BRAIN
A JOURNAL OF NEUROLOGY

SCIENTIFIC COMMENTARIES

Interleukin-4 induced 1 (IL4I1) promotes central nervous system remyelination

This scientific commentary refers to 'IL4I1 augments CNS remyelination and axonal protection by modulating T cell driven inflammation', by Psachoulia *et al.* (doi:10.1093/brain/aww254).

Multiple sclerosis is an inflammatory demyelinating disease of the CNS characterized by infiltration of immune cells and progressive damage to myelin sheaths and axons (Lorscheider *et al.*, 2016). Most patients initially develop relapsing-remitting disease (RRMS), in which acute neurological deficits are interspersed with periods of partial or complete recovery. However, up to 65% of patients with RRMS eventually develop secondary progressive multiple sclerosis (SPMS), in which disability increases continually. Conversion to SPMS is associated with a relatively poor prognosis, in part due to the limited effectiveness of disease-modifying therapies in the progressive phase (Lorscheider *et al.*, 2016). This in turn partly reflects the complex interactions between the multiple pathophysiological mechanisms responsible for demyelination and neurodegeneration. However, there is also compelling evidence that a decline in regenerative capacity occurs in the brain in chronic multiple sclerosis, possibly due to dysregulation of inflammation (Fitzner and Simons, 2010). Unravelling the mechanisms that link the immune system with CNS damage and repair therefore represents both a challenge and an opportunity for multiple sclerosis research.

In this issue of *Brain*, Psachoulia *et al.* (2016) exploit a published remyelination transcriptome obtained from laser capture microdissected CNS lesions induced in the rodent CNS by lysolecithin/lysophosphatidylcholine (LPC) (Huang *et al.*, 2011), to identify novel markers of remyelination. Of the more than 8000 differentially regulated genes, *Il4i1*, a phenylalanine oxidase secreted mainly by myeloid antigen presenting cells (APCs), was among the most strongly upregulated (4-fold increase at 14 days versus 5 days post-lesion). *In situ* hybridization demonstrated that *Il4i1* was predominantly expressed in cells with morphology reminiscent of foamy macrophages, beginning as early as 10–14 days post-lesion, a time point in which M2-like alternatively activated microglia/macrophages (AAMs) prevail (Miron *et al.*, 2013). *In vitro* work with primary and commercial microglia/macrophage cell lines confirmed that *Il4i1* expression is specific for interleukin (IL)-4-activated M2-like AAMs.

In mice lacking the IL-4 receptor alpha (*Il4ra*$^{-/-}$)—which are deficient in both type 1 and type 2 IL-4 receptor signalling (Wynn, 2015)—*in vivo* LPC-induced demyelination led to much lower *Il4i1* expression than in wild-type mice. This effect was paralleled by increased activation of iNOS$^+$CD11b$^+$ M1-like classically activated microglia/macrophages (CAMs), reduced remyelination and increased axonal dystrophy. Similarly, LPC-induced demyelination in mice lacking IL4I1 (*Il4i1*$^{-/-}$) resulted in chronic activation of CD11b$^+$iNOS$^+$ M1-like CAMs, a significant reduction of CC1$^+$ oligodendrocytes, and again increased axonal dystrophy. These data suggest that AAMs require IL-4 signalling to secrete IL4I1 both *in vitro* and *in vivo*; and that the absence of IL4I1 significantly delays the physiological switch of CAMs into AAMs described in experimental LPC-induced demyelination (Miron *et al.*, 2013), therefore leading to a prolonged non-permissive environment for efficient remyelination.

To further explore the observations arising from the *in vivo* loss-of-function experiments in the LPC demyelination model, Psachoulia *et al.* developed complementary rescue/drug delivery approaches. First, co-injection of LPC and IL4I1 into the spinal cord of *Il4ra*$^{-/-}$ mice rescued the number of CC1$^+$ oligodendrocytes and reduced the number of CD11b$^+$iNOS$^+$ M1-like CAMs at the lesion site versus controls. Second, the intraparenchymal injection of recombinant IL4I1 (co-injected with LPC) in wild-type mice induced a very early (5 days post-lesion) increase in the number of Nkx2.2$^+$Olig2$^+$ oligodendrocyte progenitor cells (OPCs) and CC1$^+$Olig2$^+$ oligodendrocytes, and a delayed (10 days post-lesion) reduction in the number of CD11b$^+$iNOS$^+$ M1-like CAMs. Thus, recombinant IL4I1 is a potentially novel therapeutic option to

promote remyelination in focal CNS demyelination (likely) via modulation of specific AAM responses.

