Update on systemic lupus erythematosus: autoantibodies and apoptosis

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Systemic lupus erythematosus (SLE) is a relapsing and remitting multisystem disease associated with the formation of autoantibodies[1,2]. It predominantly affects women (ca 1 in 2,000 in the UK) and is more common in certain racial groups, such as Afro-Caribbeans, irrespective of place of birth[3]. Why patients with SLE make autoantibodies to their own cellular constituents has puzzled physicians and biologists for many years. The importance of both genetic and environmental factors in the predisposition to the disease is reflected by 25% concordance for the disease in identical twins. Factors that contribute to the development of the disease and trigger relapses (flares) include exposure to ultraviolet (UV) light, infection, oestrogen hormones and stress. The mechanisms by which these factors can influence the disease have been elusive until recently. This article will discuss the link between autoantibodies and apoptosis (programmed cell death), a ubiquitous biological process implicated in lupus disease, particularly disease induced by exposure to UV light and infection.

Autoantibodies in systemic lupus erythematosus

The production of antibodies directed against self-antigens (autoantibodies) is typical of SLE[2,4]. There are at least 40, and possibly as many as 2,000, target antigens; they may be membranous, intracellular or nuclear[4,5]. Why these antibodies are formed has been a long-standing mystery. Nevertheless, the detection of antinuclear antibodies (ANAs) is a valuable screening aid for SLE. The presence of anti-double stranded (ds) DNA antibodies is a specific diagnostic test, and is also used to monitor lupus activity. Also specific for SLE are antibodies to Sm (Smith antigen), a soluble nuclear component. Each of these autoantibodies is used in the American Rheumatism Association (ARA) criteria for the classification of SLE[6]. For many years, a 'false positive serological test for syphilis' was used in the classification criteria for SLE. This is now known to reflect anticardiolipin antibodies in the serum, the most common form of antiphospholipid antibody, and the criteria have been modified accordingly[7]. Antiphospholipid antibodies are associated with thrombotic complications, but paradoxically can interfere with phospholipid-dependent coagulation tests (resulting in the term 'lupus anticoagulant').

Why do SLE patients make antibodies to their own cellular constituents? This process is normally thought to be inhibited by the deletion or inactivation of lymphocytes that recognise self-antigens: the process of immunological tolerance[8]. To understand this, it is necessary to review how B lymphocytes mature into memory B cells and become antibody secreting plasma cells.

Generation of antibodies

The autoantibodies in SLE are predominantly immunoglobulins (Ig) of the IgG type. Igs are present on the B cell surface where they act as a receptor for antigen and are secreted by plasma cells. Although antibodies can be produced without T cell help, the antibodies in SLE demonstrate classical features of a T cell-dependent process: class-switching (IgG, not just IgM), high affinity, and formation from somatic hypermutation. Their production requires the generation of specific memory B cells in the germinal centres of lymph nodes[5]. Initially, B cells (centroblasts) divide rapidly and spontaneously mutate their antibody genes in the dark zone. Eventually, they move to the light zone, stop dividing, and die by apoptosis unless rescued through specific cell interactions (see below). Random mutations to the Ig variable genes rarely improve affinity, are usually ineffective and occasionally dangerous (autoantibody generated). The key function of the germinal centre is to select high affinity mutations. To achieve this, follicular dendritic cells act as a reservoir of antigen. The hypermutated B cell (centrocyte) removes surface-bound antigen from a follicular dendritic cell, ingests, processes and incorporates it into major histocompatibility (MHC) class II molecules on its surface (Fig 1). If a T cell recognises the antigen presented by this B cell, it expresses a cell surface signalling molecule (CD40-ligand) that binds to its ligand (CD40) on the surface of the centrocyte. This interaction induces the necessary signals to stop apoptosis in the centrocyte. It becomes a stable mature memory B cell, and can subsequently be reactivated or differentiated into an antibody secreting plasma cell[5].

Immune tolerance

Tolerance is the process whereby immune responses to self-antigens are prevented. It is mediated by three main processes[9]:

Central tolerance

Central tolerance refers to mechanisms during lymphocyte development in the thymus (T cells) or bone marrow (B cells) which eliminate or inactivate lymphocytes that express receptors for self-antigens before they develop into functional cells.

