Hypoxia/ischemia impairs CD33 (Siglec-3)/TREM2 signaling: Potential role in Alzheimer’s pathogenesis

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ABSTRACT

Recent genetic and molecular studies have indicated that the innate immune system, especially microglia, have a crucial role in the accumulation of β-amyloid plaques in Alzheimer’s disease (AD). In particular, the CD33 receptor, also called Siglec-3, inhibits the TREM2 receptor-induced phagocytic activity of microglia. CD33 receptors recognize the α2,3 and α2,6-linked sialic groups in tissue glycocalyx, especially sialylated gangliosides in human brain. The CD33 receptor triggers cell-type specific responses, e.g., in microglia, CD33 inhibits phagocytosis, whereas in natural killer cells, it inhibits the cytotoxic activity of the NKG2D receptor. Nonetheless, the regulation of the activity of CD33 receptor needs to be clarified. For example, it seems that hypoxia/ischemia, a potential cause of AD pathology, increases the expression of CD33 and its downstream target SHP-1, a tyrosine phosphatase which suppresses the phagocytosis driven by TREM2. Moreover, hypoxia/ischemia increases the deposition of sialylated gangliosides, e.g., GM1, GM2, GM3, and GD1, which are ligands for inhibitory CD33/Siglec-3 receptors. In addition, β-amyloid peptides bind to the sialylated gangliosides in raft-like clusters and subsequently these gangliosides act as seeds for the formation of β-amyloid plaques in AD pathology. It is known that sialine plaques contain sialylated GM1, GM2, and GM3 gangliosides, i.e., the same species induced by hypoxia/ischemia treatment. Sialylated gangliosides in plaques might stimulate the CD33/Siglec-3 receptors of microglia and thus impede TREM2-driven phagocytosis. We propose that hypoxia/ischemia, e.g., via the accumulation of sialylated gangliosides, prevents the phagocytosis of β-amyloid deposits by inhibiting CD33/TREM2 signaling.

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease involving the accumulation of β-amyloid plaques and neurofibrillary tangles associated with an inflammatory microenvironment. Recent studies have demonstrated that the innate immune system, especially microglia, has a key role in the pathogenesis of AD (Hansen et al., 2018; Griciuc and Tanzi, 2021). Genetic studies have revealed several risk genes connected to the function of microglia, e.g., CD33, TREM2, CR1, SHIP1, and APOE. There is an immune dysfunction in AD brains since it seems that although microglia are activated, they are unable to cleanse the accumulating β-amyloid deposits. The persistent low-grade inflammation disturbs the homeostasis in AD brains ultimately leading to neuronal loss and brain atrophy. Interestingly, Griciuc et al. (2013) demonstrated that the CD33 receptor has a crucial role in the control of β-amyloid phagocytosis. This is an intriguing observation since microglial CD33 receptors, also called Siglec-3, are included in the Siglec family which regulates the functions of immune cells by recognizing the sialylation state of tissue glycocalyx (Pillai et al., 2012). There is substantial evidence that host tissues can educate immune cells, e.g., by secreting immunoregulatory factors or modulating the structures of glycocalyx. Most siglec receptors, such as CD33, are inhibitory receptors suppressing the activation of immune cells. Recently, Griciuc et al. (2019) revealed that TREM2 receptors regulate the phagocytosis of microglia under the control of inhibitory CD33 receptors. This means that the changes in the expression of sialic acids in glycocalyx structures regulate the phagocytic activity of microglia. It is known that there exist significant alterations in the sialylated structures in AD pathology, e.g.,
in the expression of sialylated gangliosides (Section 5.2).

Neuroimaging studies have revealed a significant decline in cerebral blood flow in those brain regions vulnerable to AD pathology (Verclytte et al., 2016; Nielsen et al., 2020). Sporadic hypoperfusion induces regional hypoxia/ischemia which increases the amyloidogenic processing of β-amylloid precursor protein (APP) (Pluta et al., 2013a; Salminen et al., 2017). Interestingly, there are mass spectrometric imaging studies indicating that the presence of focal ischemia increased the deposition of sialylated gangliosides, e.g., GM1, GM2, and GM3 (Section 5.3), which enhanced the formation of β-amylloid plaques (Section 5.2). An increased level of sialic ligands has been claimed to stimulate the inhibitory CD33 signaling and consequently disturb the TREM2-mediated microglial phagocytosis (Fig. 1). Moreover, hypoxia/ischemia stimulates the expression of CD33 receptors, thus inhibiting signaling through the CD33/TREM2 pathway (Section 4). We will propose that sporadic hypoxia/ischemia impairs the function of CD33/TREM2 signaling in microglia and thus enhances the pathogenesis of AD.

