SUPPLEMENT

Table 1. Antigens cross-reacting with anti-DNA antibodies

Antigen	Cross-reactivity with anti-dsDNA	Cross-reactivity with anti-ssDNA
Z-DNA	+ [1]	+ [2]
Cardiolipin	+ [3, 4]	+ [4, 5]
Phosphatidylglycerol	ND*	+ [5]
Bacterial phospholipids	+ [6]	ND
Pneumococcal phosphorylcholine	+ [7]	ND
Mycobacterial glycolipid	+ [8]	+ [8]
Klebsiella polysaccharides	ND	+ [9]
Meningococcal polysaccharides	+ [10]	+ [10]
Pneumococcal polysaccharides	+ [11, 12]	ND
E. coli polysaccharides	+ [10]	+ [10]
Hyaluronic acid	+ [13]	ND
Chondroitin sulfate	+ [13]	ND
Heparan sulfate	+ [14]	ND
Laminin	+ [15]	+ [15]
Cell surface proteins	+ [16-20]	ND
Vimentin	+ [21]	+ [21]
Ribosomal proteins S1, P0, P1	+ [22-24]	ND
Small nuclear ribonucleoprotein A and D	+ [25, 26]	ND

* ND, not defined.

Polynucleotides and phospholipids were the first molecules for which cross-reactivity of anti-DNA antibodies was demonstrated. Immunization of autoimmune line MRL mice with β2-glycoprotein I induced the formation of anti-DNA antibodies whose interaction with DNA was inhibited by cardiolipin micelles, indicating that these antibodies are bound to cardiolipin [3]. Using hybridoma technology, it was shown that monoclonal anti-DNA antibodies from the blood of SLE mice can bind not only cardiolipin, but also other phosphorylated molecules [5]. Phospholipids lacking regular phosphate groups do not bind to the anti-DNA antibodies. This indicates that Abs recognize in the structure of DNA and phospholipids the regularly spaced repetitive phosphate groups [4, 27].

Anti-DNA antibodies react with cell surface molecules that are expressed on some human and murine cells including Raji cells [16], B-cells [17], T-cells, red blood cells [18], neurons [19], epithelial cell cultures of human skin and kidney [17], as well as embryonic stem cells [20]. Treatment of these cells with proteases denies them the ability to bind anti-DNA antibodies, while treatment with the enzymes hydrolyzing the sugar has no effect on binding [17]. These data indicate that components of the cell wall interacting with anti-DNA antibodies are proteins and not the carbohydrate components of glycoproteins and proteoglycans. Both murine and human anti-DNA antibodies cross-react with NR2 subunit of N-methyl-D-aspartate (NMDA) receptor of the neuronal surface. Cerebrospinal fluid of SLE patients containing anti-DNA and anti-NR2 cross-reactive antibodies induced neuronal apoptosis [19]. While NMDA-receptors

are expressed on the surface of many types of cells, such anti-DNA antibodies can cause damage to many organs of the human body.

Anti-DNA antibodies may also cross-react with intracellular proteins. Human and mouse monoclonal anti-DNA antibodies react with the intermediate filament vimentin, and possibly with other cytoskeletal proteins such as α-actin [28] and tubulin [29]. Anti-dsDNA antibodies bind the ribosomal proteins S1 [22], P0 [23], and P1 [24], as well as A and D polypeptides from small nuclear ribonucleoproteins [25, 26].

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Table 2. Proteins associated with SLE etiology

Gene category	Specific features of gene expression
Antigen clearance genes	defects in complement: C1q, C1r and C1s, C4 >> C2; deficit of Sap, deficit of IgM in blood, deficit of DNase I
Tolerance induction genes	defects in the expression of genes coding for protein lymphocyte activation threshold proteins: deficit of Lyn, deficit of Shp-1, deficit of CD22, PD-1; defects in the expression of genes coding proteins participating in deletion of auto-reactive lymphocytes: deficit of Fas and Fas-ligand, heterozygous deficit of Pten, defects in expression of cell cycle inhibitor p21
Genes of organ-specific autoimmune manifestations	polymorphism of FcγRIII receptor (receptor that binds an IgG molecule via its Fc domain [1]) playing a key role in onset of kidney disease

The first category includes genes coding for proteins implicated in the physiological pathways aimed at clearing of the organism from gene products that cause the loss of immune tolerance towards nuclear autoantigens and trigger autoimmune reactions of the organism. SLE animal models are obtained by the deletion of three genes (see below). One gene codes for a protein of the complement, C1q, which together with other proteins of the complement helps to clear the organism of immune complexes and apoptotic cells [2]. Deletion of the gene encoding serum amyloid P component (SAP) of blood, a protein, which binds to chromatin and masks it to the immune system, leads to the development of antinuclear autoimmunity and glomerulonephritis [3]. A similar phenotype is observed in mice with defects in secretion of IgM [4, 5] involved in clearing the body of depleted cells.

The second category of genes whose absence may lead to the development of SLE codes for proteins that regulate thresholds and sensitivity to tolerance and

activation of T- and B-lymphocytes, such as Fas, Fas-ligand, SHP-1 [6], CD22 [7] PD-1 [8], p21 [9], and Pten [10].

The third category of genes codes for proteins that can alter the progression of the disease in individual organs as shown by correlation between polymorphic variation and SLE-nephritis [11]. It has been shown that in the blood of SLE patients the DNase I activity is low (especially in case of nephritis); such low activity correlates with high concentration of actin, a strong inhibitor of DNase I, in the blood [12]. Successful treatment of patients whose blood contained anti-DNA antibodies with bovine DNase was reported [13]. One of the problems during the treatment of patients with DNase I was the presence of high concentrations of actin, probably resulting from the degradation of platelets. Thus, for successful treatment DNase I devoid of a fragment responsible for interacting with actin was obtained [14].

It is believed that the polyclonal activation of T- or B-cells can also lead to the induction of generation of anti-DNA antibodies [15]. Polyclonal activation of immune cells can be influenced by cytokines released by cells of the innate immune system. Increased concentration of IFN-α in blood and elevated expression of specific IFN-α-induced genes in blood cells are constantly observed in SLE patients and have been shown to correlate with disease severity [16-19]. High levels of IFN-α may play a direct role in pathogenesis, as far as patients with non-autoimmune diseases treated with IFN-α may contain in their blood antibodies against nuclear antigens or anti-dsDNA antibodies, and sometimes they can develop SLE [20]. According to published data [21], the source of high levels of IFN-α may be activated plasmacytoid dendritic cells (PDC) – the major blood cells synthesizing IFN-α. Activation of PDC may be caused by signals that are recognized by TLR (Toll-Like Receptors; receptors that interact with specific nucleic acid sequences), as far as the most efficient inducers of IFN-α synthesis in PDC are the synthetic ligands for TLR7 and TLR9 receptors, as well as DNA- and RNA-containing viruses, which probably also act through these receptors [22, 23]. Recently, it was shown that mammalian DNA is also able to activate TLR7 and TLR9 [21]. These data support the hypothesis that increase in IFN-α level may result from endogenous stimulation of PDC by endogenous immune complexes containing endogenous RNA or DNA. FcRII (receptor that binds to the Fc domain of IgG molecules [1]), expressed on PDC may facilitate the absorption of immune complexes by delivering them to endosomal compartments containing TLR7 and TLR9 receptors [24-26].

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