Neuropsychiatric Manifestations Associated with Anti-Endothelial Cell Antibodies in Systemic Lupus Erythematosus

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ABSTRACT: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multisystem involvement due to immune dysregulation. Neuropsychiatric systemic lupus erythematosus (NPSLE) includes neurological syndromes involving the central, peripheral and autonomic nervous system, as well as psychiatric syndromes observed in patients with SLE in which other causes have been excluded. The pathogenesis of NPSLE has been attributed to many different mechanisms. In particular, autoantibody-mediated vasculopathy seems to play a major role in the pathogenesis of the clinical features. Several autoantibody specificities have been reported in the serum and cerebrospinal fluid of NPSLE patients. Recently, we demonstrated an association between serum anti-endothelial antibodies (AECA) and psychosis or depression in SLE patients, strengthening the notion of a possible role of this class of autoantibodies in the pathogenesis of the disease. The study of these autoantibodies could be a useful diagnostic and prognostic tool in patients with NPSLE.

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KEY WORDS: systemic lupus erythematosus (SLE), neuropsychiatric systemic lupus erythematosus (NPSLE), serum anti-endothelial antibodies (AECA)

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multisystem involvement with a broad spectrum of clinical manifestations due to immune dysregulation causing production of autoantibodies, generation of circulating immune complexes, and activation of the complement system. Neuropsychiatric manifestations in SLE (NPSLE) include psychiatric and neurologic syndromes involving the central, peripheral and autonomic nervous system observed in patients with SLE in which other causes have been excluded. These manifestations can precede the onset of SLE or occur at any time during the course of the disease [1]. They may present as single or multiple neurologic events in the same individual. A large variety of neuropsychiatric disorders are reported in NPSLE patients, including depression, psychosis, anxiety, mood disorders, cognitive dysfunction, memory deficit, delirium/encephalopathy, and acute confusional states. The reported prevalence of psychiatric disorders in SLE ranges from 14% to 75% [2], reflecting the different methods of patient selection and assessment, the different professional orientation of clinicians, and the lack of an accepted consensus for diagnosing active NPSLE. Moreover, it appears that neuropsychiatric syndromes could even be prevalent in pediatric patients with SLE, manifesting early in the disease course and with significant morbidity.

Table 1. NPSLE syndromes defined by the ACR committee in 1999

Central nervous system	Peripheral nervous system
• Aseptic meningitis • Cerebrovascular disease • Demyelinating syndrome • Headache (including migraine and benign intracranial hypertension) • Movement disorder (chorea) • Myelopathy • Seizure disorders • Acute confusional state • Anxiety disorder • Cognitive dysfunction • Mood disorder • Psychosis	• Acute inflammatory demyelinating polyradiculoneuropathy (Guillain-Barré syndrome) • Autonomic disorder • Mononeuropathy, single/multiplex • Myasthenia gravis • Neuropathy, cranial • Plexopathy • Polyneuropathy

In 1999, the American College of Rheumatology (ACR) ad hoc committee, adopting the terminology of DSM IV, defined 19 NPSLE syndromes [3] [Table 1]. However, due to the reported inadequate performance of the proposed ACR criteria, as well as difficulties in differentiating SLE patients from controls and neuropsychiatric SLE patients from other SLE patients, some authors criticized the lack of criteria based on neuropathogenic features. Since no single diagnostic test is sensitive or specific for NPSLE, the proper diagnosis depends on the combination of physical examination, brain imaging, immunoserological, psychiatric and neuropsychological tests, and presence of lupus disease activity. Recommendations for the use of computed tomography (CT), standard magnetic resonance imaging (MRI), angiography, electroencephalography (EEG), echocardiography, and duplex ultrasound were included. In particular, MRI was demonstrated to be useful.

The most common findings are small spot T2-hyperintense lesions (gliotic foci) in the white subcortical matter, mainly concentrated in frontal-parietal regions. Other signs can be cortical atrophy, periventricular lesions, dilation of ventricular chambers, and ischemic lesions [4]. While gadolinium is extremely useful for the characterization of acute inflammatory lesions, only 19% of NPSLE patients are recognized by MRI. On the other hand, single photon emission-computed tomography (PET-CT) and in vivo magnetic resonance spectroscopy have demonstrated a sensitivity of almost 100% in active NPSLE, but low specificity, compared to MRI.

