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Crosstalk Between the Immune and Central Nervous Systems with Special Reference to Drug Development

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1. Introduction

Although the understanding of disease mechanisms becomes rapidly progressed in recent years, there remain a lot of unmet medical needs in a number of disease fields. Especially in the degenerative diseases of the central nervous system (CNS) such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, stroke and traumatic brain injury, currently available drugs only manage the symptoms, and there exist few disease-modifying therapies. Therefore, novel therapeutic interventions that modify and delay the disease progression are highly demanded. Drug development for the CNS diseases is particularly challenging (Kola I and Landis J, 2004). One of the hurdles to develop drugs for the CNS diseases is poor translation from animal models to human diseases (Pritchard JF, 2008).

One strategy to overcome this hurdle is to examine therapeutic potential of a target molecule in a number of experimental settings, assuming that a molecule that modulates pathophysiologic mechanisms underlying a range of neurodegenerative disease has a higher chance to work in human disease (Mehal WZ, Iredale J & Friedman SL 2011). Although this strategy requires much resource, it enables us to eliminate candidates that act only in a particular experimental setting that might have a lower chance to be effective in humans. One example for this strategy is a kynurenine 3-monooxygenase that catalyzes the conversion of kynurenine to 3-hydroxykynurenine in the kynurenine pathway of tryptophan degradation (Stone TW & Darlington LG, 2002; Schwarcz R, 2004). A small molecule inhibitor of kynurenine 3-monooxygenase increased the levels of neuroprotective kynurenic acid and decreased the levels of neurotoxic quinolinic acid/3-hydroxykynurenine in mice. Furthermore, the treatment with the kynurenine 3-monooxygenase inhibitor ameliorated neurodegeneration in two types of mouse neurodegenerative models for Alzheimer's disease and Huntington's disease (Reinhart PH & Kelly JW, 2011; Zwilling D et al., 2011). These observations suggest that the kynurenine 3-monooxygenase would be more promising therapeutic target than conventional therapeutic ones that are effective in animal models of a single disease.

Another strategy to increase the probability of success of drug development in the CNS diseases is to target a molecule that possess multiple pharmacological actions and hence

might affect different pathophysiological phases of the disease at once, because the CNS diseases often arise from multiple pathological steps and/or factors and conventional approaches to treat one disease phase has limited efficacy in CNS disorders.

There are at least two types of approaches for this strategy. First one is to develop multi-specific therapeutics, in which two or more different mechanisms of actions are given in one molecule. Several classical drugs are known to exhibit multiple therapeutic actions in a single molecular form (e.g. aspirin, thalidomide etc.), and this strategy has been developed in industries, especially in a field of biologics such as bi- or multi-specific antibodies and co-agonistic peptides (Day JW et al., 2009; Fitzgerald J & Lugovskoy A, 2011). For example, a marketed antibody Herceptin, which targets human epidermal growth factor receptor 2 (HER2), was modified to have the simultaneous interaction to HER2 and vascular endothelial growth factor (VEGF) with high affinity in order to develop more efficient antibody-based therapeutics (Bostrom et al., 2009). Actually, the modified bi-specific antibodies inhibited the growth of both human colon cancer and human breast cancer in animal models, whereas an antibody mono-specific for HER2 or VEGF are only effective for either of them, indicating that the modified bi-specific antibodies are therapeutically effective for a range of types of cancers (Bostrom et al., 2009). In addition, it is speculated that co-inhibition of HER2 and VEGF in breast cancer is more beneficial than single inhibition of either molecules, because tumor proliferation mediated by HER2 and tumor angiogenesis mediated by VEGF are expected to be concurrently inhibited (Bostrom et al., 2009). Theoretically, bi- or multi-specific therapeutics are expected to be more effective and useful than most of mono-specific therapeutics. However, it is generally more difficult to develop bi- or multi-specific therapeutics than mono-specific therapeutics, since simultaneous optimization for multiple biological actions in one molecule are required.

Second strategy is to target a single molecule that plays critical pathological roles in the multiple phases of the disease progression, which is consistent with our current hypothesis that a molecule that possesses multi-functions and hence modulates multiple pathophysiologic phases of a disease will be a more promising therapeutic target for neurodegenerative diseases than current drugs that acts only a single phase of the disease process.

In recent years, we pursue the latter strategy, especially focusing on the CNS diseases that consist of an inflammatory/immune-mediated pathological phase and a neurodegenerative phase such as multiple sclerosis and spinal cord injury. Given the recent progress of understandings of the mutual crosstalk between the immune and the central nervous systems, we speculate that a number of molecules could play physiological roles in both immune and the central nervous systems, and some of them would be involved in the inflammatory/immune-mediated pathological phase and a neurodegenerative phase.

