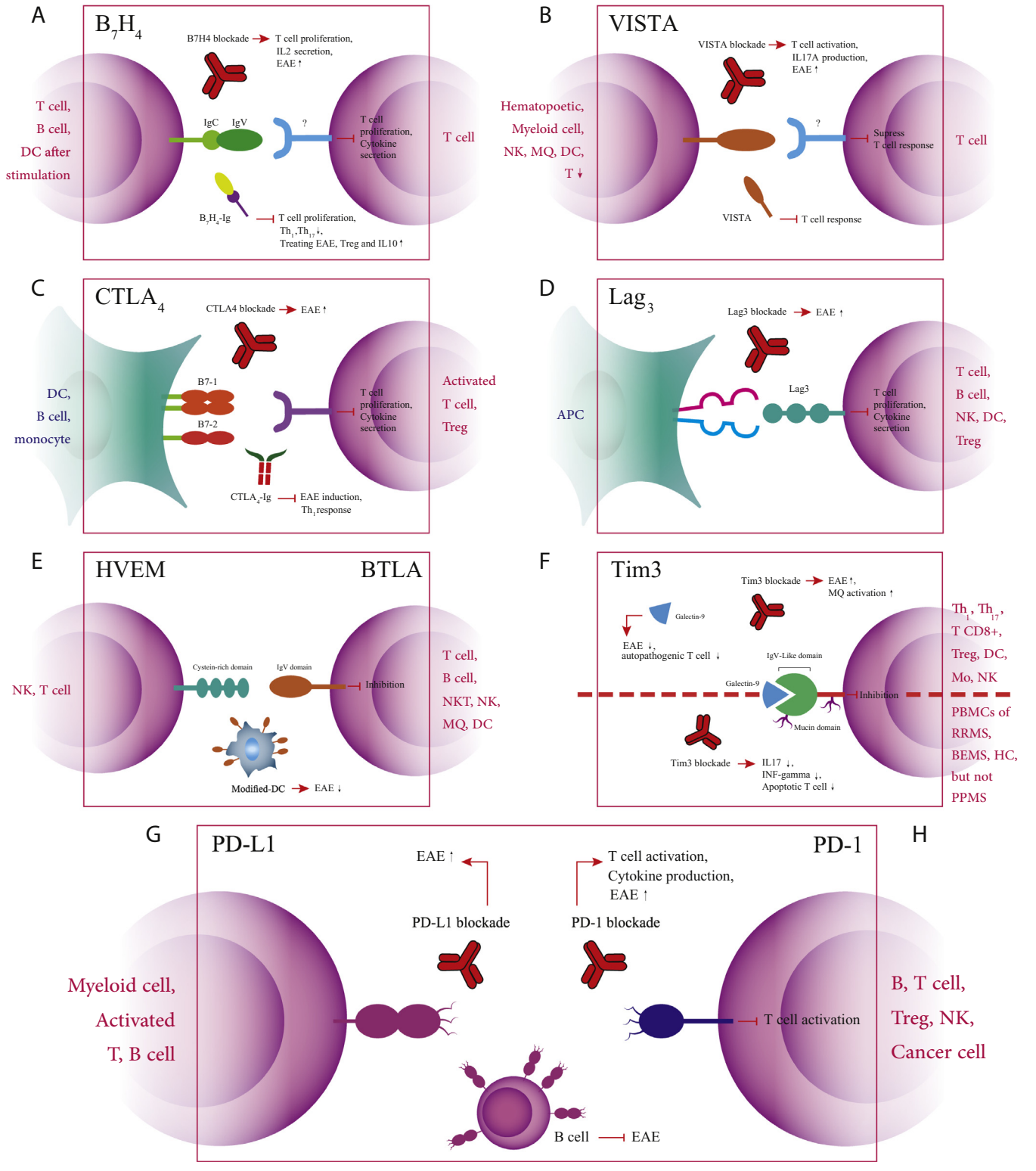
and their outcome in EAE are described.

