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ScienceDirect
Neuroscience Letters 422 (2007) 114–118
Neuroscience Letters
www.elsevier.com/locate/neulet

Changes in neuronal protein expression in LP-BM5-infected mice

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Received 30 January 2007; received in revised form 18 May 2007; accepted 21 May 2007

Abstract

Murine acquired immunodeficiency syndrome (MAIDS) induced by LP-BM5 murine leukemia virus is used as a model of human immunodeficiency virus (HIV)-related neurologic dysfunction. Mice infected with LP-BM5 have mnemonic abnormalities (i.e., spontaneous alternation behavior in the Y-maze and performance in the Morris water maze) and biochemical alternations (i.e., cytokines, platelet-activating factor, quinolinate, glutamate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) that produce neurologic symptoms similar to those observed in HIV-related neurologic dysfunction. To identify proteins associated with dysmnesia in the MAIDS model, we examined the expression of neuronal proteins in LP-BM5-infected mice using two-dimensional polyacrylamide gel electrophoresis (2-DE). Neuronal protein expression in LP-BM5-infected mice was compared with that in non-infected mice using the Image Master 2D. We detected approximately 800 protein spots, of which 35 were distinguishable between non-infected and LP-BM5-infected mice. Most of these spots were downregulated in LP-BM5-infected mice. Three of the spots were identified as 14-3-3 protein zeta/delta, synapsin 2 and protein disulfide isomerase using a capillary nanoliquid chromatography tandem mass spectrometric system. We verified the expression levels of these proteins by Western blot. Analysis of these 35 spots could provide insight into mechanisms of dysmnesia in the MAIDS model of HIV-related neuronal dysfunction.

Keywords: Murine acquired immunodeficiency syndrome; Neurologic dysfunction; Two-dimensional polyacrylamide gel electrophoresis

Murine acquired immune deficiency syndrome (MAIDS) is induced by infection with the LP-BM5 murine leukemia retrovirus mixture. LP-BM5-infected mice have impaired cognition and attention [5]. Similarities between the neurologic and immunologic characteristics of LP-BM5 and human immunodeficiency virus type 1 (HIV-1) infection suggest that LP-BM5-infected mice might also model some aspects of human acquired immune deficiency syndrome (AIDS) dementia complex [6]. HIV-1 is the causative agent of AIDS, which affects multiple systems, including the central nervous system (CNS). The syndrome of cognitive and motor dysfunction observed after infection with HIV-1 is termed HIV-associated dementia. Mice infected with LP-BM5 show the mnemonic abnormalities and biochemical changes. Alterations in neuropeptide levels observed in LP-BM5-infected mice might result from increased neuronal activation by glutamate or quinolinic acid [2]. An increase in quinolinic acid occurs in response to cytokines, which are produced by the host in response to viral infection [12]. In addition, activation of *N*-methyl-D-aspartate receptors increases in brain platelet-activating factor [11]. Finally, there is evidence that tumor necrosis factor-α has a major role in causing or promoting neuronal damage, leading to cognitive deficits [4]. These results indicate that LP-BM5-infected mice sustain significant neuronal damage, which might contribute to their behavioral deficits. In the present study, we applied proteome analysis using two-dimensional polyacrylamide gel electrophoresis (2-DE) coupled with mass spectrometry to identify proteins associated with AIDS encephalopathy.

C57BL/6 mice (5–6 weeks old) were used in this study. Mice were infected by intraperitoneal (i.p.) injection of 0.2 ml LP-BM5 murine leukemia retrovirus mixture. Non-infected mice were injected with 0.2 ml culture media from LP-BM5-infected SC-1 cells. After 10 weeks after virus infection, hippocampi from LP-BM5-infected and non-infected mice were harvested.

Tissue samples in LP-BM5-infected ($n = 3$) and non-infected mice ($n = 3$) were homogenized with lysis buffer (tissue weight:lysis buffer volume = 0.1 g:0.5 ml) containing 20 mM Tris–HCl pH 7.0, 7 M urea, 2 M thiourea, 4% CHAPS, 1 mM

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0304-3940/$ – see front matter © 2007 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2007.05.059

EDTA, 1 mM phenyl methyl sulfonil fluopide, 10 mM DTT, protease inhibitors (pepstatinA, aprotinin, leupeptin, and antipain). The suspension was sonicated twice for 5 s and incubated for 30 min at room temperature. Sonicated samples were centrifuged at 150,000 × *g* for 45 min. Protein concentration was estimated using Bradford's protein assay (Bio-Rad). The supernatant was mixed with delipidating solution. The pellet was mixed in 9 M urea, 4% CHAPS, 10 mM DTT, and 0.001% bromophenol blue for 30 min at room temperature. The sample was then centrifuged at 15,000 × *g* for 15 min, and the supernatant was used for isoelectric focusing (IEF). For silver staining, 60 μg of protein was used, and approximately 400 μg was used for protein identification.