2. CD33 (Siglec-3)/TREM2 signaling in Alzheimer’s pathogenesis

CD33/Siglec-3 receptors are members of a superfamily called sialic acid binding immunoglobulin-like lectins (Siglecs). Siglec receptors recognize the sialic groups in tissue glycoalyx structures, mostly present in glycoproteins, glycolipids, and gangliosides (Pillai et al., 2012; Linnartz-Gerlach et al., 2014a; Siddiqui et al., 2019). A total of sixteen different Siglecs recognize the specific linkage of sialic acids, e.g., human CD33/Siglec-3 binds to the α2,3 and α2,6 sialic acids on a sugar backbone, whereas Siglec-11 recognizes the α2,8 linkage and polysialic structures (Hane et al., 2021). Many Siglec receptors contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domain and thus they transduce inhibitory downstream signals in immune cells. For instance, human Siglecs 3 and 5–12 contain the inhibitory ITIM sequence, whereas human Siglecs 1, 4, and 14–16 lack the ITIM domain and are unable to trigger inhibitory responses (Siddiqui et al., 2019). Siglec receptors are almost exclusively expressed in immune cells, e.g., human macrophage express inhibitory Siglec-3 and 11 receptors (Linnartz-Gerlach et al., 2014b; Hane et al., 2021). Interestingly, human Siglec-11 and Siglec-16 receptors display an identical extracellular region but Siglec-11 contains the inhibitory ITIM domain, whereas Siglec-16 associates with DAP12 which contains the immunoreceptor tyrosine-based activation motif (ITAM) and thus Siglec-16 transduces activating signals (Schwarz et al., 2017). The sialic acid-induced activation of the ITIM-driven CD33/Siglec-3 and Siglec-11 receptors triggers the phosphorylation of the ITIM motif which consequently activates two SH2 domain-containing protein tyrosine phosphatases 1 and 2 (SHP-1/SHP-2) (Taylor et al., 1999). The human CD33 receptor triggers specific immune responses in a cell-type dependent manner. For instance, CD33 receptor inhibits phagocytosis and β-amyloid uptake in human microglia (Griciuc et al., 2013). In human natural killer (NK) cells, the activation of CD33 signaling inhibits the cytotoxic activity of NKG2D receptor (Hernandez-Caselles et al., 2019). Moreover, SHP-1 and SHP-2 can inhibit STAT3 signaling and thus affect many important functions of myeloid cells (Kim et al., 2018; Jiang et al., 2020). In summary, it seems that the ITIM-driven inhibitory Siglec receptors prevent immune responses, such as inflammation as well as the maturation and activation of myeloid cells (Pillai et al., 2012).

In their seminal study, Griciuc et al. (2013) demonstrated that the expression of the CD33 (Siglec-3) gene was significantly increased in AD brains, observed both at the mRNA and protein levels. They also reported that the number of CD33 positive microglia was not only up-regulated but also the expression level of CD33 receptors was significantly increased in the microglial cells in AD brains. Siddiqui et al. (2013) also revealed that the increased levels of CD33 protein inhibited the uptake of β-amyloid peptides into microglia, whereas the inactivation of CD33 receptor promoted β-amyloid uptake by microglia. The depletion of CD33 protein in transgenic AD mice significantly reduced the accumulation of β-amyloid peptides in transgenic AD mice. Several genetic studies have revealed that the CD33 gene is a risk factor for AD pathogenesis. Interestingly, Malik et al. (2013) demonstrated that the SNP rs12459419 was located in the exon 2 and it regulated the splicing of exon 2 in the AD-risk allele. The exon 2 encodes the sialic acid binding domain of CD33 protein in such a way that the shorter splice variant (CD33m) is unable to inhibit immune responses. In fact, Siddiqui et al. (2017) reported that the CD33m isoform did not localize to the cell surface unlike the full-length CD33m isoform but it was accumulated in peroxisomes in human microglia. Recently, Butler et al. (2021) revealed that the AD-protective CD33m variant stimulated phagocytosis in mouse BV-2 microglia, whereas CD33m inhibited microglial phagocytosis. Moreover, the CD33m isoform inhibited the proliferation and migration of microglial cells, opposite to that induced by the CD33m variant. These studies clearly indicated that the CD33m variant was a gain-of-function isoform capable of preventing AD pathogenesis. The CD33/Siglec-3

Fig. 1. The activation of CD33 receptor inhibits the TREM2-induced phagocytosis in microglia. The sialic acids of glycoalyx act as ligands and activate the inhibitory CD33 receptor in microglia. The CD33 receptor activates SHP-1 protein phosphatase which inhibits the signaling induced by TREM2/DAP12 via the SYK/Pi3K pathway. The TREM2 receptor has several of its own ligands which can activate microglia and induce phagocytosis. Hypoxia/ischemia stimulates the expression of PU.1 factor which is a potent inducer of CD33 expression. Hypoxia/ischemia also increases the expression of SHP-1, thus enhancing the inhibitory signaling of CD33 receptor. In addition, hypoxia/ischemia disturbs the sialylation of glycoalyx, e.g., increasing the level of sialylated GM1, GM2, and GM3 gangliosides which enhance the signaling of CD33 receptors. Abbreviations: CD33 (Siglec-3), Sialic acid binding immunoglobulin-like lectin 3; DAP12, DNAX-activating protein 12; PI3K, Phosphatidylinositol 3-kinase; PU.1, Spi-1 factor; SHP-1, SH2 domain-containing protein tyrosine phosphatase-1; SYK, Spleen tyrosine kinase; TREM2, Triggering receptor expressed on myeloid cells 2.
3. Hypoperfusion in Alzheimer’s pathology

