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## Deranged removal of apoptotic cells: its role in the genesis of lupus

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**Keywords:** apoptosis; autoantibodies; phagocytosis; systemic lupus erythematosus

lupus nephritis, the most serious manifestation of SLE. The evidence for dysregulation of the apoptotic process and removal of apoptotic cells for the development of SLE will be discussed in this Editorial Comment.

### Introduction

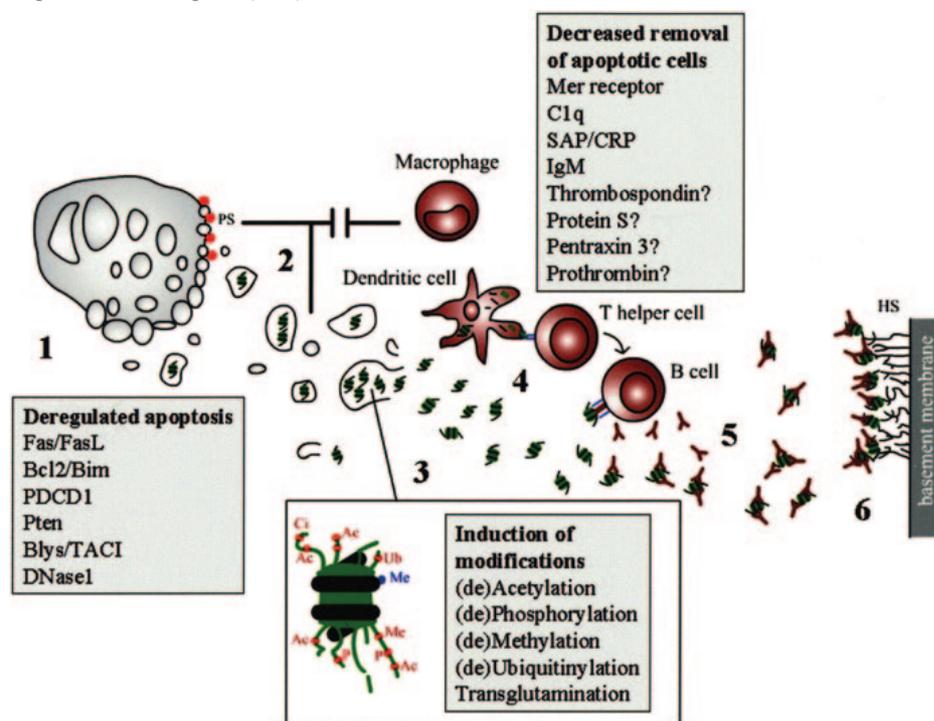
The formation of anti-nuclear autoantibodies is the main feature of the autoimmune disease systemic lupus erythematosus (SLE). We do not know why these nuclear components become autoantigens in the disease. Recently, evidence has emerged which points to an important connection with apoptosis and the removal of apoptotic cells. The process of apoptosis, or programmed cell death, contains a series of pathways leading to the regulated removal of unwanted cells, including the induction of tolerance for autoantigens. This process is strictly regulated. In particular, the adequate removal of early apoptotic cells is of great importance. Since nuclear autoantigens become clustered in apoptotic blebs [1], an impaired removal could lead to the release of nuclear structures, possibly also modified during apoptosis (Figure 1). The release of (modified) nuclear structures could then induce an immune response to these autoantigens leading to the production of autoantibodies. Chromatin, a complex of proteins and double-stranded (ds) DNA, is an autoantigen that is clustered in apoptotic blebs. Autoantibodies against chromatin (including anti-dsDNA, anti-histone and nucleosome-specific antibodies) are a hallmark of SLE [2]. Moreover, the formation of anti-chromatin/chromatin complexes can lead to the binding of these complexes to basement membranes, including the glomerular basement membrane (GBM). Here, the positively charged histone tails of chromatin bind to negatively charged molecules in the GBM, such as heparan sulphate proteoglycans [3]. This binding induces inflammation, which leads to

