Chapter

Metabotropic Receptors 4 and the Immune Responses

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Abstract

Neurotransmitters (NTs) have recently received increasing appreciation as important immune modulators. The immune cells express receptors for many classes of NTs and the communication between NTs and their receptors establish neuro-immune interactions for regulating effective immune response in both central nervous system (CNS) and peripheral tissues. Metabotropic Glutamate Receptor 4 (mGluR4) is expressed at high level in CNS and plays a role in various physiological and pathophysiological processes in CNS. Recently, mGluR4 has been reported to be expressed on immune cells and have an impact on regulating the immune system. This chapter summarized the works associated with the immunogenic function of mGluR4 and its potential underlying mechanisms.

Keywords: metabotropic glutamate receptor (mGluR4), immune response, peripheral tissues, central nervous system (CNS), cancer, autoimmune diseases

1. Introduction

Neurotransmitters (NTs) have recently received increasing appreciation as important immune modulators. The immune cells express receptors for many classes of NTs and the communication between NTs and their receptors establish neuro-immune interactions for regulating effective immune response in both CNS and peripheral tissues [1]. Interestingly, the role of NTs is very complicated and the same NTs can even exert opposing effects for promoting or inhibiting tissue immunity in different contexts [2–6].

Studies of the NTs and their receptors in modulating immunity are limited and therein are important areas of investigations. L-Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian CNS [7]. It acts via two classes of receptors, ligand gated ion channels (ionotropic receptors (iGluRs))-regulating rapid responses upon activation, and G-protein coupled (metabotropic) receptors-modulating signal transduction cascades. Eight different types of mGluRs, mGluR1 to mGluR8 are divided into groups I, II, and III on the basis of their intracellular signal transduction mechanisms, agonist pharmacology, and sequence homologies (see **Figure 1**) [8]. Group I includes mGluR1 and mGluR5, coupled to Gq protein; group II includes mGluR2 and mGluR3, coupled to Gi and Go proteins; group III includes mGluR4, mGluR6, mGluR7 and mGluR8, coupled to Gi and Go proteins in heterologous expression systems.

mGluR4 is expressed at high levels in CNS and plays a role in various physiological and pathophysiological processes in CNS [9, 10], such as learning, memory, and cognitive impairment. In addition, growing evidence indicates that mGluRs