Neuropsychological tests have been standardized to examine five major areas: general intelligence, verbal learning/memory, visual-spatial skills, psychomotor speed/manual dexterity, and attention/mental flexibility. Furthermore, most of the immunochemical tests studied in NPSLE are considered investigational, except testing for antiphospholipid antibodies (aPL) and anti-ribosomal P antibodies (anti-P), which seem to play an important role in the pathogenesis of NPSLE.

PATHOGENESIS

Over the years, several groups have expanded our knowledge on the nature of NPSLE pathogenesis. Multiple mechanisms were proposed, including antibodies, vasculitis, thrombosis, hemorrhage, hypertension, accelerated atherosclerosis, choroid plexus dysfunction, neuroendocrine immune effects, direct central nervous system (CNS) tissue injury, and cytokine-mediated damage [2]. Rhiannon [5] distinguished primary from secondary mechanisms. The primary mechanisms include vascular occlusion/hemorrhage, autoantibody-mediated (primarily anti-neuronal antibodies), choroid plexus dysfunction, cytokine effects, neuroendocrine-immune imbalances, and direct neural tissue injury (mediated by oxidative stress, excitatory amino acid toxicity and matrix metalloproteinase injury). On the other hand, secondary mechanisms include infections, medications, thrombotic thrombocytopenic purpura, hypertension, uremia, fever, electrolyte imbalances, thyroid disease, berry aneurysm, fibromyalgia, cerebral lymphoma, subdural hematoma, atherosclerotic cerebrovascular accident/stroke (CVA), reactive depression, sleep apnea, and another primary psychiatric or neurologic disease. A variety of autoantibodies has been implicated in the pathogenesis of the disease [Table 2]. These were found in the serum and cerebrospinal fluid (CSF). Anti-neuronal antibodies are directed against brain synapses, neurofilaments, GFAP, MAP-2, and NMO. Non-neural-specific antibodies include anti-P, antiphospholipid antibodies (aPL), anti-dsDNA, anti-N-methyl-D-aspartate (NMDA, also known as anti-NR2), anti-gangliosides (GM-10) lymphocytotoxic antibodies, and anti-endothelial cell antibodies (AECA).

A proper and quick diagnosis is essential for managing neuropsychiatric manifestations in SLE patients

Table 2. Autoantibodies associated with NPSLE

Autoantibodies	Reference
Anti-ribosomal P protein P0 (anti-P)	[7-15]
Anti-glial fibrillary acid protein antibodies (anti-GFAP)	[13]
Anti-N-methyl-D-aspartate (NMDA) antibodies (anti-NMDA)	[13,15]
Antiphospholipid antibodies (aPL)	[13,16-20]
Anti-Nedd5 antibodies (anti-Nedd5)	[21]
Anti-NR2 glutamate receptor (anti-NR2)	[14]
Anti-endothelial cells (AECA)	[13,19,25-27]
Anti-neuronal	[33]
Anti-brain-reactive antibodies (BRAA)	[34]
Anti-microtubule-associated protein 2 (anti-MAP-2)	[35]
Anti-ganglioside (AGA)	[36]
Anti-serum lymphocytotoxic	[37]
Anti-neurofilament	[38]
Anti-Ro/SSA	[39]
Anti-Sm	[40]

THE ROLE OF AUTOANTIBODIES

The reported prevalence of autoantibodies in NPSLE is highly variable, depending on different ethnic background, sensitivity and specificity of the assays, and the time of the analysis with respect to the manifestation of the clinical event. In 1979, Bresnihan et al. [6] first suggested the potential pathogenic relevance of anti-neuronal, anti-P and anti-glial fibrillary acid proteins, and aPL in the psychiatric manifestation of SLE. More recently AECAs have been studied and correlated with the occurrence of such clinical events. The etiopathogenic role of autoantibodies in NPSLE remains unclear. Anti-neuronal antibodies can be found in the brain of NZB/W mice, supporting a direct pathogenic role. Given their higher reactivity in the CSF of patients with NPSLE, an intrathecal synthesis has been proposed. Nevertheless, the finding of cross-reactivity of lymphocytotoxic antibodies, anti-neuronal antibodies and mycobacterial glycolipids, together with impairment of the blood-brain barrier (BBB) frequently observed in NPSLE, may suggest the hypothesis of an extrathecal synthesis of the autoantibodies.