2. B7H4

B7H4, also known as B7x, B7S1, VTCN1, is a costimulatory protein which is mostly expressed on the surface of antigen presenting cells and belongs to the B7 family (Sica et al., 2003). Like other B7-family members, B7H4 has IgV and IgC domains. B7H4 is the first GPI-linked protein in the B7 family and a highly conserved molecule, however the identity between murine and human orthologues is approximately 87% (Zang et al., 2003; Prasad et al., 2003). Although the expression of mRNA is widespread in human tissues, but B7H4 as a protein is rarely expressed on the peripheral tissue and normal cells. B7H4 can be expressed on human T, B, Macrophages, and dendritic cell (DC) upon stimulation. B7H4 expressed on the T cells act as an inhibitory molecule and arrests G0/G1 transition and subsequently proliferation of T-cells and cytokine secretion (Sica et al., 2003). B7H4-Ig impedes IL-2 production accordingly abate T cell proliferation. Reciprocal an anti-B7H4 antibody can cause increasing of T-cells growth and IL-2 secretion (Prasad et al., 2003). In-vivo administration of B7H4-Ig impairs the growth of T-cell, though monoclonal antibody therapy via blocking of endogenous B7H4 that cause an increase in T-cell responses (Sica et al., 2003). The first study describing the impact of B7H4in EAE reported that blockade of B7H4 led to enhanced T cell responses and disease severity (Prasad et al., 2003). A study considered the therapeutic efficacy of B7H4-Ig in treating EAE and identified that B7H4-Ig inhibited the growth of CD4+ T-cell as well as differentiation of Th1 and Th17 cells whereas promoting regulatory T (Treg) cells function and IL-10 production. B7H4-Ig treatment ameliorates EAE progression through reduction Th1, Th17 cells, and increases in the number of functional Tregs (Podojil et al., 2013). EAE in B7x knockout mice resulted in exacerbated disease via increasing in the number of Th1 and Th17 cells and cytokine expression (Wei et al., 2011) (Fig. 1A). Podojil et al. also introduced B7-H4 as a modulator of regulatory CD4+ T cell via ligation of a Semaphorin 3a/Plexin A4/Neuropilin-1 complex. This suggests that targeting the B7-H4/ Sema3a/Nrp-1/PlxnA4 pathway can be an important therapeutic target in autoimmune disease (Podojil et al., 2018). These data demonstrated that B7H4 negatively affect the T-cells activation and suggest it has great potential for target therapy in MS disease.

3. VISTA

V-domain Ig suppressor of T cell activation (VISTA), belongs to B7-



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family and known as PD-1 homolog (PD-1H), C10orf54, differentiation of ESC-1 (Dies1), DD1α and platelet receptor Gi24 precursor (Flies et al., 2014, 2015; Oliveira et al., 2016; Sakr et al., 2010; Yoon et al., 2015). The expression of VISTA proteins are mainly on haematopoietic cells, and myeloid cells including macrophages, natural killer (NK) cells and dendritic cells (DCs) and expressed weakly on T cells but not on B cells (Flies et al., 2011; Wang et al., 2011). This molecule has dual functions and acts as receptor well as ligand. VISTA that acts as a co-inhibitory ligand on T-cells is limiting the naive T-cell activation. On the other hand, it has been found that in VISTA expressed by APCs can interact with an unknown T-cell receptor and suppress T-cells responses (Flies et al., 2014; Wang et al., 2011). In addition, application of recombinant VISTA-Ig fusion protein showed inhibitory effects on T-cell responses (Flies et al., 2014; Flies et al., 2011). CD4+ T-cells in VISTA-