In this chapter, we introduce our recent activities to develop novel and efficient therapeutic interventions by targeting a molecule that play multiple pathological roles in the inflammatory and degenerative phases of neurodegenerative diseases. In the following sections, first we briefly describe the background information and recent updates on the physiopathological interactions between the immune and nervous systems, and then illustrate the pathogenesis of the inflammatory/immune-mediated CNS diseases such as multiple sclerosis and spinal cord injury. Finally we introduce our efforts on a multi-functional molecule and its therapeutic potential in neurodegenerative disease.

2. The mutual interactions between the immune and the nervous systems

The CNS was previously recognized as an immune privileged site, meaning the complete absence of immunosurveillance within the CNS. This concept had been supported by the fact that the CNS is devoid of the classical lymphatic drainage and has the endothelial blood-brain barrier that is a tight barrier between the cerebrospinal fluids (CSF) surrounding the CNS parenchyma and the systemic circulation, being isolated from immune system. However, accumulating evidences have demonstrated that a mutual interaction between the immune system and central nervous system does exist both in the physiological and pathological situations (Steinman L, 2004; Schwartz M, 2009&2010).

The CNS influences and controls the immune system at least partly through the autonomic nervous system (Steinman L, 2004). Anatomically, the autonomic nervous system via the vagal nerve and sympathetic nerve innervates the sites of the immune system such as spleen, bone marrow, thymus, lymph nodes and gastrointestinal system, and directly regulates the functions of the immune system. Similar to the central neural circuit where neurons communicate with each other via many types of neurotransmitter, the parasympathetic nervous pathways communicate with the immune system via the neurotransmitter acetylcholine, and the sympathetic nerves utilize norepinephrine as the neurotransmitter for the communication with the immune system. Lymphocytes express the receptors not only for these neurotransmitters but also for other neurotransmitters such as histamine, serotonin, substance P, vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide and so on (Steinman L, 2004). Receptors for neuroendocrine mediators such as corticotrophin-releasing factor, a-melanocyte-stimulating hormone exist on lymphoid tissue (Steinman L, 2004). In addition, corticotrophin-releasing factor released by hypothalamus stimulates the secretion of ACTH from the pituitary gland, and consequently induces the release of glucocorticoids from the adrenal glands, which systemically suppress the inflammation (Sternberg, 1997). The wide variety of mediators enables the brain to properly coordinate the immune system and to keep the homeostasis of the whole body by responding to the environmental changes such as infections in an appropriate manner. For example, endotoxins such as lipopolysaccharide (LPS) produced by gram-negative bacteria activate inflammatory responses e.g. release of proinflammatory cytokines that are potentially lethal when overproduced. Acetylcholine, the principle vagal nerve transmitter, effectively suppressed LPS-induced inflammatory cytokine production. In addition, direct electrical stimulation of vagal nerve inhibited the *in vivo* shock response induced by LPS in rats possibly through the secretion of acetylcholine. These observations indicate that the brain negatively regulates systemic inflammatory responses to endotoxin via a parasympathetic vagal nerve pathway by limiting the inflammatory responses (Borovikova et al, 2000; Tracey KJ, 2002).

In some cases, the influences of immune cells on the CNS are more manifested under physiological and pathological conditions. A typical example of regulation of the brain functions by the immune systems is the fever onset and the subsequent behavioural responses such as sleep, feeding and appetite (Watkins LR & Maier SF, 1999; Steinman L, 2004). When infected with virus, bacteria and parasites, the immune system feels the danger to the body and releases pro-inflammatory cytokines such as interleukin 1 (IL-1), tumor necrosis factor (TNF) and IL-6. Regarding the fever onset, these cytokines boost the fever response through the neurons in the preoptic area of the hypothalamus, acting directly on these neurons and/or generating prostaglandin E2 (PGE2) that also regulates the fever

responses of these neurons (Morrison SF & Nakamura K, 2011). Consistent with the involvement of PGE2 in the fever response, cyclooxygenase inhibitors, which block PGE2 production, are widely used as fever reducers. Of note, fever itself dampens the production of IL-1 from immune cells in the blood by modulating the proteolytic processing of this cytokine, again indicating the existence of the negative feedback loop that suppresses the overload of the inflammatory responses to keep the homeostasis of the body (Boneberg EM & Hartun T, 2003).

In addition to the influences of the immune system on the CNS functions under pathological conditions, it has recently been hypothesized that the immune system play physiological roles in the maintenance of the fundamental CNS functions such as adult neurogenesis and spatial learning and memory (Schwartz M & Shechter R, 2010).