AUTOANTIBODIES TO BRAIN COMPONENTS

• ANTI-RIBOSOMAL P PROTEIN Po ANTIBODIES (ANTI-P)

Anti-P are directed against three phosphoproteins located on the larger 60 S subunit of eukaryotic ribosomes (P0, P1, P2). The prevalence of anti-P in SLE patients ranges from 6% to 36% [7] and their specificity for SLE was recently estimated at 99.4% [8]. Only a few studies have noted their presence in other diseases, e.g., autoimmune hepatitis and autism. The association between anti-P and lupus psychosis was first described by Bonfa and coworkers [9]. Yoshio et al. [10] suggested the existence of a strong association between immunoglobulin (Ig) G and IgM anti-P with CNS disease, excluding lupus psychosis, in SLE patients. Moreover, several studies have demonstrated a decrease in verbal memory, psychomotor speed, and olfaction in SLE patients compared with healthy subjects, correlating with disease activity and CNS involvement. Interestingly, a relationship between smell impairment and anti-P has been observed. These data were confirmed by a recent study in which affinity-purified human anti-P were injected intracerebroventricularly (ICV) in mice [11]. The results showed that these antibodies induce both depression-like behavior and impaired olfactory function. The ribosomal P0 protein has been identified by molecular cloning strategy as an endothelial autoantigen in SLE patients [12]. Moreover, purified IgG anti-P derived from patients with SLE seems to activate human umbilical vein endothelial cells (HUVEC), as well as monocytes, leading to intrathecal B lymphocyte activation upon increased interleukin-6 (IL-6) production. In our cohort, 7.8% of SLE patients were anti-P positive but no association with psychiatric disturbances was found [13]. Remarkably, all patients with lupus psychosis tested seronegative for these autoantibodies. Finally, a significant difference was documented between the enzyme-linked immunosorbent assay (ELISA) kits used for the detection of anti-P; hence, the need for standardization of laboratory assays in the future which will enable better assessment of both the presence of anti-P and their clinical significance [14].

• ANTI-GLIAL FIBRILLARY ACID PROTEIN ANTIBODIES (ANTI-GFAPS)

The GFAPs are 50 kDa intracytoplasmic filamentous proteins of the astrocytes, which stabilize the cytoskeleton and maintain astrocyte cell shape through the interaction with nuclear and plasma membranes. At present, these are the most specific markers to recognize cells of astrocytic origin in both normal and pathologic conditions. GFAPs are up-regulated in gliotic hypertrophy and perivascular inflammation of Alzheimer's disease and multiple sclerosis. In addition, anti-GFPA antibodies were observed in patients with these conditions. Recently, it was demonstrated that anti-GFAPs are increased in the CSF of NPSLE patients. Although we found anti-GFPAs in 15% of sera from our SLE cohort, no significant correlations with neurologic or psychiatric morbidity were observed.

Antiphospholipid and anti-P ribosomal antibody testing can be useful in NPSLE diagnosis

• ANTI-N-METHYL-D-ASPARTATE (NMDA) ANTIBODIES

DeGiorgio et al. [15] demonstrated in vitro that a subset of anti-DNA antibodies cross-reacts with N-methyl-D-aspartate (NMDA) and induces neuronal cell injury. These autoantibodies can be found occasionally in the CSF of SLE patients. Furthermore, it was shown that sera showing reactivity to DNA and NMDA receptor obtained from lupus patients can elicit cognitive impairment when intravenously injected in mice [16].

