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Secondary progressive multiple sclerosis and the gut-brain axis

This scientific commentary refers to 'Gut microbiota-dependent CCR9$^+$ CD4$^+$ T cells are altered in secondary progressive multiple sclerosis', by Kadowaki *et al.* (doi:10.1093/brain/awz012).

The gut microbiota and the brain make strange bedfellows. Although radically different in nature and physically separated, they entertain most intimate functional relations. They regulate each other's functions in health, but in disease each may drive pathology in the other. This is the case in multiple sclerosis, where accumulating evidence indicates that signals from the gut microbiota spark an autoimmune attack on the white matter, while at the same time, the brain triggers changes in intestinal functions. In this issue of *Brain*, Kadowaki and co-workers shed new light on the immune pathogenesis of secondary progressive multiple sclerosis (SPMS) (Kadowaki *et al.*, 2019). They identify a subset of CD4$^+$ 'helper' T cells that are decreased in number and that tend to switch from a regulatory to a pro-inflammatory function in patients. These cells have two structures on their surface that indicate their affinity for the gut-associated lymphoid tissue (GALT), the immune organ that surrounds and directly interacts with the intestinal microbiota. The results of Kadowaki *et al.* suggest that a T cell population that may have been educated by gut microbes is altered in SPMS. This is an intriguing observation in two respects. First, it suggests an immune regulatory effect in SPMS, a disease stage with a disputed immune pathogenesis, and second, it invokes a gut-homing T cell class as a cellular messenger of deviant signalling in the brain (Fig. 1).

In most patients, multiple sclerosis proceeds in two distinct phases. The disease commonly begins as serial bouts interspersed with remissions [relapsing-remitting multiple sclerosis (RRMS)]. A decade or so later, the disease may assume a progressive course without remissions (SPMS). SPMS and RRMS differ in key ways. First, the brain lesions change their structural patterns: in RRMS, inflammatory infiltrates sit mostly within the demyelinating lesions and the surrounding normal-appearing white matter, while in SPMS the invading cells accumulate preferentially in perivascular and leptomeningeal spaces (Lassmann, 2019). Most importantly, however, immune cell therapies, which substantially mitigate RRMS, are largely inefficient in SPMS. This has been ascribed to a change of pathomechanism, from adaptive to innate immune response or to autonomous neurodegeneration (Weiner, 2008). The discovery by Kadowaki *et al.* of an aberrant immune regulation redirects attention to the possible role of immune mechanisms also in SPMS.

A second key observation of the current work is of a link between immune regulation of SPMS and the GALT. Kadowaki *et al.* report a decrease in the CCR9$^+$ T cell subpopulation in the blood of patients with SPMS. These lymphocytes make up ~5% of the healthy circulating lymphocyte pool, but in patients with SPMS, this proportion drops to roughly 3% on average, a subtle but significant change. In addition, in SPMS this reduced population of gut-experienced lymphocytes changes its functional phenotype. In contrast to their counterparts in healthy people, in SPMS most of the remaining CCR9$^+$ T cells assume an effector signature with increased expression of the transcription factor RORC, and increased production of interferon-γ and the pro-inflammatory cytokine IL-17.

T cells are divided into numerous subclasses, dependent on their effector functions and their migratory behaviours. The SPMS-affected immune population is part of the CD4$^+$ T cell pool, which express on their membrane the chemokine receptor CCR9, along with the cell adhesion molecule, $\alpha4\beta7$. Both structures indicate a special affinity of migratory T cells for the GALT. CCR9 binds and responds preferentially to the chemokine CCL25 (TACK), which is produced in the gut by intestinal wall epithelial cells. It attracts CCR9$^+$ immune cells with the complementary receptor from the circulation to the gut (Hernandez-Ruiz and Zlotnik, 2017). Most CCR9$^+$ T cells also express the integrin $\alpha4\beta7$, a cell adhesion molecule that binds to the complementary gut-specific addressin MAdCAM, and by so doing captures incoming T cells within the gut milieu (Habtezion *et al.*, 2016). Thus, most blood circulating T cells co-expressing both CCR9 and $\alpha4\beta7$ likely had a 'gut history', but it remains unclear how the CCR9$^+$ T cells later home to the brain. This question also arose a few years ago, when the

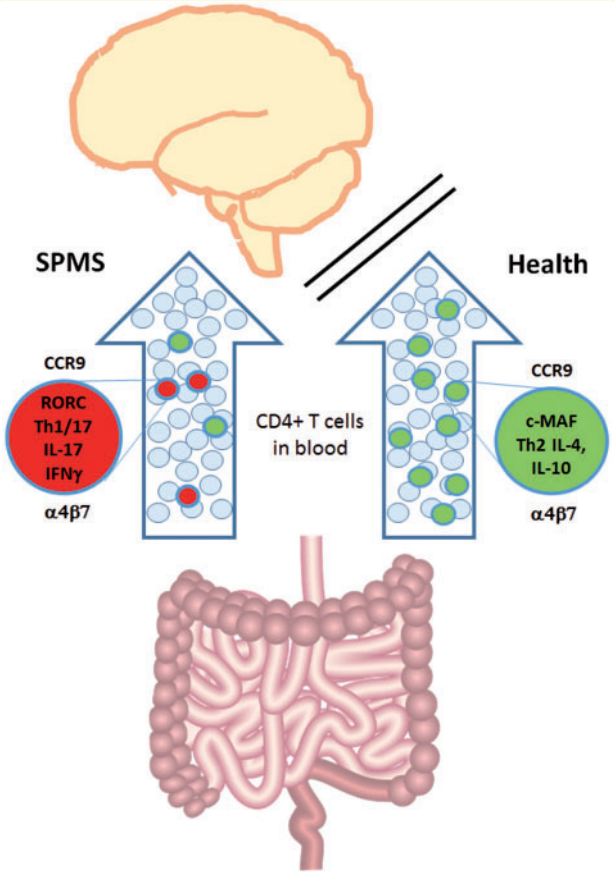

Figure 1 SPMS is marked by a reduction in peripheral CCR9$^+$ CD4$^+$ T cells, and a shift to a pro-inflammatory phenotype driven by signals from the intestinal microbiota. Image of intestines from DBCLS 統合TV.

