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Understanding the neurobiology of CD200 and the CD200 receptor: a therapeutic target for controlling inflammation in human brains?

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Abstract

CD200 and its receptor, CD200 receptor (CD200R), have unique roles in controlling damaging inflammatory processes. At present, the only identified function for CD200 is as a ligand for CD200R. These proteins interact resulting in the activation of anti-inflammatory signaling by CD200R-expressing cells. When this interaction becomes deficient with aging or disease, chronic inflammation occurs. Experimental animal studies have demonstrated the consequences of disrupting CD200–CD200R interactions in the brain, but there have been few studies in human brains. Deficiency in neuronal CD200 may explain the chronic inflammation in human neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis; however, deficits in the microglial expression of CD200R may also be of functional significance. The purpose of this review is to assess the data regarding the role of CD200–CD200R interactions in relation to the brain in order to determine if this could be a therapeutic target for human brain diseases with inflammatory components, and what additional studies are needed.

Keywords

aging; alternative activation; anti-inflammatory signaling; cell surface protein; endogenous inflammatory regulator; inflammation; neurodegenerative disease; neuropathology

Identification of CD200 (OX-2) & the CD200 receptor

The discoveries of the CD200 protein and the CD200 receptor (CD200R), and the demonstration of their functions have fascinating scientific histories that add to the interest of what is now appreciated as a critical endogenous inflammatory regulatory system. For a number of years, CD200 was an 'orphan' protein referred to as OX-2 (or MRC OX-2) in earlier publications, based on the recognition of cellular structures by a monoclonal antibody produced by immunizing mice with a partially purified preparation of rat thymocyte membranes [1–3]. Using this reagent, a protein of 41 kD was purified from a rat brain, and one of 47 kD from rat thymocytes. The difference in molecular weight was owing to the increased carbohydrate content of CD200 produced by thymocytes compared with the brain [2]. This initial study demonstrated CD200 to be a relatively abundant protein in the brain; a

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subsequent study showed that the protein could be localized to subsets of neurons and vascular endothelial cells in the rat brain, as well as to B lymphocytes and smooth muscle cells [4]. Using the OX-2 antibody and oligonucleotide sequences deduced from the CD200 protein sequence, cDNA clones coding the gene for CD200 were identified, which coded for a protein of 248 amino acids in rats. Sequence data showed CD200 to be a member of the immunoglobulin superfamily (IgSF) of proteins [5,6].

Subsequent to these findings, the human and mouse equivalents of CD200 were identified and their genes cloned [7]. Based on its distribution within tissues, initial findings suggested that CD200 would have cell adhesion properties. The immunoregulatory properties for CD200 were later identified in a series of cell transfer studies, which showed that the interaction of CD200-positive cells with dendritic cells resulted in reduced cytokine secretion and increased survival of transplanted tissues [8–10]. Blocking this interaction with a CD200 antibody reduced graft survival and prevented a reduction in cytokine secretion, but addition of a soluble recombinant form of CD200 had the same consequence as that due to cell–cell contact [8]. The immunoregulatory properties of CD200 became further apparent upon the identification of the CD200R. In 2000, OX-2 was given the cluster of differentiation number of 200 by the 7th Workshop and Conference on Human Differentiation Antigens (Harrogate, UK) [11]. Both nomenclatures were used in some of the earlier papers.

The amino acid sequence of CD200 revealed the presence of two immunoglobulin domains, a transmembrane region and a small cytoplasmic domain identifying it as a member of the IgSF of cell surface proteins. The homology between human and mouse CD200 is 77.6% for protein and 81.7% for DNA, while the homology between human and rat CD200 is 77.2% for protein and 80.7% for DNA [101]. The structure of the cytoplasmic domain suggested the absence of an intracellular signaling function for this protein [12]. In rodents, the *CD200* gene codes for a protein of 248 amino acids [6,7], while in humans, two transcript variants for *CD200* have been identified. Transcript variant *CD200a* codes for a protein of 269 amino acids, while transcript variant *CD200b* codes for a protein of 294 amino acids [101]. The additional amino acids are at the N-terminal of the polypeptide, starting at amino acid 5. The predominant transcript and protein appears to be the CD200a form, but there have been no reports on expression of the longer CD200b protein. As the amino acid sequences of CD200 required for interaction with CD200R are at the N-terminal, it is unclear whether the longer CD200 form will efficiently bind and activate CD200R [13,14]. The peptide sequence TAsLRCSLKTSQE present in the N-terminal of mouse CD200 was the most effective in blocking CD200R activation by soluble CD200 [15].