Although the CNS was considered to be an immune privileged site under healthy conditions as mentioned above, accumulating experimental evidences have demonstrated that continuous leukocyte trafficking into the CNS occurs even in healthy subjects, although the penetration of the leukocytes is mostly observed in CSF, the choroid plexus and the meninges, but not in the brain parenchyma (Ransohoff RM et al., 2003; Engelhardt B & Ransohoff RM, 2005; Schwartz M & Shechter R, 2010). Furthermore, recent investigations have revealed that specialized immune cells such as resident microglia, bone marrow-derived monocytic lineage cells, do exist in the brain parenchyma from embryonic developmental stage (Chan WY, 2007; Hanisch UK & Kettenmann H, 2007).

The physiological functions of the leukocytes trafficking into the CNS have remained unclear. However, it has been hypothesized that these CNS-migrating leukocytes, especially autoimmune T lymphocytes that react with self-antigens in the CNS, contribute to the physiological CNS functions such as adult neurogenesis in the dentate gyrus of the hippocampus and spatial learning and memory (Ziv Y et al., 2006).

In the hippocampus, an essential region for the formation of certain types of memories such as episodic memory and spatial memory, continuous generation of neurons is observed even in the adulthood, suggesting the importance of the adult hippocampal neurogenesis for fundamental neural functions (Deng W, 2010). With regard to the involvement of immune system in the hippocampus function, immune-deficient mice devoid of T cells (severe combined immune deficiency (SCID) mice) showed less adult neurogenesis in the dentate gyrus of the hippocampus compared to that in normal mice (Ziv Y et al., 2006). Conversely, transgenic mice engineered to produce autoimmune T cells, which react with the CNS antigen (myelin basic protein) and are therefore activated in the CNS, display more adult hippocampal neurogenesis than that in their wild-type counterparts (Ziv Y et al., 2006). Furthermore, hippocampus-dependent spatial learning and memory was impaired in immune-deficient mice devoid of T cells, and was enhanced in transgenic mice with a large number of autoimmune T cells (Kipnis et al., 2004; Ziv Y et al., 2006). Collectively, these observations suggest that autoimmune T cells play critical roles in the maintenance of fundamental function of hippocampus through the regulation of neurogenesis. In addition, other groups also reported the impacts of T cells on adult hippocampal neurogenesis (Wolf SA et al., 2009a & 2009b; Huehnchen P et al., 2011), further supporting the concept that the immune system contributes to certain types of brain functions.

On the contrary, in certain types of the degenerative CNS diseases, inflammatory and autoimmune responses by immune cells against the nervous system are considered to be harmful. Under pathological conditions such as autoimmune neurological diseases like multiple sclerosis and inflammatory brain/spinal cord traumatic injuries, a large number of

immune cells directly migrate to the CNS possibly due to the breakdown of BBB and these pathological infiltration of immune cells to the CNS mostly causes detrimental impact on the disease progression (Donnelly DJ & Popovich PG, 2008; Rezai-Zadeh K et al., 2009). Along with this concept, anti-inflammatory steroids and immunosuppressive therapeutic interventions are currently used to treat autoimmune neurological diseases and brain/spinal cord injuries. However, these therapies have limited clinical benefits while showing severe side effects (Kim Y et al., 2009; Barten LJ et al., 2010; Hilas O et al., 2010; Samantaray S et al., 2010). In contrast, several evidences have demonstrated that the immune cells rather have beneficial impacts on the pathology of the CNS diseases (Wyss-Coray T & Mucke L, 2002; Donnelly DJ & Popovich PG, 2008). For example, the implantation of activated macrophages into the injured CNS exhibits therapeutic benefits rather than harmful actions (Lazarov-Spiegler O et al., 1996; Rapalino O et al., 1998).

These observations suggest that the effects of the immune system on the CNS under pathological conditions are more complex than originally thought, depending on nature of pathologic mechanisms of respective disease. Development of a novel therapeutic intervention beyond the classical anti-inflammatory/immunosuppressive therapeutic approaches is essential for the treatment of these CNS diseases (Donnelly DJ & Popovich PG, 2008; Rezai-Zadeh K et al., 2009; Schwartz M et al., 2009).

In the following sections, we describe more detailed characteristics of multiple sclerosis and spinal cord injury, especially focusing on the pathological mechanisms and discuss on benefits and limitations of currently available drugs and desired profiles of novel therapeutic interventions.

