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miRNAs in Multiple Sclerosis: Regulating the Regulators

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The exact etiology and underlying cause of multiple sclerosis (MS) remains obscure, but a dysregulation of the T cell response to myelin antigens appears to play a critical role in MS pathogenesis (Frohman et al., 2006). In healthy individuals, self-reactive T cells are controlled by a highly specialized T cell subset- the regulatory T cell or Treg- but this process appears to be impaired in MS. Reduced Treg activity in MS was first described by Viglietta et al. (2004), who isolated $CD4^{+}CD25^{high}$ T cells from peripheral blood of healthy controls and relapsing-remitting (RR) MS patients and compared their ability to suppress the proliferation of polyclonally activated CD4+CD25- T cells (Viglietta et al., 2004). The suppressive activity of MS Tregs was far less than those from healthy controls when moderate (anti-CD3) stimulation was used. Other groups expanded these findings using myelin antigen-specific stimulation (Haas et al., 2005; Kumar et al., 2006). In addition, MS Tregs have reduced Foxp3 expression (Huan et al., 2005; Venken et al., 2008b) possibly explaining the reduced suppressive activity of these cells. These findings have been challenged, based on the fact that the $CD4^{+}CD25^{high}$ population is not entirely specific for Tregs. Tregs were first found to be enriched in the $CD4^{+}CD25^{high}$ subset by the Sakaguchi group (Sakaguchi et al., 1995). Since CD25, the high affinity IL-2R chain, is also expressed by recently activated T cells, a great deal of effort has been undertaken to find Treg-specific markers. Foxp3 was identified as a transcription factor required for Treg development and function that distinguishes Tregs from other cells (Hori et al., 2003). However, the detection of this nuclear transcription factor requires membrane permeabilization and is therefore incompatible with the isolation of live cells required for further functional testing. In 2006, several groups reported that the $CD4^{+}CD25^{+}CD127^{low/-}$ population distinguishes human Tregs from activated T cells (Liu et al., 2006; Seddiki et al., 2006). Michel et al did not find a functional defect in MS patients' Tregs after eliminating $CD127^{high}$ cells (Michel et al., 2008). However, some groups localize the MS Treg defect to the naïve recent thymic emigrant Treg population and attribute the aforementioned disagreement to differences in the analyzed naïve versus memory Treg populations (Venken et al., 2008a).

Despite the clear involvement of genetic and environmental factors in the development of MS, the specific genetic elements mediating the immune pathology of MS have remained elusive (Milo and Kahana, 2010). Myelin-specific T cell responses may be initiated upon exposure to infectious agents, which could explain the characteristic northern geographical distribution of MS. The propensity of MS patients' T cells to activation, pathogenic Th cell

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differentiation or reduced Treg activity upon antigen exposure is influenced by genetics as well. Increases in genetic material sharing, from distant family members to monozygotic twins, result in dramatic increases in the odds ratio of developing MS (Compston and Coles, 2008). Although considerable effort has been devoted to the identification of MS susceptibility genes, only a handful of gene polymorphisms, including the MHC, IL-2R and IL7R, have been clearly linked to MS (Zuvich et al., 2009). Combined, the identified genes can only explain a small percentage of the genetic component of MS, leaving an important gap to fill. A twist in our understanding of how genetics influence disease comes from the recent recognition that miRNAs, a class of non-coding RNAs, have a profound impact on gene expression through mRNA destabilization and/or protein translation inhibition (Ambros, 2004). miRNAs are encoded in the genome and are therefore genetic elements, but many miRNAs are also up-regulated in response to viral infections (Yin et al., 2008). Therefore, miRNA expression may be altered by both gene polymorphisms and environmental factors.