The original publication on CD200 identified a protein of 41 kD in rat brains [2]; the predominant form of CD200 in human brains had a molecular weight of 43 kD. Until very recently, a physio-logical species of soluble CD200 had not been identified. Earlier work had defined a species of CD200 mRNA that contained an N-terminal deletion resulting from the loss of exon 2 in mouse cells. This resulted in a protein that could antagonize the immune suppressive activity of CD200 [16]. The truncated form of CD200 was expressed in the mouse brain, but at very low levels compared with the full length species. Recently, a form of cell surface released soluble CD200 (sCD200) from chronic lymphocytic leukemia cells has been characterized in plasma [17]. Increased levels of sCD200 correlated with severity of chronic lymphocytic leukemia disease and plasma with high levels of sCD200 promoted the establishment of transplanted chronic lymphocytic leukemia cells in mice. In addition, increased levels of sCD200 were detected in plasma from patients with systemic lupus erythematosus, an autoimmune disease [18]. At present, there have been no reports of a soluble form of CD200 in the brain. The authors' findings seemed to show that CD200 in fractionated samples of human brains was almost exclusively localized to the membrane

fraction and absent from the cytosolic fraction [19]. Similar results demonstrating exclusive membrane localization for CD200 were seen with rodent brain CD200 [20]; however, the recent studies that demonstrate a secreted form of CD200 in the plasma suggest that further studies in brain samples are needed.

Critical to the identification of the function of CD200 was the identification of its receptor, but what became apparent from these earlier studies, and still true today, is that CD200 is a ligand rather than a receptor, and its only known function is to interact with and activate CD200R. There was a gap of 15 years between identification of the CD200 protein [2] and the critical experiments that identified a receptor that pointed to the function of this orphan protein that is now known to critically control many aspects of inflammation and immune responses. Using a recombinant preparation of the CD200 protein bound to fluorescent spheres, specific binding to a receptor was demonstrated that was present only on macrophages. This antibody identified macrophages by immunostaining of mouse spleen. A recent extensive review of this field that focused on CD200–CD200R regulation of peripheral immunity highlights recent discoveries [21].

Biology of CD200 & the CD200R

The first functional characterization of CD200–CD200R interactions was demonstrated with a CD200R blocking antibody (designated OX-102). CD200R was shown to be highly glycosylated, with a DNA sequence coding for a protein with homology to CD200. CD200R resembles CD200, with both containing two IgSF domains with the typical variable and constant arrangements and a single transmembrane region, but with CD200R having a larger cytoplasmic domain than CD200 [22]. This cytoplasmic region of CD200R was subsequently shown to contain cellular signaling domains, while the CD200 cytoplasmic domain does not appear to [23]. Understanding the molecular signaling mechanisms of CD200R has been recent work; these pathways were elucidated using peripheral non-neural myeloid cells. In brief, CD200R lacks the common immunoreceptor tyrosine-based inhibitor motifs, but instead functions through a unique inhibitory pathway. This involves direct interaction of the adaptor protein Dok2 with a CD200R cytoplasmic domain, which results in binding and activation of RasGAP [24]. This process inhibits Ras activation, resulting in inhibition of other downstream inflammatory signals. Dok2 function has been shown to be essential for regulating CD200 modulation of inflammation of activated brain microglia [25]. This system, which appears to be regulated by Dok1 itself, binds to another phosphotyrosine residue on CD200R and inhibits the effects of Dok2–RasGAP inhibition of Ras signaling (Figure 1) [23,24]. These signaling mechanisms are suggested based on the findings of Mahrshahi, but have not been verified for microglia–neuron interactions [23,24].

Using the OX-102 blocking antibody in the multiple sclerosis (MS) disease model experimental allergic encephalomyelitis (EAE) in rats, it was shown that inhibiting cellular interactions resulted in significantly greater EAE disease scores and enhanced inflammation [22]. A similar functional consequence of preventing CD200–CD200R interactions was shown with a line of mice that were engineered to be deficient for CD200 [26]. These CD200-deficient mice developed constitutively greater levels of inflammation, including enhanced CD11b and CD45 immunoreactive microglia in brain. Inflammatory responses were enhanced in *CD200* knockout mice following experimental insults that included facial nerve transection and EAE [26]. Following neuronal damage as a result of facial nerve transection, an unregulated microglia response resulted in *CD200* knockout mice; and confirming the results of Wright *et al.*, using the EAE model, microglial inflammation and clinical disease was enhanced in the CD200 knockout mice [22]. In these mice, the severity of collagen-induced arthritis was also enhanced. It should be noted that this publication was the first report to adopt the nomenclature CD200 and CD200R instead of OX-2 and the

OX-2 receptor [26]. These two publications were landmark studies establishing the significance of CD200 and CD200R interactions as key regulators of inflammation in the brain. Since then, the field has grown significantly and has become specialized in studying the role of CD200 in a number of peripheral cell-mediated inflammatory and immune conditions, including arthritis [27,28], transplantation [29], viral infections [30,31], in the field of cancer [32,33] and also as a feature of neuroinflammation. CD200 is now known to be a significant player in cancer metastasis, with upregulation of CD200 being a negative factor as it promotes the evasion of cancer cells, including cancer stem cells, from the innate immune system [34].