• ANTIPHOSPHOLIPID ANTIBODIES (APL)

Antiphospholipid antibodies are a heterogeneous group of antibodies directed against anionic phospholipids, phospholipid-binding plasma proteins, and phospholipid-protein complexes [17]. The association between aPL and NPSLE was first reported in 1984, and it is clear today that their main pathogenic effect results in thrombosis. aPL may contribute to neurologic damage by reacting with brain cells via β_2-glycoprotein I (β_2GPI) interaction. We have demonstrated the expression of β_2GPI mRNA by astrocytes and neuronal and endothelial cells, suggesting that these cells can be a target of autoantibodies in antiphospholipid syndrome (APS) [18,19]. No significant association was found between aPL and AECA [13], although it has been postulated that AECA reactivity might be partly caused by the binding to a complex of β_2GPI with phospholipids on endothelial cells. Nonetheless, Meroni et al. [20] found AECA positivity in 5 of 14 SLE patients with CNS involvement. The authors demonstrated that aPL and AECA are associated with neurological manifestations in SLE patients.

In another study, human endothelial cells were incubated with mouse AECA monoclonal antibodies, and the translocation of phosphatidylserine (PS) was established through the binding of annexin V, which binds specifically to PS. A rabbit β_2GPI antibody and biotin-conjugated F(ab')2 aPL derived from three patients were also used to detect β_2GPI on the cells. The authors found that 20–36% of the cells expressed anionic PL following incubation with AECAs, suggesting that some of these may be pathogenic and may even have the potential to induce production of aPL [21]. These in vivo observations were further validated by studies conducted on mouse models of APS, showing the development of neurological dysfunction and hyperactive behavior associated with aPL, anti-β_2GPI and AECA after passive immunization with human anticardiolipin (anti-CL) monoclonal antibodies. Interestingly, we recently investigated whether cognitive impairment in SLE is associated with serum autoantibodies, disease activity and chronic damage. Antinuclear antibodies (ANA), anti-dsDNA, anti-CL, anti-β_2GPI, anti-P, AECA and anti-Nedd5 antibodies were evaluated, and SLEDAI-2000 and SLICC were used to assess disease activity and chronic damage, respectively. We reported a significant association between aPL, disease activity, and chronic damage with cognitive dysfunction in SLE. In particular, anti-CL IgM were found associated with visual-spatial domain impairment ($r = 0.331$, $P = 0.005$) [22], strengthening the view of aPL involvement in NPLE manifestations.

• ANTI-NEDD5 ANTIBODIES

Nedd5 is a septin that plays an essential role in cytokinesis in mammalian cells. During apoptosis, Nedd5 moves from the cytoplasm to the cell surface, a process that might explain its increased immunogenity [23]. Although anti-Nedd5 autoantibodies are not specific to SLE, they are significantly associated with NPSLE and could serve as an immunological marker of psychiatric manifestations.

• ANTI-NR2 GLUTAMATE RECEPTOR

As mentioned earlier, in 2001 DeGiorgio et al. [15] showed that anti-dsDNA autoantibodies from sera of SLE patients cross-react with NR2 glutamate receptors and mediate apoptotic death of neurons in vivo as well as in vitro. Following these results, it was demonstrated that IgG anti-NR2 antibodies in the CNS of SLE patients induce EC activation. Moreover, by activating the nuclear factor kB (NF-κB) signaling pathway, these antibodies may lead to inflammation of the blood-brain barrier, thereby possibly initiating the pathogenesis of NPSLE [24]. Most studies found anti-NR2 antibodies in blood in approximately one-third of SLE patients, at a higher ratio than in patients with other autoimmune diseases. Anti-NR2 antibodies are observed more often in the CSF of NPSLE patients than in the CSF of patients without autoimmune, neurologic or psychiatric diseases. Moreover, SLE patients with acute CNS manifestations or septic meningitis may present higher intrathecal concentrations of anti-NR2 antibodies than SLE patients hospitalized for other reasons. Nonetheless, their role in cognitive dysfunction and psychiatric manifestation of SLE is still under debate.