There is convincing evidence that the pathogenesis of AD is associated with a reduced cerebral blood flow (CBF) leading to the hypoxia/ischemia-induced disturbances in neuronal metabolism (Pluta et al., 2013b, 2021a; Vercliever et al., 2016; Nielsen et al., 2020). Especially, the decline in CBF is extensive in those brain regions vulnerable to AD pathology. Moreover, a reduced level of CBF already appears in the patients with mild cognitive impairment (MCI). There are also observations that the diseases displaying a diminished CBF, e.g., heart failure, obstructive sleep apnea, and idiopathic normal pressure hydrocephalus, are associated with an AD-type of pathology (Lahtera et al., 2014; Cermakova et al., 2015; Andrade et al., 2018). Sporadic hypoxia/ischemia impairs mitochondrial energy production and augments glycolysis and lactate production. Many neuroimaging studies have demonstrated that aerobic glycolysis was significantly increased in the brain regions which were vulnerable to AD pathogenesis (Vlassenbroeck et al., 2010). It has been demonstrated that a chronic cerebral hypoperfusion (CH) of normal rats disturbed glucose metabolism and increased the amyloidogenic processing of APP protein to β-amyloid peptides and consequently, CCH induced the deposition of β-amyloid plaques and provoked brain atrophy (Pluta et al., 2013a; Salmén et al., 2017; Park et al., 2019). In addition to APP processing, hypoxia/ischemia also impaired the expression of some genes controlling autophagy and apoptosis in the hippocampus of ischemic rats representing an AD model (Ulamek-Kozioł et al., 2019). Microglia and astrocytes were significantly activated in rat hippocampal CA1 and CA3 layers, for as long as two years following a global cerebral ischemic insult (Radovovic et al., 2020). The chronic hypoxia/ischemia animal models have revealed that brain hypoperfusion clearly causes pathological responses which are surprisingly similar to those present in AD patients (Pluta et al., 2013a, 2021b; Park et al., 2019).

Cerebral amyloid angiopathy (CAA) is a typical hallmark of AD pathology (Thal et al., 2008). The appearance of CAA involves the deposition of β-amyloid peptides within the wall of blood vessels, especially on the capillaries supplying vulnerable brain regions. Interestingly, Okamoto et al. (2012) demonstrated that the experimental CCH in mice accelerated the formation of capillary CAA and induced cortical microinfarcts in those vessels containing β-amyloid deposits. They also reported that the frequency of microinfarcts increased with the severity of CAA in the postmortem samples of human AD brains. There is convincing evidence that the integrity of the blood-brain-barrier (BBB) is disturbed in AD brains, probably due to the accumulation of β-amyloid peptides and the loss of pericytes (Zlokovic, 2011). Sagare et al. (2013) demonstrated that a deficiency of pericytes augmented AD pathology in transgenic mice, e.g., it increased β-amyloid accumulation into capillaries, induced tau protein pathology, and promoted neuronal loss. Liu et al. (2019) revealed that CCH treatment reduced pericyte coverage and increased the permeability of BBB in mouse corpus callosum. Evidently, the disruption of BBB has an important role in maintaining the persistent neuroinflammation in the pathogenesis of AD (Zlokovic, 2011). It is known that chronic low-grade inflammation induces the counteracting anti-inflammatory/immunosuppressive response, i.e., the recruitment of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) as well as the polarization of macrophages/microglia into anti-inflammatory M2 phenotypes (Kanterman et al., 2012; Stubbe et al., 2013; Cherry et al., 2014; Salminen, 2020). This kind of immunosuppressive response seems to be present also in the inflamed brains of AD patients (Salminen et al., 2018; Salminen, 2021). For instance, it has been demonstrated that an immunosuppressive microenvironment inhibited the clearance of β-amyloid peptides, thus enhancing the formation of senile plaques and neuronal atrophy in AD brains (Wyss-Coray et al., 1997; Chakrabarty et al., 2015). It is known that hypoxia is a powerful inducer of an immunosuppressive network (Corzo et al., 2010; Escribese et al., 2012) which is potentially involved in the pathogenesis of AD (Salminen, 2021). Currently, it is not known whether the AD-related immunosuppression is caused by the hypoxia-induced infiltration of immunosuppressive cells or the activation of the inhibitory immune receptors in microglia, e.g., the inhibitory Siglec receptors (Section 2).

There is substantial evidence that in AD, microglial cells are hyporesponsive in promoting the phagocytosis of β-amyloid peptides (Monesoglu et al., 2001; Hickman et al., 2008; Krabbe et al., 2013). The deficiency in the phagocytic activity enhances the deposition of β-amyloid plaques. Currently, the cause of inefficient microglial activity in AD is unknown although an increased expression of CD33 protein might attenuate the phagocytic activity of these cells (Section 4). Interestingly, there are observations indicating that acute hypoxia/ischemia disturb the functions of microglia, e.g., their migration and survival potentials (Yenari and Giffard, 2001; Weinstein et al., 2010). However, intermittent CCH induced hypothalamo-hypophyseal system called preconditioning, induced adaptations which were able to protect against destructive hypoxic/ischemic insults (Chen et al., 2015; Tantingco and Ryu, 2020). For instance, Tantingco and Ryu (2020) demonstrated that IHT enhanced the phagocytic activity of mouse microglia. IHT also reduced the generation of ROS by mouse microglia. Currently, it is not known whether long-term IHT increases the phagocytic activity of microglia by affecting the CD33/TREM2 signaling pathway. Intriguingly, Wu et al. (2017) demonstrated that the expression of TREM2 significantly

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increased in mouse microglia in the ischemic penumbra after middle cerebral artery occlusion (MCAO). They also reported that the silencing of TREM2 aggravated the outcome of MCAO, e.g., it exacerbated inflammation and increased the extent of neuronal apoptosis. Instead, an overexpression of TREM2 reduced the inflammatory response and protected neurons against ischemic insult. These studies clearly indicated that signaling through the CD33/TREM2 axis seems to have an important role in the cerebral pathology induced by hypoxia/ischemia insults.