The significance of CD200 and CD200R in brain inflammatory pathology is now widely appreciated. In comparison to cancer, enhancement of CD200 and CD200R interactions in controlling neuroinflammation has become a therapeutic strategy. The key features of the neuro-biology of these molecules are the interactions of CD200, mainly on neurons, and CD200R, primarily on microglia, but also macrophages, which are shown in Figure 1. These pathways are suggested based on the findings of Mahrshahi [23,24], but have not been verified in microglia. It is from this premise of controlling microglial inflammatory interactions with neurons that this field has developed.

Neurobiology of CD200 & the CD200R

The localization of these molecules on the surface of peripheral blood cells has been demonstrated in multiple publications employing flow cytometry techniques. In addition, immunocytochemistry for CD200 in human nervous system materials showed CD200 localization on neurons, myeloid and nonmyeloid cells, select endothelial cells and a subset of astrocytes in diseased and normal human retinas [35]; results were confirmed by flow cytometry. The authors' study of human brains showed similar findings of CD200 immunoreactivity in neurons, vessel occupying myeloid cells and a subset of astrocytes [19]. In the human brain, endothelial staining was not observable, while distinct localization in presumptive myelinated fibers was seen. To date, there have been no reports on CD200R localization in human CNS materials, probably owing to its low level of expression and absence of suitable reagents that can identify CD200R in fixed materials.

Since the initial discovery of CD200, its expression by neurons has been consistently observed [4,5,36]. There appeared to be relatively high levels of CD200 in the brain, particularly by neurons. These data suggested that CD200 may act as a protective layer for neurons by deactivating 'activated' CD200R-expressing microglia with which they come into direct contact. Is this the factor keeping microglia or perivascular macrophages in a resting, ramified or surveillance state, or at least in an alternatively activated state? In *CD200* gene-deficient mice, aggregates of activated microglia were seen in the spinal cord, even though there was no injurious stimulus [26]. This suggested that microglia activation was being actively suppressed. A number of other endogenous anti-inflammatory proteins, such as fractalkine, TREM-2, CD22 and CD47/CD172a, have been identified that seem to function similarly to CD200-CD200R by the activation of anti-inflammatory signals following ligand receptor interactions [37]. These systems appear to have complementary functions in maintaining microglia/macrophages in a resting state, or more importantly returning proinflammatory activated microglia to this state; however, the work with *CD200* knockout mice would suggest that there is not a redundancy in this system. *CD200* knockout mice, which presumably express normal levels of other inflammation regulatory systems, demonstrate higher levels of baseline inflammation and significant amplified responses to injurious stimuli or viral infection [26,31]. If there was redundancy in these control systems, one could argue that the consequences of *CD200* knockout would be compensated. Identifying which are the most important proteins is still a matter of debate, but a range of

studies that used various tools to inhibit CD200–CD200R interactions and have shown significantly enhanced inflammation in the brain and greater degrees of pathology will be discussed later in this article. By comparison, tools that activate CD200R in the absence of cellular contact have reduced neuroinflammation and tissue damage. Key findings are discussed below. This raises the question as to why this process is not fully functional to control neuro-inflammation, particularly with aging. Several key findings have revealed that CD200 levels in the brain and expression by neurons become reduced with age, and that certain proinflammatory stimuli also downregulate expression levels of CD200 and CD200R [38–40].