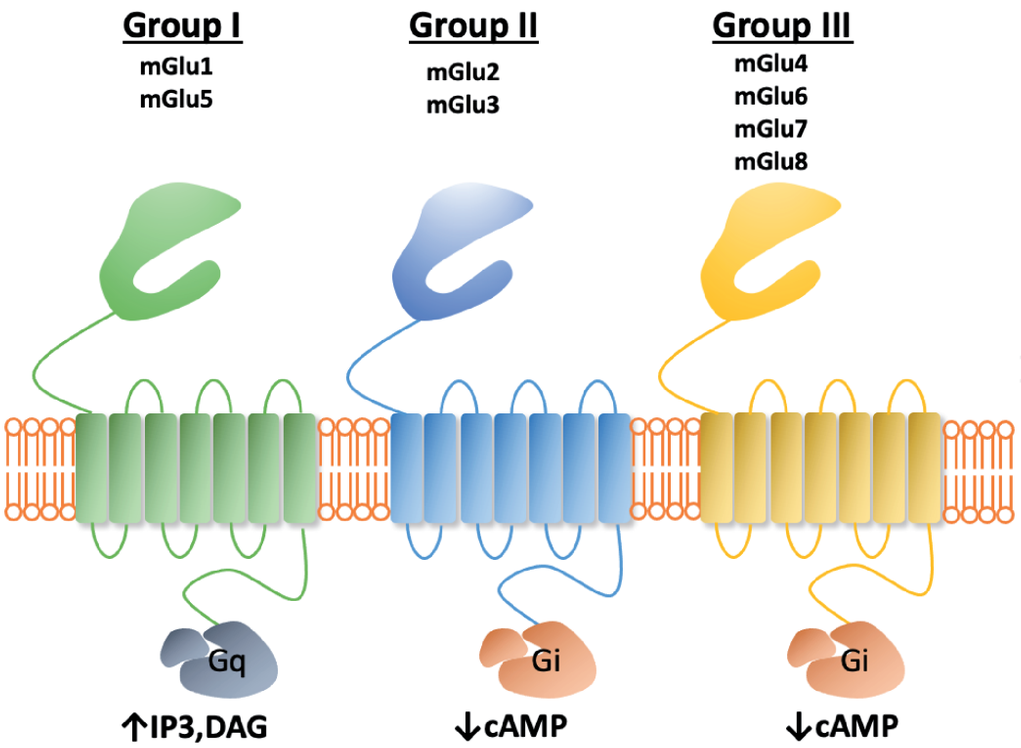

Figure 1.
The summary of mGluRs families. mGluRs are classified into three families: group I, group II, and group III. In the CNS, activation of mGluRs from group I leads to the induction of phosphoinositide hydrolysis with formation of inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). The activation of groups II and III receptors induce a decrease on the intracellular levels of cyclic adenosine monophosphate (cAMP) [7].

are expressed in the peripheral such as thymus and lymphocytes [11]. These results suggest a potential role of mGluR4 in immune regulation. In this chapter, we summarized the association of mGluR4 with immune responses and its role in different diseases. The potential of mGluR4 as a novel therapeutic target in immune-related diseases was also discussed.

2. Expression of mGluR4 on immune cells

Clinical data indicated that elevated plasma concentrations of Glu are associated with immune deficiency [12, 13]. In addition, in vitro assays showed that high concentration of Glu (>100 uM) can inhibit mitogen-induced T-cell proliferation [12, 13]. Therefore, it is not surprising that immune cells express mGluRs. It has been proposed that mGluRs can mediate an emergency mechanism once high levels of Glu are reached.

Using immunostaining and Western blot analysis, Rezzani et al. observed the expression of mGluR4 in rat thymic cells [14]. The expression of mGluR4 was abundant in dendritic cells (DCs) and lymphocytes of the thymic medulla but was weak in lymphocytes of the cortex. It is interesting to note that a rapid inhibition on the expression of mGluR4 was induced in the rat thymus after treatment with cyclosporine (an immunosuppressant). The mGluR4 expression reached undetectable levels after a longer treatment regimen of cyclosporine.

Other evidence also pointed out that the expression of mGluRs is not exclusive to young immune cells because mature lymphocytes are activated by selective mGluRs ligands. In addition, rat peripheral lymphocytes responded by producing reactive oxygen species (ROS) when they were exposed to the group III mGluRs

agonist L-2-amino-4-phosphonobutyric acid (L-AP4) [15]. ROS play important roles in T-cell biology and participate in activation-induced T cell apoptosis and hence in the termination of the immune response [16]. Moreover, DCs are capable of secreting glutamate when interacting with T lymphocytes, a process that might be essential for the function of lymphocyte. This hypothesis is based on the fact that the absence of glutamate led to impaired Th1 (Interleukin-2 (IL-2) and interferon-γ) and proinflammatory (IL-6 and tumor necrosis factor-alpha) cytokine production. However, these changes were not correlated with a decrease in T-cell proliferation.

3. mGluR4 and Autoimmunity in CNS

A role of mGluR4 in immune modulation was first described in an autoimmune disease model [17]. Fallarino et al. [17] reported that mGlu4 knockout mice ($Grm4^{-/-}$) were highly susceptible to experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. More specifically, $Grm4^{-/-}$ mice and their wild-type (WT) counterparts were immunized with myelin oligodendrocyte glycoprotein (MOG35–55), which can induce EAE in C57BL/6 mice. The EAE clinical scores were recorded periodically and a lack of mGluR4 was found to be associated with earlier onset, more severe, and ultimately fatal EAE in >40% of the hosts. Along with these changes, white matter demyelination and inflammatory infiltrates were more prevalent in the spinal cord of MOG-vaccinated $Grm4^{-/-}$ mice in comparison to their WT counterparts, according to the morphological changes. The phenotype has also been characterized in littermates as well (heterozygote breeding—with cohorts of mice being matched for gender and age) and the disease indications were also more severe in $Grm4^{-/-}$ and $Grm4^{+/-}$ mice than in WT mice. In contrast, treatment of N-Phenyl-7-(hydroxyimino) cyclopropa [b] chromen-1a-carboxamide (PHCCC), an Grm4-positive allosteric modulator led to increased resistance to EAE. This was in agreement with previous reports demonstrating that long-term treatment of L-AP4 can increase the recovery rate from EAE in Lewis rats [17, 18].

