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Cross-reactivity between human cytomegalovirus peptide 981-1003 and myelin oligodendroglia glycoprotein peptide 35-55 in experimental autoimmune encephalomyelitis in Lewis rats

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ABSTRACT

Multiple sclerosis (MS) has been documented to have various clinical and pathological presentations. However the underlying mechanisms remain unknown. Viral infections may play a certain role in the etiopathogenesis of MS. This study was designed to explore whether different phospholipid peptides and viral mimic peptides induce antigen specific lesion in experimental autoimmune encephalomyelitis (EAE), an MS animal model. In the present study, Lewis rats immunized with myelin basic protein (MBP) 82-99 or MBP68-86 exhibited clinical signs of EAE and inflammatory infiltrates throughout CNS. Immunization with myelin oligodendroglia glycoprotein (MOG) 35-55 also induced inflammatory infiltrates in spinal cords. Although cytomegalovirus (CMV) 981-1003 failed to induce clinical signs of EAE and inflammatory infiltrates, immunological examination revealed that CMV981-1003 cross-reacted with serum from rats immunized with MOG35-55, and vice versa. Further, MOG35-55 triggered CMV981-1003 specific lymphocytes recruitment in spleen. Together these, this study provides certain evidences for various pathological manifestations of EAE and the linkage of viral mimic peptides with phospholipid peptides. Molecular mimicry may be an explanation the pathogenesis of MS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), characterized by perivascular inflammatory infiltrates, demyelination and axon injury [1]. As the clinical manifestations and pathological features of MS vary among patients [2], the mechanisms underlying the diversity of pathological features are far to be elucidated. Recent studies have suggested that the symptoms and prognosis of MS are correlated with inflammatory response in the specific regions of the CNS [3,4]. Experimental autoimmune encephalomyelitis (EAE) is the most available animal model of human MS so far. In this model, animals are immunized with myelin components, including myelin basic protein (MBP) and myelin oligodendroglia glycoprotein (MOG) to develop inflammation, and at times demyelination and axon damage [5]. It has been demonstrated without doubt that different genetic backgrounds produce different distribution of lesions and clinical symptoms/courses [6]. Some studies demonstrated that the properties of antigen specific-T cells determined disease sites, clinical presentations, and cellular pathology in mice [7].

The etiology of MS has been always in disputed. Autoantibodies and autoreactive T cells, were detected in MS patients [8]. Although to date no virus has been recognized as a causative factor of MS, viral infections may play an important role in the autoimmunity of the disease [9,10]. Molecular mimicry was proposed to explain how viruses might trigger such autoreactive immune responses in MS [11], which involved de novo activation of autoreactive T cells, due to the cross-reactivity between self epitopes and viral epitopes during virus infection [12]. EBV DNA polymerase 627-641 (EBV627-641) shares similar amino acid sequence with MBP82-99, while human cytomegalovirus major capsid protein UL86 981-1003 (CMV981-1003) shares with rat MOG 35-55. There also exists sequence similarity between core B-cell epitope (MBP86-95) and EBV627-641, and rat T-cell epitope (MOG44-55) and CMV981-1003. These findings trigger a question whether immunization with EBV627-641 and CMV981-1003 induces antigen specific lesion topology through molecular mimicry.

Thus, in the present study, we immunized Lewis rats with different myelin antigens and viral mimic peptides to explore whether they induced different pathological features, and whether there exists cross-reactivity between the myelin antigens and viral mimic peptides.

Abbreviations: EBV627-641, EB virus DNA polymerase 627-641; CMV981-1003, cytomegalovirus major capsid protein UL86 981-1003.

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2. Materials and methods

2.1. Animals

Female Lewis rats (6–8 weeks, 160–180 g, specific pathogen free) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), and housed individually in room with specific pathogen free standards at a constant humidity and temperature, with food and water available *ad libitum*. The animal room was on a 12/12 h light/dark cycle. This study was strictly in accordance with institutional animal care procedures of Capital Medical University. All operations were performed under sodium pentobarbital anesthesia.

2.2. Preparation of phospholipid antigens and viral peptides

MBP82-99 (DENPVVHFFKNIVTPRTP), MBP68-86 (YGSLPQKSQRSQDENPV), MOG35-55 (MEVGWYRSPFSRVVHLYRNGK), EBV627-641(TGGVYHFVKKHVHES), and CMV 981-1003 (HEYNWLRSPFSRYSATCPNVLH) were synthesized by Ji'er Biochemical Company (Shanghai, China). The amino acid sequences were analyzed and the peptides were purified with high pressure/performance liquid chromatography. The purity was $\geqslant$98%.

2.3. Induction and clinical assessment of EAE

Lewis rats were divided randomly into seven groups (group A–G, 10–20 rats/group). Group A served as normal control. Rats in group B were subject to bilateral subcutaneous injection of 100 μl Freund's complete adjuvant (CFA; Sigma, St. Louis, MO, USA) containing 4 mg/ml *Mycobacterium tuberculosis* (BD Difco, Detroit, MI, USA) and 100 μl PBS (pH 7.4) at the base of the tails. Rats in group C–G were immunized with MBP82-99 (100 μg), MBP68-86 (100 μg), EBV627-641 (250 μg), MOG35-55 (200 μg), or CMV981-1003 (200 μg), in addition of 100 μl CFA and 100 μl PBS. Neurological signs were evaluated and body weights were measured daily in a blinded fashion by two investigators. Clinical scores of EAE were graded according to the following criteria: 0, healthy; 1, limp tail; 2, mild to moderate hind limb paraparesis; 3, hind limb paraplegia; 4, quadriplegia; and 5, moribund or dead [13].

