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Stroke induces specific alteration of T memory compartment controlling auto-reactive CNS antigen-specific T cell responses

Juliane Klehmet [a,*], Sarah Hoffmann [a], Gerrit Walter [b], Christian Meisel [c,d,1], Andreas Meisel [a,1]

[a] Department of Neurology (JK, SH, and AM), Charité Universitaetsmedizin, Charitéplatz 1, Berlin, Germany
[b] Department of Orthopedics, Helios Klinikum Buch, Schwanebecker Chaussee 50, Berlin, Germany
[c] Department of Medical Immunology (CM), Charité Universitaetsmedizin, Berlin, Germany
[d] Department of Immunology (CM), Labor Berlin Charité Vivantes, Sylter Strasse 2, Berlin, Germany

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ABSTRACT

Whether and when auto-reactivity after stroke occurs is still a matter of debate. By using overlapping 15mer peptide pools consisting of myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) we show increased frequencies of immunodominant MOG- and MBP T cell responses in acute ischemic stroke which were associated with reduced frequencies of naïve T cells as well as CD8+ TEMRA cells. Auto-reactive CNS antigen-specific T cells responses as well as alterations of T cell subpopulations normalized in long-term follow up after stroke. Our findings suggest that stroke-induced immunodepression might function as an adaptive mechanism in order to inhibit harmful and long-lasting CNS antigen-specific immune responses.

1. Introduction

Stroke might induce a suppression of innate and adaptive immunity predisposing to bacterial infection, mainly stroke associated pneumonia [1–4]. Pneumonia is considered as a contributing factor for increased mortality and morbidity in patients with acute stroke [5–8]. Infections are considered to be caused by dysphagia facilitating aspiration and secondary immunodeficiency (CNS-injury induced immunodepression, CIDS) which is caused by severe CNS injury or stroke [4,9–12]. CIDS is characterized by lymphopenia and immune dysfunctions, in particular by functional deactivation of T-helper (Th)-1/natural killer (NK) cells as well as of antigen-presenting cells (APC) such as monocytes, which is mediated by the sympathetic nervous system and the hypothalamus-pituitary axis [2]. Moreover, CIDS deactivates alveolar epithelial cells as well as macrophages in lung via cholinergic pathways [13]. Previously, we demonstrated that stroke leads to rapid T lymphopenia and long-lasting suppression of lymphocytic IFNγ production [14]. Recently, an acquired hypogammaglobulinemia has been detected in stroke as well as in the MCAO model of experimental stroke following ischemia [15]. Damage of the brain parenchyma as well as of breakdown of the blood brain barrier function as conditions for the development of auto-antigen driven inflammatory process [16,17]. We know, that stroke-induced activation of the adaptive immune system, including T and B cells, regulatory T cells, as well as γδT cells can lead to deleterious antigen-specific autoreactive responses but can also have cytoprotective effects [1]. Higher frequencies of CNS-auto-antigen specific T cells such as myelin basic protein (MBP) and other antigens have been found in the blood of stroke patients in comparison to healthy controls, associated with worse clinical outcome [18;19]. Experimental data suggest that animals subjected to a systemic inflammatory stimulus at the time of stroke are predisposed to develop an autoimmune response to brain, and that this response is associated with worse outcome [20]. Accordingly, induction of MBP-induced tolerance prevented central nervous system autoimmunity and improved outcome in experimental stroke [21]. On the other side, we have shown that blocking of the sympathetic nervous system led to increased auto-antigenic immune responses [22].

The immunological memory is one of the key factors of the adaptive immune system leading to a fast and vigorous immune response to antigens that have been encountered previously. According to their expression of CD45RA and the lymph node homing receptor CCR7 T cells are divided into naïve and memory/effector T cells [23]. The T cell memory compartment influence self-antigen responses in chronic

* Corresponding author at: Department of Neurology, Charitéplatz 1, 10117 Berlin, Germany.
E-mail addresses: juliane.klehmet@charite.de (J. Klehmet), sarah.hoffmann@charite.de (S. Hoffmann), gerrit.walter@helioskliniken.de (G. Walter), christian.meisel@laborberlin.com (C. Meisel), andreas.meisel@charite.de (A. Meisel).
[1] These authors contributed equally to this work.

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autoimmune diseases [24–26]. Here we aim at investigating whether the ischemic stroke induce autoreactive T cell responses against CNS antigens, such as MBP and myelin oligodendrocyte glycoprotein (MOG), and induces changes in the T cell memory compartment.

2. Methods

2.1. Patients and controls

Between April 2009 and November 2011 twenty-eight patients with ischemic stroke admitted to the Charité Universitätsmedizin Berlin were enrolled within 36 h of symptom onset. Individuals with ongoing therapy for infectious diseases of clinical or subclinical signs for infection, and those taking immunomodulatory drugs were excluded. Blood was drawn as soon as possible after stroke onset within 36 h (baseline = day1) and at 72 h (day3), 7 days (day7), and at least 3 months after stroke onset (follow up, range 3–11 months). Healthy controls were age- and sex-matched (n = 10 for elispot assay and n = 26 for flow cytometry).

2.2. Standard protocol approvals, registration, and patients consents

The present study has been approved by the local ethics committee (Ethikkommission, Charité Universitätsmedizin Berlin) and clinical investigations were conducted according to the Declaration of Helsinki. All patients or their surrogates gave written informed consent to the study.

2.3. Peripheral blood monocytes (PBMC) preparation

Peripheral blood was obtained within 36 h after stroke onset, at day 3, at day 7 and at follow up (3 months at minimum) and sampled into CPT tubes. PBMC were isolated within 5 h after venipuncture by density gradient centrifugation at 1500*g* for 20 min.

