

SCIENTIFIC COMMENTARIES

Cytotoxic T cells go awry in inclusion body myositis

This scientific commentary refers to ‘Association of inclusion body myositis with T cell large granular lymphocytic leukaemia’, by Greenberg *et al.* (doi:10.1093/brain/aww024)

Inclusion body myositis (IBM) is the most prevalent acquired myopathy in adults beyond the age of 50. The disease is relentlessly progressive and largely resistant to immunosuppressive therapy (reviewed by Hohlfeld, 2011). In IBM, and less frequently also in polymyositis, non-necrotic muscle fibres are focally surrounded and invaded by CD8+ cytotoxic T cells (CTL) (Fig. 1) (Engel and Arahata, 1986). The CTLs show immunohistological features of activation and form close contacts with muscle fibres. The muscle fibres that are attacked express major histocompatibility complex (MHC) class I molecules on their surface, and are therefore capable of presenting MHC class I-bound peptides to CD8+ T cells (Fig. 1). It is thus assumed that the muscle-invading CTLs recognize an unknown antigen (or antigens) on muscle fibres. This is further supported by the observation that CTLs tend to orient their cytotoxic granules towards the contacting muscle fibre, providing tell-tale evidence that an immunological synapse has formed between the CTL and its target cell (Goebels *et al.*, 1996). Furthermore, the muscle-infiltrating CTLs are clonally expanded, and identical clones of CTLs may be detected in muscle and blood for pro-

longed periods of time, providing evidence for chronic antigen-driven proliferation of the CTLs (Hohlfeld, 2011). In this issue of *Brain*, Greenberg and co-workers report that ~50% of patients with IBM harbour clonally expanded populations of CTLs that meet the haematological criteria for ‘T cell large granular lymphocytic leukaemia’ (T-LGL leukaemia) (Greenberg *et al.*, 2016). These novel, somewhat provocative findings could have implications not only for understanding the pathogenesis, but also for improving the diagnosis and treatment of IBM.

The term ‘large granular lymphocyte’ (LGL) refers to a population of white blood cells containing azurophilic cytotoxic granules. LGL comprise both CTL and natural killer (NK) cells. Normally, the number of LGLs in peripheral blood is below $0.25 \times 10^9/l$ (Steinway *et al.* 2014). In T-LGL leukaemia, CD8+ CTLs are elevated and clonally expanded. Despite the term T-LGL ‘leukaemia’, the disorder really belongs to a grey zone between chronic lymphocytic proliferation and malignancy. Accordingly, the spectrum of T-LGL leukaemia ranges from relatively benign ‘clonal T-cell expansions of unknown significance’ to a more aggressive haematological disorder accompanied by anaemia, neutropenia or thrombocytopenia. Furthermore, T-LGL leukaemia may be associated with autoimmune diseases, such as rheumatoid arthritis, in particular presenting with extra-articular clinical signs characteristic of Felty’s syndrome,

e.g. splenomegaly and neutropenia (Steinway *et al.*, 2014).

Greenberg *et al.* (2016) investigated 38 IBM cases for features of T-LGL leukaemia. In all, 22/38 (58%) of patients met the criteria of an abnormal expansion of LGL in association with an autoimmune disorder (assuming that IBM is indeed an autoimmune disorder), and 13/38 (34%) met the more stringent criteria of an absolute LGL count of $>500/\mu l$, presence of clonal T cell receptor rearrangements, and association with an autoimmune disorder (Greenberg *et al.*, 2016). The expanded LGLs had phenotypic features of T-LGL leukaemia cells, as demonstrated by flow cytometry. Moreover, the authors looked for haematological changes that can be assessed with routinely available laboratory tests. They found that elevated absolute counts of CD8+ T cells and a reduced CD4/CD8 ratio closely correlated with the presence of T-LGL clonal expansions, and interestingly, also with the intensity of the histopathological changes in patients’ muscle. Although muscle biopsy remains the gold standard for the diagnosis of IBM, haematological markers such as those described here might assist in the diagnosis and be useful for monitoring the response to immunosuppressive treatment (see below).