Peripheral tolerance

Peripheral tolerance refers to mechanisms acting on mature lymphocytes after they have left the primary lymphoid organs. This is necessary because not all self-antigens are expressed in the thymus; thus, not all T cells capable of recognising self-antigens are deleted.

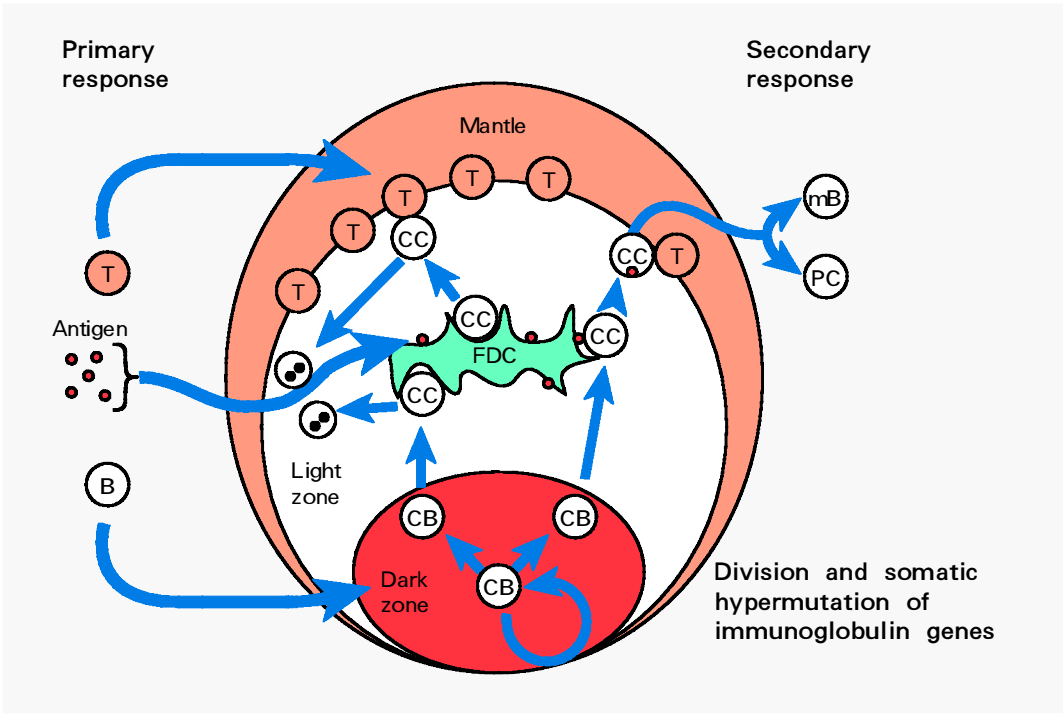

Fig 1. Somatic hypermutation and selection of B cell antigen receptors (antibody) in a germinal centre
(B = B cell; T = T cell; CB = centroblast; CC = centrocyte; mB = memory B cell; PC = plasma cell; FDC = follicular dendritic cell).

Also, somatic hypermutation in B cells may generate B cells specific for self-antigen.

Inactivation (anergy or suppression)

Peripheral deletion involves signalling pathways mediated by cell surface molecules called Fas and Fas-ligand. Another molecule, CTLA-4, is involved in making cells unresponsive in the presence of antigen (clonal anergy). Genetic deficiency of these molecules is associated with autoimmune disease in mice and may contribute to autoimmunity in humans. Suppression is probably mediated by specific T cells that secrete certain signalling molecules (cytokines) that damp down immune responses.

As described above, only a B cell that has produced a high-affinity mutation in its Ig genes can retrieve a specific antigen from a follicular dendritic cell and survive by interacting with a T cell which recognises part of the antigen through its own specific T cell receptor. Although tolerance to self-antigens can be mediated by the absence of specific B cells, it is the absence of T cells that recognise self which largely prevents autoimmune responses. As described below, the breakdown in tolerance in SLE is largely due to the immune system normally being ignorant of self-antigens.

Systemic lupus erythematosus: a disease of too little or too much apoptosis?

