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SCIENTIFIC COMMENTARIES

Immune dysbalance in childhood multiple sclerosis: a 'chicken or the egg' conundrum

This scientific commentary refers to 'Abnormal effector and regulatory T cell subsets in paediatric-onset multiple sclerosis', by Mexhitaj *et al.* (doi:10.1093/brain/awz017).

> 'The problem about the egg and the chicken, which of them came first, was dragged into our talk, a difficult problem which gives investigators much trouble. And Sulla my comrade said that with a small problem, as with a tool, we were rocking loose a great and heavy one, that of the creation of the world' Plutarch, Moralia.

Paediatric-onset and adult-onset multiple sclerosis presumably share similar, if not identical, mechanisms of pathogenesis. Like adult-onset multiple sclerosis, childhood-onset multiple sclerosis has a prodromal phase that precedes the clinical onset by an unknown, variable time span. In paediatric-onset multiple sclerosis, this prodromal phase may be considerably shorter, although there is no formal proof for this assumption. Furthermore, children with multiple sclerosis are less likely to receive treatment immediately after clinical onset. For these reasons, paediatric multiple sclerosis offers a very special window into early disease mechanisms. In this issue of *Brain*, Mexhitaj and co-workers—on behalf of the Canadian Pediatric Demyelinating Disease Network (CPDDN)—report that children with multiple sclerosis have pronounced changes in circulating immune cells (Mexhitaj *et al.*, 2019). Specifically, the authors observed decreased frequencies and reduced function of putatively anti-inflammatory, regulatory T cells, as well as increased activity of putatively pro-inflammatory T cells (Fig. 1). These results indicate that an 'immune dysbalance', especially of T cells, represents an early, and therefore probably important, element of the immune pathogenesis of paediatric, and by extrapolation, adult multiple sclerosis.

In a logistical and methodological *tour de force*, the investigators longitudinally collected blood samples from 23 children with multiple sclerosis, 26 with monophasic demyelinating disease, and 22 healthy control subjects. The diagnosis of multiple sclerosis, as opposed to monophasic illness, required a sufficient length of prospective follow-up. Furthermore, the investigators had to develop and implement rigorous standard operating procedures to guarantee high-quality conditions for the sophisticated immunological studies, which included multiparametric flow cytometry as well as functional *in vitro* assays of selected immune cell subsets. This formidable task would not have been possible without the infrastructures of the CPDDN.

The core findings are shown in Fig. 1: putatively pro-inflammatory subsets of T cells [CD4+ effector T cells and CD8+ mucosal-associated invariant T cells (MAIT cells)] are increased in frequency and function, whereas putatively anti-inflammatory CD4+ regulatory T cells are decreased. These findings are consistent with earlier reports of disturbed 'T-cell homeostasis' in adult multiple sclerosis (reviewed in Venken *et al.*, 2010; Kleinewietfeld and Hafler, 2014). Although some of the previous studies have remained controversial, the present results lend further support and credence to the notion that disturbances of T cell homeostasis may be an early and fundamental mechanism in the pathogenesis of multiple sclerosis across the entire age spectrum.

The most obvious next question is whether the observed changes constitute a primary mechanism, or are merely secondary to a still undefined triggering event. In fact, pondering this issue poses a classical 'chicken or the egg' conundrum. It may be helpful to approach the issue of a primary versus secondary 'immune dysbalance' by considering different settings of viral infection as an analogy (Table 1). An acute viral infection of a healthy person results in massive activation of pro-inflammatory immune mechanisms and (at least transient) downregulation of anti-inflammatory mechanisms to overcome the infection with maximal speed and efficiency (scenario A in Table 1). For example, in infectious mononucleosis, primary infection of B cells with Epstein-Barr virus (EBV) leads to a massive defensive anti-EBV T cell response. Eventually the immune system of the host is left with lifelong immunity against EBV, and the virus remains dormant in B

cells without harmful consequences for the host.

Progressive multifocal leukoencephalopathy (PML) provides an example of a distinctly different scenario of viral infection (scenario B in Table 1). Here, the disease-triggering mechanism is not the acute primary infection, but rather a profound disturbance of the immune system, namely a state of immunodeficiency caused by infection (HIV), immunosuppressive therapy (e.g. natalizumab), or genetically determined alterations. During such a prolonged state of immunodeficiency, a latent, otherwise harmless infection with John Cunningham (JC) virus turns into a devastatingly destructive process. The latent archetype virus mutates into a pathogenic variant that infects the brain and causes PML (Major *et al.*, 2018).