We do not know which of the two disorders, T-LGL leukaemia or IBM, developed first in these patients. The authors suggest a scenario whereby chronic antigenic stimulation and

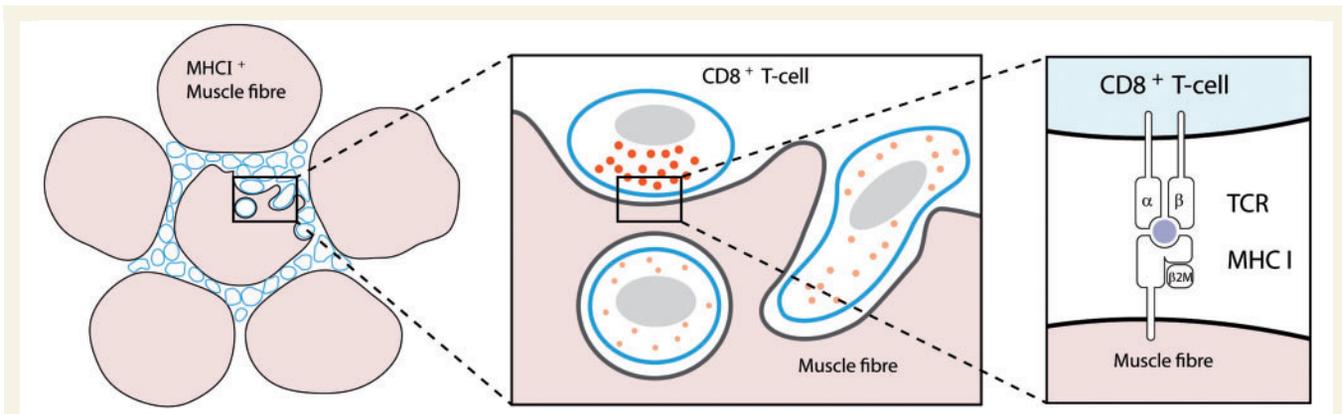


Figure 1 Inflammatory infiltrate in IBM muscle (schematic). Clonally expanded cytotoxic CD8+ T cells (CTL, blue) focally surround, indent and deeply invade non-necrotic muscle fibres (*left*) (Engel and Arahata, 1986). The invaded muscle fibres are strongly positive for major histocompatibility class I (MHC I +). It should be noted that this type of lesion is not specific for IBM, as it may also be observed in polymyositis (Engel and Arahata, 1986; Dalakas, 2015), nor is it specific for CD8+ alpha-beta T cells, as it was also observed in a case of gamma-delta T cell mediated myositis (Hohlfeld *et al.*, 1991). As was shown for polymyositis, some of the CTLs orient their cytotoxic cytoplasmic granules (red) towards the contacting muscle fibre (*middle*) (Goebels *et al.*, 1996). This provides indirect evidence that their antigen-specific T cell receptor (TCR) recognizes an MHC-class I-bound peptide on the muscle fibre (grey circle, *right*). $\beta 2M$ = beta-2-microglobulin.

Glossary

Inclusion bodies: Congophilic inclusions in vacuolated muscle fibres are one of the histopathological hallmarks of IBM.

STAT-3: STAT (Signal Transducer and Activator of Transcription)-3 mediates cytokine and growth factor-directed transcription. It is persistently activated in many cancers.

proliferation of CTL, as occurs in IBM muscle, eventually leads to the systemic changes of T-LGL leukaemia. Assuming this is true, what could be the mechanism? Might antigenic stimulation in the milieu of muscle fibres confer longevity and therapeutic resistance to the proliferating CTLs? CTLs from patients with T-LGL leukaemia may harbour somatic mutations of the transcription factor STAT-3, which plays an important role in cell survival and apoptosis (Koskela *et al.*, 2012). Although STAT-3 mutations were absent in the IBM patients investigated by Greenberg *et al.* (2016), it is possible that other signalling pathways might be altered in the proliferating CTLs at the genomic or post-transcriptional level. One approach to address this crucial, unresolved question would be to perform genomic and transcriptomic profiling of CTLs isolated from muscle and blood of patients with IBM.