4. Hypoxia/ischemia regulates the function of CD33/TREM2 signaling pathway

An exposure to hypoxia/ischemia has profound effects on the function of the immune system, affecting both innate and adaptive immunity (Krzywinska and Stockmann, 2019; Chen and Gaber, 2021). For instance, hypoxia/ischemia regulates the polarization, phagocytosis, and cytokine secretion of microglia and macrophages (Fumagalli et al., 2015; Zhang et al., 2017). An increase in phagocytosis in ischemic conditions can be associated with both the pro-inflammatory M1 phenotype (toxicity) and the anti-inflammatory M2 polarized state (protection/recovery) (Fumagalli et al., 2015). The inhibitory immune checkpoint proteins, e.g., CD33, LILRB2, PD-L1, TIM3, and VISTA, have an important role not only in maintenance of self-tolerance but also in the inhibition of excessive inflammatory responses to prevent tissue damages. It is known that hypoxia/ischemia is a strong stimulator of PD-L1, TIM3, and VISTA expression (Koh et al., 2015; Otta, 2018; Deng et al., 2019). For instance, the hypoxic environment surrounding a glioma triggers a robust expansion of CD33-positive myeloid cells, such as macrophages, generating an immunosuppressive microenvironment (Pinton et al., 2019). It seems that hypoxia/ischemia is a potent enhancer of immunosuppression through the stimulation of inhibitory checkpoint proteins in myeloid cells (Otta, 2018).

4.1. Hypoxia/ischemia increases the expression of inhibitory CD33 protein

Given that the sialylation of glycocalyx acts as a crucial checkpoint for innate immunity in the brain (Klaus et al., 2021) (Section 5), it seems that the inhibitory CD33 checkpoint has an important role in the suppression of pro-inflammatory activity of myeloid cells. There are very few studies on the changes in the expression level of CD33 protein in immune cells since the CD33 receptor has been considered to be a myeloid lineage marker in cell sorting assays. The hypoxic conditions associated with inflammation modify the properties of many immune cells; hypoxia especially regulates the expression profiles in human mature dendritic cells (Bosco et al., 2011; Winning and Fandrey, 2016). Bosco et al. (2011) demonstrated that chronic hypoxia increased the expression of several immunoregulatory receptors in human mature dendritic cells. The expression of CD33 displayed a 4.7-fold increase in hypoxic culture as compared to normoxic conditions. Blumenau et al. (2020) demonstrated that subacute hypoperfusion induced a robust upregulation of CD33 expression in mouse hippocampus after a bilateral common carotid artery stenosis (BCCAS) although they did not characterize the origin of the elevated CD33 expression. Moreover, Zhang et al. (2013) reported that in human chronic obstructive pulmonary disease (COPD), the expression level of CD33 on the surface of circulating granulocytes negatively correlated with the serum level of C-reactive protein (CRP), a pro-inflammatory marker. This indicates that the CD33 receptor present in granulocytes might inhibit excessive inflammation in COPD.

4.2. Hypoxia/ischemia stimulates the expression of PU.1 factor

The promoter of CD33 does not contain the hypoxia-responsive element (HRE) but it contains several binding sites for hypoxia-responsive transcription factors, such as PU.1 (also called Spi-1) and Sp-1 factors (Bodger and Hart, 1998). Bodger and Hart (1998) demonstrated using functional analyses that the PU.1 site was crucial to the expression of the CD33 protein in human THP1 and HEL cell lines (Fig. 1). Transcription factor PU.1 has been demonstrated to be expressed in immune cells in which it controls lineage commitment, e.g., myelopoiesis, and immune cell diversity (Guerrero et al., 2000; Dakic et al., 2005). PU.1 protein is not only a transcription factor but it can also induce epigenetic chromatin remodelling, e.g., in the differentiation and activation of myeloid cells in response to environmental insults (Ivashkiv and Park, 2016; Yeh and Ikezu, 2019). The PU.1 factor is robustly expressed in human microglia and it is involved in the maintenance of microglial viability through transcriptional and epigenetic regulation (Walton et al., 2000; Smith et al., 2013; Yeh and Ikezu, 2019). Interestingly, several investigators have revealed that the expression of PU.1 factor is significantly upregulated by hypoxic/ischemic treatments (Walton et al., 2000; Ghosh et al., 2010; Lin et al., 2015). Lin et al. (2015) demonstrated that the ischemic stroke (MCAO) of rats caused a significant increase in the expression of PU.1 factor in brain tissue and in the cells of peripheral blood. In addition, Ghosh et al. (2010) reported that hypoxic treatment increased the DNA-binding activity of PU.1 factor in human endothelial cells (HUVEC) and this might well enhance the expression of some target genes, e.g., GM-CSF and IL-8 receptor 1 (CXCR1). Huang et al. (2017) revealed that a common haplotype in AD patients reduced the expression of the PU.1 gene in monocytes and macrophages and delayed the onset of AD pathology. They also reported that PU.1 factor was able to bind to the cis-regulatory elements of several AD risk genes, including the CD33 gene. However, the role of PU.1 protein in the regulation of CD33/TREM2 axis needs to be clarified.