### Apoptosis and SLE

Apoptosis not only plays a critical role in removing damaged cells, but is also very important in inducing and maintaining self-tolerance. Tolerance of self antigens requires the deletion of autoreactive T- and B-cells by apoptosis and, therefore, defects in inducing apoptosis could lead to the persistence of autoreactive T- or B-cells. The first evidence for the relation between apoptosis and SLE came from a mouse model involving a defect in an apoptosis-inducing system, the Fas/Fas ligand (FasL) system [4]. Mice deficient for either Fas (*lpr*) or FasL (*gld*) develop lymphoproliferation and autoimmunity. It is important to note that *lpr* or *gld* mutations alone do not cause lupus-like syndromes. Depending on the genetic background, they can accelerate the disease expression. Overexpression of the apoptosis inhibitor Bcl-2 resulted in an increased number of autoreactive B-cells and autoimmunity, which again was dependent on the genetic background [5]. Other apoptotic signal molecules have also been associated with lupus. Bim, a member of the Bcl-2 protein family, plays an important role in the induction of apoptosis in thymocytes and loss of this molecule leads to the development of fatal autoimmune glomerulonephritis [6,7]. Mice heterozygous for Pten, a phosphatase involved in apoptosis, have an impaired Fas response and develop disease comparable to Fas-deficient mutants [8]. Recently, BLys, a member of the tumour necrosis factor ligand superfamily and known to be important in B-cell survival, and TACI, an inhibitor of BLys, have been associated with lupus-like disease. Overexpression of BLys [9] and loss of TACI [10,11] resulted in the formation of autoantibodies to nuclear antigens and immune-complex deposits in the kidney.

In human lupus and in the (NZB × NZW)F1 mouse model, however, the evidence for disturbed apoptosis is

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**Fig. 1.** Hypothesis for the development of anti-chromatin responses in SLE. (1) Disturbed apoptosis either at the wrong time-point and/or at the wrong microenvironment. (2) Defective removal of apoptotic cells leading to the release of apoptotic blebs containing chromatin. (3) Apoptosis induced chromatin modifications. (4) Stimulation of the immune response by chromatin. (5) Formation of anti-chromatin/chromatin complexes. (6) Binding of the complexes to heparan sulphate in basement membranes due to the charge interaction between cationic N-termini of the core histones and the anionic charged heparan sulphate.

less clear. Only a quarter of patients with a defect in Fas or FasL develop anti-nuclear antibodies [12]. In humans with SLE, the Fas-dependent pathway seems to be unaffected [12], but some evidence has been found for upregulation of soluble Fas [13,14] and increased expression of Bcl-2 [15]. In addition, increased levels of apoptotic cells have been found in SLE patients [16,17], but this did not correlate with disease activity and was also found in non-SLE patients. Moreover, these elevated levels may also be explained by an elevated number of circulating lymphocytes prone to apoptosis. Recently, a polymorphism of a new apoptosis-related gene, programmed cell death 1 gene (*PDCD1*), was linked to SLE [18]. A single-nucleotide polymorphism altering a binding site for run-related transcription factor X1 (*RUNX1*), was found in Nordic SLE patients. The *PDCD1* gene is located in a locus previously linked to SLE [19]. In summary, especially in certain lupus mouse models there is a clear association between abnormal apoptosis and lupus development. In humans, however, this is less evident.

### Removal of apoptotic cells

In addition to a disturbed apoptosis, as described above, elevated levels of autoantigens might result from an impaired removal of apoptotic cells. Important for this hypothesis was the observation of Rosen *et al.* [1] that autoantigens, such as DNA, cluster in blebs on the

surface of apoptotic keratinocytes after exposure to UV light. Normally, apoptotic cells are swiftly removed by neighbouring cells or, in places where the load of apoptotic cells is high, by macrophages or other professional phagocytes. This uptake does not induce a pro-inflammatory response, but rather generates an anti-inflammatory reaction. This rapid non-phlogistic removal prevents the formation of unstable apoptotic blebs, which might result in the release of potential autoantigens. These autoantigens may become extra immunogenic by modifications obtained during apoptosis.