2.4. Histology and immunohistochemistry

At the 14th day post immunization, rats were sacrificed and perfused intracardially with saline and 4% paraformaldehyde. Cerebrums, brain stems, cerebellums, and spinal cords were removed and immersed in 4% paraformaldehyde for 4 h at 4 °C [14]. These tissues were then fixed in paraffin and cut into 4-μm-thick sections.

Ten equidistant sections from cerebrum, brain stem, cerebellum, and spinal cord of each rat were stained with hematoxylin and eosin (H&E). The number of perivascular inflammatory infiltrates with 20 or more aggregated cells was evaluated blindly at 200× magnification, and the number of perivascular inflammatory infiltrates per square centimeter (cm2) was calculated. Ten additional sections containing specific brain region from each animal were selected for Luxol Fast Blue staining.

Neuronal axons were evaluated with immunohistochemistry according to the manufacturer's instructions. Briefly, the sections were treated with 3% hydrogen peroxide for 10 min to quench endogenous peroxidase. The sections were incubated with primary antibody (anti-neurofilament-200, 1:50, Abcam) at 4 °C overnight. The sections were then incubated with horseradish peroxidase(HRP)-conjugated secondary antibody for 30 min at room temperature. Immunohistochemical reaction was revealed by using 0.05% 3,30-diaminobenzidine and 0.03% hydrogen peroxide as chromogen. After each incubation, the sections were thoroughly washed with PBS. Control sections were incubated with secondary antibody alone [15]. Digital images were taken using Adobe Photoshop (Adobe Systems), and analyzed by a blinded observer.

2.5. Real-time RT-PCR

Real-time RT-PCR was performed to examine mRNA expression of CCL-7, vascular cell adhesion molecule-1 (VCAM-1) and neurofilament medium (NEFM) according to previous study [16]. At the 10th day post immunization, cerebrums, brain stems, cerebellums, and spinal cords were removed and total RNA was extracted and reversely transcribed into cDNA respectively. The resultant cDNA was amplified by real-time PCR with designed primers. CCL7 primers were sense: 5′GGGACCAATTCATCCACTTGC3′ and antisense: 5′TCAGCACAGACTTCCATGCC3′. VCAM-1 primers were sense: 5′GGAAATGCCACCCTCACCTT3′ and antisense: 5′CAC CTGA GATCCAGGGGAGA3′. NEFM primers were sense: 5′TCTGTACACAC ACCGACAGC3′ and antisense: 5′CTGTGAGGGCGTCTTCCATT3′. Melting curve, which was measured immediately after amplification, showed single peak, indicating good product specificity. Results were presented as the levels of expression following normalization to housekeeping gene glyceraldehyde-3-phosphate dehydrogenase using the ΔΔCt method.

2.6. Determination of antibody cross-reactivity

Antibody cross-reactivity between phospholipid peptides and viral mimic peptides was tested with indirect enzyme linked immunosorbent assay (ELISA) according to previous study [17]. Briefly, microtiter plates were coated with 4 μg/ml of MBP68-86, MBP82-99, EBV627-641 or CMV981-1003 overnight at 4 °C. A total of 100 μl serum sample from group A, B, C, D, E, F, G diluted in bovine serum albumin (1:100) was added in wells in duplicate for 2 h. After wash, HRP-conjugated anti-rat IgG was added for 1 h, followed by 3,3′5,5′-tetramethyl benzidine dihydrochloride (TMB). Optical density (OD) was measured at 450 nm using ELISA reader. Absorbance values were corrected by subtracting the OD value obtained in wells without antigen. Anti-MOG35-55 antibody in serum was detected with anti-MOG35-55 IgG quantitative ELISA kit (Anaspec, Fremont, CA, USA) following the manufacturer's instructions. The results were expressed as ng/ml.

2.7. Preparation of spleen mononuclear cells (MNCs) and enzyme linked immunospot assay (ELISpot)

Spleens were dissected under aseptic conditions, and spleen MNCs were enriched with a 1.083 g/ml Percoll gradient centrifuge (Pharmacia, Piscataway, NJ, USA) for 20 min at 450 g. Vital cells were counted by means of trypan blue dye exclusion staining.

An ELISpot was performed to examine the cross-reactivity in MNCs between phospholipid peptides and viral mimic peptides, according to the manufacturer's instructions. In brief, the Multi-Screen$_{HTS}$ filter plates were coated with IFN-γ antibody and blocked with RPMI 1640 medium containing 10% fetal calf serum for 4 h. The MNCs suspension (4 × 105 cells/well) containing different myelin peptide or viral peptide (10 μg/ml) was added. The plates were then incubated for 20 h in a humidified incubator with 5% CO_2 at 37 °C. TMB was added in the plates until the distinct spots emerged. The spots were inspected and counted with an ELISpot reader. Data were presented as mean spot-forming units per 0.4 × 106 cells. Concanavalin A (5 μg/ml; Sigma, St. Louis, MO, USA) was used as positive control.