Fig. 1. The role of co-inhibitory molecules in multiple sclerosis.
(A) B7H4: B7H4-Ig impairs T cell proliferation, Th1, Th17 differentiation, and treating EAE and promoting T reg function and IL-10 production. Anti-B7H4 antibody increase T cell proliferation, IL-2 secretion and accelerated EAE severity. (B) VISTA: VISTA-Ig inhibited T cell responses. Anti mAb targeting VISTA exacerbated EAE through activations of CD4+ T cells and IL-17A production. (C) CTLA-4: CTLA-4 Ig inhibits the induction of EAE and Th1 response. Anti-CTLA-4 mAb accelerated and exacerbated EAE. (D) Lag-3: Lag-3-blocking mAb considerably increased EAE severity molecule. (E) BTLA: Administrated modified DC containing MOG peptide with BTLA into EAE induced mice ameliorated CNS inflammation and demyelination, and reduced severity of EAE. HVEM (herpes virus entry mediator) is BTLA ¨s ligand. (F) Tim-3: Anti-Tim-3 antibody exaggerate EAE and increase macrophage activation. Administration of galectin-9 alleviated EAE through reducing autopathogenic T cells. Tim-3/Gal-9 interaction was blocked by a specific mAb, lead to decreased T-lymphocyte apoptosis, and elevated the production of IFN-γ and IL-17 in the BEMS, RRMS, and HC groups, but not in the PPMS group. (G) PD-L1: PD-L1-blocking mAb eventuate the rapid onset of EAE and severity. Adoptive transfer of PD-L1^{hi} B-cell suppresses EAE. (H) PD-1: Blockade of PD-1 led to accelerated EAE severity via elevated antigen-specific T cell expansion, activation, and cytokine production. EAE, experimental autoimmune encephalomyelitis; IL, interleukin; Ig, immunoglobulin; mAb, monoclonal antibody; IFN-γ, Interferon gamma; BEMS, benign MS; RRMS, relapsing-remitting MS; HC, healthy control; PPMS, primary progressive MS; DC, dendritic cells; MOG, myelin oligodendrocyte glycoprotein; CNS, central nervous system.

deficient mice exhibited increased proliferation and cytokine production. It has been reported that Vsir−/− mouse model develops multi-organ inflammatory phenotypes. In addition, Vsir−/− CD4+ and CD8+ T cells showed hyper-responsiveness towards self- and foreign antigens. VISTA regulates the inflammatory responses mainly by mediating DCs and IL-17-producing TCRγδ+ and CD4+ Th17 T cells (Li et al., 2017; Nowak et al., 2017). Administration of a neutralizing monoclonal antibody (mAb) targeting VISTA exacerbated EAE through activations of CD4+ T cells and IL-17A production (Wang et al., 2011). A recent study reports that defect in VISTA, causes developing of autoimmune disease that is mediated by Th1 and TH17. Wang et al. developed EAE mice model with VISTA deficiency by using 2D2 TCR transgenic strain, and observed an accelerated disease onset and severity. In these mice, IL-17A and interferon (IFN)-γ produced by CD4+ T-cells are accumulated in the CNS and spleen and consequently causes disease's development (Wang et al., 2014) (Fig. 1B). Overall, the consensus from the various studies suggest that VISTA functions as an inhibitory receptor and has a vital role in immune system regulation. In line with these data, it is one of the goals in the therapeutic target for MS treatment.

4. CTLA-4

Cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152) belongs to the immunoglobulin superfamily and is a member of the membrane-bound single V domain subfamily (Dariavach et al., 1988), and it is located on the chromosome 2q33 (Thompson and Allison, 1997). CTLA-4 is mainly expressed on the T-regs and activated T cells and it is homologous of CD28, which they both can bind to B7 ligands: B7-2 (CD86) and B7-1 (CD80). (Lindsten et al., 1993). CTLA-4 is an inhibitory molecule that limits the T-cells' proliferation, and IL-2 secretion (Krummel and Allison, 1996; Walunas et al., 1994). In CTLA-4 deficient mouse model polyclonal T cell activation and proliferation via CD4+ T cells activation have been observed (Chambers et al., 1997; Waterhouse et al., 1995). In addition, one study reports protective effects of CTLA-4-Ig on CTLA-4−/− mice (Tivol et al., 1997). Accumulative number of research studies indicate a critical immune regulatory role for CTLA-4. Some publications have shed light into the correlation of MS and CTLA-4 polymorphisms (Dinčić et al., 2006; Flinstad Harbo et al., 1999; Kantarci et al., 2003; Ligers et al., 1999; Mäurer et al., 2002). While some researches have demonstrated that CTLA-4 polymorphisms are not a risk factor of MS developing (Čizmarević et al., 2011; Dilmec et al., 2008; Dyment et al., 2002; Greve et al., 2008; Heidari et al., 2010; Luomala et al., 2003; van Veen et al., 2003; Wray et al., 2008). A recent meta-analysis done by Liu et al., regarding the correlation of MS and CTLA-4 gene, showed no significant correlation of -318C/T, CT60A/G or +49A/G polymorphism with MS susceptibility (Liu and Zhang, 2014). A study identified a reduction of CTLA-4 gene expression according to certain alleles of SNP -658 in MS patients. It suggested that epigenetic changes in the disease process could affect the expression of CTLA-4 thus interfere in T regs function (Palacios et al., 2008). A case report study has shown, high MS activity via CD4+ and CD8+ T cells clonal expansions on a melanoma patient who was given ipilimumab, CTLA-4 blocking antibody (Gerdes et al., 2016). In-vivo administration of anti-CTLA-4 monoclonal antibody in EAE model demonstrates increased severity of the disease (Hurwitz et al., 2002; Karandikar et al., 1996; Perrin et al., 1996). Khoury et al. showed that CTLA-4 Ig inhibits the EAE induction and Th1 response (Khoury et al., 1995) (Fig. 1C). Consequently, albeit CTLA-4 polymorphisms do not correlate with MS susceptibility, CTLA-4 function is important and play as an immunological checkpoint that could be critical in autoimmune disease such as MS.

