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Diagnostics of autoimmune neurodegeneration using fluorescent probing

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The discovery of antibody-mediated catalysis was a breakthrough that showed antibody function is not limited to specific binding interactions, and that immunoglobulins (Igs) may also chemically transform their target antigens. Recently, so-called "natural catalytic antibodies" have been intimately linked with several pathologies, where they either protect the organism or contribute to the development of autoimmune abnormalities. Previously, we showed that myelin-reactive autoantibodies from patients with multiple sclerosis (MS) and mice with experimental autoimmune encephalomyelitis (EAE) exhibit the ability to recognize and hydrolyse distinct epitopes within myelin basic protein (MBP). Further, the antibody-mediated cleavage of encephalitogenic MBP peptide 81–103, flanked by two fluorescent proteins, can serve as a novel biomarker for MS. Here, we report the next generation of this biomarker, based on the antibody-mediated degradation of a novel chemically synthesized FRET substrate, comprising the fluorophore Cy5 and the quencher QXL680, interconnected by the MBP peptide 81–99: Cy5-MBP$_{81–99}$-QXL680. This substrate is degraded upon incubation with either purified antibodies from MS patients but not healthy donors or purified antibodies and splenocytes from EAE but not from non-immunized mice. Data presented herein suggest the elaboration of potential specific, rapid, and sensitive diagnostic criteria of active progressive MS.

B cells contribute to the immune response via presentation of antigens, release of various cytokines, secretion of antibodies and also may have immunosuppression functions[1]. In turn, the major functions of antibodies, produced by B cells, include pathogen neutralization, antibody-mediated phagocytosis, antibody-dependent cellular cytotoxicity, and complement-mediated lysis of pathogens and infected cells[2]. In addition to these functions, the catalytic activity of immunoglobulins is elevated during pre-B-cell acute lymphoid leukaemia, acute myeloid leucosis, acquired immune deficiency syndrome[3], infections[4,5], and, especially, autoimmune disorders[6–9]. In contrast to the dozens of chemical reactions catalysed by artificial catalytic antibodies[10–12], natural catalytic antibodies were initially shown to exhibit limited hydrolysis ability (restricted to amide[13] and phosphodiester[6] bonds); however, numerous alternative activities were further observed[14–17]. Therefore, the hydrolysing activity of antibodies may be important in their function, either in host defence or autoimmune progression[18].

One of the most socially significant autoimmune diseases worldwide is multiple sclerosis (MS). This disease is characterized by chronic inflammation, demyelination, axonal loss, and oligodendrocyte loss; it is caused by activation and migration of immune cells, such as T cells, B cells, and macrophages, into the central nervous system (CNS)[19]. Early diagnosis is necessary for successful MS treatment. Although the diagnosis of MS is based predominantly on clinical and magnetic resonance imaging (MRI) findings, a variety of para-clinical laboratory tests can support clinical observations[20,21]. Since 2005, MRI of CNS has been widely used for MS diagnosis, following the so-called McDonald Criteria[21,22]. Concomitantly, biochemical and immunological diagnostic markers continue to undergo development. These include (i) detection of intrathecal synthesis of oligoclonal bands and quantitative IgG index[23,24]; (ii) an elevated titre of autoantibodies[25] and (iii) cytokine levels[26,27]. Previously, we developed a diagnostic marker for MS based on the ability of serum autoantibodies from experimental

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