Apoptosis is a fundamental process involved in growth and differentiation of all tissues that leads to the ordered destruction of cells, thus avoiding the release of intracellular contents into the extracellular microenvironment which would otherwise cause severe inflammation[5]. Apoptotic cells undergo a series of distinct physical changes, including alteration of the surface lipid membrane, cytoskeletal disruption, cell shrinkage and a characteristic pattern of DNA fragmentation involving the formation of nucleosomes[10]. Apoptosis can be actively induced through ligation of specific receptors such as Fas, or passively through lack of essential survival signals[11]. All cells in the body require continuous positive signals to stay alive. In the absence of a positive signal for survival, caspase 3 becomes activated, and its autocatalytic activity sets off a catastrophic chain reaction that activates all the caspase 3 in the cell[5,12]. This is the point of irrevocable commitment to apoptosis, rather like the pivotal role of C3b in complement activation. The active caspase 3 subsequently activates a range of downstream mediators that co-ordinate the ordered destruction of the cell. *In vitro*, the terminal phase of apoptosis is 'blebbing', in which minute balloon-like membrane extrusions bud off from the surface of the cell, incorporating nuclear and cytoplasmic material[13]. *In vivo*, this rarely happens because apoptotic cells are effectively removed by phagocytes that use a range of recognition systems to identify them[14].

The first model to link apoptosis and SLE was the MRL/lpr mouse, in which autoantibodies and immune-mediated glomerulonephritis are found. The observation that the *lpr* gene is a defective form of Fas, a surface receptor that transduces an active signal for apoptosis, suggested that the failure of self-tolerance in SLE would be associated with defective Fas-mediated apoptosis[15]. This hypothesis was supported when the *gld* abnormality (another mouse gene producing a similar disease phenotype to *lpr*) was identified as a non-functional Fas ligand gene. However, the lpr and gld mice suffer from a severe lymphoproliferative disorder, which is not characteristic of patients with SLE who are often

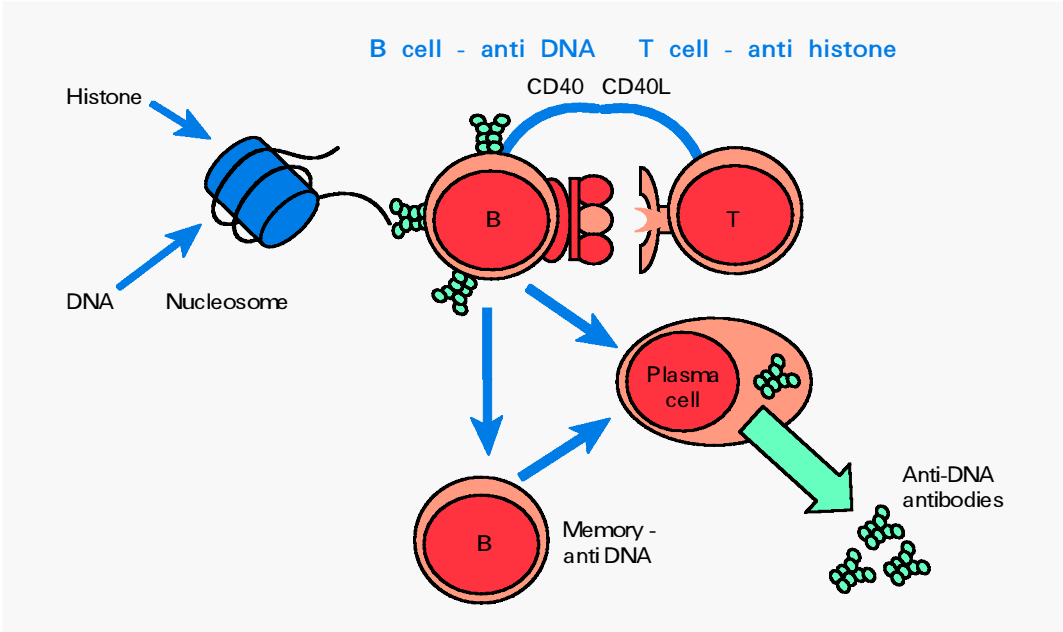

Fig 2. The hapten-carrier mechanism responsible for selecting high-affinity immunoglobulin (Ig) G antibodies to non-protein autoantigens in SLE. Nucleosomes derived from apoptotic cells are pinocytosed by B cells (centrocytes) which bind to DNA wound around a histone core. This protein core is processed and presented to a T cell whose T cell receptor recognises a specific histone-derived antigen. The T cell delivers a rescue signal through CD40 to the B cell, which can then become a stable memory B cell or differentiate into an anti-DNA antibody secreting plasma cell.

profoundly lymphopenic. Furthermore, both the expression and function of Fas and its ligand are normal or increased in SLE. Finally, a few individuals with an lpr-type genetic abnormality have been identified as having a lymphoproliferative disease like the MRL/lpr mouse but which is not much like SLE[16,17].