CD200 & the CD200R in experimental neuroinflammation

It had been consistently observed that microglial activation and inflammation become enhanced with aging in rodents [41–44]. In humans, a similar enhancement of inflammation with aging was observed, where gene-expression profiling demonstrated a significant increase with aging of many different inflammatory-related genes [45]. However, a possible role for CD200 in this chronic age-associated inflammation was not reported in this particular study [45]. Comparing the gene-expression profiles of the hippocampus from young (3 months) rats with elderly (24 months) rats demonstrated significant upregulation of inflammatory markers MHC class II, CD86, CIITA and IFN- γ , but downregulation of anti-inflammatory molecules IL-10 and CD200 [38]. These results were confirmed at the protein level in a similar study. Comparing neuronal CD200 immunoreactivity and total brain protein levels between rats aged 3–4 months (young) and 22–24 months (elderly) showed significant reduction in both features, while in these same animals, there was a significant age-associated increase in activated microglia and expression of MHC class II mRNA [39]. In this same model, intraventricular infusion of aggregated amyloid (A β) peptide (1–42) resulted in a large increase in microglial activation and secretion of proinflammatory cytokines, but a reduction in CD200 levels [39]. This observation provided a potential mechanism as to why CD200–CD200R inflammatory control may be deficient under circumstances when it is most needed. The proinflammatory stimuli could result in the induction of cytokine/factors that downregulate CD200 expression, or alternatively loss of cytokine/factors that increase or maintain its expression. The latter situation is indicated as this same study using *in vitro* cultured neurons, demonstrated that there was strong induction of CD200 by the anti-inflammatory cytokine IL-4 [39]. *In vitro* studies involving coculture of microglia with neurons showed that the presence of the neurons was required to downregulate the secretion of inflammatory cytokines by A β -activated microglia; this effect was inhibited if blocking antibody to CD200 was added to the cultures [39]. Using the same experimental model, this group had shown in a previous publication that there was an age-associated decrease in hippocampal IL-4 levels, which correlated with increased levels of IL-1 β [46]. The *IL-4* gene-deficient mouse model has proven useful for further studying the role of neuronal expression of CD200 in controlling inflammation. *IL-4* knockout mice have significantly reduced levels of neuronal CD200 compared with wild-type mice. Injection of wild-type mice with intraperitoneal doses of lipopolysaccharide (LPS) upregulated many inflammatory markers, but downregulated CD200 expression, while the *IL-4* knockout mice developed an enhanced inflammatory response to LPS injection, but did not show a further decline in CD200 levels [40]. Using the *in vitro* microglia/neuronal coculture system, neurons derived from *IL-4* knockout mice could not downregulate inflammatory cytokine expression by LPS-treated microglia, while neurons from wild-type animals were effective in this assay [40]. Enhanced neuroinflammatory responses were also seen *in vivo* and *in vitro* with glial cells derived from *CD200* gene-deficient mice [20].

Using *CD200* gene-deficient mice, enhanced and accelerated pathology, developed in the retina in response to injection of an autoimmune inducing peptide in the model of

experimental autoimmune uveoretinitis compared with CD200-expressing wild-type mice. There were constitutively higher numbers of nitric oxide synthase-expressing microglia in the retina of the knockout compared with wild-type mice [47]. A subsequent study using this experimental autoimmune uveoretinitis model showed that administration of a CD200R-activating (receptor crosslinking) antibody into *CD200* knockout mice resulted in downregulation of inflammation, reduced pathology and shorter disease duration [48].

Increased expression of neuronal CD200 is a feature of the *Wlds* mutant strain of mice [49]. This mouse strain has a triplication in the *Ube4b/Nmnat* gene that results in defects in the ubiquitin–proteasome degradation system. This mouse strain demonstrated significantly reduced severity of EAE compared with wild-type mice owing to the enhanced levels of CD200. *Wlds* mice maintained higher levels of CD200 in spinal cord lysates of EAE-treated mice, owing to reduced ubiquitination of CD200 that resulted in reduced proteasomal degradation. In this model, immunostaining demonstrated increased neuronal, astrocytic and oligodendrocytic immunoreactivity for CD200 and appears to be the only study to demonstrate astrocyte and oligodendrocyte, along with microglial, immunoreactivity for CD200R [49].

Measurement of CD200 and CD200R levels was made in the hippocampus using a rodent model of surgical trauma to induce postoperative cognitive dysfunction. The animals were subjected to partial hepatectomy as the surgical procedure. Results showed a significant early decrease in CD200 levels at day 1, but levels were restored to normal levels by day 3. There were no changes in CD200R levels. In this model, there were increased levels of IL-1, IL-6 and TNF- α at days 1 and 3 after surgery, along with significant cognitive dysfunction. This model also demonstrated a significantly greater increase for these proinflammatory cytokines in aged rats (20–24 months), and a significantly greater decrease in CD200 levels compared with treated younger rats (3–6 months) [50].

CD200 knockout mice showed impaired long-term potentiation owing to enhanced levels of TNF- α . Glial cells derived from knockout mice responded more strongly to ligands for TLR-2 and TLR-4 than cells derived from wild-type animals [20]. This study, for the first time, provided evidence for a role for astrocyte expression of CD200 in controlling microglia inflammation. Astrocyte expression of CD200 has been identified in both animal and human studies, but it appears to be only a subset of these cells showing immunoreactivity. As astrocytes are present in greater numbers than neurons, further investigations of the significance of astrocyte CD200 are required.

