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T cell reactivities to myelin protein-derived peptides in neuromyelitis optica patients with anti-aquaporin-4 antibody

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Abstract

We previously reported the establishment of major myelin protein-derived T cell lines from 11 patients with multiple sclerosis. In the present study, we determined anti-aquaporin-4 (AQP4) antibody status in these patients and classified them into five patients with anti-AQP4 antibody who met the criteria for neuromyelitis optica (NMO) or NMO spectrum disorders, and six patients without anti-AQP4 antibody who fulfilled the revised McDonald criteria for multiple sclerosis. T cell lines reactive to myelin oligodendrocyte glycoprotein, proteolipid protein and myelin basic protein were detected in 5/5, 3/5 and 3/5 of the anti-AQP4 antibody-positive patients, respectively, and in 5/6, 4/6 and 4/6 of the anti-AQP4 antibody-negative ones, respectively. T cell lines from most of these patients showed inter- or intra-molecular epitope spreading, irrespective of anti-AQP4 antibody status. These findings suggest that T cells are stimulated in vivo against major myelin proteins in anti-AQP4 antibody-positive patients with NMO/NMO spectrum disorders.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that is generally considered to be mediated by myelin-autoreactive T cells. By contrast, neuromyelitis optica (NMO) is characterized by severe and selective involvement of the optic nerves and spinal cord. Recently, a specific IgG against NMO, designated NMO-IgG, targeting aquaporin-4 (AQP4), was described.[1,2] Because of the high specificity of NMO-IgG/anti-AQP4 antibody and the selective loss of AQP4 from acute lesions in autopsied NMO spinal cord specimens[3], NMO has been claimed to be a distinct disease entity with a fundamentally different causal mechanism from MS. The demyelination in NMO is proposed to be secondarily produced following damage to the astrocyte foot process, where AQP4 is localized.[3]

In Asians, selective and severe involvement of the optic nerves and spinal cord is characteristic, and there are two distinct subtypes of MS: the opticospinal form of MS (OSMS), which has similar features to the relapsing-remitting form of NMO in Western populations[4], and the conventional form of MS, which is associated with disseminated lesions in the CNS[4], similar to classical MS in Western populations. Because a fraction of OSMS patients also have anti-AQP4 antibodies[1,5], OSMS is suggested to be the same disease as NMO. Although both NMO and OSMS are claimed to be primary astrogliopathies, it remains unknown how anti-AQP4 antibody present in the peripheral blood enters into the CNS across the blood brain barrier (BBB) to initiate parenchymatous inflammation, and how astrocyte foot process damage produces extensive demyelination.

We previously reported the establishment of T cell lines (TCLs) reactive to major myelin proteins, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), by stimulating peripheral blood T cells from MS patients with a myelin peptide mixture, before the discovery of NMO-IgG.[6] Therefore, in the present study, we aimed to

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clarify anti-AQP4 antibody status in these patients and compare differences in T cell reactivities to myelin proteins between anti-AQP4 antibody-positive and -negative conditions.

METHODS

Subjects and antigen-specific responses of established T cell lines

T cell lines were originally established from 11 patients with MS (three men and eight women) according to the McDonald criteria and seven healthy controls.[6] The median age was 48 years (range 28–64 years), while the median disease duration was 8.5 years (range 1–23 years). Briefly, T cell lines specific to the myelin self-peptides were established from peripheral blood mononuclear cells (PBMCs)[6], using 64 overlapping peptides of 16- to 21-amino acids in length, corresponding to the primary sequences of ^{196}MBP (amino acids 1–196), ^{276}PLP (amino acids 1–276), and ^{218}MOG (amino acids 1–218), including the exon 1–3 and exon 4–6 junctions of MBP.[6] Antigen-specific proliferation of the T cell lines was determined using peptide-pulsed PBMCs, as follows: The T cell lines ($3x10^4$) was cultured with irradiated (3,000 cGy) PBMCs for 72 h and pulsed with 1 μCi/well of [^{3}H] thymidine for the last 16 h. Test wells were considered to be positive with a stimulation index >2.0 and with a Δcpm (antigen-stimulated cpm minus non-stimulated cpm) >1,000 and at least three standard deviations above the mean cpm of unstimulated control wells. Blocking of the proliferative response was investigated by adding the following anti-HLA class II monoclonal antibodies (mAbs): Hu-4 (anti-HLA-DRB1+DRB5 monomorphic), 1a3 (anti-HLA-DQ monomorphic) and B7/21 (anti-HLA-DP monomorphic). The original T cell reactivity data, which have been previously described[6], were used for the present analyses.