The authors sought to dissect out the functional effects downstream of IL4I1 signalling in OPCs/oligodendrocytes, microglia/macrophages and spleen-derived lymphocytes (splenocytes) *in vitro*. Exposure of OPCs/oligodendrocytes to recombinant IL4I1 had no effect on cell survival and proliferation. Likewise, the same treatment applied to lipopolysaccharide-stimulated microglia/macrophages did not change the expression of the pro-inflammatory gene *Nos2*. Conversely, exposure of activated splenocytes [with phorbol 12-myristate 13-acetate (PMA) + ionomycin] to IL4I1 led to significant reduction of interferon gamma (*Ifng*) expression *in vitro*. *In vivo*, co-injection of LPC and IL4I1 into the spinal cord of wild-type mice produced a significant reduction in expression of *Ifng* and *Il17*, but not *Il4*, at the level of the lesion. Hence, the most likely mechanism of IL4I1-induced promotion of remyelination requires signalling by T cells and reduction of prototypical inflammatory gene expression programmes.

To provide further evidence in support of their proposed mechanism of action (Fig. 1), the authors conducted a final *in vivo* experiment in which they administered recombinant IL4I1 therapeutically to mice with full blown myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an *in vivo* model of multiple sclerosis. IL4I1 was injected intravenously on two occasions, first when an EAE score of 2/2.5 had been reached and then again 4 days later. Behavioural, *ex vivo* immunological, and post-mortem tissue pathology and axonal responses were analysed up to 30 days post-immunization. IL4I1-treated EAE mice showed a slightly lower (albeit significant) EAE score and diminished accumulation of non-phosphorylated neurofilaments in dystrophic axons, when compared with control EAE mice injected with vehicle. *Ex vivo* FACS-based analysis of CD4+ T cells from the CNS and spleens of IL4I1-treated EAE mice confirmed an effect on CD4$^+$ T lymphocytes with prevention of the expansion of t-bet$^+$ (Th1), Ror γt^+ (Th17) and Gata3$^+$ (Th2) cells.

This paper provides an exciting perspective on the modulation of adaptive immune responses with a novel therapeutic protein capable of fostering the intrinsic regenerative capability of the CNS. Nonetheless, some key controls and additional conditions might have lent further support to the authors' conclusions and helped address some outstanding questions.

First, *in vitro* exposure to IL4I1 was not examined in Th1- or Th2-polarized spleen-derived lymphocytes, thus raising questions about the specificity of IL4I1 action on T cells and to what extent the reduction of T cell expansion observed in EAE-treated mice might depend on undesirable off-target effects of the protein. Similarly, the authors did not investigate whether IL4I1 has any pro- (as opposed to anti-) polarizing effects on M0-like (resting) macrophages/microglia. This additional information would have been helpful in developing a treatment for the disease model, which is intrinsically dynamic as per immune cell responses, both in the periphery as well as in the brain.

Second, most of the *in vivo* work on knockout mice was conducted in animals that ubiquitously lack the candidate gene(s), which makes it difficult to dissect the specific role of IL-4 and IL4I1 signalling pathways in myeloid cells versus OPCs/oligodendrocytes. Tissue/lineage-specific mutant mice would have enabled more specific questions to be asked.