The role of CD200–CD200R interaction was studied in a rodent model of Parkinson's disease (PD). Rats were injected with 6-hydroxydopamine to induce loss of substantia nigra (SN) neurons leading to parkinsonism-like movement behavior. One group of 6-hydroxydopamine-treated rats had been infused with a CD200R-blocking antibody into the striatum 1 day prior to lesioning, while another group was treated with a control antibody. Blocking of CD200R resulted in significantly more severe PD-like movement dysfunction and a significantly greater loss of tyrosine hydroxylase-positive dopaminergic neurons in SN [51]. CD200R blocking antibody-treated rats showed an approximately fourfold greater number of activated microglia compared with control-treated animals, combined with three- to four-fold higher levels of TNF- α and IL-6. These results provide data justifying further investigations whether manipulating CD200R-expressing microglia in human SN, where in PD there is a strong inflammatory response, may be able to slow the rate of loss of the crucial dopamine-producing neurons. Similar enhanced inflammatory effects following the blocking of CD200R with an antibody were seen in mice using the EAE model of MS. CD200R blockade resulted in a more severe clinical course of EAE and greater numbers of infiltrating T lymphocytes and activated macrophages in the spinal cord [52]. Two

experimental rodent studies, one using a model of ischemia/ stroke to induce brain injury [53], and the other using kainic acid administration into the hippocampus to induce excitotoxic neurodegeneration [54], identified transient expression by microglia or macrophages of CD200 in the immediate periods after lesioning. The significance of this is unclear at present. The latter study also observed that treatment of microglia cultured from aged rodents with IL-4 also resulted in the induction of CD200 [54].

Considering the divergent nature of each of these experimental animal models and the neurological insults used, it can be seen from these studies that manipulating CD200–CD200R interactions has significant involvement and common effects on modifying neuropathological consequences.

CD200 therapeutics & neuroinflammation

The therapeutic potential of manipulating CD200–CD200 interactions to modulate neuroinflammation has been demonstrated using a soluble form of CD200 recombinant protein (CD200-Fc) [55]. Injection of this protein into the hippocampus of aged or LPS-treated rats resulted in the reduced expression of microglia inflammatory markers MHC class II and CD40, and attenuated the deficit in inflammation-associated long-term potentiation. A related study demonstrated that activating CD200R with this protein in rat brains responding to the inflammatory effects of A β administration significantly reduced the expression of inflammatory proteins IL-1 β , TNF- α , CD68 and CD40 [25]. Using a similar experimental rodent model, rats that had been treated with methamphetamine and administered soluble CD200-Fc to activate CD200R showed reduced numbers of activated microglia and reduced loss of striatal dopamine compared with rats treated with methamphetamine alone. These data also confirm that dopaminergic neuronal toxins have a mechanism of action, including the induction of inflammation and microglial activation [56]. Preventing this inflammation was protective to this vulnerable population of neurons. The protective effects of CD200-Fc were also demonstrated in methamphetamine-treated neuron–microglia cocultures. A recent study employing the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model to induce lesions in the SN and PD symptoms in mice showed a significant increase in SN microglial activation markers, along with the loss of tyrosine hydroxylase-expressing neurons in lesioned animals, and also significant decreases in CD200 and CD200R levels in the ventral midbrain. As a treatment strategy, the 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine/probenecid-treated mice were subjected to a 4-week period of treadmill exercise after the last lesioning injection [56]. These animals demonstrated a significant improvement in clinical tests compared with the control lesioned group. In this group as well, as a result of the exercise, the loss of tyrosine hydroxylase neurons was reduced, the degree of microglial activation was reduced and there were significantly increased levels of CD200 and CD200R [57]. Although this study did not investigate whether the exercise-induced increased CD200 and CD200R was responsible for the reduced inflammation and neuroprotection, it illustrated a novel way for manipulating levels of CD200 and CD200R that may have significant translational potential. In this study, they showed that exercise treatment alone, without the neurotoxic lesion, also significantly increased CD200 and CD200R levels in mice brains [57].

Neurobiology of CD200 & the CD200R in humans

This article has discussed how manipulating CD200 and CD200R in various experimental animal models can affect disease pathology. However, the literature on CD200 and the CD200R in the human brain is extremely limited at present, and there are now indications that these genes are regulated differently in humans compared with rodents [19,58]. The types of experimental manipulations of CD200–CD200R interactions that have been

performed on animals can obviously not be carried out on humans, so there is a need to further consider the similarities and differences in CD200 and CD200R-expression patterns between animals and humans, particularly in the brain, before human therapeutics are attempted. Initial characterization of human CD200 localization showed a similar distribution of cell surface expression by neurons and vascular endothelial cells, as well as B and T lymphocytes [36].