5. Lag-3

Lymphocyte activation gene-3 (Lag-3, CD223) gene expression occurs on activated CD4+ and CD8+ T-cells and a number of NK cells (Prigent et al., 1999), DCs (dendritic cells) and B-cells (Kisielow et al., 2005; Workman et al., 2009). The human Lag-3 gene is located on chromosome 12p13. Lag-3 belongs to the Ig superfamily with a four extracellular domain similar to CD4 (Bruniquel et al., 1997; Triebel et al., 1990). It binds to a human MHC class II molecules non-polymorphic region and the affinity of this binding is higher than CD4 (Baixeras et al., 1992; Huard et al., 1995, 1996). In-vitro and in-vivo studies have also demonstrated that Lag-3 negatively regulates the expansion of CD4+ and CD8+ T cell and regulates the size of the memory T cell pool (Grosso et al., 2007; Workman et al., 2004; Workman and Vignali, 2003). Blockade of Lag-3 inhibits the function of Treg both in-vivo and in-vitro. In agreement with this, Treg cells from Lag-3 knockout mice had reduced regulatory activity (Huang et al., 2004). Lag-3 is further expressed in type 1 regulatory T cells (Tr1) (Gagliani et al., 2013). These data demonstrated that Lag3 is important for negative regulation of T cell proliferation, activation, and homeostasis. Zhang et al. showed the association between Lag-3 gene and MS susceptibility (Zhang et al., 2005). The first study of the critical role of Lag-3 in the EAE suppression has demonstrated that CD4+ 2D2-IEL-T cells decrease the EAE severity and Lag-3 has an essential role since Lag3 gene has a regulatory role among the 2D2-IEL-T cells that infiltrate the CNS.

Additionally, blocking of Lag-3 with monoclonal antibodies in the mice, considerably causes increased severity of EAE as well as increased number of CNS infiltrated mononuclear cells (Kadowaki et al., 2016). A recent study reports, Lag-3 is highly expressed on the Tregs which are present in the CNS of EAE mice.

Furthermore, more lesions in both brain and spinal cord of Lag-3 deficient mice had identified (Chihara, 2018). It was shown that disease severity was affiliated to T cells producing elevated levels of IFN-γ and granzyme B (Thaker et al., 2018) (Fig. 1D). Conclusively, current data supports that modulating Lag-3 can affect the autoimmune disease as well as MS and suggests that deficiency in immune regulatory receptor-like lag-3 can promote MS autoimmunity.

6. BTLA

BTLA (B and T lymphocyte attenuator) that known as CD272 belongs to immunoglobulin superfamily. This molecule in its cytoplasmic region has two immunoreceptor tyrosine-based inhibitory motifs (ITIM) (Watanabe et al., 2003). Variety of cells including T and B lymphocytes, NKT cells, NK cells, macrophages, DCs, and TFH cells express BTLA (M'hidi et al., 2009; Murphy et al., 2006). Its ligands are B7H4 and herpesvirus entry mediator (HVEM). Interaction between BTLA and B7H4 results in inhibition of interleukin-2 (IL-2) production and T-cell activation (Carreno and Collins, 2003). Also, HVEM signaling through BTLA has an inhibitory effect on the activation of T-cell and cause anti-inflammatory cytokines production such as IL-10 (Sedy et al., 2005).