The evidence has been increasing that SLE is associated with too much apoptosis rather than too little[10,13,18]. Normally, apoptotic cells and their contents are cleared effectively by phagocytes[14]. This is important because, for example, up to 10^{11} neutrophils are produced and subsequently die each day by apoptosis. Patients with SLE often have high levels of circulating DNA, usually present as short stretches of DNA wound around a histone core forming a nucleosome[10], the classical cleavage product of apoptosis. Antibodies to nucleosomes are common in SLE and predate anti-dsDNA antibodies[10]. Furthermore, haematoxylin bodies, found in the glomeruli in lupus nephritis, are actually 'blebs' from fragmented apoptotic cells[19]. The LE cell, until recently an ARA criterion for SLE (but now rarely sought)[6,7] is itself a neutrophil that has phagocytosed apoptotic material. Apoptotic blebs can be processed by dendritic cells as foreign antigen, and are presented by MHC class II molecules to T cells. Apoptotic blebs occur in the skin after UV exposure and contain proteins normally found in the nucleus, including Ro, La and nucleosomes, all classical autoantigens of SLE. The formation of some of these autoantigens may depend on the activities of certain proteases that are active during apoptosis such as granzyme B[13,18].

Deficient clearance of apoptotic cells and the generation of autoantigens

The problem with immunological tolerance is that we are usually ignorant of, rather than functionally tolerant to, our cell contents. Intracellular proteins do not usually encounter the immune system because phagocytes remove them intact, but deficient phagocyte-mediated clearance of intact apoptotic cells occurs in SLE. This results in fragmentation and the release of intracellular antigens that can trigger an immune response[13,18]. However, there is a conundrum: T cells can recognise only peptides, but the most characteristic antibodies in SLE are to DNA and phospholipids. This is easily solved in the context of the following model.

In an SLE patient with circulating nucleosomes, a germinal centre forms with B cells specific for dsDNA. The centrocytes use their anti-DNA antibody to remove whole nucleosomes from the follicular dendritic cells. Nucleosomes are processed, and the protein core is incorporated into the grooves of MHC class II molecules and presented to histone-specific T cells (Fig 2). Experimental evidence for this model has been reported[20]. Similarly, membrane phospholipids such as phosphatidylserine are linked to lipid-binding proteins that can act as carriers. This provides a simple hapten-carrier model similar to that used in generating conjugate vaccines.

A complete failure of the clearance mechanisms for apoptotic cells would be fatal in days, so SLE patients must have a partial defect that reduces the threshold for overloading the system. Patients with homozygous deficiencies in the early complement proteins (C2, C4 and especially C1q) develop a severe lupus-like disease early in life. Deficiencies of C3 produce a much less severe phenotype than deficiencies in C1q[21]. Recent studies have shown that C1q receptors on the surface of phagocytes are an extremely important mechanism in clearing apoptotic cells (but not the only one)[22,23]. Patients or mice with homozygous C1q deficiency develop autoantibodies and a lupus-like syndrome. This appears to be due to deficient clearance of apoptotic cells and presentation of antigens derived from them to the immune system[19,21,24].

It is less clear *why* patients with SLE develop defective clearance of apoptotic cells, as genetic C1q deficiency is rare. Antibodies to C1q are present in many patients, particularly those with renal disease[21,23]. This probably results in a functional deficiency of C1q protein. C1q antibodies are not likely to be the primary abnormality in most patients with SLE, but this provides a mechanism for persistent disease and relapses. Exposure to UV irradiation and infection are the most common triggers of lupus flares, and are associated with a marked increase in the number of apoptotic cells in the skin[13] and blood[11]. This could overload inefficient clearance mechanisms of the reticuloendothelial system, leading to the release of large amounts of antigen. This, in turn, would drive the immune response to produce more antibodies and lead to the formation of immune complexes, producing inflammatory reactions in highly vascularised tissues, especially the skin and kidneys. The immune complexes would also cause massive consumption of classical pathway complement, including C1q, exacerbating the initial problem.