AECA AND NPSLE

AECAs were first described in 1971 by Lindqvist and Osterland [25] in chronic tuberculosis. These antibodies target a heterogeneous group of antigens directed against different structural endothelial proteins, ranging from 10 [25] to 200 KDa, as well as adhering to endothelial cells detected in a variety of diseases sharing vessel wall damage. AECAs have been detected in healthy individuals, as well as in autoimmune and systemic inflammatory diseases including nervous system diseases. The prevalence of AECA in SLE patients ranges from 17% to 75%. We recently reported an association between serum AECA with psychosis and depression in patients with SLE [13], strengthening the view of a possible role of AECA in the development of psychiatric disorders.

AECA were shown to be highly associated with NPSLE and their pathogenic role has been demonstrated

• ANTIGEN CHARACTERIZATION

AECAs are generally not specific for endothelial cells and a variety of antigens can be found on different substrates. AECA antigenic specificity is also observed in fibroblasts, leukocytes and monocytes. AECAs recognize antigens expressed constitutively, other cryptic antigens that are cytokine-induced, as well as adhesion molecules. Even the human leucocyte antigen class I and II (HLA) determinants as well as extracellular matrix components (i.e., collagen types II, IV and VII, vimentin or laminin) constitute endothelial cell antigens. Autoantibodies against these antigens have been reported in systemic sclerosis (SSc) and SLE. Several molecules could bind to endothelial cells and become the so-called planted target antigens for AECA via presumed charge-mediated mechanisms, a DNA-histone bridge, or a specific receptor. Some examples are myeloperoxidase, DNA and β_2GPI, which might adhere to endothelial cells incubated with patient's sera. AECAs can recognize antigens present solely in microvascular but not macrovascular endothelial cells. It seems that phenotypic and functional differences – such as nutritional requirements and responses to growth and migration stimuli – between endothelial cell antigens from microvascular and macrovascular sites may be responsible for this phenomenon. Thus, the evaluation of endothelial cells from vessels of different sizes seems advised in AECA assays.

The development of techniques to identify new antigens targeted by AECAs has allowed the building of expression libraries of complementary DNA to messenger RNA extracted from endothelial cells and then transfected into prokaryotic or eukaryotic cells. Belizna et al. [26] characterized the putative target antigens for AECA, recognizing a role for EC-specific plasminogen activator inhibitor, ribosomal P protein P0, ribosomal protein L6, elongation factor 1a, adenylcyclase-associated protein, DNA replication licensing factor, profilin II, and human EC-associated lupus autoantigens 1 and 255. Furthermore, it was shown that the levels of these antibodies directly correlate with AECA levels and clinical findings in patients affected with SLE and systemic vasculitis. Another technique, two-dimensional electrophoresis, seems to be promising. Indeed, combined with western blot analysis using protein extracts from a hybridoma cell line, it enables the identification of antigens such as calreticulin, tubulin, vimentin and Hsp70. Recently, RLIP76 was identified as a new AECA autoantigen. RLIP76 catalyzes the ATP-dependent transport of glutathione (GSH) conjugates including GS-4-hydroxy-*t*-2,3-nonenal (GS-HNE) [27]. ELISA detected IgG specific to RLIP76 in 30% of patients with Behçet disease (BD), 17% with SSc, 21% with rheumatoid arthritis (RA), and 30% with SLE, but in none of the sera from patients with mononucleosis or from healthy subjects. These results suggest that RLIP76 may play a major role in endothelial dysfunction in distinct autoimmune diseases.

Another newly discovered endothelial antigen is RABPT5, an essential and rate-limiting component of early endosomal fusion that regulates the release of neurotransmitter and neuritis outgrowth. RABPT5 is massively redistributed in the cytoplasm of endothelial cells during apoptosis, possibly explaining its immunogenicity. Anti-RABPT5 antibodies bind and hinder the function of the RABPT5 protein in the neurons. This chain of events leads to the inhibition of endosomal membrane fusion which performs a protective function against amyloid deposit. In addition, anti-RABPT5 might cross-react with neurocrescin, hampering its ability to regenerate damaged neurons, suggesting a pathogenic role for these antibodies. There is evidence for a specific clone of AECA directed to actin, particularly in patients with atherosclerosis. It is well known that the humoral immune response to endothelium has a pivotal role in the development of atherosclerosis. In a previous study by our group [22], we performed immunoscreening of a human umbilical artery endothelial cells (HUAEC) expression library with IgG from two patients with carotid atherosclerosis and identified a clone specific to actin. The actin-specific IgG reactivity was evaluated in patients with carotid atherosclerosis, and the results were compared with those obtained from SLE and T1DM patients and from healthy subjects. Actin-specific IgG reactivity was detected in a significantly higher percentage of sera from patients with atherosclerosis and SLE than from healthy subjects (26% and 39%, respectively, vs. 5% of healthy subjects; $P = 0.012$ and $P < 0.0001$). Therefore, actin can represent an autoantigenic molecule of potential clinical interest in carotid atherosclerosis and SLE vasculitis.