Piancone et al. indicated that B cells expressing BTLA are significantly reduced in patients with MS disease. In addition, it is shown that CD19+/BTLA+/IL-10+ cells in MS patients were decreased compared to healthy control. Furthermore, this study indicated that patients who received fingolimod had increased B-regs, CD19+/BTLA+, and CD19+/BTLA+/IL-10+ B lymphocytes (Piancone et al., 2016). In another study, it was indicated that BTLA expression on CD8+ T cells was increased in multiple sclerosis patients (Brenu et al., 2016).

It has been shown that BTLA- and HEVEM-deficient mice are more susceptible to peptide antigen-induced EAE and they develop exacerbated disease (Wang et al., 2005; Watanabe et al., 2003). Yuan et al. administrated modified DC containing MOG peptide with BTLA into EAE induced mice. They showed that modified DC ameliorated CNS inflammation and demyelination, and reduced severity of EAE (Yuan et al., 2014) (Fig. 1E).

Considering these studies, BTLA and its ligand have a regulatory role in maintaining tolerance and autoimmunity. More studies in this field seem to be needed, but the importance of the regulatory role of BTLA in MS is undeniable.

7. TIM3

T cell immunoglobulin and mucin domain 3 (TIM-3) that known as HAVCR2 (Hepatitis A virus cellular receptor 2) belong to the TIM gene family. Expression of TIM3 is occurred on Th1, Th17, CD8+ T cells, Treg, DCs (dendritic cells), NK cells, and monocytes (Anderson et al., 2007; Gao et al., 2012; Gleason et al., 2012; Hastings et al., 2009; Monney et al., 2002). TIM3 can bind to Galectin9, PtdSer (phosphatidylserine), HMGB1 (High Mobility Group Protein 1) and CEACAM1 (Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1) (Chiba et al., 2012; DeKruyff et al., 2010; Huang et al., 2015; Wada and Kanwar, 1997). TIM3 negatively regulates immune responses by triggering apoptosis and suppressingTh1 and Th17 activities and inducing the immune tolerance through interaction with galectin9 (Zhu et al., 2005, 2010).

It was reported that a polymorphism of TIM3 gene was associated with multiple sclerosis (Pouladian et al., 2017). The analysis indicates that CD4+ and CD4 + CD25+ T cells percentage that expressed TIM3 was reduced in PBMCs of MS patients; in addition, there is an increase in serum concentrations of IFN-γ, galectin-9 and IL-17 (Feng and Feng, 2016)

In another study, it was confirmed that MS patient's cerebrospinal fluid (CSF) clones secreted high level of IFN-γ and expressed low amount of TIM3 mRNA. In addition, polarization with IL-12, substantially induced high level of IFN-γ secretion but low amount of TIM3 in CSF clones of MS patients compared with control clones,. Finally, blocking of TIM3 expression by a siRNA on CD4 + T Cells leads to an enhanced proliferation and IFN-γ production which all together supports the role of TIM3 in MS (Koguchi et al., 2006). In accordance to this, using a mAb targeting TIM-3 resulted in an increased secretion of IFN-γ in control group but not in MS patients. However, treatment with IFN-γ or glatiramer acetate reversed this functional defect (Yang et al., 2008). It was indicated that there is a correlation between the TIM3 expression level and concentration of TNF-α and IFN-γ in patients' CSF with active disease forms (Khademi et al., 2004). Alternatively, molecular adaptor human leukocyte antigen B (HLA-B)-associated transcript 3 (Bat3) as a cellular factor, can bind to the intracellular tail of TIM3and increase the production of proinflammatory cytokines and proliferation.

It has been shown in benign MS patients, MBP-specific CD4+ T cells that are expressing TIM3 or Gal9 as well as apoptotic T cells were increased but in primary progressive MS patients CD4+/Tim-3+/AV + T cells were reduced and CD4+ and CD8+ T cells expressing Bat3 were increased. Blocking Tim-3/Gal-9 interaction by a specific mAb, lead to a reduction of apoptosis in T-lymphocytes, and increasing of IL-17 and IFN-γ production in the RRMS, BEMS, and HC groups, but not in the PPMS group.