Expression of functional CD200R in human retinal microglia was demonstrated; these cells isolated from retinal explants, responded to treatment with a CD200 soluble ligand by modulating their migratory and proinflammatory response [59]. Studies of CD200 and the CD200R in human brains in relation to MS [60,61] and Alzheimer's disease (AD) [19] have been reported. Micro-dissecting areas of MS plaques, both from chronic active lesions and inactive lesions, followed by gene-expression analysis demonstrated decreased CD200 mRNA expression in the rim and center of MS lesions, as well as in adjacent normal white matter compared with matched controls. By comparison, CD200R mRNA expression was not significantly changed [61]. A follow-up study demonstrated that CD200 immunoreactivity could be detected in neurons and oligodendrocytes in human brains by immunostaining, although there was also considerable diffuse CD200 immunoreactivity in neuropil [60]. Interestingly, CD200R immunoreactivity could only be detected in perivascular or infiltrating macrophages, not in microglia, in control and MS brains. Flow cytometry of freshly isolated microglia from brain tissue demonstrated very low levels of CD200R expression. An additional study also showed CD200 protein in neurons in human MS brains, but could only show CD200R in macrophages, not microglia [52]. The authors carried out similar studies focusing on AD, but also with the aim of characterizing CD200 and CD200R expression in elderly human brains [19]. Using a custom antibody specific for the cytoplasmic domain of CD200, the authors showed distinct staining of neurons in the cortex and hippocampus, but with a heterogeneous distribution, as many neurons in elderly brains did not show CD200 immunoreactivity. There was also strong CD200 staining of myelinated white matter areas. Small subsets of highly activated astrocytes were immunoreactive for CD200, particularly in AD brains in astrocytes associated with A plaques. Biochemical measurements of AD and age-matched control brains demonstrated a significant decrease in expression of CD200 and CD200R mRNA in AD brains in the hippocampus and temporal cortex, brain regions affected by AD pathology, but not in the cerebellum, an AD-spared region. The decrease in both measures correlated with the severity of AD pathology. The authors also demonstrated a reduction of CD200 protein in the AD hippocampus and cortex, but not the cerebellum. Similar to results presented by Koning *et al.*, the authors were unable to show microglia staining for CD200R in human brains [60]. *In vitro* studies using isolated microglia from human brains demonstrated low levels of expression of CD200R mRNA and protein compared with CD200R levels in blood-isolated macrophages [19]. Of significance from these *in vitro* studies was the demonstration that CD200R expression in microglia and macrophages was highly induced if cells were treated with the antiinflammatory cytokines IL-4 or IL-13. Interestingly, expression of these cytokines was undetectable in most of the human brain samples that the authors tested [19]. As discussed earlier, it had been demonstrated that IL-4 levels decreased with age in rats [46]; a similar situation may be occurring in the aged and pathologically affected human brain.

Indication of differences in the regulation of CD200 and CD200R between humans and rodents can be seen. The authors' findings on IL-4 and IL-13 effects on CD200R mRNA expression by human microglia and macrophages were confirmed at the mRNA and protein levels in human macrophages and other white blood cell types, and were extended by demonstrating that these cytokines have no effect on inducing CD200R expression on rodent macrophages [58]. The specific induction of the CD200R by IL-4 and IL-13 identifies it as a

marker for alternative activation in human myeloid cells [58,62]. Increasing the alternative activation phenotype in microglia using gene vector induction of IL-4 in transgenic mouse models promoted their phagocytosis of the A β peptide, and has been shown to be neuroprotective by downregulating expression of proinflammatory cytokines [63]. Although it had been shown that IL-4 induces neuronal expression of CD200 in rodent neurons in culture [39], the authors could not observe this using IL-4-treated cultured human neurons [WALKER DG *ET AL.*, UNPUBLISHED DATA].

Changes in CD200 and CD200R interactions ongoing in PD may be occurring in immune cells of the peripheral circulation. Monocyte-derived macrophages (MDMs) from young and old controls were compared with those from PD patients for changes in CD200R expression [64]. Although constitutive CD200R levels were the same between all of the groups, MDMs from PD cases had impaired induction of CD200R expression in response to different inflammatory stimuli. In addition, levels of TNF- α secretion negatively correlated with induced levels of CD200R in MDMs from young controls, while this correlation was positive in MDMs from PD cases. This suggested a potential loss-of-function for CD200R in regulating inflammation in PD.