3. The inflammatory/immune-mediated CNS diseases

3.1 Multiple sclerosis (MS)

Multiple sclerosis (MS) is a complex CNS disease and typically begins between age 20 and 50. Women are affected twice more often than men, and 50% of the patients will need help walking within 15 years after disease onset. Its clinical symptoms include sensory and visual deficits, balance and gait disturbances, limb weakness, neurogenic bladder and bowel problems (Noseworthy JH et al., 2000). Also, in some patients, mental problems such as emotional liability, depression and cognitive impairment are associated.

MS is classified into four distinct types: (1) relapsing-remitting MS; (2) secondary progressive MS; (3) primary progressive MS; and (4) progressive-relapsing MS (Noseworthy JH et al., 2000; Hilas, O et al., 2010). Approximately 80 % of the patients are categorized in relapsing-remitting MS, in which patients develop the symptoms over a period of several days, and then stabilize or even improve the symptoms within weeks. In some cases, neurological deficits persist after a relapse, and the disease progressively becomes worse between relapses (secondary progressive MS). Within 6-10 years from the disease onset, almost half of the patients with relapsing-remitting MS progress to secondary progressive MS. Twenty percent of the patients display primary progress MS that is characterized by a gradual and progressive disease course from the onset without an obvious remission stage.

In general, this type of MS shows poor prognosis (Noseworthy JH et al., 2000; Hilas, O et al., 2010). A fourth type of MS, referred to progressive-relapsing MS, is unusual and shows a progressive phenotype with obvious relapses with or without recovery. However, discrimination of primary progressive MS and progressive-relapsing MS on their clinical

characteristics is often unclear and remains controversial (Lublin FD & Reingold SC, 1996; Andersson PB et al., 1999).

The precise etiology of MS remains uncertain, but it is assumed that immune responses against self-antigens such as myelin constituents are dysregulated in genetically susceptible subjects and cause destructive inflammation and autoimmune responses to the CNS components. This autoimmune reactions lead to demyelination and subsequent neurodegeneration in the CNS. Traditionally, therapeutic interventions of MS exacerbations in an acute phase are conducted with an anti-inflammatory steroid such as methylprednisolone to suppress neuroinflammation and to shorten the duration of exacerbation (Barten LJ, 2010). In order to reduce the frequency of relapses and slow the disease progression, disease-modifying drugs are also used. Current disease-modifying therapeutics include interferon-β (IFN-β) products and glatiramer acetate, which show modest efficacy with 30-40% reduction in MS relapse rates compared with placebo (Brinkmann V et al., 2010). These agents have a number of immune-related actions including anti-inflammatory functions, but the precise mechanisms of therapeutic actions are incompletely understood (Noseworthy JH et al., 2000). Although they are established first-line therapies, they are unable to reverse existing CNS damage and have no influence on the development of permanent disability (Compston A & Coles A, 2002). Another marketed MS drug, Natalizumab is a humanized monoclonal antibody that is specific for α-4 subunit of very late antigen 4 integrin expressed on lymphocytes. Natalizumab blocks the migration of leukocytes from the blood stream into the CNS across the BBB, and decreases the relapse rates of MS by 68% at 1 year (Polman CH et al., 2006). Despite its higher clinical efficacy compared to other approved therapeutics, Natalizumab is currently used only as a second-line treatment due to its association with progressive multifocal leukoencephalopathy, which is a rare but life-threatening demyelinating neurological disorder caused by the reactivation of John Cunningham (JC) virus under immunosuppressive conditions (Barten LJ, 2010; Brinkmann V, 2010). Fingolimod (FTY720), an orally active drug with immunomodulatory actions, is recently approved as a first-line treatment for relapsing MS (Brinkmann V, 2010). Fingolimod influences the leukocyte trafficking through the modulation of sphingosine 1-phosphate receptors expressed on the leukocytes. In phase III trials, fingolimod demonstrated greater pharmacological efficacy in the reduction of the relapse rates as compared with placebo and IFN-β (Cohen JA et al., 2010; Kappos L et al., 2010). However, fingolimod treatment causes some adverse events such as infections, cardiovascular and ocular events in several cases, requring long-term follow-up clinical studies in order to provide further information on the benefit-risk profile of fingolimod as a novel oral treatment for relapsing MS (Brinlmann V, 2010).