2.4. Flow cytometry

Flow cytometric analysis of human lymphocyte subsets in EDTA whole blood was performed as described recently [27]. Briefly, the following mouse anti-human fluorescently-labelled monoclonal antibodies were used for quantification of lymphocytes and T cell subsets: cluster of differentiation (CD)3 Allophycocyanine-Alexa Fluor 750 (APC-A750), CD4 energy coupled dye (ECD), CD8 APC, CD14 Fluorescein isothiocyanate (FITC), CD16 Phycoerythrine (PE), CD19 PE-Cy5.5, CD56 PE, CD45RA Pacific-Blue (PB), CD45 Krome-Orange (KrO), (all from Beckman Coulter) and CCR7 Phycoerythrine (PE) (R&D Systems). Stained samples were acquired on a ten-color Navios flow cytometer and analyzed using Navios Software (Beckman Coulter).

2.5. Myelin proteins and peptides

Synthetic myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) peptides which were 15 amino acids long overlapped by 10 single amino acids spanning the whole sequence of MBP (Table 1A) and MOG (Table 1B) were synthesized by Jerini Peptides technology (JPT) Berlin.

2.6. Elispot assay

Human IFN-γ ELISPOT assays were performed as previously described [31]. Briefly, ELISPOT plates were coated overnight at 4 °C with IFN-γ-specific capture antibody (eBioscience, San Diego, CA, USA) diluted in sterile phosphate buffered saline PBS (PAA, Velizy-Villacoublay, France) at 4 μg/ml. Blocked plates (1% in PBS—PBS/BSA) were washed, PBMC were then added to individual wells in complete RPMI medium (Biochrom, Berlin, Germany) in the presence of anti-CD28 (monoclonal) antibody (eBioscience, San Diego, CA, USA) at 1 μg/ml. The plated cell number was 3×10^5 PBMC/well for ELISPOT plates (Millipore, Billerica, MA). Peripheral myelin peptides as well as synthetic MBP peptides were added at 10 μg/ml. As a positive control for T cell responses, CEF, a peptide pool containing viral antigens was added at 10 μg/ml [32]. The cells were incubated at 37 °C for 18–24 h in the presence of 5% CO_2 and washed afterwards with PBS followed by four washes with PBS with 0.025% Tween (Serva Electrophoresis, Heidelberg, Germany). The detection antibody, mouse anti-human IFN-γ-biotin (eBioscience, San Diego, CA, USA), was diluted in PBS-BSA-Tween and added at a concentration of 2 μg/ml in 100 μl. After an overnight incubation at 4 °C, plates were washed again with PBS-Tween and incubated for 2 h with Streptavidin-HRP (Biolegend, San Diego, CA, USA) at 1:1000 followed by development with 3-Amino-9-ethyl carbazole reagent (Sigma Aldrich, St. Louis, MO, USA). The resulting spots were counted using a computer-assisted ELISPOT image analyzer (Cellular Technologies, Cleveland, OH, USA) and custom software. Antigen-specific frequencies were calculated by subtracting the number of detected spots in the absence of antigen from the frequency of spots obtained in the presence of relevant antigen (performed in triplicate).

2.7. Statistical analysis

All statistical tests were performed using GraphPadPrism 5.0 software. Patient measurements were compared with HC using Kruskal-Wallis Test followed by post-hoc unpaired *t*-test or Mann-Whitney-test, were appropriate. Level of significance was defined as $p < 0.05$ for all comparative tests. For quantitative analysis of MOG and MBP responses mean ± SD, median as well as range were calculated.

3. Results

3.1. Study population

Mean age of the patients (n = 28) was 70.0 (range 46–87), of whom 10 (35.7%) were female and 18 (64.3%) were male. Mean age of the patients tested in the Elispot assay (n = 18) was 70.7 (range 46–87) of whom 8 (44.4%) were female. Mean NIHSS at admission was 10.3 (range 7–18). Modified Rankin Scale at admission was 4.0 (range 1–5) and 3.7 (range 0–6) at follow up (range 3–21 months). After admission 5 patients developed pneumonia (17.9%). According to TOAST criteria 11 patients (39.3%) had large artery thrombosis, 8 cardioembolic (28.6%), one patient (3.5%) other cause, 2 patients undefined cause (7.1%) and one patient concurrent etiology (3.5%), from 5 (17.8%) patients information were missing. Mean age of HC (group for Elispot n = 9) was 67.6 (range 58–76) of whom were 5 (50.0%) were female. For FACS analysis (n = 26) mean age was 59.56 (range 40–84) with a female proportion of 16 (61.6%).

3.2. Autoreactive MOG- and MBP-specific T cell response in patients early after stroke onset

Tables 1A and 1B show the overlapping peptides we have used in this study with human immunodominant epitopes (marked in bold) for MBP (Table 1A), [28,29] and for MOG (Table 1B), [28,30]. Autoreactive MBP and MOG-specific IFNγ responses tended to be

Table 1A
MBP peptide pool used for the Elipot assay with immunodominant peptides (marked in bold) [28–30].

Pool 1	1-15, 5-19, 9-23, 13-27, 17-31, 21-35, 25-39, 29-43, 33-47,37-51
Pool 2	41-55, 45-59, 49-63, 53-67, 57-71, 61-75, 65-79, 69-83, **73-87, 77-91**
Pool 3	**81-95**, **85-99**, **89-104**, 93-107, 97-111, 101-115, 105-119, 109-123, 113-127, 117-131
Pool 4	121-135, 125-139, **129-143**, **133-147**, 137-151, 141-155, 145-159, 149-164, 153-167, 157-171