Therapeutic potential for CD200–CD200R interactions in human brain disease

There is a need for further studies to define the cellular components expressing CD200 and CD200R in the human aged and diseased brains, as some important differences have become apparent compared with experimental rodent models. The animal studies have suggested that a decrease in levels of CD200 due to age or inflammatory manipulation is the central reason why CD200–CD200R interactions are not functional in downregulating microglia inflammation in the human brain. Supplementing cell-expressed CD200 with soluble recombinant CD200 protein has been effective in reducing inflammation in certain animal models, but the central question is whether there is actually a functional deficit of CD200 on neurons and other cell types in the human brain. Both *CD200* and *IL-4* gene-deficient mice have artificially low levels of CD200 in the brain; a feature that one would not expect to find in any human condition. Therefore, supplementing CD200 in these models would expect to be effective in activating CD200R, but do they point to the same rationale for human diseases? However, it should be added that supplementing CD200 in aged or treated normal animals was also effective. Even though the authors showed a significant decrease in CD200 levels between AD and control brain samples, overall this protein is still relatively abundant in human brain. CD200 levels in the cingulate and temporal cortices of aged human brains ranged from 25 to 50 ng CD200/mg total brain protein [WALKER DG *ET AL.* CHANGES IN CD200 AND INTERCELLULAR ADHESION MOLECULE-1 (ICAM4) IN BRAINS OF LEWY BODY DISORDER CASES ARE ASSOCIATED WITH ALZHEIMER'S DISEASE PATHOLOGY (2013), MANUSCRIPT IN PREPARATION]. Therefore, what the authors can suggest from their results that have been indicated by the findings of others, is that in human brains there may be a deficit of CD200R as well as CD200. In AD brains, microglia are primarily in a proinflammatory classical activated state, rather than in an alternative activated state, and a therapeutic strategy for humans may require enhancing CD200R expression through promoting alternative activation. At present, it is not known how much CD200 expression is sufficient to activate CD200R, but as the level of expression of CD200R in the human brain appears to be several orders of magnitude lower than that of CD200, boosting CD200R may be more effective. There have been no convincing reports showing microglial expression of CD200R in human brains and limited results from animal studies. As its expression can be detected on perivascular and infiltrating macrophages in human brains, these results suggest the failure to detect CD200R in human brain microglia is due to its absence and not due to technical reasons. In experimental *in vitro* studies, the

effectiveness of the suppression of inflammatory responses by CD200 was proportional to the levels of cellular CD200R expression [65].

Conclusion

The neurobiology of CD200 and CD200Rs is a relatively unexplored field, with many additional studies that can be proposed. This applies, in particular, to studies of CD200 and CD200R in human brains and human neural-derived cells. A seminal series of studies, particularly by the group of Lynch, have elucidated many aspects of the neurobiology of CD200 in rodent disease models [20,25,39,40,55]. These data clearly provide rationale for further investigations of the manipulation of CD200 and the CD200R in human neurological diseases. We have shown that there is a decrease in both components in the human brain enhanced by AD pathology, but whether CD200R signaling is deficient in this disease remains to be proven. Owing to the potential differences between rodents and humans in the expression of CD200 and CD200R, the development of 'humanized' transgenic mice that express the human genes under their native promoters appears to be a requirement for further investigation of CD200 therapeutics. As most of the experimental studies have involved the surgical introduction of CD200 protein or CD200R-blocking antibodies, an approach that cannot be used in human therapy, development of suitable gene therapy vectors that can be targeted for neural cells is also indicated.

Although this is still a new field of study, the significance of CD200 and CD200R in many different human conditions has been definitively established. Critical mechanistic studies have been carried out using animal models of human disease, but there are many more that need to be performed, particularly those that can model the species differences in gene regulation between humans and rodents. These models are crucial for the field, as they can be used to study the outcomes of CD200 and CD200R interactions, which have similar anti-inflammatory effects. Overall, there is a strong indication that this system could be a target for therapeutic enhancement in human neurological disease.