Another endothelial antigen targeted by AECA is Sip-1. This is a nuclear splicing factor containing an arginine/serine-rich domain and a RNA-binding motif probably involved in transcription and pre-mRNA splicing. In a previous study, we demonstrated that IgG, IgM, and IgA specific to factor Sip1 C-ter were detectable in patients with several autoimmune diseases characterized by the presence of serum AECA, such as BD, SLE, SSc, and primary vasculitis as well as in patients with diseases that share some clinical features with BD (inflammatory bowel disease and uveitis). We also found that IgM immunoreactivity was significantly higher in patients with BD and in patients with primary vasculitis than in the other tested groups [28].

Finally, the presence of AECA has even been demonstrated in a small percentage of healthy individuals. The so-called natural AECAs (NA) include polyreactive IgM, IgG, and/or IgA, which recognize a restricted and conserved set of endothelial antigens and express low affinity for their target antigens in most of the cases. NA may bind to circulating molecules such as hormones and cytokines, as well as lymphocytes and endothelial cells. Normal IgG interacts with living endothelial cells and is internalized with a mechanism involving microtubules and resembling that of ligand-receptor internalization. IgG-endothelial cell interaction appears to be dependent on the variable region of antibodies and is followed by modifications of endothelial cell function. Natural AECAs increase anti-inflammatory properties of endothelial cells through the selective inhibition of thromboxane A2, endothelin and metalloproteinase-9 secretion, as well as through the inhibition of endothelial cell pro-inflammatory response to tumor necrosis factor-alpha (TNFα) [29]. Recently, Servettaz et al. [30] identified the targets of NA in cytoskeletal proteins (β-actin, vimentin, α-tubulin), prolyl-4 hydroxylase β-subunit (a member of the disulfide isomerase family) and two glycolytic enzymes (glyceraldehyde-3-phosphate-deshydrogenase and α-enolase). All these are ubiquitary proteins implicated in cell-cycle regulation, membrane fusion, microtubule bundling, nuclear RNA export and DNA replication and repair, regulation of coagulation (α-enolase is an activator of plasminogen), and cell growth. Thus, NA may participate in host defense against pathogens by opsonization, contributes to the clearance of senescent cells and immune complexes, and/or exerts anti-inflammatory and anti-thrombotic properties, together with preventing endothelial cell activation by TNFα. Finally, these antibodies may play a role in fetal tolerance and impaired immune regulation, such as diminished levels of serum IgM-AECA detected in SLE patients that may contribute to impaired reproductive function commonly seen in SLE [31].

