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REVIEW

Midkine and multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune neurological disease characterized by inflammatory demyelination with subsequent neuronal damage in the CNS. MS and its animal model, experimental autoimmune encephalomyelitis (EAE), have been thought as autoreactive Th1 and Th17 cell-mediated diseases. $CD4^+CD25^+FoxP3^+$ regulatory T-cell (Treg) plays a pivotal role in autoimmune tolerance, and tolerogenic dendritic cells (DCreg) drive the development of inducible Treg cells. Thus, a dysfunction in the development of Treg and DCreg leads to the development of autoimmune diseases. However, the factors that regulate Treg and DCreg are largely unknown. We recently showed that removal of midkine (MK) suppressed EAE due to an expansion of the Treg cell population as well as a decrease in the numbers of autoreactive Th1 and Th17 cells. MK decreased the Treg cell population by suppressing the phosphorylation of STAT5, which is essential for the expression of Foxp3, the master transcriptional factor of Treg cell differentiation. Furthermore, MK reduces the DCreg cell population by inhibiting the phosphorylation of STAT3, which is critical for DCreg development. Blockade of MK signalling by a specific RNA aptamer significantly elevated the population of DCreg and Treg cells and ameliorated EAE without detectable adverse effects. Therefore, the inhibition of MK may provide an effective therapeutic strategy against autoimmune diseases including MS.

LINKED ARTICLES

This article is part of a themed section on Midkine. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-4

Abbreviations

DCreg, tolerogenic dendritic cell; EAE, experimental autoimmune encephalomyelitis; MK, midkine; Treg, regulatory T-cell; MS, multiple sclerosis

Introduction

Multiple sclerosis (MS) is an autoimmune neurological disease characterized by inflammatory demyelination and neuronal damage in the CNS (Hemmer *et al.*, 2002). MS is characterized by a wide-range of clinical features, such as sensory and motor paralysis, blindness, pain, incontinence and dementia. The prevalence rate of MS in the Western countries is ~50–100 per 100 000 making it one of the most common neurological diseases. Experimental autoimmune encephalomyelitis (EAE), an animal model of MS, is usually induced in rodents using active immunization of myelin components such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein and proteolipid protein. MOG-induced EAE mice show paralysis from approximately 2 weeks after the initial immunization, the disease then develops reaching a peak approximately 1 week later; this is followed by a period of remission when they only exhibit mild symptoms of the disease. Typically, EAE paralysis starts with weakness of tail tonus, followed by a progression up the body to affect the hind limbs and finally the forelimbs.

Until recently, the mechanism of autoimmunity was explained by a dualistic model involving Th1 and Th2 cells. However, Th17, a newly discovered lineage of $CD4^+$ T-cells that secretes IL-17 and granulocyte macrophage colony-stimulating factor, has recently been shown to play a critical role in the development of chronic inflammation and autoimmune diseases, so that MS and EAE are now considered as Th1/Th17-mediated diseases (Bettelli *et al.*, 2006; Ivanov *et al.*, 2006; Mangan *et al.*, 2006; Weaver *et al.*, 2006; Codarri

et al., 2011; El-Behi *et al.*, 2011). Moreover, $CD4^+CD25^+$ regulatory T-cell (Treg) has also been recently identified as a new lineage of $CD4^+$ T-cells. Treg usually regulates Th1, Th2 and Th17 to maintain the immunological balance, resulting in peripheral tolerance and autoimmune suppression. Recent reports have revealed that abnormalities in Treg function cause autoimmune diseases (Sakaguchi, 2004; Sakaguchi, 2005; Liu and Leung, 2006), and Treg has been shown to suppress the activation of Th1/Th17 and act as a negative regulator of the pathogenesis of MS (Kohm *et al.*, 2002; Baecher-Allan and Hafler, 2004; Viglietta *et al.*, 2004; Matarese *et al.*, 2005). These findings suggest that the expansion of Treg is a promising therapeutic strategy for MS (Kohm *et al.*, 2002; von Herrath and Harrison, 2003; Mills, 2004; Viglietta *et al.*, 2004; Matarese *et al.*, 2005). The differentiation of Th cells requires antigen presentation by antigen-presenting cells such as dendritic cells (DCs). Recent studies have revealed that DCs stimulated with TGF-β and IL-10 differentiate into tolerogenic DCs (DCreg), which induce the differentiation of Treg from naïve CD4 T-cells (Lan *et al.*, 2006; Fujita *et al.*, 2007; Sato *et al.*, 2009). These results suggest that the regulation of DCreg may provide another therapeutic approach for autoimmune diseases including MS.