There was significant infiltration of $CD4^+$ T cells, $CD8^+$ T cells and $B220^+$ B cells in both peripheral lymphoid organs and the CNS in both $Grm4^{-/-}$ and WT mice, but the percentages of $CD4^+$ and $CD8^+$ T cells as well as $CD11b^+$ and $CD11c^+$ cells were significantly higher in the CNS of $Grm4^{-/-}$ mice at the peak of disease [17]. Extended studies using littermates from heterozygote breeding further showed that the disease course was more severe in $Grm4^{-/-}$ and $Grm4^{+/-}$ mice than in WT mice. The cytokine profiling of sorted $CD4^+$ T cells from brain-infiltrating leukocytes (BILs) and pooled lymph nodes demonstrated a significant increase in *Rorc* transcripts (encoding the T_H17 specification factor), a reduction in *Foxp3* (Treg) transcripts, and no change in Tbx21 (coding for Tbet; a T_H1 maker) in $Grm4^{-/-}$ mice during the neurologic signs. No changes were observed in *Gata3* (a T_H2 marker) in both groups. These data suggested that $Grm4^{-/-}$ tipped the balance of transcriptional activation in favor of inflammatory genes in response to MOG vaccination. In particular, $Grm4^{-/-}$ favored the emergence of T_H17 over Treg cells, which would sustain inflammation and exacerbate EAE [18].

Expression of mGluR4 was confirmed in several immune subpopulations, such as $CD4^+$ T cells, $CD8^+$ T cells, γδ T cells, $B220^+$ B cells, $CD11b^+$ and $CD11c^+$ cells, particularly in $CD4^+$ T and $CD11c^+$ cells, suggesting those cells as potential targets for Grm4 mediated effects. The expression of mGluR4 was also confirmed in DC subsets in splenocytes, including conventional DCs (cDCs; $CD11b^+CD11c^{high}$) and plasmacytoid DCs (pDCs; $mPDCA1^+$ $CD11c^{low}$). They have further shown that

treatment with toll-like receptor ligands such as lipopolysaccharide (LPS) and CpG-oligonucleotide (CpG-ODN) led to increased Grm4 expression. Modulation of mGluR4 expression in activated nTreg cells ($CD4^+$ $CD25^+$) and LPS-stimulated cDCs was also confirmed, further supporting that mGluR4 activation within an immunologic synapse contributes to the crosstalk and reciprocal influence between T and accessory cells [17].

IL-17-producing T helper (Th17) cells are considered mediators of autoimmunity in multiple sclerosis and EAE. The accumulation of Th17 cells in the CNS as well as in the periphery is also associated with the development of demyelinating plaques of multiple sclerosis [19]. Fallarino et al. [17] also pointed out that the absence of mGlu4 in dendritic cells is key to inducing a differentiation of T helper cells toward the Th17 phenotype. More specifically, possible regulatory function of mGluR4 in the interaction between $CD4^+$ T cells and DCs has been examined. Both cDCs and pDCs from $Grm4^{-/-}$ mice produced higher amounts of IL-6 and IL-23, but less IL-12 and IL-27, compared to their WT counterparts in response to LPS or CpG-ODN, respectively [17].

The notable results of coculturing of WT $CD4^+$ T cell and $Grm4^{-/-}$ DCs demonstrated an increase of IL-$17A^+$ $CD4^+$ T cells, along with a significantly reduction of IFN-γ producing $CD4^+$ T cells (a portion of which also expressed IL-17A). However, they failed to see this effect when the coculture consisted of WT DCs and $Grm4^{-/-}$ T cells, suggesting that the effect of mGluR4 depletion was largely dependent on DCs in this *in vitro* system. The cytokine production in culture supernatants has been examined and there are decreased amounts of T_H1-associated IL-2 in coculture system involving $Grm4^{-/-}$ cDCs. IL-27 is known to counter the effect of IL-6 in directing TH17 cell development, which can limit the EAE progression. The decrease in IL-27 during activation of naïve $CD4^+$ T cells might be another reason for favoring the emergence of Th17 cells [17].