Monney et al. have identified that anti-TIM3 antibody exaggerates EAE and increases macrophage activation (Monney et al., 2002). Lee et al. reported that blocking TIM3 signaling in CD4+ T cells changed the inflammatory pattern in the CNS due to differential effects on Th1 versus Th17 cells. In contrast, blocking TIM3 signaling during CD8+ T cell-mediated EAE exacerbated disease (Lee and Goverman, 2013). In-vivo administration of galectin-9 alleviated EAE through reducing autopathogenic T cells. It has been shown, knockdown of galectin-9 expression with siRNA exacerbated EAE (Zhu et al., 2005) (Fig. 1F).

Conclusively TIM3–galectin-9 pathway associated with auto-reactive T cells; therefore, it could be a therapeutic target for autoimmune diseases as well as MS.

8. PD-L1

Programmed death-ligand 1 (PD-L1) that known as CD274 (cluster of differentiation 274) or B7-H1 (B7 homolog 1) is a member of the B7 family (Dong et al., 1999). This molecule is presented on activated T-cells, B-cells, and myeloid cells (Arrieta et al., 2017; Khan et al., 2015; Lu et al., 2016). PD-L1 binds to PD-1 as a ligand and provide inhibitory signals (Sheppard et al., 2004). Experiments showed that the level of PD-L1 mRNA was significantly reduced in PBMCs of MS patients compared to control group (Javan et al., 2016), however in MS lesions PD-L1 was up-regulated, and T cell responses were restricted (Ortler et al., 2008). In a different form of MS, it was indicated that PD-L1-expressing CD19+ cells, and PD-L1+/IL-10+/CD14+ and CD19+ cells were significantly higher in stable MS patients. Moreover, MBP-specific and apoptotic T cells were increased, and proliferating cells were reduced in stable MS compared to acute MS patients. Furthermore, PD-L1+/IL-10+ over B7-H3+/IFN-γ + ratio significantly decreased in AMS compared to SMS patients. Finally, the expression of PD-L1 was decreased in immune cells of patients that treated (Trabattoni et al., 2009). Before this, it was showed that Interferon-β enhances PD-L1 expression on monocyte and dendritic cell in MS (Schreiner et al., 2004).

It has been demonstrated that expression of PD-L1 in T cells limits the autoreactive responses of CD4 T cells in EAE. PD-L1 Knockout mouse model (129S4/SvJae a resistant mouse to development EAE) notably leads to developed EAE. Moreover, blocking of PD-L1 by using monoclonal antibody (10F.9G2) in wild type C57BL/6 mice in the course of the induction of EAE eventuate the rapid onset of severe clinical disease. In addition, adoptive transfer of MOG35–55-specific T cells into PD-L1 deficient mice recipients developed clinical EAE (Latchman et al., 2004). PD-$1^{-/-}$ and PD-$L1^{-/-}$ mice developed severe EAE through increasing the pro-inflammatory cytokine level including TNF, IFN-γ, IL-17 and IL-6 while PD-$L2^{-/-}$ causes EAE similar to wild type control mice (Carter et al., 2007). Schreiner et al. indicated that PD-L1 expressed by CNS myeloid APC negatively regulates the activation of T-cell during acute relapsing EAE (Schreiner et al., 2008). Another study reported that adoptive transfer of PD-$L1^{hi}$ B-cells suppresses EAE (Khan et al., 2015) (Fig. 1G).

Critical immune-modulatory role of PD-L1 in CNS diseases has been discussed comprehensively by Chauhan and Lokensgard (2019). They concluded that the expression of PD-L1 by neurons, microglia, oligodendroglia and astrocytes provides accumulating evidence for susceptibility for chronic CNS dis orders such as MS. Conclusively several studies has been showing PD-L1 deficiency in MS patients and its correlation with disease progression and susceptibility to develop MS. Therefore, modulation of this pathway holds promise in MS therapeutic potential.