miRNAs are encoded in the genome and are transcribed in the nucleus as primary RNAs (pri-miRNA) of several hundred nucleotides containing a hairpin structure (Cullen, 2004). Pri-miRNAS are then cleaved by the Drosha/DGCR8 complex into ~70 ntd hairpin precursor miRNAs (pre-miRNAs) that are exported into the cytoplasm by Exportin5. Once in the cytoplasm, pre-miRNAs are processed by Dicer to yield 19–24 nucleotide double stranded (ds) RNA. Only one strand is the mature miRNA that is loaded onto the RISC complex to mediate target mRNA destabilization and translation inhibition (Cullen, 2004). As of Sept 2009, over 1000 mature miRNAs had been identified in humans (http://www.mirbase.org). miRNAs are highly conserved between species, attesting to their functional importance (Tanzer and Stadler, 2006) and modulate a myriad of cellular processes, from differentiation to proliferation or apoptosis. miRNAs influence immune cell development, and their role in the maintenance of tolerance is just beginning to be recognized (Tili et al., 2008). miRNAs have been recently sought as biomarkers in MS. Two studies have found differences in miRNA expression in peripheral blood mononuclear cells (PBMC) of RRMS patients (Keller et al., 2009; Otaegui et al., 2009). Although there was little overlap between the identified miRNAs in both studies, possibly due to differences in the patient treatment status, they highlight the potential of miRNAs as MS and/or treatment effectiveness biomarkers. More recently, Du et al and Lindberg et al have found miRNAs differentially expressed in MS T cells and implicated them in pathogenic Th17 differentiation (Du et al., 2009) and activation (Lindberg et al., 2010), respectively. In this issue, De Santis *et al* identify miRNAs altered in regulatory T cells of MS patients that may mediate the reduced anti-inflammatory activity of this T cell population observed in MS patients {De Santis}.

DeSantis et al isolated CD4+CD25^{high} Treg cells from 12 MS patients and 14 healthy donors and analyzed the expression of 723 miRNAs by microarray analysis. They found 23 miRNAs differentially expressed (more than 1.5 fold change) in MS vs healthy Tregs. Most of these miRNAs were up-regulated in MS vs healthy controls and only a fourth of them were down-regulated. This might indicate that increased silencing (of Treg-specific genes) in MS Tregs is associated with reduced suppressive activity. Several of these miRNAs were confirmed to be up-regulated in a second set of MS patients and healthy donors when utilizing the more stringent CD4^{+}CD25^{high}CD127^{low} Treg definition, albeit the detected changes were less remarkable, perhaps reminiscent of the more similar functional activity detected in Tregs defined by low CD127 expression. Several of the up-regulated miRNAs (miR106b, miR-19a, miR19b, miR25) belong to the miR-106b/25 cluster that is involved in modulating the TGFbeta pathway by silencing CDKN1A/p21 and BCL2L11/Bim (Petrocca et al., 2008). The miR-17-92 cluster that has also been proposed to contribute to TGFbeta pathway inhibition was also found to be up-regulated in MS (Petrocca et al., 2008). Taken

together, these miRNAs may collaboratively suppress Treg development and/or function and explain the reduced Treg activity in MS.

There are several important diagnostic and therapeutic implications of these findings. First, MS-associated miRNAs may be of use in identifying MS in its early stages. The Treg defect in MS appears to be present during the RRMS phase of the disease, but not during the secondary progressive phase (SPMS) (Venken et al., 2006) and the associated miRNAs may prove to follow a similar pattern. It would be interesting to determine whether the miRNA pattern is present in individuals that respond to therapies that reportedly improve Treg function, such as glatiramer acetate (Cui et al., 2009; Haas et al., 2009; Jee et al., 2007; Venken et al., 2008a), as it could be used as a predictor of treatment efficacy. Finally, significant effort is being devoted as we speak to development of miRNA inhibitors as therapeutic agents for several diseases, and inhibitors targeting those miRNAs dysregulated in MS may be able to restore Treg activity while having few side effects, particularly if Treg specific delivery can be achieved.

A small piece of the puzzle on how miRNAs modulate the immunopathogenesis of MS has been revealed. We expect more discoveries to come. It will be particularly interesting to learn not only how miRNAs modulate T cells in MS but also how we can limit or harness their power for therapeutic purposes.

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