4.3. Hypoxia/ischemia activates SHP-1 phosphatase

The activation of CD33 receptor recruits protein tyrosine phosphatase SHP-1 to its ITIM domain which subsequently suppresses not only the TREM2-driven phagocytosis but also several important signaling pathways, e.g., T-cell receptor (TCR) (Taylor et al., 2007). This means that SHP-1 has an important immunosuppressive role in immune cells. It is known that hypoperfusion/stroke induces both inflammatory and immunosuppressive responses (Jiang et al., 2021; Salminen, 2021). There is extensive evidence that hypoxia/ischemia upregulates the expression and activation of SHP-1 phosphatase which prevents the development of excessive inflammatory responses (Wishcamper et al., 2003; Huang et al., 2020; Peng et al., 2020; Peng et al., 2020). However, the signaling mechanisms of the induction and activation of SHP-1 during hypoxia/ischemia are mostly unknown. It is known that several immunosuppressive cytokines, e.g., IL-10 and TGF-β, present in hypoperfused brain can also activate SHP-1 phosphatase (Park et al., 2005; Taylor et al., 2007). Taylor et al. (2007) demonstrated that the IL-10-activated SHP-1 dephosphorylated the costimulatory receptors of TCR, i.e., CD28 and ICOS, thus inhibiting the activation of human T cells. They also observed that the activation of SHP-1 prevented the binding of P13K to CD28 receptor. Accordingly, Myers et al. (2020) reported that the loss of SHP-1 in mouse and human macrophages triggered a 2-3-fold increase in the phagocytic activity of these cells. Considering the activation of SHP-1, it seems that hypoxia/ischemia can activate SHP-1 signaling in a CD33-dependent and -independent manner.

4.4. Hypoxia/ischemia enhances the expression of TREM2 protein

There is clear evidence that hypoxia/ischemia stimulates the expression of both TREM2 and DAP12 receptors (Sieber et al., 2013; Albertsson et al., 2014; Kawabori et al., 2015; Zheng et al., 2021). For instance, Sieber et al. (2013) demonstrated that in mice subjected to an experimental stroke (MCAO) there was a robust increase in the number
of TREM2 positive microglia within the peri-infarct zone. The response peaked after 7 days from ischemia although a considerable upregulation was still present at one month after the ischemia. They also demonstrated that a comparable stroke significantly reduced the microglial activation and inflammatory response in the TREM2-knockout mice. Kawabori et al. (2015) reported that the phagocytic activity of microglia was clearly reduced in the TREM2-knockout mice and that these animals also experienced elevated cerebral damage after the ischemic insult. Interestingly, Zhao et al. (2013) demonstrated that miR-34a targeted the TREM2 mRNA 3′-UTR and it downregulated the expression of TREM2 in mouse microglia. This might explain the hypoxia/ischemia-induced upregulation of TREM2 expression since several investigators have reported that hypoxia down-regulated the expression of miR-34a in different cell types (Du et al., 2012; Wang et al., 2016). However, in AD, the expression levels of both TREM2 (Frank et al., 2008; Matarin et al., 2015) and miR-34a (Sarkar et al., 2016; Jaber et al., 2017; Herrera-Espejo et al., 2019) were clearly upregulated. Sarkar et al. (2019) also demonstrated that the over-expression of miR-34a induced a cognitive impairment and AD-like pathology in mouse brain. It is known that the over-expression of miR-34a attenuated the clearance of apoptotic bodies, i.e., efferocytosis, in mouse and human macrophages (McCubrey et al., 2016). The expression of miR-34a is under a very complex regulatory network involving many signaling pathways, transcription factors, epigenetic regulation, feedback loops, and epistatic control between neurons and immune cells. It seems that the TREM2-driven phagocytosis is not only dependent on the expression level of TREM2 but many other factors, e.g., the activity of inhibitory CD33/SHP-1 signaling (Section 2), regulate microglial phagocytosis in a context-dependent manner.

5. Sialylation of glycocalyx regulates the functions of immune cells

The glycoproteins and -lipids of extracellular glycocalyx are sialylated containing one or more terminal sialic acids which regulate the functions of immune cells (Daniotti and Iglesias-Bartolome, 2011; Pillai et al., 2012; Sandhoff and Sandhoff, 2018; Liao et al., 2020). Sialylated glycocalyx components control the functions of immune cells via inhibitory and activating Siglec receptors (Section 2). Siglec receptors of cell membranes recognize the sialylated ligands expressed on both the cell-surfaces of immune cells themselves (cis-ligands) and the surfaces of neighbouring cells and the matrix of host tissues (trans-ligands). Most sialylated ligands activate inhibitory Siglecs and thus they maintain the tolerance of immune system, e.g., they inhibit inflammation, control phagocytosis, affect T cell activation and polarization, and regulate the cytotoxicity of NK and CD8+ T cells (Pillai et al., 2012; Lübbers et al., 2018). It is known that the sialylation state of glycans is altered in many pathologies, e.g., in Alzheimer’s disease (Section 5.2), which might well disturb the function of immune cells and lead to pathological changes.

5.1. Overview of glycan sialylation

Sialic acids are nine carbon monosaccharides which form the terminal caps on the glycoproteins and glycolipids in the glycocalyx. Secreted glycoproteins can also be sialylated. In humans, N-acetylneuraminic acid (Neu5Ac) is the most frequent sialic acid which can be modified in several ways, e.g., methylated, sulphated, and lactylated. There are several sialyltransferases which conjugate sialic acids into the acceptor sugars through the α2,3, α2,6, or α2,8 linkages (Bhide and Colley, 2017). Sialic acids can also form homopolymers, e.g., polysialic acids (PSA) contain 10-200 Neu5Ac monomers connected through the α2,8-linkages. In mammals, brain tissue and immune cells contain an abundance of sialylated structures. Gangliosides and NCAM are the most common sialylated compounds in mammalian brains (Schnaar, 2016; Liao et al., 2020). The major gangliosides in human brain comprise the GM1, GD1a, GD1b, and GT1b structures (Vajn et al., 2013) (Fig. 2). The cleavage of the terminal sialic groups, i.e., the desialylation process, involves four sialidases/neuraminidases (NeU1–NeU4) (Pshezhetsky and Ashmarina, 2018). The NEU enzymes are located in different cellular compartments and they have specific properties, e.g., in cleaving specificity. All neuraminidases can cleave the α2,3-conjugates, whereas the α2,6-linked sialic acids are cleaved by NeU1 and NeU3. The α2,3 and α2,6-linked glycans are the specific ligands for CD33 receptors (Section 2). NeU3, which is bound to plasma membrane, is the main sialidase involved in the desialylation of gangliosides. It is known that inflammatory insults increase the activity of NEU1 and NEU4 and reduce the sialylation of glycans (Demina et al., 2018). Moreover, regulatory complement factor H (CFH), a soluble inhibitor of complement, binds to the α2,3-linked sialylated glycoconjugates and the desialylation of these sites prevents the binding of CFH to glycocalyx leading to the activation of the alternative complement system (Schmidt et al., 2018; Liao et al., 2020). Moreover, CFH displays the α2,6-sialylation (Schmidt et al., 2018), a ligand to CD33. Interestingly, CFH and CD33 and some other Siglecs share binding to the same sialylated targets that might affect the outcomes of cerebral pathological processes.