They also suggest that activation of mGlu4 (as a result of elevated levels of glutamate during the neuroinflammation) might exert a protective effect by preventing the unbalance in T helper cells. Such mechanism presents a clear therapeutic potential for treating autoimmune related disorders.

The underlying mechanism for Grm4-mediated immune regulation is not clear at present. However, there appears to be a cross-talk and reciprocal influences between Grm4 and indoleamine 2,3-dioxygenase 1 (IDO-1) pathways [20]. IDO1 has been well known to be involved in generating an immunosuppressive environment through catalyzing the metabolism of tryptophan, resulting in tryptophan depletion and accumulation of kynurenine [21]. A protective role of IDO-1 has been shown in mice with different forms of EAE including acute, relapsing–remitting, and adoptively transferred disease [22]. Interestingly, in addition to the direct immunosuppressive effect of kynurenine through inhibition of $CD8^+$ T cells and activation of Treg cells, kynurenine metabolites such as cinnabarinic acid (CA) act as selective, although weak, orthosteric agonists of mGluR4 [23]. The therapeutic effect of CA in acute EAE was attenuated in $Grm4^{-/-}$ mice [24]. On the other hand, activation of Grm4 could positively impact the IDO1 pathway. Treatment of DCs with ADX88178, a positive allosteric modulator (PAM) of Grm4, led to both increased expression levels of IDO-1 and phosphorylation of IDO-1 [20]. These effects require a Gi-independent, alternative signaling pathway that involves phosphatidylinositol-3-kinase (PI3K), Src kinase, and the signaling activity of IDO1. Moreover, the effect of ADX88178 on the expression of several cytokines was impaired in $IDO1^{-/-}$ DCs [20]. Therefore, Grm4 and IDO1 constitute a loop that provides a positive feedback mechanism to amplify the immune-protective effect in EAE and possibly other immune-related diseases [20].

4. mGluR4 and cancers

Most studies on the role of glutamate receptor in cancers have been focused on iGluRs [25, 26]. Tumor cells originated from neuronal tissues express iGluRs subunits and iGluRs antagonists have shown inhibitory effect on the proliferation of the tumor cells. Similarly, iGluRs subunits have been shown to be expressed in several peripheral cancers and blockade of the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) inotropic glutamate receptor subtypes leads to decreased cell proliferation and migration [26].

mGluRs are also expressed in several cell lines derived from human tumors, including neuroblastoma, thyroid carcinoma, rhabdomyosarcoma/medulloblastoma, lung carcinoma, multiple myeloma, glioma, colon adenocarcinoma, astrocytoma, T cell leukemia, and breast carcinoma [27]. In particular, mGluR1 has been shown to be expressed in subsets of human melanomas [28]. Ectopic expression of mGluR1 in melanocytes drives the development of melanoma in mouse models. Pharmacological inhibition of mGluR1 led to inhibition of tumor cell growth both in vitro and in vivo [28]. Riluzole, an antagonist of mGluR1 signaling has advanced to phase II clinical trial in patients with advanced melanoma [29, 30].

The studies on the roles of mGluR4 in cancers are very limited and controversial. Change et al. studied the expression pattern of mGluR4 in several healthy and diseases-derived human tissues [31]. mGluR4 receptor expression was identified in 68% of colorectal carcinomas, 50% of laryngeal carcinoma, and 46% breast carcinomas. In the case of colorectal carcinoma, overexpression of mGluR4 was correlated with poor prognosis, and cell lines derived from human colorectal carcinomas showed increased cell invasiveness when treated with L-AP4. In another study, comparative proteomics was used to characterize a human colon cancer cell line that was resistant to 5-fluororacil (5-FU, a common chemotherapy agent). Interestingly, 5-FU resistant cells were found to overexpress mGluR4 in comparison with parental cancer cells. It has been demonstrated that cell survival was increased by the group III mGlu receptor agonist L-AP4 in the nonresistant parent cancer cells; conversely, survival was synergically decreased by 5-FU and the group III receptor antagonist MAP4 in 5-FU-resistant cells. It is noteworthy to mention that 5-FU downregulated mGluR4 expression, and MAP4 has a dose dependent cytotoxic effect in both cell lines [32].