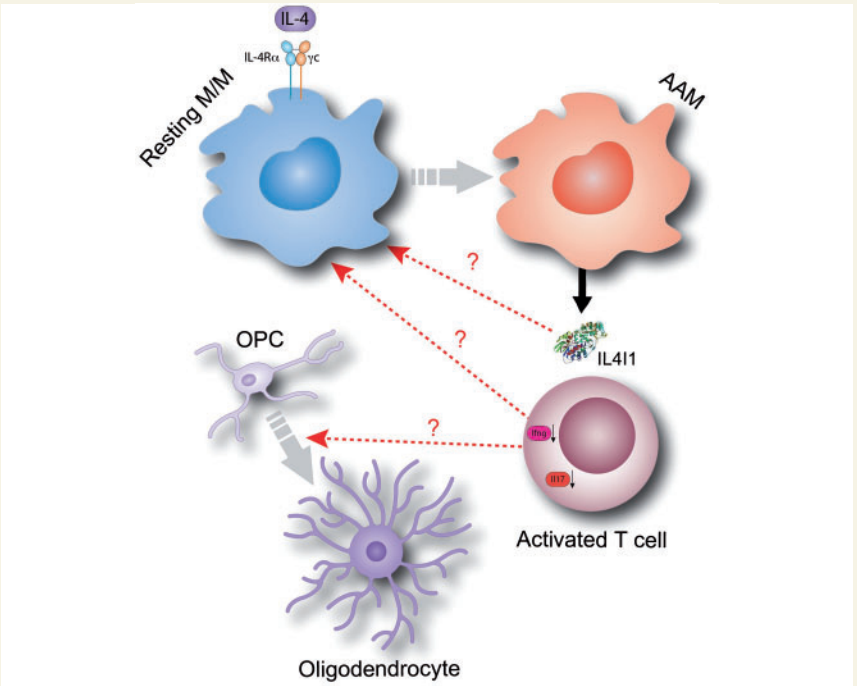

Figure 1 Proposed mechanism of action of IL4I1 in promoting CNS remyelination. IL-4 signalling on resting macrophages/microglia (Resting M/M) induces an M2-like, alternatively activated (AAM) phenotype that is associated with IL4I1 release. IL4I1 signalling on activated T cells reduces the expression of *Ifng* and *Il17*, thus creating a favourable environment for CNS remyelination. Red dashed arrows with question marks indicate the main mechanistic questions remaining following the work by Psachoulia *et al.*: the effects of IL4I1 signalling on (M0-like) resting M/M, as well as the ability of IL4I1-licensed peripheral T cells to affect M/M phenotype and OPC differentiation directly. *Ifng* = interferon gamma; OPC = oligodendrocyte progenitor cell.

Finally, the degree of functional improvement observed after therapeutic injection of recombinant IL4I1 in EAE was moderate. The emphasis on IL4I1 as a preclinical treatment that reduces clinical disability, while suppressing all spleen-derived T cell responses (with no major difference between Th1, Th2 and Th17 subsets), seems excessive. It also leaves several questions unanswered with respect to which pathways are activated by IL4I1-licensed peripheral T cells in microglia/macrophages and/or OPCs within the CNS. Intracerebroventricular injection of IL4I1 would have helped to distinguish between peripheral and central effects of the therapeutic protein.

Besides the general criticisms that can be levelled at systemic interventions with recombinant, highly immunogenic, short half-life proteins (Boyko, 2010), one wonders about the chances of promoting remyelination via a peripheral (non-specific) T cell targeting approach and its applicability to progressive multiple sclerosis. In fact, accumulation of brain damage in progressive multiple sclerosis is mainly the result of mononuclear phagocytes attacking myelin sheaths in the CNS (Tannahill *et al.*, 2015), while peripheral adaptive immune responses are minimal, and the repurposing of molecules or principles that are efficacious in RRMS for the treatment of SPMS has yet to prove successful (Mallucci *et al.*, 2015).

In conclusion, whilst this work provides compelling evidence that IL4I1 reduces T cell expansion in a way that supports OPC survival and remyelination *in vivo*, more work is required to understand its precise mechanism of action, the therapeutic niche of this novel molecule, and to fully assess its functional impact in animal models of progressive multiple sclerosis.

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doi:10.1093/brain/aww266

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Default mode network, connectivity, traumatic brain injury and post-traumatic amnesia

This scientific commentary refers to 'Disconnection between the default mode network and medial temporal lobes in post-traumatic amnesia', by De Simoni *et al.* (doi:10.1093/brain/aww241).

In the individual who has sustained a traumatic brain injury (TBI), three clinical metrics are typically ascertained: the presence and duration of post-traumatic amnesia (PTA), whether loss of consciousness occurred, and a score on the Glasgow Coma Scale. These common metrics are used to determine injury severity and to triage the patient, without particular reference to what the underlying neuropathology might be. Previous neuroimaging studies have attempted to identify particular patterns of lesions or abnormalities associated with each of these metrics; however, efforts have generally been unsuccessful because the research has been limited by a focus on lesion type, location and/or burden, and has typically involved only a single imaging modality. A more contemporary approach is to examine the patient with multiple types of imaging and image analysis methods to explore not so much the location of identifiable lesions, but how brain networks and systems have been altered by the injury (Aerts *et al.*, 2016; Hayes *et al.*, 2016). Advanced neuroimaging methods have revolutionized how brain