These two different settings of viral infection may help us to imagine potential scenarios of autoimmunity in multiple sclerosis. In scenario A (Table 1), a definable 'auto-sensitizing event' coaxes the immune system into attacking the patient's own body. The hypothetical initial triggering event may involve some viral or bacterial infection, and it may occur at different immunologically important sites, such as lymph nodes, gut or lung. This triggering event will induce a state of heightened pro-inflammatory and dampened anti-inflammatory immunity, resulting in autoimmune destruction of the CNS.

In an equally hypothetical autoimmune scenario B (Table 1), the pathogenic cascade of multiple sclerosis is triggered by an initial state of 'immune dysbalance', characterized by dampened anti-inflammatory and enhanced pro-inflammatory mechanisms. This state of dysbalance could be caused by genetic factors or by environmental influences (e.g. vitamin D deficiency), or by any combination of genetic and environmental factors. The initial state of immune hyperreactivity would set the stage for—perhaps even multiple independent—auto-sensitizing events, possibly involving different peripheral organs and tissues.

When thinking about these hypothetical scenarios, it becomes clear that scenarios A and B pose a classical 'chicken or the egg' dilemma: the postulated triggering events and the postulated secondary mechanisms are so intimately intertwined that they can only be separated in certain

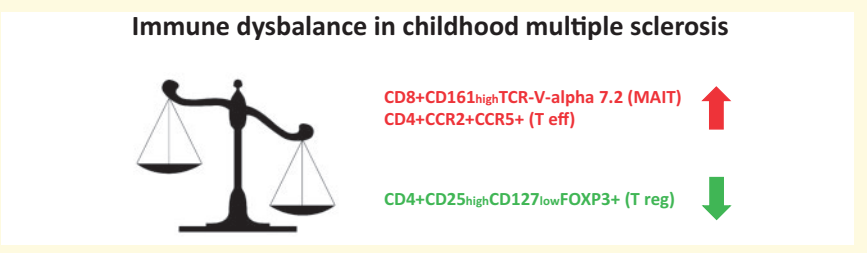

Figure 1 Immune dysbalance in childhood multiple sclerosis. As reported by Mexhitaj *et al.*, putatively pro-inflammatory subsets of T cells [marked red; CD4+ effector T cells (T eff) and CD8+ mucosal-associated invariant T cells (MAIT cells)] are increased in frequency and function, whereas putatively anti-inflammatory CD4+ regulatory T cells (T reg; green) are decreased. The different T cell subsets are defined by a combination of cell surface markers revealed by monoclonal antibody staining and flow cytometry (Glossary). The markers include differentiation and activation molecules (CD4, CD8, CD161, CD127), chemokine receptors (CCR2, CCR5), transcription factors (FOXP3), and T cell receptors for antigen (TCR-V-alpha 7.2). For a detailed review of T cell immunity, see Korn and Kallies (2017).

Table 1 Hypothetical analogies between scenarios of viral infection and autoimmunity (multiple sclerosis)

Scenario	Example	Primary (triggering) event or mechanism	Secondary/tertiary events or mechanisms
Viral infection A	Infectious mononucleosis (primary EBV infection of young adults) ('kissing disease')	Infection/transformation of B cells	**Immune activity** ↑ → Clearance of acute infection, lifelong latency of EBV
Viral infection B	Progressive multifocal leukoencephalopathy (PML)	**Immune activity** ↓ Development of a state of immune deficiency in subjects latently infected with JCV	Latent archetype virus mutates into pathogenic virus → Infection/destruction of oligodendrocytes and other types of brain cells
Autoimmunity A	Multiple sclerosis A	Hypothetical auto-sensitizing event [e.g. aberrant exposure of immune cells to viral or bacterial antigens in conducive environments (e.g. gut, lung, lymph nodes)]	**Immune activity** ↑ → Autoimmune destruction of CNS
Autoimmunity B	Multiple sclerosis B	**Immune activity** ↑ **('immune dysbalance', see Fig. 1)** The pro-inflammatory state may be caused or facilitated by genetic predisposition or environmental factors (e.g. Vitamin D deficiency)	Autoimmune destruction of CNS

In scenarios A (viral infection A or autoimmunity A), a definable event (acute viral infection or 'auto-sensitization') triggers secondary immunological changes, which eventually result in viral clearance or autoimmune destruction of CNS tissue. In scenarios B, a primary alteration of the immunological state ('dysbalance') leads to viral or autoimmune destruction of the CNS. The results reported by Mexhitaj *et al.* lend support to 'autoimmunity scenario B', but would also be consistent with 'autoimmunity scenario A'.