Collectively, current therapeutic interventions for MS are mainly focusing on suppression of the immune system; inhibition of lymphocyte activation and the blockade of leukocyte trafficking into the CNS. Histopathological analyses in MS patients and MS animal models such as autoimmune encephalomyelitis (EAE), suggest that certain subsets of $CD4^+$ T lymphocytes (helper T (Th) cells) and antigen presenting cells (APCs) such as dendritic cells and macrophage/microglia (CNS tissue macrophage) play key roles in the pathogenesis of MS (Chastain EML et al., 2011). Upon encountering CNS self-antigen such as myelin antigen, APCs become maturated and migrate to lymph nodes where they activate antigen-specific $CD4^+$ T cells by presenting the CNS self-antigen (Guermonprez J et al., 2002; Bailey SL et al., 2007). During the activation process, $CD4^+$ T cells differentiate into mature effector subsets such as Th1, Th2, Th17 and Treg cells. Direction of $CD4^+$ T cell differentiation is at

least partly dependent on cytokines produced by APCs (Weaver CT et al., 2006; Zhu J & Paul WE, 2010). Accumulating evidences suggest that Th1 and Th17 cells are involved in the pathogenesis of MS (Olsson T, 1992; Segal BM, 2010). Once activated, $CD4^+$ T cells penetrate into the brain and spinal cord by crossing the BBB, and are re-activated by APCs located in the CNS, which triggers the disease induction and progression by attacking myelin structures of the CNS neurons (Chastain EML et al., 2011). Mild but significant clinical efficacy of the existing therapeutic interventions for MS via immunosuppressive actions supports the autoimmune-mediated onset of MS pathology.

However, current therapeutics such as IFN-β and glatiramer acetate are ineffective in reversing axon degeneration, another hallmark of certain types of MS such as secondary progressive MS (Compston A & Coles A, 2002; Bjartmar C et al., 2003). Thus, a novel intervention that concurrently inhibits autoimmune reactions and neuronal damages such as axon degeneration is a desirable treatment for MS patients.

3.2 Spinal cord injury

Traumatic injuries to the adult mammalian CNS often cause serious and long-lasting sensory and motor problems, because the CNS shows very poor regenerative ability. One of the reasons for poor regenerative ability of the CNS is that the injured CNS axons following brain trauma and spinal cord injury show very limited regeneration in contrast to those in the peripheral nervous system. The lack of appropriate axon regeneration in the CNS results in permanent neuronal deficits such as paralysis, and the permanent neuronal deficits have great impacts on quality-of –life of the injured subjects.

The pathology of the CNS injuries, particularly spinal cord injuries, has been studied at a molecular level in animal models, and the lack of regeneration of injured CNS axons is attributed, at least partly, to the CNS environment itself rather than to any intrinsic disability of CNS nerve fibers (Richardson et al, 1980; David and Aguayo, 1981). Among myelin proteins of the CNS, there exist axon outgrowth inhibitors such as Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp), which are suggested to inhibit the regenerative axon growth around the lesioned site after traumatic injury (Mueller et al, 2005; Yamashita et al, 2005; Kubo et al, 2007).

During our effort to identify a new member of axon outgrowth inhibitors, we discovered that the repulsive guidance molecule a (RGMa), which is expressed in neurons and oligodendrocytes in the CNS, also functions as a myelin-associated neurite outgrowth inhibitor (Hata et al, 2006; Yamashita et al, 2007). RGMa significantly inhibited neurite outgrowth in cultured neurons (Hata et al, 2006; Kubo et al, 2008). Furthermore, a neutralizing anti-RGMa antibody exhibits therapeutic effects on rat spinal cord injury by enhancing spinal axon outgrowth, suggesting that RGMa plays a key pathological role in suppressing axon regeneration and functional recovery after spinal cord injury (Hata et al, 2006; Yamashita et al, 2007).

In addition to the myelin-associated proteins that inhibit axonal regeneration, immune components play critical roles in the recovery process of the injured CNS. Traumatic injuries expose the CNS directly to the immune cells by destruction of the BBB, and are followed by the infiltration of a variety of immune cells around the damaged sites post injury (Jones TB et al., 2005; Donnelly DJ & Popovich PG, 2008). Given the immune-mediated pathology of the CNS diseases such as MS, it had been assumed that the penetrating active immune cells were harmful, and therefore should be eliminated or suppressed (Popovich PG, 2000; Dheen

St et al., 2007). Along with this concept, anti-inflammatory steroids are currently used for the treatment of spinal cord injury. However, the therapeutic efficacy of steroids such as methylprednisolone is limited, and therefore, the original concept that the CNS inflammation is simply harmful is currently re-considered.

Among the immune cells that accumulate at the lesion site, macrophages and microglia are reported to have both beneficial and detrimental impacts on the recovery from CNS injury (Jones TB et al., 2005; Donnelly DJ & Popovich PG, 2008; Popovich PG & Longbrake EE, 2008). The beneficial role of macrophages/microglia at the lesion site is the clearance of debris of the damaged axon and myelin that inhibit axon regeneration, through their phagocytotic actions and protease secretion. The concurrent secretion of neurotrophic factors that promote axon elongation/neuronal survival is potentially beneficial for the recovery of the damaged CNS (Jones TB et al., 2005; Donnelly DJ & Popovich PG, 2008; Popovich PG & Longbrake EE, 2008). Of note, it was reported that the implantation of activated macrophages promoted axon growth and functional recovery following CNS injury (Lazarov-Spiegler O et al., 1996; Rapalino O et al., 1998).

