# The Enigma of Antigen Selection and Antigen Recognition in Systemic Lupus Erythematosus

Dror Mevorach MD and Yaakov Naparstek MD

Department of Medicine, Hadassah University Hospital and Hebrew University Medical School, Jerusalem, Israel

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Systemic lupus erythematosus is a disease characterized by the occurrence of autoantibodies against intracellular antigens, including DNA, nucleoproteins and cytoplasmic antigens. Although the structure of these antibodies as well as the genes that encode them have been well characterized in the last decade, the enigma of the role of these intracellular self molecules as the *immunogens* that induce the production of autoantibodies and their role as the *target antigens* for the pathogenic antibodies has not been deciphered.

Mammalian DNA, the classical self-antigen for lupus autoantibodies, is a weak immunogen and most probably cannot induce the production of anti-DNA antibodies via the classical adoptive immune response. Immunization of either normal or lupus-prone mice with mammalian DNA not only fails to induce autoantibody production but even prevents the production of immunostimulatory cytokines and thereby has an immunosuppressive effect. The bacterial, non-self DNA differs from that of the mammalian DNA by the presence of a larger number of CpG motifs that have an immunostimulatory function. In this issue of the journal, Pisetsky [1] shows that these motifs induce anti-DNA antibodies in both normal and lupus-prone mice. These findings may suggest that bacterial DNA rather than the self-DNA may be the immunogen that drives the autoimmune response.

Another possibility is that it is the whole nucleosome rather than the pure DNA that drives the autoimmune response. Indeed, it was shown that nucleosomes are the major immunogen for pathogenic autoantibody-inducing T cells of lupus [2]. The mechanism by which nucleosomes and several other intracellular antigen targets can be immunogenic in SLE may lie in the process of programmed cell death. Cleavage of DNA-histone complexes to subunits consisting of different numbers of nucleosomes is one of the hallmarks of apoptosis, leading to the typical laddering phenomenon in DNA-electrophoresis. Casciola-Rosen et al. [3] demonstrated that exposure of keratinocytes to ultraviolet light induces apoptosis, and that the cell surface expression of Ro and La, nucleosomes and ribosomes, can be explained by the translocation of certain intracellular particles to the apoptotic surface blebs. Another important translocation that occurs during apoptosis is that of phosphatidylserine, an acidic phospholipid that normally resides in the inside of the cell but flips to the outside of the cell membrane when the cell undergoes apoptosis [4]. Phosphatidylserine, like cardiolipin, is a major autoantigen for anti-phospholipid antibodies in SLE. Taken together, these findings provide the basis for a unifying hypothesis to explain antigen selection in SLE, suggesting that SLE patients respond to exposure and modifications of intracellular proteins during apoptosis [3,5].

Post-translational modification [reviewed in 6] of autoantigens may represent one mechanism of acquiring immunogenicity, and Zampieri and colleagues [7] have suggested that dephosphorylation of ribosomal P proteins during FasL apoptosis may trigger autoimmune response. However, other proteins may undergo dephosphorylation without becoming immunogenic, and it was shown that during stress-induced apoptosis phosphorylated rather than dephosphorylated proteins are common targets for autoantibody production in patients with SLE [8]. The interesting suggestion of a role for protein dephosphorylation [9] should be examined further.

The next question that arises is why SLE patients develop an immune response to apoptotic material? There are many possible explanations, but one unifying hypothesis suggests that SLE patients have impaired clearance of apoptotic cells [3,10,11] that may lead to pro-inflammatory presentation and clearance of secondary necrotic cells and lysates [12]. Increased levels of nucleosomes were found in plasma of patients with SLE [13], which may support altered or inefficient phagocytosis of apoptotic cells in SLE. Nucleosomes are formed in the process of programmed cell death; however, in efficient phagocytosis, nucleosomes are generally created within a phagocyte following ingestion of cells undergoing early apoptosis [14]. In this way nucleosomes are not released to the plasma, where another safety mechanism in the form of nucleases exists. In contrast to mammalian DNA [1], the pathogenicity of nucleosomes was demonstrated by their ability to induce an immunoproliferative response [15] and interleukin-6 secretion [16]. Recently, it was shown that SLE-like disease develops in mice deficient in DNAse I, which is the major nuclease present in the serum [17]. In addition, in animal models, mice deficient in C1q [18], C4 [19], ABC1 cassette transporter [20] and Mer [21] are deficient in receptors or factors required for interaction with apoptotic cells, and due to their absence develop autoimmunity or lupus-like disease. The observation that freshly isolated and maturing monocytes showed accelerated Fas-dependent apoptosis with an *in vitro* reduced capacity of the remaining mature macrophages to clear

SLE = systemic lupus erythematosus

apoptotic cells [22] suggests at least the existence of *in vitro* alteration of clearance of apoptotic cells. As suggested by Pisetsky [1], the role of bacterial and mammalian DNA may be relevant as a "natural adjuvant" (bacterial DNA) shifting an immunosuppressive response to a pro-inflammatory response, and as a natural immunosuppressor (mammalian DNA). These effects may be expressed in phagocytosis and antigen presentation.

In summary, while the role of adoptive immune response is established for the formation of T cell-dependent generation of autoantibodies, the role of innate immunity is revisited and may provide an important link for the development of autoimmunity.

The second puzzle regarding the lupus autoantibodies lies in the nature of their *in vivo* target antigen [23] Most lupus autoantibodies are charactetized by their binding to intracellular antigens by a variety of *in vitro* assays. This is shown for example by indirect immunofluorescence using fixed mammalian cells in which the membrane has been disrupted, or by solid-phase assays in which the naked intracellular antigens are used as the target. Binding to intracellular antigens that are present in living cells has usually not been demonstarted for most lupus autoantibodies and is not seen by direct immunofluorescence studies of involved tissues from lupus patients. It has recently been suggested by various groups that direct penetration to the cells can lead to the pathogenicity of some lupus autoantibodies. Ghirardello et al. [9], also in this issue of IMAJ, suggest that anti-P antibodies have a direct pathogenic role and penetrate the cells after binding to a membrane form of the ribosomal P protein. Whether it is penetration to cells, binding to free nucleosomes, cross-reactivity with extracellular antigens, or opsonization with apoptotic cells [10,23] that leads to the damage in SLE has yet to be clarified.

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**Correspondence:** Dr. D. Mevorach, Dept. of Medicine, Hadassah University Hospital, P.O. Box 12000, Jerusalem 91120, Israel. Phone: (972-2) 6777-111, Fax: (972-2) 643-3935,
email: mevdm@netvision.net.il