9. PD-1

Programmed cell death 1 (PD-1) that known as CD279 belongs to the CD28/CTLA-4 family. PD-1 is an important regulatory molecule that expresses on a variety of cells such as activated T cell, Tregs, T follicular regulatory (TFR) cells, T follicular helper (TFH) cells, tolerant T cells, memory T cells, B-cells, NK cells, cancer cells and some myeloid cells (Sharpe and Pauken, 2018). PD-1 ligands are PD-L1 (B7- H1; CD274) and PD-L2 (B7-DC; CD273) which are expressed on APCs (antigen presenting cells) and various parenchymal cell types (Freeman et al., 2000; Latchman et al., 2001). PD-1 and PD-L1 or PD-L2 interaction cause initiation of inhibitory signals which regulates T-cells activation, immune tolerance and tissue damage that mediate by the system (Chen and Flies, 2013).

Studies have implied the importance of PD-1 gene polymorphism in MS. Kroner et al. demonstrated that PD-1 gene polymorphism is correlated with the progressive form of MS (Kroner et al., 2005). In another study, Pawlak-Adamska et al. showed that polymorphism variation of PD-1 gene has a relationship with the MS first symptoms and relapsing-remitting form of MS severity (Pawlak-Adamska et al., 2017).

The expression level of PD-1 and PD-L1 in PBMCs from the patients with MS disease was significantly lower than the healthy control group, and the low level of PD-1 expression is significantly associated with increased expanded disability status scale (EDSS) score of the MS patients (Javan et al., 2016).

Analysis of PD1 expression in the different form of multiple sclerosis indicated that MBP-stimulated and PD-1-expressing CD4+ and CD8+ T lymphocytes in peripheral blood of stable MS (SMS) is significantly higher than acute MS (AMS) patients. These data showed that MS quiescent phases have a strong relationship with significantly up-regulated PD-1/PD-L1 expression. Notably, this study indicated higher production of IL-10 as an anti-inflammatory cytokine in MBP-stimulated cells, as well as higher rate of apoptosis and a lower proliferation rate in MBP-specific CD4+ and CD8 + T lymphocytes. It has been shown that PBMC incubation of AMS patients with specific antibodies neutralizing PD-1, cause increased proliferation in MBP-specific CD4+ and CD8+ T lymphocytes (Trabattoni et al., 2009). Expression of PD-1 on T cells, present in lesions in the brain, was significantly lower and mainly present in acute or relapsing MS patients (Machado-Santos et al., 2018).

Moreover, studies suggested elevated PD-1 Treg in CSF of MS patients. In particular, it has been reported that PBMSs from stable form of this MS contains higher rate of PD1-Treg cells compared to acute form of it. Studies regarding PD1 + Treg cells in CSF and peripheral blood of MS patients showed significantly higher activity of the Treg cells in SMS and COPA- or IFN-β-responsive compared to AMS- and COPA-unresponsive individuals (Saresella et al., 2008). Another study indicated that Treg cells from MS patients have high PD-1 expression compared to healthy controls (Sambucci et al., 2018).

Salama et al. reported after immunization of mice with MOG (myelin oligodendrocyte glycoprotein), PD-1 and PD-L1 ligands expression were increased within the central nervous system (CNS). PD-1 blocking led to acceleration and severity of disease via increasing expansion and activation of antigen-specific T cell and cytokine production. These data showed that PD-1 has a pivotal role in regulating EAE (Salama et al., 2003). Another study indicated that EAE could be commenced in PD-1KO mice, and the expression level of PD-1 was crucial to maintain the frequency and function of Treg cells. Thus, autoreactive CD4 T cells activate. PD-1 checkpoint molecule is vital for the auto-reactive T cells activation and infiltration into CNS (Wang et al., 2010) (Fig. 1H). This finding suggested that PD-1 is an important regulatory molecule in multiple sclerosis. The different pattern of expression of PD1 in a different stage of disease indicates that this molecule can suppress the activation of autoreactive cells. PD-1 is a potential therapeutic target for multiple sclerosis.

10. Toll-like receptors

Toll-like receptors (TLR) as a group of receptors family that involved in host defense and recognition of pathogens and accordingly they also classified in pattern recognition receptors (PRRs) (Yu et al., 2010). TLRs are expressed on the several peripheral immune cells, also resident cells of the CNS. There have been different studies showing pivotal role of TLRs in modulating MS, as well as EAE (Racke and Drew, 2009; Azizi et al., 2017).