Fig. 2. The metabolic pathways in the sialylation of gangliosides. There are three sialylation series of α-c denoting mono-, di-, and trisialogangliosides. Blue circles represent the number and position of sialic acids bound to the core molecule composed of glycosphingolipid. GM3 synthase (GM3S, lactosylceramide α2,3-sialyltransferase) transfers a sialic acid residue to Gal-Glc-Cer via the α2,3-linkage to form monosialoglycosyl GM3 ganglioside. GD3 synthase (GD3S, STα2,8-sialyltransferase) sialylates GM3 via the α2,8-linkage to form disialoganglioside GD3. GM2 synthase (GM2S) transfers N-acetylglactosamine to form a monosialoglycosyl GM2 ganglioside. The sialylation pathways of gangliosides have been presented in detail elsewhere (Daniotti and Iglesias-Bartolome, 2011; Sandhoff and
5.2. Role of sialylated glycans in Alzheimer’s pathology

AD pathogenesis is associated with crucial alterations in the levels and functions of sialylated glycans, especially those of gangliosides (Ariga, 2017; Rawal and Zhao, 2021; Yang et al., 2021). Briefly, it seems that the content of complex gangliosides, e.g., GM1, GD1a, GD1b, and GT1b is reduced, whereas those of simple gangliosides, such as GM2, GM3, and GD3, are significantly increased in the brains of AD patients (Kracun et al., 1990, 1992; Kalanj et al., 1991; Fukami et al., 2017) (Fig. 2). In transgenic AD mice, a similar tendency has been observed, especially the levels of simple gangliosides, e.g., GM2 and GM3, were robustly increased (Barrier et al., 2007; Caughlin et al., 2018; Strnad et al., 2020). The changes were localized to the regions vulnerable to AD pathology and they correlated with the appearance of β-amyloid deposits. Several gangliosides, e.g., GM3, GM2, and GD3, are the targets of CD33 and CD33-related siglecs (Rapoport et al., 2003). Fukami et al. (2017) reported that the expression of cholinergic neuron-specific gangliosides GT1ac and GQ1bα was also significantly increased in the brains of AD patients. The molecular basis of the alterations and their role in AD pathogenesis still need to be clarified although there are clear indications that dysfunctions in sialometabolism are involved in AD pathology. For instance, Oikawa et al. (2009) demonstrated that the deletion of the GM2 synthase (GM2S) gene induced the accumulation of GM3 gangliosides, whereas GM1 gangliosides were lacking in the brain of transgenic APP<sup>ST3/−/−</sup> mice (Fig. 2). The absence of the GM2S enzyme significantly increased the level of β-amyloid peptides and elevated the deposition of senile plaques, especially within vascular tissues. Duhkinova et al. (2019) crossed the SxPAd mice with the S3gal5-deficient (ST3<sup>−/−</sup>) mice which lacked major brain gangliosides (GM1, GD1a, GD3, GT1b, and GQ1b). They reported that the transgenic ST3<sup>−/−</sup>/SxPAd mice displayed a reduced level of β-amyloid deposits and neuroinflammation and furthermore there were no signs of neuronal loss or synaptic degeneration. Accordingly, Bernardo et al. (2009) revealed that the elimination of GD3 synthase (GD3S) gene, which downregulated the synthesis of di- and trisialogangliosides (Liu et al., 2018) (Fig. 2), significantly reduced the accumulation of β-amyloid plaques and improved the memory of transgenic APP/PSEN1 mice. These studies indicate that an increased level of sialylated gangliosides, especially monosialogangliosides, might enhance AD pathogenesis.

Interestingly, there exists a mutual crosstalk between the processing of amyloid precursor protein (APP) and the metabolism of sialylated compounds. Grimm et al. (2012) demonstrated that the intracellular domain of APP protein (AICD), a transcription factor, strongly down-regulated the expression of GD3 synthase in cultured neuronal cells. As discussed above, GD3S inhibits the formation of complex gangliosides and thus APP processing might disturb sialometabolism. These investigators also revealed that β-amyloid peptides were able to bind to GM3 gangliosides, i.e., the substrate of GD3S, and thus they were able to inhibit the activity of GD3S enzyme and the synthesis of complex sialo-compounds. On the other hand, they reported that the GD3 ganglioside side increased the production of β-amyloid peptides, whereas GM3 decreased its production, evidence of a feedback process between APP processing and ganglioside metabolism. Annunziata et al. (2013) demonstrated that the deficiency of lysosomal NEU1 sialidase stimulated the amyloidogenic APP processing in mice. They observed that the deletion of NEU1 induced the accumulation of oversialylated APP proteins in lysosomes as well as an increase in the release of β-amyloid peptides from neurons through the lysosomal exocytosis. Moreover, Kitarzumi et al. (2003) revealed that the β-site APP-cleaving enzyme 1 (BACE1) cleaved α2,6-sialyltransferase (ST6Gal-I) and subsequently, a shedded, soluble ST6Gal-I enzyme stimulated the α2,6-sialylation of secreted proteins (Sugimoto et al., 2007). The increased activity of BACE1 also enhanced the α2,6-sialylation of plasma proteins in mice. In addition, Nakagawa et al. (2006) reported that the overexpression of STGal-I stimulated the α2,6-sialylation of endogenous APP protein and increased the secretion of β-amyloid peptides in Neuro2a cells. Given that Siglec receptors regulate the immune functions of microglia, it seems that changes in sialylation could well be closely associated with pathological disturbances present in the AD brain.