The hypothesis that CTLs might acquire survival-fostering properties in IBM could have implications for

therapy. Various immunosuppressive treatments have been tried for IBM, including corticosteroids, methotrexate, cyclosporine, azathioprine, mycophenolate, intravenous immunoglobulins, anakinra, rituximab and alemtuzumab, but outcomes have overall been disappointing (reviewed by Aggarwal and Oddis, 2012; Dalakas, 2015). Indeed, the notorious treatment-resistance of IBM is often taken as evidence that the disease is driven by a myo-degenerative process or chronic viral infection (reviewed by Dalakas and Schmidt, 2016). Should the new findings spur efforts to explore new immunosuppressive approaches for IBM? As a note of caution, one should keep in mind that the expanded CTLs observed in IBM might be quite different from the 'T-LGL' described in the oncological and rheumatological literature. In those settings, T-LGL is at least partly responsive to certain immunosuppressive agents (but not steroids) (Steinway *et al.*, 2014). Furthermore, the form of T-LGL sometimes associated with rheumatoid arthritis is typically

accompanied by haematological complications such as anaemia, neutropenia and splenomegaly, i.e. features that are not typical for IBM. So it seems that a lot more work is needed to better define the properties of the 'CTL going awry in IBM'.

Acknowledgement

We thank Mr Robert Schorner for help with preparing the figure.

Funding

R.H. is funded by the Deutsche Forschungsgemeinschaft (DFG; TRR 128 and EXC 1010) and German Federal Ministry of Education and Research (BMBF; KKNMS). H.S.K. is funded by the Deutsche Forschungsgemeinschaft (DFG SCHU 1683/9-1), the German Federal Ministry of Education and Research (BMBF ArthroMark projects 1 and 7, OIEC100913) and the K + R Wucherpfennig Foundation.

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doi:10.1093/brain/aww053

Cerebellum in Alzheimer's disease and frontotemporal dementia: not a silent bystander

This scientific commentary refers to 'Network-selective vulnerability of the human cerebellum to Alzheimer's disease and frontotemporal dementia' by Guo *et al.* (doi: 10.1093/brain/aww003)

The network theory of neurodegeneration builds upon the Hebbian notion that neurons that fire together wire together. In this vein, they also die together. Interconnected neural networks in the non-human primate were identified initially with physiological and lesion-degeneration techniques, and refined through anatomical tract tracing studies. Interconnected networks are now identified in the human brain through resting state functional connectivity MRI, and intrinsic connectivity networks have been defined that bind distributed neural hubs according to putative functional relevance (Fox *et al.*, 2005). At the mechanistic level, it

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has been proposed that neurodegeneration occurs within these interconnected networks as a result of self-propagating/prion-like spread of neurotoxic agents along neural pathways linking distributed nodes into functional modules (Prusiner, 2013). In this issue of *Brain*, Guo and co-workers provide evidence that network-based degeneration in Alzheimer's disease and frontotemporal dementia also extends to the cerebellum (Guo *et al.*, 2016).

The cerebellum is a critical node in the distributed neural circuits subserving not only motor function but also autonomic, limbic and cognitive behaviours. Dysmetria of movement that characterizes the cerebellar motor syndrome results from lesions of the motor cerebellum, mostly in lobules III–V in the anterior lobe and the secondary sensorimotor region in lobule VIII. Dysmetria of thought in the realms of intellect

and emotion (Schmahmann, 1991, 1996) manifests as the cerebellar cognitive affective syndrome (Schmahmann and Sherman, 1998), which occurs following lesions of the cognitive and limbic cerebellum in the posterior lobe, represented in lobules VI, VIIA (including lobules VIIAf and VIIAt at the vermis, and crus I and crus II in the hemispheres) and VIIB, and possibly in lobule IX as well. The vestibular cerebellum is localized to lobule X and adjacent parts of lobule IX (Schmahmann *et al.*, 2000). The deep cerebellar nuclei are also incorporated into this motor-cognitive dichotomy. Cerebellar functional topography has been demonstrated by tract tracing studies of the cerebrocerebellar circuit in non-human primates (Schmahmann and Pandya, 1997; Strick *et al.*, 2009) (Fig. 1), and in physiological and behavioural studies in rodents, cats and monkeys (see Schmahmann, 1991 for