Conclusion

The current model for the perpetuation of SLE involves deficient clearance of apoptotic cells under conditions of stress, partly due to a functional deficiency of C1q. This produces large amounts of antigen to which the immune system is not tolerant. Periodic episodes of infection or UV exposure produce high levels of apoptotic cells which will fragment and drive the disease process by the formation of immune complexes causing inflammation and consumption of C1q, rendering the protein even more deficient and less able to clear apoptotic cells. In individuals with homozygous C1q deficiency, provision of recombinant C1q may have therapeutic benefit. However, in SLE patients with antibody-mediated functional deficit, provision of C1q may exacerbate lupus by providing antigen to drive the specific immune response.

This model is useful for explaining the persistence of SLE and the association between sunlight/infection and flares, but is not sufficient to explain all the multifactorial causes of the disease. Understanding the processes involved in the generation of autoantibodies is important in the development of new therapies for lupus, for example anti-CD40 ligand monoclonal antibody which is currently undergoing clinical trials.

Key Points

Autoantibodies in systemic lupus erythematosus

- **Autoantibodies are directed against membranous, intracellular and nuclear antigens**
- **The antinuclear antibody test is a valuable screening aid for systemic lupus erythematosus (SLE), but is not specific**
- **Testing for anti-double stranded (ds) DNA antibodies is a specific diagnostic test, but is positive in only about 60% of patients with SLE**
- **Antinucleosome antibodies predate anti-dsDNA antibodies**
- **Antiphospholipid antibodies can cause false positive tests for syphilis and prolongation of activated partial thromboplastin time and other phospholipid-dependent coagulation tests**

Apoptosis in systemic lupus erythematosus

- **Reduced apoptosis predisposes to lymphoproliferative disease**
- **Ultraviolet light and infection result in increased apoptosis and can trigger SLE flares**
- **Impaired clearance of apoptotic cells results in the presentation of self-antigens and autoantibody formation**
- **C1q deficiency may be genetic or acquired; it is associated with impaired clearance of apoptotic cells and with immune-mediated glomerulonephritis**

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Anticardiolipin syndrome: antiphospholipid syndrome

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Anticardiolipin syndrome, more appropriately called antiphospholipid syndrome (APS), is an autoimmune disorder characterised by recurrent venous or arterial thrombosis, fetal loss, thrombocytopenia, and some other clinical complications associated with antiphospholipid (aPL) antibodies[1] (Table 1). Some components of this syndrome have been known since the 1950s, but the whole syndrome was recognised in 1983 when simple and reproducible methods for the detection of aPL antibodies became available[2–4].

Epidemiology

APS was first described in patients with systemic lupus erythematosus (SLE) (secondary APS), but it may occur in the absence of any other disorder (primary APS). Major clinical features are similar in both forms. The syndrome has been reported in all ethnic groups, but seems to be both less common and less severe among patients from the Indian subcontinent and in African Americans and African Jamaicans. It was first described in adults, but is also found in children. The female to male ratio is 6:1.

Pathogenesis

The close association of aPL antibodies with thrombosis and pregnancy loss strongly suggests these antibodies have a causative role. This is supported by induction of these complications in normal mice following passive transfer of aPL antibodies from APS patients[5,6]. Several mechanisms by which aPL antibodies may cause these complications have been suggested:

- Activation of endothelial cells and increased expression of adhesion molecules, resulting in increased adherence of platelets and monocytes to endothelial cells.
- Alteration of prostacyclin/thromboxane balance.
- Impairment of antithrombin III activity via cross-reactivity with glycosaminoglycans.
- Inhibition of thrombomodulin protein C-protein S activation.

Key Points

Antiphospholipid syndrome (APS) is defined as recurrent thrombosis or recurrent fetal death associated with anticardiolipid (aCL) or lupus anticoagulant (LA) antibodies

APS may occur in the absence of systemic lupus erythematosus (SLE) or any other autoimmune disorder

Untreated thrombosis in APS has a high risk of recurrence, unless long term aggressive anticoagulation is administered

Anticoagulation for thrombosis in APS may need to be continued for life

A pregnant woman with a past history of pregnancy loss, and high levels of IgC aCL antibodies without treatment, has a very high risk of pregnancy loss

Catastrophic APS has a mortality rate of over 50% and may be triggered by discontinuation of anticoagulation, by surgery or by infection