On the other hand, the excessive inflammatory responses induced by macrophages/microglia upon CNS injuries are detrimental to the recovery of the injured CNS (Jones TB et al., 2005; Donnelly DJ & Popovich PG, 2008; Popovich PG & Longbrake EE, 2008). For example, pro-inflammatory cytokines such as TNF-α and/or IL-1 expressed by activated macrophages/microglia enhance neuronal degeneration after spinal cord injury (Lee YB et al., 2000; Nesic O et al., 2001; Genovese T et al., 2006).

These lines of evidences described above suggest that the complex crosstalk between the immune system and CNS exists through a variety of molecules in the inflammatory/immune-mediated CNS disease. Interestingly, we found that RGMa is expressed not only in neurons and oligodendrocytes, but also in accumulated and activated macrophages/microglia in the injured spinal cord, suggesting RGMa might play multi-pathophysiological roles in the degenerative CNS disorders through neural and immune systems. This hypothesis prompted us to uncover the function of RGMa in the immune system and the involvement of this molecule in the immune-mediated degenerative CNS diseases.

In the next section, we describe our recent data showing the functions of RGMa in the immune system and its potential involvement in the pathogenesis of one of the immune-mediated CNS diseases, MS by using an animal model of MS, EAE.

4. Repulsive guidance molecule a (RGMa) that has pivotal roles both in the CNS and the immune system is a promising drug target

RGMa was originally discovered as an axon guidance molecule in the chick visual system (Stahl B et al., 1990). Recently, it was reported that RGMa regulated cephalic neural tube closure in mouse embryo and neuronal apoptosis (Niederkofler V et al., 2004; Matsunaga E et al., 2004; Yamashita T, 2007). In the pathological context, we demonstrated that RGMa inhibited neurite outgrowth and hindered functional recovery after spinal cord injury (Hata et al., 2006; Kubo T et al., 2008). While the functions of RGMa in the CNS have been well-established, its functions in other organs have remained uncertain. As described in the previous section, we found that RGMa was also expressed in macrophages/microglia in the injured spinal cord, implying the pathological roles of RGMa in the immune system. In order to address this possibility, we further evaluated RGMa expression in dendritic cells,

which are key components to evoke the immune reactions as APCs through the activation and differentiation of $CD4^+$ T cells (Weaver CT et al., 2006; Zhu J & Paul WE, 2010). We found that bone marrow derived dendritic cells produced RGMa upon inflammatory stimulation with lipopolysaccharide (LPS), suggesting the involvement of RGMa in the immune system, especially in the activation process of $CD4^+$ T cells triggered by dendritic cells (Muramatsu R et al., 2011). The activation process of $CD4^+$ T cells includes the enhancement of the adhesion property of $CD4^+$ T cells to intercellular adhesion molecules such as ICAM-1. Enhanced adhesion of $CD4^+$ T cells to intercellular adhesion molecules enhanced the penetration of circulating $CD4^+$ T cells into tissues including the CNS under pathological conditions such as MS. Actually, *in vitro* exposure of $CD4^+$ T cells to RGMa led to increased adhesion to ICAM-1, showing the direct activation of $CD4^+$ T cells by RGMa. These data strongly suggest that RGMa, which inhibits axon regeneration in the CNS, also plays regulatory roles in the immune system.