We performed 2-DE in a horizontal electrophoresis system, IPG phor™ (GE Healthcare Biosciences) for the first-dimensional IEF using 13 cm immobilized nonlinear pH 3–10 gradient (IPG) strips (GE Healthcare Biosciences). A multi-step IEF voltage program was applied to the strips on an Ettan IPGphor IEF System (GE Healthcare Biosciences): 30 V for 2 h, 100 V for 2 h, 200 V for 5 min, a 200 V to 5000 V gradient (3 V/min) for 24 h, and 8000 V for 3 h. Strips were reduced by incubation in the equilibration buffer (6 M urea, 50 mM Tris pH 8.8, 2% SDS, 30% glycerol, and 1% (w/v) DTT) and then alkylated in the same buffer containing 2.5% (w/v) iodoacetamide instead of DTT. The second dimension was accomplished by running the strips on 1.5-mm thick 12.5% (w/v) SDS polyacrylamide gels using the Protean II XL Multi-Cell (Bio-Rad). The electrophoresis unit was cooled to 20 °C with a water circulation system and 40 mA constant current was applied to the gels. After two-dimensional separation, the gels were stained with silver stain or GelCode Blue Stain (Pierce). Image analysis was performed by using the Image Master 2D platinum 6.0 (GE Healthcare Biosciences).

For protein identification, individual spots were digested with trypsin. Digested proteins were analysed using a nanoscale capillary liquid chromatography system (Shimadzu) coupled to an ion-trap tandem mass spectrometer LCQ Advantage Max (Thermo Electron). The proteins were identified by MASCOT (MATRIX SCIENCE), and the parsing software DBParser (http://www.proteomecommons.org/archive/1109121060785-/DBParserMain.html).

Western blot analysis of protein expression was performed according to standard procedures. Samples of protein extracts from individual animals, LP-BM5-infected ($n = 6$) and non-infected mice ($n = 6$), containing 50 μg of proteins were migrated on 10% polyacrylamide gels and transferred onto PVDF membranes. Antibodies to 14-3-3 protein zeta/delta (rabbit polyclonal antibody, BioLegend), synapsin 2 (rabbit polyclonal antibody, abcam) and protein disulfide isomerase (PDI) (mouse monoclonal antibody, Stressgen) were used as primary antibodies; anti-rabbit IgG antibody, anti-mouse IgG antibody conjugated to HRP were used as secondary antibody conjugates. Membranes were treated with ECL plus mixture (GE Healthcare Biosciences), and the chemiluminescence signal was recorded using a Hyperfilm ECL (GE Healthcare Biosciences). The quantitative analysis of the resulting images was performed using the KODAK ID 3.5.4 software (KODAK) and the results were normalized using the GAPDH signal detected on the same membrane. The optical density of each image was determined by converting the gray scale values to absolute optical density units using a calibrated film scale.

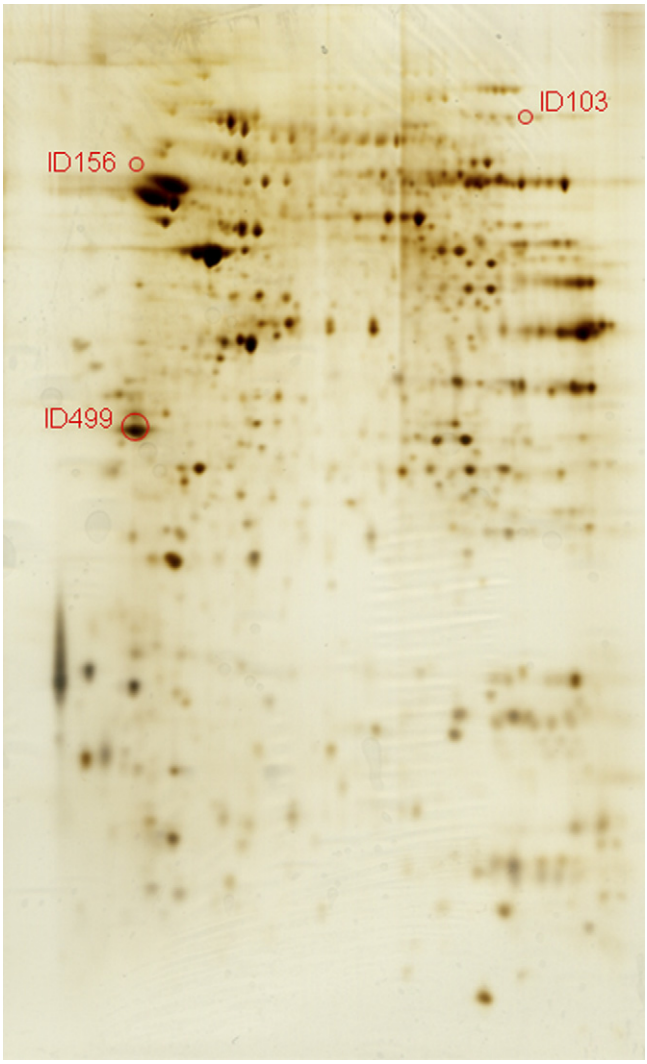

Fig. 1. Silver-stained 2-DE gel of proteins extracted from a hippocampus of non-infected mice. Proteins with p*I* from 4 to 9.5 and mass from 12 to 250 kDa were resolved. The differences in expression of three protein spots were pointed out with circle.

Statistical analysis of the difference between groups was assessed using Student's *t*-test.

The extent of LP-BM5 infection and immunosuppression in mice at 10 weeks post-infection was assessed by spleen weight. Spleen weight was approximately six-fold greater in LP-BM5-infected mice compared to non-infected mice. The development of spatial learning and memory deficits in LP-BM5-infected mice is preceded by inflammation in the CNS and neurodegeneration [7–9]. We demonstrated that hippocampus is covered with certain memory functions in LP-BM5-infected mice and non-infected mice in this study.

To compare proteome profiles of LP-BM5-infected and non-infected mice, we performed 2-DE combined with mass spectrometry. We extracted proteins from mouse hippocampi