BRAIN
SCIENTIFIC COMMENTARY

Cytotoxic T cells and plasma cells dominate early in temporal lobe epilepsy with GAD antibodies

This scientific commentary refers to 'Temporal lobe epilepsy with GAD antibodies: neurons killed by T cells not by complement membrane attack complex' by Tröscher *et al.* (https://doi.org/10.1093/brain/awac404).

Antibodies (Abs) against the intracellular protein glutamic acid decarboxylase (GAD) are associated with a spectrum of neurological disorders, including stiff person syndrome, cerebellar ataxia, limbic encephalitis (LE), and temporal lobe epilepsy (TLE).[1] But while these associations were first identified several decades ago, the pathogenesis of these disorders, including the specific role of GAD-Abs and the identity of the immune mediators driving the diseases, remain largely enigmatic.

In this issue of *Brain*, Tröscher and colleagues[2] provide new insights into the longitudinal pathomechanism in GAD-TLE, by studying 15 adults who underwent temporal lobe surgery. The authors combined clinical, MRI, CSF and neuropathological examinations with immunohistochemistry, multiplex fluorescent microscopy, and transcriptomic analysis in order to identify the inflammatory mediators driving neurodegeneration. The various assessment strategies revealed an early active inflammatory 'encephalitic' stage (≤6 years) and a distinct immunological inactive or 'low-active' stage (>6 years). The early inflammatory stage was characterized by mediotemporal swelling and T_2 signal increase, intrathecal synthesis of immunoglobulin G, as well as infiltration of large numbers of plasma cells into the brain parenchyma—albeit without signs of complement-mediated tissue damage. Additionally, an even greater number of CD8+ cytotoxic T cells (CTLs) showing signs of local proliferation were observed, as well as signs of CTL-mediated neuronal destruction. Whole transcriptome analysis supported a dominant role of T cell immunity. The late inactive stage was characterized by decreasing numbers of infiltrating lymphocytes and by ongoing seizures, presumably due to irreversible structural damage to the temporal lobe as reflected by hippocampal sclerosis. The study thus demonstrates a key role for CTL-mediated neuronal destruction in GAD-TLE, and illustrates how the contribution of immune responses to disease can vary with disease duration (Fig. 1).

A dominant role of T-cell mediated neuronal destruction has previously been proposed in GAD-LE, based on immunopathological analysis of three patients.[3] The immunopathology of GAD-LE differed from that of classic paraneoplastic encephalitis with antibodies against intracellular antigens, in that there was less intense inflammation; however, apposition of granzyme-B+ T cells to neurons was observed in GAD-LE.[3] The study by Tröscher *et al.*[2] extends these findings, particularly by showing that the extent of T-cell mediated neuronal destruction depends in part on the disease duration. The authors also observed a large number of infiltrating plasma cells at early stages of GAD-TLE, although the antigenic target of these local plasma cells is unknown. The intrathecal production of GAD-Abs argues for GAD-specificity of at least some of them. We have previously observed high levels of GAD-reactive memory B cells in the peripheral blood of patients, as well as production of GAD-Abs by rituximab-resistant plasma cells in the bone marrow.[4] In addition, we found that monoclonal antibodies can be derived from memory B cells, plasmablasts and plasma cells in the CSF at early stages of GAD-Ab spectrum disorders, with a sizeable fraction of these antibodies being GAD-reactive.[5] GAD-reactive B cells therefore presumably transgress to plasma cells and remain in the CNS parenchyma, thereby contributing to the intrathecal production of GAD-Abs.

Why are so many plasma cells observed in the brain parenchyma in the early stages of GAD-TLE? One possibility discussed by the authors is that the plasma cells are simply bystanders of disease.[2] Alternatively, the plasma cells may produce not only GAD-Abs, but also pathogenic antibodies against surface antigens.[3] Another possibility is that plasma cells and GAD-Abs are directly involved in disease pathogenesis via effects on synaptic signal transduction, as discussed in synaptopathies associated with antibodies against proteins such as synapsin 1, amphiphysin and septin-5.[6] However, convincing evidence for a direct functional effect of GAD-Abs is lacking as both *in vivo* and *in vitro* studies using GAD-Abs have generated inconsistent results.[7] Likewise, in the present study, no specific Ig staining of synaptic structures on the surface of neurons was detected.[2] Autoantibodies may also be pathogenic by enhancing activation of local cognate $CD4^+$ T cells, as we demonstrated for Abs against myelin oligodendrocyte glycoprotein.[8] Tröscher and colleagues[2] provide evidence for a pathogenic role of $CD8^+$ T cells (which are presumably GAD-specific). The role of GAD-specific $CD4^+$ T cells is less clear; one of their footprints is the isotype of the GAD-65 antibody (largely IgG), typically requiring the help of $CD4^+$ T cells. An interplay between GAD-specific $CD4^+$, $CD8^+$ T cells and GAD-Abs may contribute to the local inflammatory network (Fig. 1).