This encouraged us to further explore the pathological involvement of RGMa in the immune-mediated CNS disease, mouse EAE where neurodegenerative symptom was evoked by immunization with myelin antigens thereby dendritic cells and activated $CD4^+$ T cells plays pivotal roles in its pathogenesis. Immmunohistochemical analyses demonstrated that RGMa expression was upregulated in dendritic cells located in spinal cords, lymph nodes and spleen after the induction of mouse EAE. In order to evaluate the functional involvement of RGMa in the pathogenesis of EAE, we produced pharmacological effects of a neutralizing antibody against RGMa (Hata et al., 2006). The administration of the neutralizing antibody significantly suppressed the pathological changes such as demyelination of spinal axons and the paralysis of hind limbs in association with reduction of immune cell infiltration into the spinal cord and decline of inflammatory cytokine (IL-2, IFN-γ, IL-17, IL-4) production from $CD4^+$ T cells (Muramatsu R et al., 2011).In another mouse EAE models where neurological deficit was induced by adoptive transfer of activated dendritic cells that are pretreated with CNS myelin antigens *in vitro*, adoptive transfer of the activated dendritic cells lacking RGMa caused less severe neurological deficit , further suggesting the pathological role of RGMa in mouse EAE. Moreover, we found that expression levels of RGMa in dendritic cells increased in the brain and spinal cord of human subjects with MS. Moreover, the blockade of RGMa with the neutralizing antibody against RGMa inhibited the proliferation and the inflammatory cytokine (IL-2, IFN-γ, IL-17A, IL-4) production by peripheral blood mononuclear cells obtained from MS patients (Muramatsu R et al., 2011). These observations suggest that dendritic cell-derived RGMa also plays a key role in T cell activation in MS subjects. Intriguingly, RGMa polymorphisms are associated with MS and levels of pro-inflammatory cytokines (IFN-γ and TNF) in CSF of MS patients (Nohra, R et al., 2010). Collectively, these lines of evidences strongly suggest that RGMa plays critical roles in the pathogenesis of MS, especially via activating autoreactive $CD4^+$ T cells (Muramatsu et al., 2011; Flemming A, 2011).

In the current experimental settings where the neutralizing antibody to RGMa was administered via a peripheral route, the therapeutic benefits of RGMa inhibition is assumed to be mainly based on the blockade of the immune system i.e. reduction of the penetration of immune cells such as activated $CD4^+$ T cells from circulation into CNS, since the antibody did not effectively reach to the CNS (Muramatsu R et al., 2011). Nevertheless, we could not exclude the possibility that the therapeutic effect of anti-RGMa antibodies was partially mediated by the therapeutic action on the CNS because a small amount, but significant levels of the antibodies were detected in the spinal cord.

Current therapeutic interventions for MS mainly focus on anti-inflammatory effects and they are ineffective in reversing the CNS damage (Noseworthy JH et al., 2000; Compston A & Coles A, 2002; Bjartmar C et al., 2003).The approach to inhibit RGMa, an endogenous axon regrowth inhibitor, might enhance the recovery of neuronal deficits not only by suppressing excessive autoimmune responses but also by promoting regeneration of the damaged axons and restoration of the injured neural circuit in EAE. However, the pathological actions of RGMa on the axon damage in the CNS are needed to be further addressed in EAE.

Other molecules that are originally identified as regulatory factors in the CNS are also reported to have key roles in the pathogenesis of the inflammatory/immune-mediated diseases. For example, semaphorins that regulate axon guidance during neural development are reported to directly modulate the immune reactions in animal disease models including EAE (Suzuki K et al., 2008; Kumanogoh A & Kikutani H, 2010). This further supports the idea to target a molecule that acts both in the inflammatory/immune-mediated pathological phase and a neurodegenerative phase in the neurodegenerative diseases.

A therapy with a neutralizing antibody provides a highly target-selective intervention, but is also associated with critical drawbacks such as poor exposure to the CNS as mentioned and parenteral route administration. Identification of a small molecule drug that mimics antibody and interferes directly with the ligand-receptor interaction or subsequent signal transductions is an alternative strategy to overcome the drawbacks of therapeutic antibodies. However, development of a small compound that blocks direct interactions of large proteins often encounters high hurdles. In this context, we identified a key signal pathway that mediates the inhibitory action of RGMa on neurite outgrowth (Hata K et al., 2006; Kubo T et al., 2008). RGMa expresses its actions through the receptor, neogenin and its inhibitory actions are mediated by the downstream effectors, Rho, Rho kinase and myosin IIA. Therefore, small molecule that inhibits the actions of downstream molecules (Rho, Rho kinase and myosin IIA) would be alternative approaches to the therapeutic antibodies. Since inhibitors for Rho, Rho kinase and myosin IIA are currently available, it is worth examining their therapeutic potentials on EAE and spinal cord injury (Straight AF et al., 2003; Mueller BK et al., 2005; Kubo T & Yamashita T, 2007). Actually, fasudil, a Rho kinase inhibitor, exhibits therapeutic effects on experimental spinal cord injury, supporting the strategy to block downstream signals of a target molecule for the treatment of the inflammatory/immune-mediated neurodegenerative diseases (Hara M et al., 2000; Sung JK et al., 2003; Ding J et al., 2010).