group responsible for the current work identified another mucosa-associated lymphocyte population, MAIT cells, in multiple sclerosis lesions (Illés *et al.*, 2004). The signals that direct CCR9$^+$ T cells from gut to brain remain to be defined. Notably, MAdCAM/ α4β7 is not gut exclusive; for example it may aberrantly drive gut-seeking T cells into the liver (Adams and Eksteen, 2006). Moreover, the addressin α4β7 is promiscuous, also binding cerebrovascular VCAM1 (Adams and Eksteen, 2006). Another possibility, as Kadowaki *et al.* suggest, is that the immune cells use additional chemokine pathways, such as CCR6.

However, the surface phenotype *per se* is not formal proof of a functional gut link. Kadowaki *et al.* therefore sought to bolster their interpretation with more direct experimental evidence obtained using an experimental autoimmune encephalomyelitis (EAE) model. The model was induced by vaccinating C57BL6 mice with the autoantigen myelin oligodendrocyte glycoprotein in complete Freund's adjuvant. Kadowaki *et al.* showed that also in this model, a putatively gut-homing T cell set invaded the inflammatory brain tissue, and its activity depended on an intact gut flora. Some would argue, however, that mouse EAE is a highly artificial laboratory model, which reproduces certain features of early RRMS but much less so those of SPMS (Friese *et al.*, 2006). It could also be argued that the human CCR9$^+$ T cell class is not exactly duplicated in the mouse (Habtezion *et al.*, 2016). Nevertheless, the current findings would appear to strengthen the gut/brain link in SPMS.

Kadowaki *et al.* took pains to address some of the problems that complicate clinical studies of multiple sclerosis, such as issues related to sample size, participant age, and treatment history. They found, for example, that the proportion of blood CCR9$^+$ T cells also decreases with age in people without multiple sclerosis. Thus, given that individuals affected by SPMS are typically older than those with RRMS, it remains debatable to what extent the SPMS-associated decrease is attributable to ageing as opposed to the disease process *per se*. The reason for loss of these cells also remains obscure. Is it an age-related deficit of thymus-emigrant naïve T cells, as Kadowaki *et al.* propose, or is it the result of age-related changes in the gut microbiota?

The contribution of CCR9$^+$ to the immune pathogenesis of SPMS is intriguing, but still remains to be understood in detail. On the one

hand, CCR9$^+$ T cells produce mediators that downregulate inflammatory responses, which led Kadowaki *et al.* to propose that the observed numerical deficit in the CCR9$^+$ population might permit expansion of pathogenic inflammation in SPMS. On the other hand, some of the cells are diverted to generate pro-inflammatory mediators of the Th17 programme, and thus would fuel a pro-inflammatory effector function.

This innovative study raises questions for future studies to address. In addition to clarifying the regulatory role of CCR9$^+$ CD4$^+$ T cells, we must understand the origin of their changes in SPMS. The most pressing question relates to causality of the changes. Are they actively involved in the pathogenesis of SPMS, or are they secondary to the disease process? Then, we need information on the CCR9$^+$ T cell repertoire in health and disease. Is the repertoire polyclonal, or is it dominated by a few expanded clones, reflecting a response to microbial or other antigens? Which microbes signal the T cell changes, and how do they act? Whatever the answers to these questions, translating the present findings to clinical diagnostics and therapy will be as challenging as it is important.

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Competing interests

The authors report no competing interests.

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Visual neglect: getting the hemispheres to talk to each other

This scientific commentary refers to 'Theta burst stimulation in neglect after stroke: functional outcome and response variability origins', by Nyffeler *et al.* (doi:10.1093/brain/awz029).

The identification of biomarkers of recovery from the sensorimotor and cognitive consequences of stroke is important to improve clinicians' ability to stratify patients and to reduce variability in trial outcomes. Biomarkers can assist clinical decisions for individual patients, by predicting the potential for recovery and by enabling clinicians to choose an appropriate rehabilitation strategy (Boyd *et al.*, 2017). In this issue of *Brain*, Nyffeler and co-workers explore the anatomical predictors of response to treatment in patients with visual neglect, a leading cause of disability after right hemisphere strokes (Nyffeler *et al.*, 2019).

In a carefully conducted longitudinal study on a group of right brain-damaged patients, Nyffeler *et al.* assessed the effect of 'inhibitory' continuous theta burst stimulation (cTBS) over the parietal lobe of the left, undamaged hemisphere. Patients as a group showed evidence of a positive response to cTBS, with a reduction in clinical and neuropsychological signs of neglect. However, response was variable, with some patients recovering better than others. To assess whether the lesion location in the right hemisphere could explain this variability, the authors used voxel-based lesion-symptom mapping. Results showed that variability of individual responses to cTBS could be explained by the state of a portion of the splenium of the corpus callosum (Fig. 1, arrow), which connects the two parietal lobes: patients with intact interhemispheric connections tended to benefit most from cTBS. Other neuroimaging techniques based on diffusion MRI, such as tract-based spatial statistics, would have likely increased the statistical power of the anatomical analysis of the white matter, by focusing comparisons on the white matter skeleton. Nevertheless, Nyffeler *et al.*'s discovery of this anatomical biomarker of response to cTBS remains important on clinical grounds, and it also contributes to our understanding of the mechanisms of visual neglect.