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Novel brain reactive autoantibodies: Prevalence in systemic lupus erythematosus and association with psychoses and seizures

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Abstract

Autoantibodies can cause neuropsychiatric manifestations in lupus patients by altering the physiological function of neuronal cells. In this study, we identified Brain Reactive Autoantibodies (BRAAs) against murine neuronal membrane proteins (M.W. 27.5 and 29.5 kD) and found them correlating with psychosis and/or seizures in lupus patients. They were specific to neuronal membrane tissues of mammalian origin and are significantly associated with psychosis and/or seizures ($p < 0.0001$). These membrane proteins mass spectrometry profiles did not match to any published protein sequences. These BRAAs may play important roles in the pathophysiology of neuropsychiatric lupus.

Keywords: Neuropsychiatric lupus; Brain reactive autoantibodies; Psychosis; Seizures

1. Introduction

Systemic Lupus Erythematosus (SLE) is a chronic and potentially fatal autoimmune disease which attacks many organ systems during its disease course. Significant organs involved in SLE are the kidneys (lupus nephritis) and the brain (neuropsychiatric lupus) (Cameron, 1999; West et al., 1995). It was reported that between 31% and 70% of SLE patients has significant neuropsychiatric (NP) manifestations in the course of their disease (West et al., 1995; Kaell et al., 1986; Futrell et al., 1992; Sibley et al., 1992; Rood et al., 1999).

The spectrum of NP manifestation in SLE includes both neurologic and psychiatric features, many of which can be subjective to both patients and clinicians. They vary from overt neurologic dysfunctions due to psychoses, seizures, to subtle abnormalities in neurocognitive functions such as memory, intellect. (Hanly and Hong, 1993). They cannot be easily differentiated because lupus itself, drugs used in lupus management or other associated pathological conditions may be responsible for the neuropsychiatric manifestations. Currently the diagnosis of NP-lupus has to depend largely on exclusion of other causes, as the pathogenesis is yet unclear (Hanson et al., 1992).

More than 100 different autoantibodies have been found in both human and animal lupus studies (Sherer et al., 2004). Among these autoantibodies, some may become "pathogenic" against self-antigens and result in (a) pathological change in the target organ and/or (b) binding of autoantibodies to target antigen that may lead to altered physiological function of target antigens (Tung, 1994).

Pathogenic roles of many autoantibodies are not well defined in SLE. Prevalence of brain reactive autoantibodies detected in sera and cerebrospinal fluid (CSF) in both human and animal studies were reported (Hanson et al., 1992; Bluestein and Zvaifler, 1976; Wilson et al., 1979; Toh and Mackay, 1981; Hoffman et al., 1987; Moore, 1992; Zameer and Hoffman, 2001). However, not all brain reactive autoantibodies are responsible for NP-lupus, i.e. they are not

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necessarily of pathogenic importance (Khin and Hoffman, 1993).

Of these autoantibodies, some pathogenic autoantibodies may target neuronal tissue and lead to neuropsychiatric manifestations. These autoantibodies can bind to neuronal surface membranes (Koren et al., 1992) and cause injury via a direct toxic effect, or they disrupt the physiological functions of neuronal tissue when bound, but without causing cell death. In preliminary results, we identified novel antibodies present in the sera of lupus patients that were reactive to neuronal membrane antigens. We then proceeded to study these autoantibodies in an unselected group of SLE patients and evaluate whether they are associated with neuropsychiatric manifestations in lupus patients.

2. Materials and methods

2.1. Human subjects and sera collection

One hundred unselected SLE patients, fulfilling the 1997-updated ACR criteria for classification of SLE were recruited into the study (Hochberg, 1997). One hundred and thirty non-lupus patients (20 rheumatoid arthritis (RA), 20 osteoarthritis (OA), 20 ankylosing spondylitis (AS), 20 psoriatic arthritis (PsA) and 50 primary anti-phospholipid syndrome (APS)) were also recruited, and all of them satisfied current diagnostic criteria for their corresponding diseases. One hundred and two healthy controls, recruited from among healthcare workers and medical students, were also included in this study.

Five milliliters of peripheral venous blood in plain tubes were obtained after informed consent. The venepunctures were timed to coincide with review visits at the Rheumatology Clinics. They were processed to separate the sera from clotted blood using standard protocol. All sera were kept in −80 °C freezer until further experiments.

A standard protocol was used to record lupus patients' clinical data, ACR criteria and neuropsychiatric manifestations by chart review. This study was approved by the hospital's institutional review board.

2.2. Animal selection

Two 8-week-old female BALB/c mice purchased from the Animals Holding Unit, National University of Singapore, were used to extract membrane proteins from brain, heart, liver, kidney and spleen. The female Wistar rat's brain was a gift from Ms. Irene Kee of Department of Experimental Surgery, Singapore General Hospital. The brains of pig, chicken, duck, quail, frog and fish (Indian Mackerel—*Rastrelliger kanagurta*) were purchased from local markets.

Human brain membrane protein (normal adult male) was purchased from BioChain, USA.

2.3. Membrane protein extraction

All chemicals were purchased from Sigma Aldrich, unless otherwise mentioned.

The frozen/fresh tissue was thawed and cut into small pieces, followed by homogenization using mortar and pestle in 1 × PBS containing a cocktail of proteinase inhibitors (5 μg/ml leupeptin, 5 μg/ml approtinin and 1 mM phenyl methyl sulfonyl fluoride). Brain lysates were further homogenized using syringe and needles method (starting from 19 G and gradually reduced in bore sizes to 27 G). The water-insoluble pallet was separated by centrifugation at 12,000 ×*g* for 10 min at 4 °C. Repeated washings with 1 × PBS containing cocktail of protein inhibitors were done until proteins were not detected in wash buffer using BioRad protein assay. The water-insoluble protein was dissolved in 1 × PBS containing 6 M urea together with 1 mM phenyl methyl sulfonyl fluoride and incubated on shaker for 15 min at room temperature. The urea-soluble protein was separated using centrifugation at 12,000 ×*g* for 30 min at 20 °C. The protein concentration was measured using BioRad protein assay and aliquoted proteins were kept in −80 °C freezer until future use.

The membrane proteins were extracted from different mouse tissues (brain, heart, liver, kidney and spleen) using the protocol described. The same protocol was also used to extract neuronal membrane proteins from different species (rat, pig, duck, chicken, quail, frog and fish).

2.4. Immunoblotting

The sodium dodecyl sulphate–polyacrylamide gel electrophoreses (SDS–PAGE) were prepared using analytical comb or preparative comb according to the needs of experiments. The membrane proteins (20 μg proteins per well for analytical comb or 200 μg proteins per well for preparative comb) were denatured and separated in 20% SDS–PAGE. Upon completion of protein separation, the polyacrylamide gels were either visualized with Coomassie blue or silver stain or transferred to Hybond nitrocellulose membrane (Amshersham). Coomassie Brilliant Blue R-250 (BioRad) and SilverPlus silver staining kit (BioRad) were compatible with Mass Spectrometry (MS) analysis.

The electrophoretic transfer of separated proteins to nitrocellulose membrane was performed at 100 constant Volts for 2 h using buffer transfer tank in cold room. The nitrocellulose membrane, on which the proteins were transferred, was incubated in blocking solution (3% scanned milk powder in 1 × TBS-T; 1 × TBS containing 0.1% Tween 20) on shaker for 1 h at room temperature to block non-specific reactions prior to sample incubation. Depending on the purpose of experiment, nitrocellulose membrane with proteins separated using analytical combs were incubated with samples while those with proteins separated using preparative combs were cut into strips and