Detection of anti-AQP4 antibody

Anti-AQP4 antibody was detected by an indirect immunofluorescence method using green fluorescent protein (GFP)-AQP4 fusion protein-transfected human embryonic kidney cells (HEK-293), as previously described.[5,7]

RESULTS

Anti-AQP4 antibody was detected in five of the eleven patients; these patients fulfilled the criteria for NMO[8] or NMO spectrum disorders.[9] Reactivities to MOG, PLP and MBP were detected in T cell lines established from 5/5, 3/5 and 3/5 of the anti-AQP4 antibody-positive patients with NMO/NMO spectrum disorders, respectively, and from 5/6, 4/6 and 4/6 of the anti-AQP4 antibody-negative patients with MS, respectively (Table 1). T cells reactive for myelin antigens from four of the five anti-AQP4 antibody-positive patients showed inter- or intra-molecular epitope spreading, while the same was true for five of the six anti-AQP4 antibody-negative MS patients (Figure 1).

DISCUSSION

Intramolecular epitope spreading is defined as a phenomenon in which T cells initially react only to a major or dominant antigenic epitope of an immunized antigen, and later show reactivity to other secondary or cryptic epitopes of the same immunogen. Intermolecular epitope spreading is defined as a phenomenon in which T cells initially reactive to only the immunized antigen molecule later demonstrate reactivity to other non-immunized molecules. Thus, reactivity of T cell lines to multiple sites of an antigenic molecule is regarded as intramolecular epitope spreading, while reactivity of T cell lines to multiple antigenic molecules is regarded as intermolecular epitope spreading. Inter- and intra-molecular epitope spreading of T cell lines is usually observed

Table 1: Proliferative responses to each myelin protein

Myelin protein	Anti-AQP4 antibody-positive NMO/NMO spectrum disorders	Anti-AQP4 antibody-negative MS	Healthy control
MBP	3/5	4/6	2/7
PLP	3/5	4/6	2/7
MOG	5/5	5/6	3/7

MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein

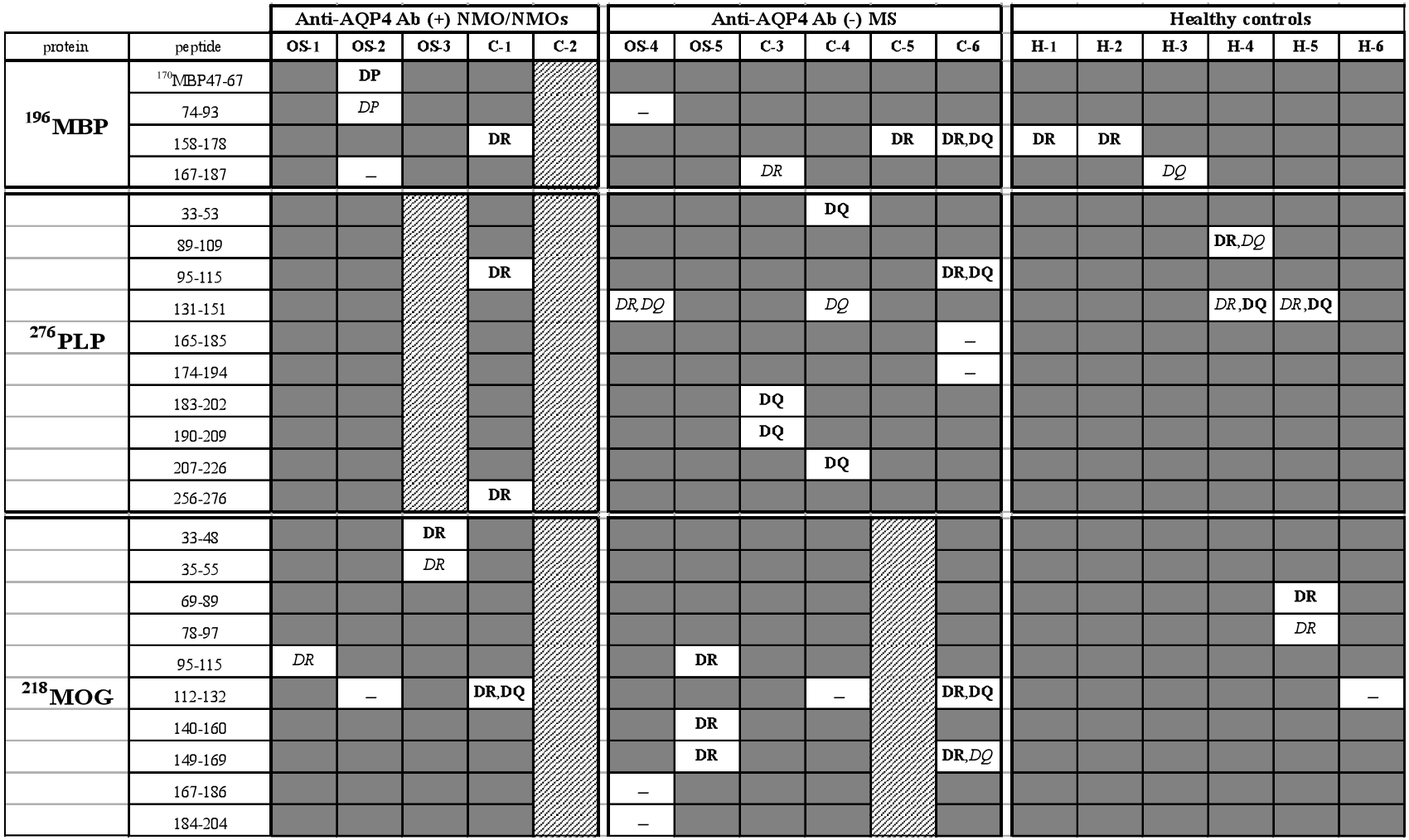

Figure 1. Myelin protein-reactive T cell lines characterized for peptide specificity and HLA restriction. T cell lines demonstrating either proliferative responses (open box) or no response (closed box) to peptides are shown. The hatched box indicates that the relevant epitope of T cell lines could not be determined because of a low response to the myelin peptide mixture. The restriction by HLA class II molecules of T cell lines is indicated in bold letters when anti-HLA class II mAb inhibited the proliferative response of T cell lines by ≥80%. Italic letters and (-) indicate that the mAb directed against the corresponding HLA class II molecules inhibited T cell proliferation by 50–80% and ≤50%, respectively.

Anti-AQP4 Ab: anti-aquaporin-4 antibody; HLA: human leukocyte antigen; mAb: monoclonal antibody; TCL: T cell lines; (+): positive; (-): negative.

in individuals whose T cells are stimulated or sensitized in vivo by specific antigen(s), whereas T cell lines stimulated or sensitized in vitro by antigen(s) during culture only react to neither multiple epitopes of the same antigen nor multiple antigens.

In the present study, T cell lines from healthy subjects also showed some reactivity to myelin proteins, but this was limited to one or two site(s) of a single myelin protein (five out of six cases demonstrated such a pattern). By contrast, T cell lines from four of five anti-AQP4 antibody-positive NMO/NMO spectrum disorders patients and five of six anti-AQP4 antigen-negative MS patients showed reactivity to multiple sites of multiple myelin proteins. It is therefore suggested that inter- and intra-molecular epitope spreading of T cell lines against myelin proteins occurs in most anti-AQP4 antibody-positive patients with NMO/NMO spectrum disorders and most anti-AQP4 antibody-negative ones with MS, but not in healthy subjects. The inter- and intra-molecular epitope spreading observed in anti-AQP4 antibody-positive patients indicates that T cells are already sensitized in vivo against major myelin proteins.

Recently, it was shown that sera from NMO patients with anti-AQP4 antibody can damage astrocytes in vivo following induction of experimental autoimmune encephalomyelitis by MBP-specific T cells.[10-12] It thus appears that encephalitogenic T cells are required for anti-AQP4 antibodies to exert their effects efficiently. Accordingly, the myelin protein-specific T cells found in anti-AQP4 antibody-positive NMO/NMO spectrum disorders patients may contribute to the initiation of CNS inflammation, and thereafter, anti-AQP4 antibody may invade the CNS and damage astrocytes in the presence of complement.

However, our T cell lines were established from the patients at certain periods after disease onset. Thus, myelin protein-specific T cell responses may have been secondarily developed after disease onset as a result of intermolecular epitope spreading. It will therefore be critical to study which CNS antigens T cells target at the time of initial attack, to elucidate the mechanisms underlying CNS anti-AQP4 autoimmunity. Nevertheless, it is possible that the myelin protein-specific T cells found in anti-AQP4 antibody-positive patients contribute to the development of inflammatory demyelination either primarily or secondarily.

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