There is robust evidence that sialylated gangliosides have a crucial role in the pathological processes induced by β-amyloid peptides. Yanagisawa et al. (1995) demonstrated that secreted β-amyloid peptides were able to bind to GM1 gangliosides in the membranes of AD brain. Subsequently, Kakio et al. (2002) revealed that β-amyloid peptides bound to the raft-like clusters rich of sialylated gangliosides, especially to the GM1 species. They reported that the ganglioside-bound β-amyloid peptides underwent a conformational transition from the α-helix structure to the β-sheet shape. Yanagisawa (2005) reported that the GM1-bound β-amyloid acted as a seed for the formation of β-amyloid fibrils and aggregates. Subsequently several mass spectrometric studies have revealed that senile plaques contain sialylated gangliosides, especially monosialogangliosides such as GM1, GM2, and GM3 species (Matsubara et al., 2017; Michno et al., 2019; Kaya et al., 2020). It seems that the structure of β-amyloid plaques is very heterogeneous with respect to the compounds in the diffuse periphery and the compact core region (Michno et al., 2019). Sialylated gangliosides were located in the diffuse periphery of plaques rather than in the core which tends to be enriched with β-amyloid peptides. Plaques contain also some other sialylated proteins associated with AD pathology, including apoE and clusterin (apoJ) (Kida et al., 1995). We proposed several years ago that the inhibitory Siglec receptors of microglia could recognize the sialylated groups of β-amyloid plaques and thus prevent their phagocytosis, i.e., senile plaques are hiding from immune clearance (Salminen and Kaarniranta, 2009).

5.3. Hypoxia/ischemia impairs tissue sialylation

Currently, it is not known whether the accumulation of β-amyloid deposits in AD pathology is caused by the hypoperfusion-induced disturbances in brain sialylation although it is known that (i) hypoxia/ischemia triggers β-amyloid deposition (Section 3), and (ii) disturbances in sialylation induce the formation of β-amyloid plaques in AD (Section 5.2). There is convincing evidence that hypoxia/ischemia disturbs the metabolism of sialic compounds. For instance, in tumors, the hypoxic microenvironment controls the extent of immunosuppression through the modification of sialoglycans in glycolaxyl (Yin et al., 2010; van de Wall et al., 2020). Studies exploiting MALDI-TOF imaging mass spectrometry have revealed that mice subjected to transient focal cerebral ischemia (MCAO) underwent clear increases in the deposition of sialylated gangliosides, e.g., GM1, GM2, GM3, GD1, and GT1, within the ipsilateral hemisphere (Whitehead et al., 2011; Caughlin et al., 2015). The highest concentrations appeared in hippocampus, cerebral cortex, and striatum between 3 and 7 days after the MCAO insult (Whitehead et al., 2011). Similar responses in gangliosides have been reported after MCAO treatment in rats (Kwak et al., 2005). The expression of these simple gangliosides is also upregulated in AD brains (Section 5.2). There are also reports indicating that the polysialylation of neural cell adhesion molecule (PSA-NCAM) sharply increased after transient global ischemia in gerbils (Fox et al., 2001) and after neonatal hypoxic-ischemic brain injury in mice (Chavez-Valdez et al., 2021). In gerbils, ischemia induced a slight increase in PSA-NCAM in hippocampal neurons, while the response was more robust in hippocampal astrocytes; it appeared in all hippocampal regions between 1 and 7 post-occlusion days. The level of immunoreactive PSA-NCAM was also significantly increased in the hippocampus of AD patients (Mikkonen et al., 1999). CD33 does not recognize PSA oligomers, whereas Siglec-11 is able to interact with PSA chains (Shahrzad et al., 2015). Shahrzad et al. (2015) demonstrated that PSA have clear anti-inflammatory effects mediated via Siglec-11 receptors in human THP macrophages. For instance, PSA prevented the LPS-induced increase in phagocytosis by macrophages. PSA also inhibited the oxidative burst of macrophages induced by fibrillary β-amyloid peptides. It seems that the increase in the level of
PSA in AD is associated with the anti-inflammatory defence. Trindade et al. (2001) demonstrated that hypoxia/ischemia in neonatal mouse brain increased the accumulation of GM3 gangliosides which was associated with a decrease in the activities of GD3 and GM2 synthases (Fig. 2). Given that there was no change in the expression of those genes, this implies that hypoxia/ischemia directly inhibited the activities of these enzymes, probably through changes in the redox state. It is also known that hypoxia and several stresses increase the level of free sialic acid in tissues and serum. For instance, in patients with coronary artery disease or stroke, the concentration of sialic acids increased in serum and positively correlated with mortality (Gopaul and Crook, 2006; Nanetti et al., 2008). Sialic acid is released from glycolcyx, e.g., gangliosides, through the activation of NEU1-NEU4 enzymes. An increase in the expression of NEU enzymes is a common hallmark of hypoxia/ischemic conditions, e.g., in myocardial ischemia/reperfusion (Hieber et al., 2020), hypoxic heart disease (Piccoli et al., 2017), and hypoxia in cultured muscle cells (Scaringi et al., 2013). Inflammation, e.g., exposure to LPS, also was a potent inducer of cerebral NEU1 expression in neonatal rats (Demina et al., 2018). The induction of NEU1 was associated with a sustained desialylation of cerebral glycoproteins and the reduction of PSA level. There are observations indicating that free sialic acids have anti-inflammatory properties and thus possess significant therapeutic potentials (Böhm et al., 2012; Xue et al., 2018). Moreover, sialic acids are potent scavengers of hydrogen peroxide (Iijima et al., 2004; Ogawara et al., 2007) and thus they can attenuate oxidative stress. The hypoxia-induced increase in the level of free sialic acids might increase the sialylation of soluble targets, e.g., that of galectin-3 which is a crucial enhancer of microglia-mediated neuro-inflammation (Tan et al., 2021) and Alzheimer’s pathology (Boza-Serrano et al., 2019). Interestingly, Zhuo and Bellis (2011) demonstrated that the sialylation of galectin proteins blocked their binding to β-galactoside residues and thus inhibited their inflammatory properties. They also reported that ST6Gal1 sialyltransferase, e.g., shedded by BACE1 (Section 5.2), was attributed to the sialylation of extracellular galectin proteins. Given that galectin-3 is an endogenous ligand of TREM-2 (Boza-Serrano et al., 2019), the sialylation of galectin-3 might suppress the TREM2-driven phagocytosis and enhance the accumulation of β-amyloid plaques in AD. It seems that hypoxia/ischemia can stimulate the inhibitory activity of CD33/TREM2 signaling by (i) increasing the level of sialoligands of the CD33 receptor, e.g., GM1, GM2, GM3, and GD1, and by (ii) releasing free sialic acids which can be used in the sialylation of galectin-3 and other soluble glycans.