By staining for C9neo, which is indicative of functional activation of the membrane attack complex, Tröscher *et al.*[2] show that

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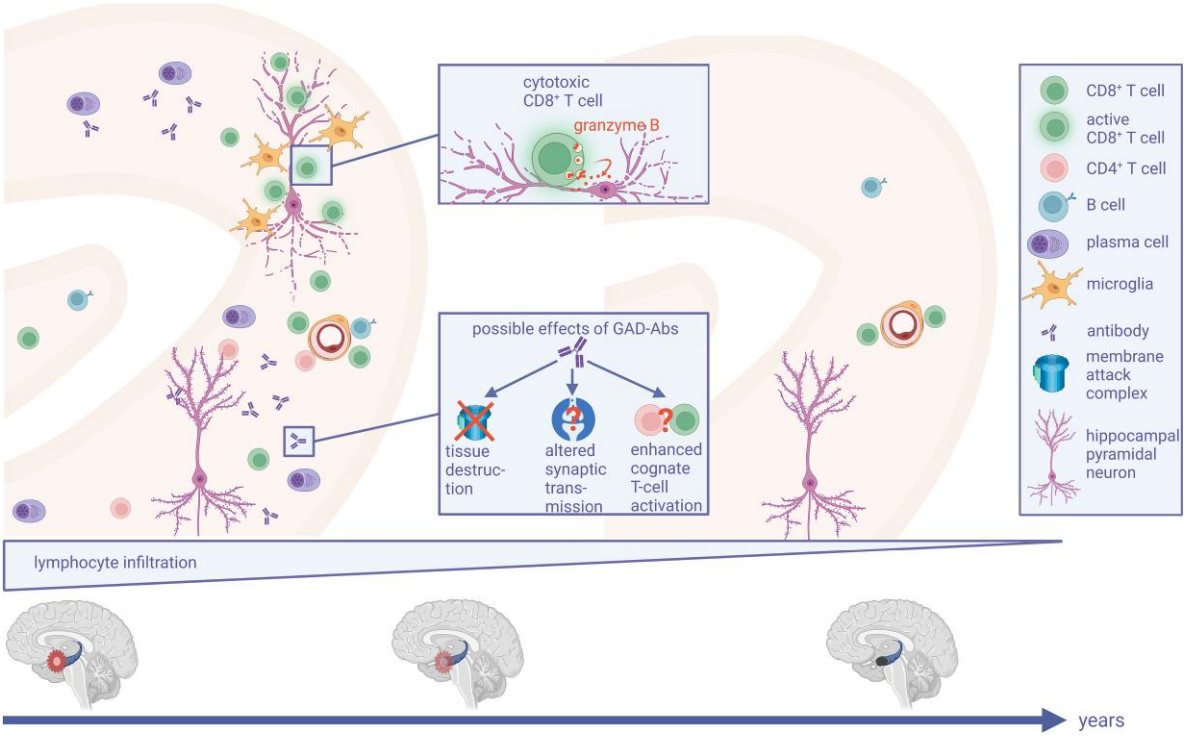

Figure 1 Temporal development of GAD-TLE. At early disease stages, large numbers of lymphocytes are observed in the perivascular space and in the brain parenchyma. These include cytotoxic CD8+ T cells with granzyme-B+ granules present in microglial nodules and in close proximity to neurons. In addition, plasma cells infiltrate the brain parenchyma, while CD4+ cells and B cells may also be present. The terminal complement cascade, as reflected by the membrane attack complex, is not involved in the pathogenesis of GAD-TLE. Possible effects of GAD-Abs include alteration of synaptic transmission or enhanced activation of cognate T cells, but neither has been convincingly demonstrated so far. At later disease stages, the number of infiltrating lymphocytes decreases. The immunopathological changes are reflected by hippocampal swelling (star) at early disease stages, followed by hippocampal sclerosis at later disease stages (black dot). Created with BioRender.com.

tissue destruction by the terminal complement complex, which is implicated in other neuroimmunological conditions such as aquaporin-4-Ab associated neuromyelitis optica spectrum disorders, does not play a major role in GAD-TLE. In another recent study, increased levels of activated complement proteins were observed in the CSF of patients with GAD-encephalitis, while enhanced transcription of genes encoding complement proteins, such as C3, C4A and C4B, were found in hippocampal specimens. Furthermore, C3d immunoreactivity was detected in neurons in various hippocampal regions.[9] The relevance of the observed C3d deposition remains to be established; it could play a role in removal of damaged axons or neurons.[9]

The postulated transition over time from an early immunological active to an inactive disease stage (Fig. 1) has clinical implications and is consistent with observations of decreasing response to immunotherapy with long-standing disease.[10] The translational implications of these results are evident, and raise the question of whether we should start or continue immunotherapy in patients with chronic disease. The need for new regenerative treatment approaches that can tackle neuronal damage is also apparent.

In summary, this study provides further evidence for the role of CTL-mediated neuronal destruction in GAD-TLE, and illustrates how the contribution of immune responses to disease can vary with disease duration. An open question is whether the mechanisms identified here also apply to other neurological disorders associated with GAD-Abs. Further studies, including in patients with stiff person syndrome and cerebellar ataxia, are required to determine whether findings in GAD-TLE and GAD-LE can be generalized to other GAD-Ab spectrum disorders.

Franziska S. Thaler[1,2,3] *and Edgar Meinl*[1,2]

1 Institute of Clinical Neuroimmunology, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany
2 Biomedical Center (BMC), Medical Faculty, Ludwig-Maximilians-Universität München, Martinsried, Germany
3 Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Correspondence to: Dr Franziska Thaler
E-mail: franziska.thaler@med.uni-muenchen.de

Correspondence may also be addressed to: Dr Edgar Meinl
E-mail: edgar.meinl@med.uni-muenchen.de

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Competing interests

The authors report no competing interests.

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