MS patients derived PBMCs (peripheral blood mononuclear cells) show reduced level of TLR1 expression however the expression level increases in the patients treated with IFN-β (Fernald et al., 2007; Singh et al., 2007). Yeast zymosan as a TLR2 agonist has been shown immune suppressive effects by inducing tolerogenic Antigen Presenting Cells (APC) which leads to the high level production of TGF-β and IL-10, and reduced IL-12 and IL-6 production (Dillon et al., 2006; Slack et al., 2007). Accordingly, it can function as a modulator of MS severity by inducing IL-10 secretion from peripheral blood DCs from MS patients which were treated with IFN-β, subsequentlyIL-10 can suppress the production of IL-1β and IL-23 and finally alleviate the severity of disease (Sweeney et al., 2011). Helminths-infected MS patients had higher expression level of TLR2 on B cells and DC, T-regs induction and therefore increased TGF-β and IL-10 levels. Their clinical and radiological outcomes were better compared with uninfected patients (Suzanne et al., 1986).

TLR3 signaling in astrocytes results in neuroprotective responses and controls the axons growth and neuronal progenitor cells. It is identified that the endogenous TLR3 ligand stathmin was expressed in microglia, astrocytes, and neurons of MS-affected human brain, and was indicated by cDNA arrays to initiate the same set of neuroprotective factors as the synthetic agonist of TLR3 polyinosinic: polycytidylic (poly I:C) acid (Bsibsi et al., 2010).

The rs5743810 SNP of TLR6 gene was correlated with the IFN-β-specific neutralizing antibodies development in men but not in women after 24 months of IFN-β therapy (Enevold et al., 2010).

In-vitro human monocyte-derived DCs treated with IFN-β1, induced the TLR7 expression and its downstream signaling pathway (TRAF6, MyD88, IRAK4) while IL-1R expression was inhibited. Using of siRNA (small interfering RNA) to knocking down the expression ofTLR7 gene, indicated that supernatants from IFN-β1a-treated DCs inhibited differentiation of CD4 + Th17 cells, with downregulation of retinoic acid-related orphan nuclear hormone receptor C (RORC) and IL-17A gene expression and IL-17A secretion (Zhang et al., 2009).

B cells are one of the MS modulators mainly due to the IL10 secretion and it has been shown that using CpG DNA as an TLR9 agonist in MS significantly decreases the production of TLR9-mediated IL-10 by B-cells, and it is likely due to decreased TLR9 expression in memory B cells (Hirotani et al., 2010).

Altogether, it is shown that some of TLRs with anti-inflammatory potent has reduced expression in MS disease. On the other hand, treatment with IFN-β induced immunomodulatory effects through TLR signaling. Therefore, by restoring the expression or function of TLRs, we can regulate the inflammation in MS.

11. Conclusion

Multiple sclerosis is the commonest inflammatory and neurodegenerative disease that affect CNS and correlate with dysregulation of systemic immune system. Since MS is an autoimmune disease, the recognition of receptors and ligands that reduce the T cells activity potentially holds great promises for targeted therapy in autoimmune disease as well as MS.

Accumulating evidence suggests that the described receptors and their ligands have a key role in the regulation of T cells activation and tolerance followed by induction and maintenance of peripheral tolerance and also they have an inhibitory effect on T cell proliferation and cytokine production. The extracellular localization of these molecules makes these cell-surface proteins very attractive targets for therapeutic application. It has been shown, blockade of these molecules lead to enhanced T cell responses and EAE severity, and mouse models deficient in these molecules develop spontaneous autoimmunity EAE.

Enhancement of the inhibitory pathways including B7H4/ VISTA/ CTLA-4/ BTLA/ Lag-3/ PD-1/ PD-L1/ TIM3 with antibodies or recombinant proteins negatively, regulate T cell-mediated immune responses. Although several studies have been done on the mouse model, current clinical evidence for their efficacy in multiple sclerosis is deficient. Hence, studying the function and molecular recognition of these immune regulatory molecules in more details might shade light onto the path finding treatment for multiple sclerosis.

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