Future perspective

The modern era of neuroinflammatory studies in human aging brain diseases is now over 25 years old. The hope from these studies was that a regular anti-inflammatory drug could cure diseases such as AD, PD or MS; however, this has proven to be unattainable. Understanding inflammation in the diseased human brain has been complex and shown not to be easily treatable. Instead of trying to stop all forms of inflammation in the human brain with a drug, the aim now should be to manipulate this process in a more discrete manner. As with most systems, there are many endogenous cellular proteins with the function to control aspects of unwanted inflammation. CD200 and the CD200R are now known to be key players in this process, including in inflammation in the specialized environment of the CNS. A range of animal studies have shown the beneficial effects of augmenting CD200 levels to activate CD200R through direct injection of therapeutic proteins into the brain; however, this approach has limited use for treating human diseases. Future functional studies of CD200 and CD200R may require the use of gene therapy with vectors that can target expression of these proteins to regions of disease-affected brains. The use of viral gene therapy for *IL-4* to manipulate microglia and/or neuronal expression of these proteins is also achievable. As it appears that rodents and humans regulate the expression of these genes differently, there is also a critical need for the preparation of humanized transgenic mice engineered to express human CD200 or CD200R under their native human gene promoters. Since the outcomes of CD200–CD200R signaling appear similar between humans and rodents, with these tools, therapy aimed at human CD200–CD200R interactions in the brain can be more definitively investigated. Although augmenting CD200 expression needs to be approached carefully, as

there might be the potential to increase the cancerous nature of other cell types, the significant neuroprotection shown in animal models indicates that this pathway should be further examined for human neurodegenerative diseases, all of which have significant inflammatory components. Based on recent publications concerning soluble forms of CD200 and their use as plasma biomarkers in systemic lupus erythematosus and transplantation, this area also needs to be re-examined in brain diseases, as cerebrospinal fluid or plasma levels of CD200 may be reflective of ongoing inflammatory changes. Cerebrospinal fluid, in particular, represents a clean biofluid that could be a reservoir for released CD200 from neurons. This is a focus of our ongoing studies with human materials. With studies on CD200 and CD200R expression in human brains with AD and MS, detailed studies of affected regions in Parkinson's disease are also a goal of the authors' laboratory.

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Executive summary

Identification of CD200 & the CD200 receptor

- CD200 was identified as an 'orphan' protein (referred to as OX-2) that was localized to the cell surface of neurons, endothelial cells and lymphocytes, among other cell types.
- CD200 is abundantly expressed in the brain.
- There is a high degree of homology between mouse, rat and human CD200 genes, suggesting a conservation of function.
- CD200 receptor (CD200R) expression by macrophages was first identified functionally by their adherence of recombinant CD200.
- A number of different cell types of myeloid origin, including brain microglia, express CD200R.
- CD200–CD200R interactions have significant roles in modulated cell-mediated immunity with significant lymphocytic involvement.

Neurobiology of CD200 & the CD200R

- CD200 as a ligand binds to CD200R through N-terminal amino acid-specific sequences, resulting in the activation of anti-inflammatory signaling by inflammatory CD200R-expressing cells.

CD200 & the CD200R in experimental neuroinflammation

- In rodent brains, CD200 expression decreases with age or inflammatory stimulation.
- In rodents, neuronal expression of CD200 is increased by the cytokine IL-4.
- Blocking CD200R in the brain results in enhanced neuropathology.

CD200 therapeutics & neuroinflammation

- Activating CD200R through injection of CD200 into the rodent hippocampus reduced neuroinflammation.

Neurobiology of CD200 & the CD200R in humans

- There are reduced levels of CD200 and CD200R in human brains affected by Alzheimer's disease.
- There is a very low expression of CD200R by microglia isolated from human brains.
- Expression of CD200R by human macrophages and microglia is increased by IL-4 and IL-13.

Therapeutic potential for CD200-CD200R interactions in human brain disease

- The major question to be answered is whether the decrease in CD200 expression in aged human brains makes CD200–CD200R interactions deficient or whether the focus should be to identify therapeutic agents that can increase CD200R expression in microglia.

Conclusion

- The significance of CD200 and the CD200R in immune regulation and tissue homeostasis is becoming more widely understood with a large increase in publications in recent years.
- As studies of CD200 and CD200Rs in human brains are extremely limited compared with those in animals, there is a critical need for further studies on their biochemical and histological properties, including understanding how these proteins are regulated in human neural cells.
- Owing to species differences in the regulation of CD200 and CD200Rs, there is a critical need for humanized transgenic mice for these genes before human therapeutics can be considered.

Figure 1. Presumptive mechanism of CD200–CD200 receptor interactions between neurons and microglia

The binding of neuronal CD200 to microglial CD200R through N-terminal amino acid sequences results in the activation of microglial CD200R through cell–cell contact. The primary mechanism of action involves activation of the adaptor proteins Dok2 and RasGAP, leading to the inhibition of Ras activation with multiple downstream effects that result in increase of multiple anti-inflammatory signals. Control of this system is mediated by the inhibition of RasGAP activation due to the activation of Dok2 and CrkL. CD200R: CD200 receptor; pY: Phosphotyrosine. Based on the findings from [23,24].