6. Conclusions

Recent genetic and molecular studies have emphasized the crucial role of microglia and a low-grade neuroinflammation in the pathogenesis of AD (Hansen et al., 2018; Leng and Edison, 2021). It is known that chronic inflammation induces a counteracting immunosuppression to prevent the harmful effects of prolonged inflammation (Kanterman et al., 2012; Wang and Dubois, 2015; Amadio et al., 2019). Hypoxia/ischemia-associated inflammatory conditions are potent inducers of immunosuppression, not only in the tumor microenvironment but also in AD (Wang and Dubois, 2015; Salminen, 2021). Interestingly, Griciuc et al. (2013) demonstrated that the expression of CD33/Siglec-3 receptor, a crucial immunosuppressive receptor, was robustly increased in the microglia of AD patients. They also revealed that the signaling of CD33 receptor inhibited the TREM2-mediated phagocytosis in mouse microglia (Griciuc et al., 2019). According, the deletion of CD33 receptors in transgenic AD mice significantly attenuated AD pathogenesis. This indicates that the activity of CD33 receptor driven by the sialylated glycans of glycolcyx is an important immune checkpoint in AD pathogenesis (Klaus et al., 2021). It is known that AD pathology is associated with accumulation of sialylated gangliosides (Section 5.2) although its mechanism needs to be clarified. There is clear evidence that brain hypoperfusion, a hallmark of AD pathogenesis, can crucially increase the inhibitory activity of CD33 signaling which consequently impedes the TREM2-related phagocytosis of microglia. Hypoxia/ischemia not only increase the level of sialylated gangliosides in the brain but also increase the expression of CD33 receptors (Section 4.1. and 5.3.). Furthermore, hypoperfusion increases the expression of neuroaminidases which reduce the level of sialylation but concurrently generate free sialic acids which possess both anti-inflammatory and antioxidative properties (Section 5.3.). It seems that the sialic ligands/CD33 receptor axis is a double-edged sword in AD pathogenesis, i.e., increased CD33/SHP-1 activity prevents microglial phagocytosis thus inducing the deposition of β-amyloid plaques in AD brains while CD33 signaling also has several beneficial functions, e.g., it suppresses excessive inflammation and maintains tissue integrity (Crocker et al., 2007; Siddiqui et al., 2019; Klaus et al., 2021). Nonetheless, there are both genetic and small molecular drug discovery projects aimed at inhibiting the expression of CD33 receptor or preventing its activation (Miles et al., 2019; Griciuc et al., 2020). Both treatments have been able to increase the phagocytic uptake of β-amyloid into microglial cells. However, it is known that the deletion of CD33 receptor from human macrophages and microglia resulted in detrimental oxidative burst and inflammatory phenotypes in these cells (Willfold et al., 2021). Accordingly, deficiency of gangliosides, a rich source of sialic acid ligands, activated the complement system and caused inflammation and neurodegeneration in mouse spinal cord (Ohmi et al., 2014). Currently, it seems that the activation of sialic acid/CD33 receptor axis in AD pathogenesis has an alleviating effect which prevents the detrimental inflammation induced by transient hypoperfusion episodes although concurrently it suppresses many functions of the immune system, thus enhancing the pathogenesis of AD.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Authors’ contributions

All authors contributed to the design of the article based on their collaborative research. AS wrote the draft which was reviewed by AK and KK. All authors approved the final manuscript.

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