Glossary

Immune dysbalance: A state of disturbed 'immune homeostasis' characterized by increased (or decreased) pro-inflammatory activity, and/or decreased (or increased) anti-inflammatory activity.

MAIT cells: Mucosal-associated invariant T (MAIT) cells express a particular T cell receptor alpha chain (TCR V alpha 7.2). Some MAIT cells recognize bacterial metabolites. MAIT cells were first identified in the gut lamina propria (Treiner and Liblau, 2015). They are found in the blood and in multiple sclerosis brain lesions.

Multiparametric flow cytometry: A method for defining and enumerating lymphocyte populations. Cells are tagged by staining with multiple monoclonal antibodies directed against different cellular differentiation or activation molecules. Inside the flow cytometer, the mixture of labelled cells is funnelled through a laser beam, and the fluorescence signals of individual cells are recorded by sensitive photomultiplier tubes. In this way, flow cytometry provides quantitative information about the composition of the cell sample.

extreme settings such as the ones discussed here.

What is also very clear is that the new Canadian study opens up many more questions than it provides final answers. Obviously, one of the most important future challenges is to determine the nature of the auto-sensitizing antigens (Hohlfeld *et al.*, 2016). Are these myelin autoantigens as might be predicted by many animal models? Are they viral antigens that might stimulate cross-reactive autoimmune responses by molecular mimicry? Or might they belong to a novel category of autoantigens, e.g. intracellular proteins released during cellular damage or decay (Brändle *et al.*, 2016; Winger and Zamvil, 2016; Planas *et al.*, 2018)? All these possibilities are completely open at the moment, suggesting an important and challenging direction for future research.

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Competing interests

The author reports no competing interests.

References

Brändle SM, Obermeier B, Senel M, Bruder J, Mentele R, Khademi M, et al. Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins. Proc Natl Acad Sci USA 2016; 113: 7864–9.

Hohlfeld R, Dornmair K, Meinl E, Wekerle H. The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research. Lancet Neurol 2016; 15: 317–31.

Kleinewietfeld M, Hafler DA. Regulatory T cells in autoimmune neuroinflammation. Immunol Rev 2014; 259: 231–44.

Korn T, Kallies A. T cell responses in the central nervous system. Nat Rev Immunol 2017; 17: 179–94.

Major EO, Yousry TA, Clifford DB. Pathogenesis of progressive multifocal leukoencephalopathy and risks associated with treatments for multiple sclerosis: a decade of lessons learned. Lancet Neurol 2018; 17: 467–80.

Mexhitaj I, Nyirenda M, Li R, O'Mahony J, Rezk A, Rozenberg A, et al. Abnormal effector and regulatory T cell subsets in pediatric-onset multiple sclerosis. Brain 2019; 142: 617–32.

Planas R, Santos R, Tomas-Ojer P, Cruciani C, Lutterotti A, Faigle W, et al. GDP-l-fucose synthase is a CD4(+) T cell-specific autoantigen in DRB3*02:02 patients with multiple sclerosis. Sci Transl Med 2018; 10: eaat4301.

Treiner E, Liblau RS. Mucosal-associated invariant T cells in multiple sclerosis: the jury is still out. Front Immunol 2015; 6: 503.

Venken K, Hellings N, Liblau R, Stinissen P. Disturbed regulatory T cell homeostasis in multiple sclerosis. Trends Mol Med 2010; 16: 58–68.

Winger RC, Zamvil SS. Antibodies in multiple sclerosis oligoclonal bands target debris. Proc Natl Acad Sci USA 2016; 113: 7696–8.

How to help cerebellar patients make the most of their remaining learning capacities

This scientific commentary refers to 'Can patients with cerebellar disease switch learning mechanisms to reduce their adaptation deficits?', by Wong *et al.* (doi:10.1093/brain/awy334).

Cerebellar disease results in well known motor deficits. Balance problems and poor limb coordination limit activities of daily living, sharply reducing quality of life. Although certain genetic cerebellar disorders may soon benefit from genetic treatments, as yet there is no causal treatment for degenerative cerebellar disease. Furthermore, no currently