AECA: MULTIPLE MECHANISMS FOR A PLEIOTROPIC PLAYER

AECA might contribute to the pathogenesis of systemic vasculitis and vasculitis-associated diseases through a) activation of ECs, b) direct cytotoxic effect due to complement-dependent cytotoxicity or indirect cytotoxic effect secondary to antibody-dependent cytotoxicity, c) induction of coagulation, and d) induction of apoptosis through the binding of phospholipids or heat-shock protein 60. Mechanisms of disease are summarized in Figure 1. Del Papa and colleagues [32] demonstrated that AECAs can activate ECs by inducing the expression of adhesion molecules, such as E-selectin, intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, in a dose-dependent fashion. Moreover, AECAs can stimulate the production of cytokines, including interleukin (IL)-1b and IL-8, and chemokines such as monocyte-chemotactic protein 1. Specifically, AECAs might promote a pro-inflammatory and pro-adhesive EC phenotype through the induction of the mitogen-activated protein kinase cascade. Additionally, a number of cytokines, e.g., TNFα, can in turn activate NF-κB pathway and c-Jun N-terminal kinase-mitogen-activated protein kinases. The result is increased leukocyte adhesion to endothelial cells induced by the release of endothelium-derived mediators rather than complement-dependent cell-mediated EC damage. AECAs have been associated with thrombosis, for instance by promoting the production of tissue factor (TF) and thereby favoring coagulation. It was also demonstrated that AECA is a genuine promoter of TF synthesis. Accordingly, high plasma levels of von Willebrand factor, thrombomodulin and tissue plasminogen activator (TPA) were detected in patients with SLE. Furthermore, TF activity, TF antigen and TF mRNA

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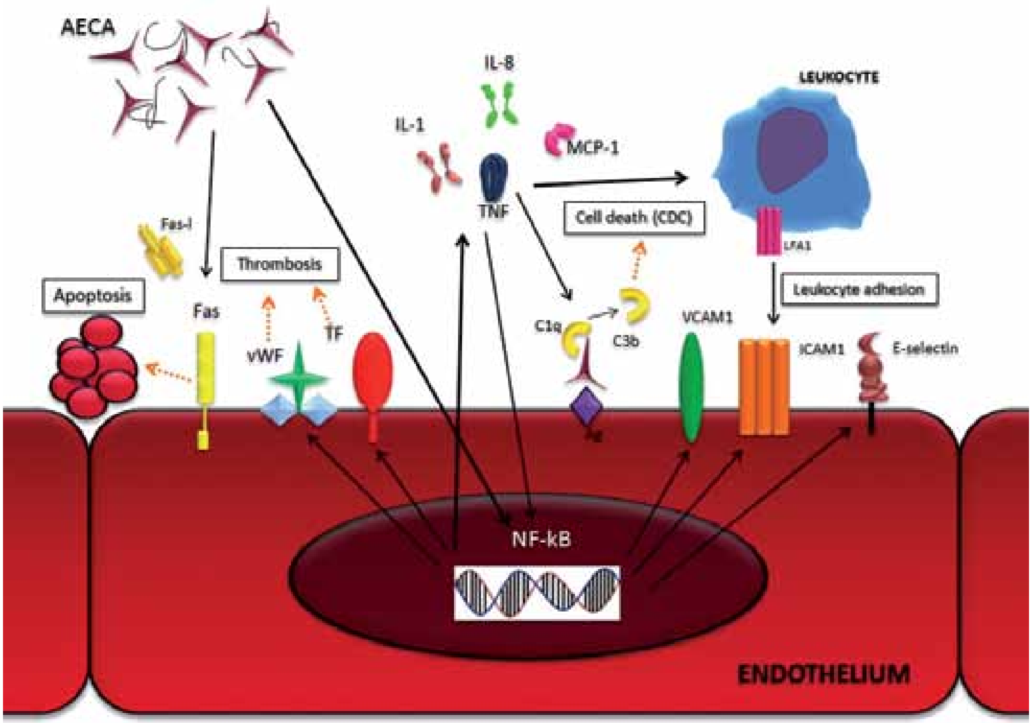

Figure 1. Mechanism of damage mediated by AECA in NPSLE
AECA might contribute to the pathogenesis of systemic vasculitis and vasculitis-associated diseases through different mechanisms. They can activate ECs by inducing the expression of adhesion molecules, such as E-selectin, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), and stimulating the production of cytokines, including interleukin IL-1 and IL-8, and chemokines, such as monocyte-chemotactic protein 1 (MCP-1), thus increasing leukocyte adhesion to ECs. Additionally, a number of cytokines, such as tumor necrosis factor-alpha (TNFα), can in turn activate the nuclear factor kB (NFkB) pathway. AECAs can also enhance the production of tissue factor (TF) and high plasma levels of von Willebrand factor (vWF), hence promoting thrombosis. Finally, AECAs can target Fas receptor or exert direct cytotoxic effect through CDC, especially when ECs are stimulated with IL-1 or TNFα