Immunological functions of midkine (MK)

MK, a heparin-binding growth factor, plays important roles in cell proliferation, cell migration, angiogenesis and fibrinolysis in a variety of tissues (Muramatsu, 2002). MK also contributes to the induction of inflammation and tissue repair. MK is most highly expressed during midgestation. In adult tissue, MK expression is limited to pathological conditions such as tumour, inflammation and injury. For instance, MK expression is increased in both the spinal cord and $CD4^+$ T-cells in peripheral lymphoid tissues during the induction and progression phase of EAE (Liu *et al.*, 1998; Wang *et al.*, 2008; Sonobe *et al.*, 2012). MK-knockout mice are resistant to ischaemic renal injury (Sato *et al.*, 2001) and neointima formation in atherosclerosis (Horiba *et al.*, 2000). Moreover, the development of a model of rheumatoid arthritis was found to be suppressed in mice deficient in MK, as MK is a key participant in inflammatory leukocyte migration and osteoclast differentiation (Maruyama *et al.*, 2004).

We have recently demonstrated novel immunological functions of MK as a negative regulator of Treg cell expansion (Wang *et al.*, 2008) and DCreg development (Sonobe *et al.*, 2012). MK-knockout mice are resistant to the development of MOG-induced EAE due to an increased population of DCreg and Treg cells in the peripheral lymph nodes, which is followed by suppression of infiltrating autoreactive Th1 and Th17 cells in the periphery and the CNS. Moreover, MK administration to MK-knockout mice abolished this suppression of MOG-induced EAE, while cessation of MK administration resulted in the restoration of this suppression of EAE. These findings suggest that the immunological effects of MK are transient and reversible. MK directly suppressed Treg cell expansion *in vitro* (i.e. without DCreg), while MK also directly inhibited DCreg cell development *in vitro*. Therefore, MK affects both Treg and DCreg equally. We also revealed that MK diminished the phosphorylation of STAT5 and the expression level of Foxp3 in $CD4^+CD25^+$ T cells. Moreover, MK suppressed the phosphorylation of STAT3 in DCs, which is an essential requirement for DCreg development. Recent studies have shown that tyrosine phosphatase SHP-2 is a critical negative regulator of STAT3 and STAT5 (Yu *et al.*, 2000). In fact, MK enhanced the expression of SHP-2. Pharmacological inhibition of SHP-2 restored STAT3 phosphorylation in DC and enhanced the development of DCreg. The SHP-2 inhibitor also restored STAT5 phosphorylation and subsequent Foxp3 expression in $CD4^+$ T cells and the expansion of the Treg cell population. These data indicate that MK impedes DCreg development and Treg cell expansion by inhibiting the phosphorylation of STAT3/STAT5 (Figure 1). Previous studies demonstrated that MK expression was upregulated in the CNS and $CD4^+$ T cells in the peripheral lymphoid tissues during EAE (Liu *et al.*, 1998; Wang *et al.*, 2008; Sonobe *et al.*, 2012). Therefore, MK may act as an exacerbater of autoimmune diseases by suppressing the development of DCreg and the expansion of Treg cells in the periphery and the CNS. Furthermore, inhibition of MK may prevent the adverse effects caused by disturbances in the homeostasis because the expression of MK in the adult is mostly restricted to pathological tissues (Muramatsu, 2002).