In contrast to the above reports, mGluR4 agonists are shown to inhibit the proliferation of human breast and bladder cancer cells in a GRM4-depenedt manner [33]. In the study by Lasek et al., the expression of mGlu4 was shown to be inversely correlated with the severity of human medulloblastoma [34]. After scoring the extent of immunoreactivity for mGlu4 in human biopsies of medulloblastoma, the absence of spinal metastases, cerebrospinal fluid spread, and tumor recurrence as well as the survival of patients were all shown to be associated with high levels of mGlu4 immunoreactivity. Treatment with PHCCC (which is considered a group I mGlu receptor antagonist but can also act as a positive allosteric modulator of mGlu4 receptor) reduced the proliferation of cultured medulloblastoma cells and inhibited the growth of medulloblastoma implants in mice. In addition, subcutaneous or intracranial injections of PHCCC during the first week of life reduced the incidence of medulloblastoma from 85 to 28% in a mutant mouse model known to develop the disease upon X-ray irradiation. This indicates that activation of mGlu4 receptors also affects early events in tumorigenesis [35].

The above studies focus on the role of tumor cell-derived Grm4. It has been reported that the plasma levels of Glu are generally elevated in patients with carcinoma and seem to correlate with an impairment in immune function [36]. However,

the role of immune cell-derived mGluR including mGluR4 has hardly been studied. Kansara et al. reported that $Grm4^{-/-}$ mice showed accelerated radiation-induced tumor development in an irradiation-induced osteosarcoma model [37]. Outside the CNS, mGluR4 is highly expressed by DCs, as well as $CD4^+$ T cells [17]. In the mouse osteosarcomas, they found that mGluR4 is predominantly expressed by $CD45^+CD11c^+MHC^+$ myeloid cells within the tumor microenvironment (TME) instead of tumor cells. Few $CD4^+$ T cells were detectable to characterize mGluR4 expression. In consistent with the study by Fallarino et al. [17] in an EAE model, $Grm4^{-/-}$ DCs isolated from the tumors showed increased expression of IL-23. Interestingly, high expression of IL-23 has been observed in primary osteosarcomas and allografted cell lines relative to normal bone, while ex-vivo cultured osteosarcoma cell lines and primary tumor cells did not express IL23. A role of IL-23 in tumorigenesis has been well established from previous studies [38]. Indeed, $IL23^{-/-}$ mice were resistant to the irradiation-induced osteosarcoma. They hypothesized that knockout of Grm4 in DCs facilitates the oncogenesis of osteosarcoma through increased production of IL-23 [37].

We have recently shown in three murine syngeneic tumor models (B16, MC38, and 3LL) that either genetic knockout ($Grm4^{-/-}$) or pharmacological inhibition led to significant delay in tumor growth (Wan et al., unpublished data). Mechanistically, perturbation of GRM4 resulted in a strong anti-tumor immunity by promoting nature killer (NK), $CD4^+$ and $CD8^+$ T cells toward an activated, proliferative, and functional phenotype. We have further shown that the antitumor activity of Grm4 antagonists can be further improved through combination with anti-PD-1 antibody. The differing role of Grm4 in different tumor models may reflect the complex functions of Grm4 in different tumor environment. More studies are needed to further define the roles of immune cells-derived Grm4 and its potential as a novel therapeutic target for cancer immunotherapy.

5. Conclusions

Although the neurological function of GRM4 in CNS has been well established, its role in modulating immune response just began to be appreciated. GRM4 is expressed in various immune cells and loss of GRM4 function in immune cells led to sensitization to EAE. GRM4 selective agonists may hold potential as a novel therapy for autoimmune disorders of CNS. GRM4 is also expressed in various cancer cells, however, conflicting results have been reported regarding whether GRM4 promotes or inhibits tumor cell proliferation. The role of immune cells-derived GRM4 in antitumor immunity is also controversial and may reflect the complex function of GRM4 in different tumor microenvironment. Further studies using more defined animal models and selective GRM4 modulators may not only advance our understanding of the complex immunobiology of GRM4 but also lead to the development of a new immunotherapy for the treatment of cancer.

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Conflict of interest

The authors declare no conflict of interest.

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