were dose-dependent on AECA titers. Taken together, these data strongly suggest that activation of ECs leads to a procoagulant status. Furthermore, anti-heparin antibodies (AHA) can be found in patients with SLE and seem to correlate with renal and neurological disease. AHAs exert complement-dependent cytotoxicity on ECs and form immune complexes with heparin, one of the major glycosaminoglycans in ECs. It has been reported that the binding of AECA to EC can provoke cleavage and release of heparan sulphate and promote pro-inflammatory and procoagulant processes and apoptosis. This effect is specific for AECA since elution studies failed to show inhibition with anti-CL, anti-DNA, hyaluronate or chondroitine sulphate. AECAs also seem to correlate with anti-CL in SLE patients. Indeed, aPL can contribute to a pro-inflammatory and procoagulant endothelial phenotype by interfering with the binding of annexin V, synthesis of endothelin I, induction of apoptosis and the protein containment/surveillance system. AECAs may increase the expression of negative ionic phospholipids such as phosphatidylserine (PS) on the surface of ECs, leading to increased binding of anti-CL. It was reported that AECAs from the sera of patients with vasculitis can trigger the translocation of anionic phospholipids, most notably PS, from the inner to the outer leaflet of the plasma membrane, and consequently the binding of FITC-conjugated annexin V. Interestingly, the accessibility of PS to annexin V was restricted to those cells recognized by AECA. Moreover, autoantibodies targeting the heat-shock protein 60 (Hsp60) were recently identified in patients with SLE. These bind endothelial cells and induce PS exposure, followed by apoptosis, thus providing a target for anti-PS. Fas receptor can also be targeted by AECA; however, further activation does not seem to be a prerequisite for AECA-mediated apoptosis of ECs, and other authors have reported Fas-independent apoptosis. Finally, AECA could exert a direct cytotoxic effect through complement-dependent cytotoxicity, especially when ECs are stimulated with IL-1 or TNFα.

DETECTION METHODS

AECAs were identified using mouse frozen kidney sections as the substrate in the standard indirect immunofluorescence (IF) technique. The binding of AECA was demonstrated in the target cells through the F(ab)2 portion, rather than the Fc portion of the antibody. The most frequently used AECA detection methods are IF, ELISA, immunoblotting, radioimmunoassay, fluorescence-activated cell sorting, immunoprecipitation, complement-dependent cytotoxicity and the antibody-dependent cell-mediated cytotoxicity. Immunofluorescence was the first technique to be developed, but today AECAs are only rarely tested with this method. ELISA, performed on HUVEC as substrate, is the most commonly used. Other substrates can be employed, such as cell membrane extracts, cells from renal or medullary microvessels, and hybridoma cell lines. Generally, confluent EC monolayers are fixed before testing to avoid non-specific IgG-binding loss of cells. Nevertheless, fixation induces permeabilization of EC membranes, which may allow AECA cross-reaction against intracellular compounds. To avoid these artifacts, ELISA with unfixed EC can be used. False-negative AECA results can be due to the lack of expression of certain target antigens on a specific substrate. For this reason, it has been recommended that several EC substrates be used simultaneously. False-positive AECA could occur owing to endogenous antibodies, such as anti-β2GPI, reacting with fetal calf serum (FCS) proteins from culture medium coated on ELISA plates. This error could be avoided by antibody absorption in FCS-containing dilution buffer or by washing cells of FCS before plating. Finally, there is a subpopulation of AECA that is likely to react with extracellular matrix components, such as collagen type II, IV, VII, and laminin, leading to false-positive results.

CONCLUSIONS

In the management of NPSLE, identification of the clinical features as well as achievement of early diagnosis is crucial for determining the optimal treatment. Several mechanisms are involved in the pathogenesis of these disorders. Most are autoantibody-dependent or related, and there is considerable evidence that AECAs might play a key role in these processes. Nevertheless, low specificity, the lack of standardized detection methods, and the possible presence of NA secondary to SLE polyclonal B cell activation limit the use of AECAs as diagnostic and prognostic markers. Clearly, further studies are necessary for fully understanding their role in SLE clinical practice.

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