Pharmacological inhibition of MK by RNA aptamer

Therapeutic antibodies are now available for treatment against a variety of diseases such as cancer, leukaemia and autoimmune diseases. Although antibodies show high affinity and specificity for target molecules, antibody therapy also has several disadvantages such as the emergence of neutralizing antibodies against the therapeutic antibodies and cross-reactivity to essential molecules. Therefore, aptamer-based therapies have been recently considered as alternatives to antibody therapies (Wilson and Szostak, 1999; Brody and Gold, 2000; Mori *et al.*, 2004). RNA aptamers are single-stranded oligonucleotides (~50-mer) synthesized *in vitro* using an RNA selection-amplification protocol referred to as systematic evolution of ligands by exponential amplification (Wilson and Szostak, 1999; Brody and Gold, 2000; Mori *et al.*, 2004;). Aptamers are isolated from randomized RNA libraries as high-affinity oligonucleotides that recognize a wide range of target molecules with affinities and specificities that are 100–1000-fold higher than those of antibodies. Moreover, they can recognize slight conformational changes in the target molecules. Aptamers usually antagonize the target molecules by molecular mimicry. These features of RNA aptamers can circumvent several disadvantages associated with therapeutic antibodies. Firstly, unlike therapeutic antibodies, aptamers do not trigger an immune response, which could neutralize the introduced molecules. Secondly, the process by which aptamers are chemically synthesized allows large quantities of aptamers to be produced expeditiously and without batch-to-batch variations. Thirdly, aptamers are amenable to various chemical modifications such as radioisotopes, fluorescent and organ-targeting signal tags. The half-life of an aptamer *in vivo* is also controllable (hours–days) by chemical modification such as cholesterol and polyethylene glycol. Fourthly, the total production cost of aptamers is relatively less than that of therapeutic antibodies. Therefore, aptamer-based therapy is a promising therapeutic strategy that may replace many antibody therapies in the future. The first therapeutic aptamer used was the anti-vascular endothe-