5. Conclusion

In this chapter, we illustrate our recent strategy to develop a novel and effective therapeutic interventions, especially focusing on the identification of potential targets that have multiple pathophysiological functions in the neurodegenerative diseases such as MS and spinal cord injury. Our recent efforts to discover a molecule that displays multiple actions in the immune system and the CNS resulted in the identification of RGMa that plays a key role in the autoimmune-mediated pathological process in the CNS disease. Furthermore, therapeutic potential of RGMa inhibitor is demonstrated by the pharmacological benefits of the anti-RGMa neutralizing antibody in EAE animal model. Although the central effects of the neutralizing antibody has remained to be addressed, current experimental data suggest that the blockade of RGMa functions might provide improved therapeutic effects on autoimmune encephalomyelitis through the inhibition of harmful autoimmune reactions

and the promotion of axon regeneration of damaged neurons (Figure 1). In addition, the effectiveness of a small molecule inhibitor of the downstream signal pathway suggests the alternative drug discovery approach with higher probability of success.
This type of strategy to target a single molecule with multiple pathological actions will give us an opportunity to develop an efficient therapeutic intervention, which will be further elucidated in the future.

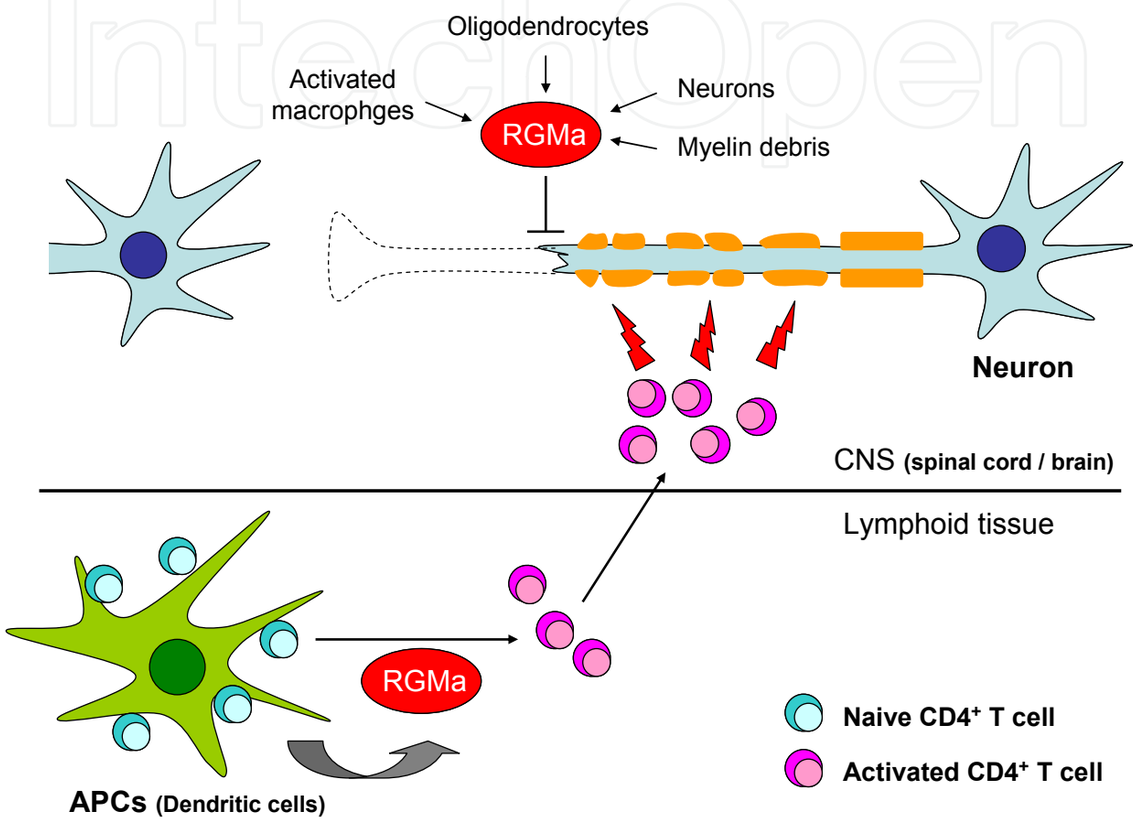

Fig. 1. RGMa plays key pathological roles both in the activation of autoreactive $CD4^+$ T cells and the inhibition of regeneration of damaged CNS axons, which renders RGMa as a promising drug target for the treatment of inflammatory/immune-mediated CNS diseases.

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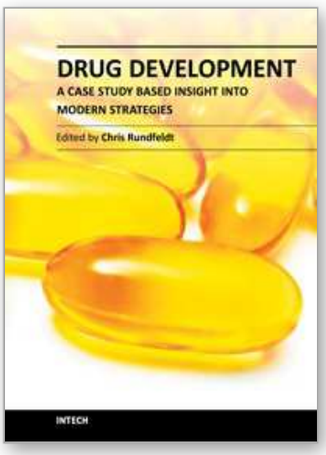

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This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

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