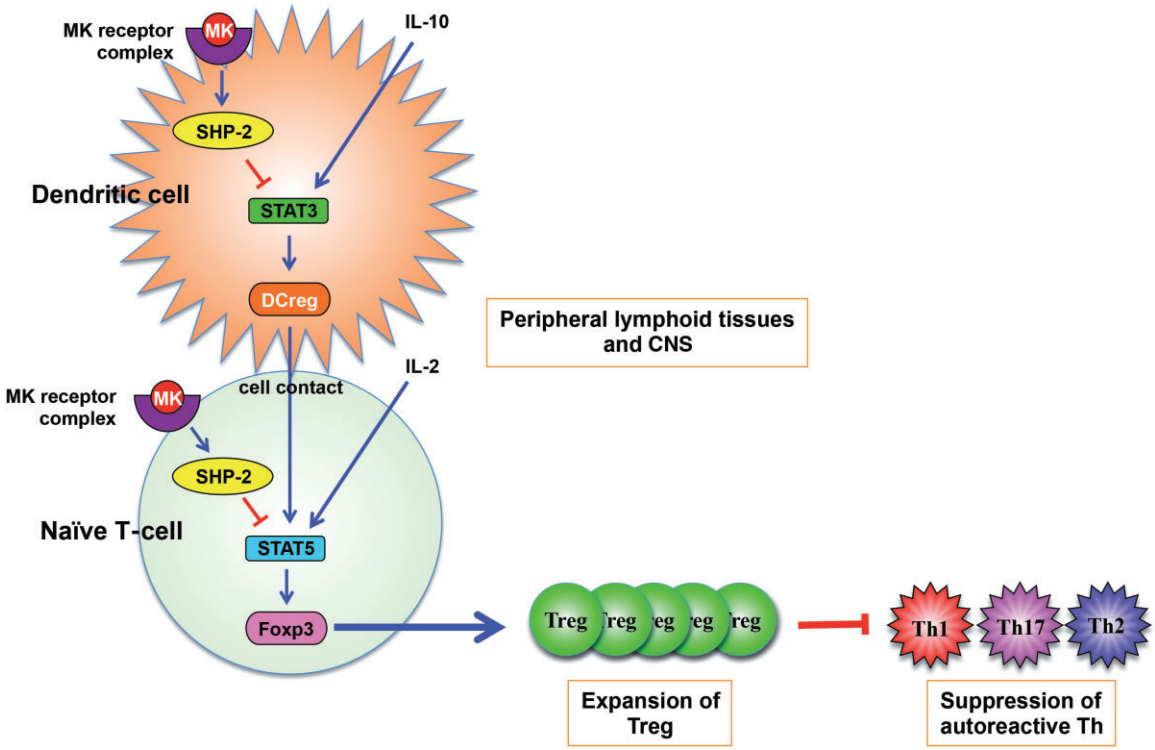

Figure 1

MK negatively regulates autoimmune tolerance via suppression of DCreg development and Treg expansion. IL-10 signalling differentiates dendritic cells into DCreg via STAT3 activation. Then, cell-to-cell interaction with DCreg and IL-2 signalling differentiates naïve T-cells into $CD4^+CD25^+Foxp3^+$Treg via STAT5 activation. MK activates tyrosine phosphatase SHP-2 that is a negative regulator of STAT3 and STAT5, leading to suppression of DCreg development and Treg expansion. Therefore, MK is a critical suppressor of autoimmune tolerance. In other words, blockade of MK effectively suppresses autoimmunity by enhancing DCreg development and Treg expansion.

lial growth factor aptamer as a treatment for age-related macular degeneration; it was approved by the US FDA in 2005 and a number of novel aptamer-based therapeutics are currently undergoing clinical trials for treating diseases such as macular degeneration, choroidal neovascularization, intravascular thrombus, acute coronary syndrome, von Willebrand factor-related disorders, von Hippel–Lindau syndrome, angiomas, acute myeloid leukemia, renal cell carcinoma, non-small cell lung cancer and thrombotic thrombocytopenic purpura (Sundaram *et al.*, 2013).

In recent studies (Wang *et al.*, 2008; Sonobe *et al.*, 2012), we blocked MK activity using anti-MK RNA aptamers. This 49-bases long oligonucleotide was stabilized by ribose-2′ modifications as well as cholesterol and inverted dT conjugations at its 5′ and 3′ ends respectively. Its ability to bind to MK was high with an apparent dissociation constant (K_d) of 0.9 nM. Administration of this anti-MK RNA aptamer enhanced DCreg development and Treg expansion in a dose-dependent manner *in vitro* (its effects reached a plateau at a dose of 125 nM). Moreover, treatment of mice with an anti-MK RNA aptamer ameliorated MOG-induced EAE in a dose-dependent manner when mice were injected either from the initial immunization day or after EAE onset (approximately 2 weeks after the initial immunization day). These effects reached a plateau at a dose of 15 mg·kg^{-1}. Administration of these anti-MK RNA aptamers expanded the Treg cell population and decreased the number of autoreactive Th cells both in the periphery and the CNS. Moreover, aptamers can penetrate into the CNS because the blood–brain barrier is disrupted in EAE mice. Thus, an anti-MK RNA aptamer can affect Treg and DCreg both in the periphery and the CNS. And as expected, blockade of MK signalling did not induce any significant adverse effects.

Currently, several monoclonal antibodies targeting the T-cell and/or B-cell (natalizumab, anti-α5 integrin; alemtuzumab, anti-CD52; daclizumab, anti-CD25; rituximab, ocrelizumab and ofatumumab, anti-CD20) have been approved or tested as the first line drugs for MS; however, excessive immunosuppression using these therapeutic antibodies (e.g. natalizumab and rituximab) often induces fatal encephalitis such as progressive multifocal leukoencephalopathy by JC virus reactivation (Diotti *et al.*, 2013). Therefore, an MK-targeted therapy seems to have the advantage of these drugs because expanded Treg cells only suppress excessive autoreactive Th cells, but not non-specific T-cells/B-cells.

Theoretically, in addition to MS, MK-targeted therapy theoretically could be an effective treatment for several autoimmune Th cell-mediated diseases (e.g. rheumatoid arthritis, inflammatory bowel disease, asthma, systemic lupus erythematosus, type 1 diabetes) although further confirmation studies are needed. The dosage regimen of the aptamer also needs to be refined for future clinical application. Lower

dosage and less frequent administration with a minimal invasive approach must be investigated, although several properties of the aptamer *in vivo*, including half-life and hydrophilicity/lipophilicity, are controllable.

Taken together, aptamer-based pharmacological inhibition of MK may be a promising therapy for autoimmune diseases such as MS.

Conclusion

MK negatively regulates autoimmune tolerance by suppressing the development of DCreg and the expansion of Treg cells. Pharmacological inhibition of MK by an RNA aptamer significantly increases DCreg and Treg and ameliorates EAE without any detectable adverse effects. Thus, blockade of MK signalling may provide an effective therapeutic strategy against autoimmune diseases including MS.

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Conflicts of interest

None.

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