Recognition of apoptotic cells is a complex process, involving many phagocytic receptors, bridging molecules and markers on the apoptotic cell [20]. The (early) apoptotic process includes chromatin condensation and also surface changes on the apoptotic cell, most notably the appearance of phosphatidylserine (PS) at the outside. This and other changes provide 'eat me' signals to phagocytic cells. These express a number of receptors able to bind apoptotic cells, including the PS receptor, CD14, the C1q receptor, the vitronectin receptor and others. The lipopolysaccharide (LPS) receptor, CD14, normally induces a pro-inflammatory response to invading organisms. However, docking of apoptotic cells results in the opposite effect, an anti-inflammatory reaction. Recently, a new receptor on the macrophage for apoptotic cells has been identified [21]. Macrophages of mice with a truncated cytoplasmic tail of the MER receptor

tyrosine kinase ( $\text{mer}^{\text{kd}}$ ) showed a defect in clearance of apoptotic cells and development of lupus. This receptor binds via the protein encoded by the growth arrest-specific gene 6 (*Gas6*) to PS. In addition, certain 'bridging molecules' play an important role in the removal of apoptotic cells. These molecules include C1q,  $\beta$ 2-glycoprotein I, pentraxins such as pentraxin 3 (PTX3), C-reactive protein (CRP), serum amyloid P protein (SAP) and thrombospondin, and the recently identified prothrombin [22] and protein S [23]. Studies involving these molecules have led to increasing evidence for a pivotal role of a disturbed removal of apoptotic cells in SLE [2]. Deficiency for C1q or SAP in mice resulted in an anti-nuclear autoimmune response and glomerulonephritis. Both molecules, along with PTX3, not only act as mediators in the removal, but most likely also play a role in the masking of apoptotic blebs and autoantigens, such as chromatin, for the immune system. Recently, this has been underlined by the binding of SAP to late apoptotic cells. A 'back-up' system seems to exist in case the removal of apoptotic cells is impaired and late apoptotic cells, blebs and/or autoantigens are released. This system also includes DNase1, which digests chromatin-derived autoantigens. Mice deficient for DNase1 also develop anti-nuclear autoimmunity and glomerulonephritis. Indeed, the involvement of C1q, CRP (functionally related to SAP) and DNase1 in the genesis of human lupus has also been found [24–26]. In lupus-prone mice, the evidence for an impaired removal of apoptotic cells is not yet clear. We found no constitutive defect in the uptake of apoptotic cells by macrophages of pre-morbid lupus-prone mice [27], but recently an impaired clearance of apoptotic cells by thioglycolate stimulated macrophages was found in MRL/Mp and (NZB  $\times$  NZB)F1 mice [27]. This corroborates our finding that in diseased lupus mice clearance of apoptotic cells is impaired.

In summary, increasing evidence is accumulating, showing that a disturbed removal of apoptotic cells leads to an anti-nuclear response and the subsequent development of lupus glomerulonephritis.

## Conclusion

The involvement in SLE development of one or more defects in the induction of apoptosis and, especially, the removal of apoptotic cells has become clear during the last decade (Figure 1). Deranged apoptosis, via an increase or a delay in the wrong microenvironment and/or a reduced clearance of apoptotic cells, leads to the release of nuclear autoantigens [2], which in turn triggers an autoimmune response by autoreactive nucleosome T-cells after presentation by dendritic cells or other antigen-presenting cells. This leads to the production of autoantibodies, such as anti-chromatin autoantibodies, which through a complex formation with chromatin become deposited in basement membranes, especially in the skin and the kidney, where they may cause glomerulonephritis [28].

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## Cyclosporin A toxicity, and more: vascular endothelial growth factor (VEGF) steps forward

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**Keywords:** calcineurin; cyclosporin A; endothelial cells; toxicity; tubular cells; vascular endothelial growth factor

Toxic effects, which involve some organs with particular intensity, are the main disadvantage in the therapeutic use of cyclosporin A (CsA). Nephrotoxicity, renal vascular damage and hypertension are most relevant among the undesirable effects. Vascular injury has generally been considered a common-ground factor of all types of CsA-induced organ damage [1–3].

Albeit that vascular smooth muscle cells initially appeared to be the main CsA target [2], the finding of multiple endothelial effects [1,3,4–6] indicates that endothelial cell (EC) toxicity is an emerging feature of CsA-induced vascular injury.

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### Cyclosporin A, endothelium and VEGF

A main issue related to the mechanisms of the effects of CsA is the putative involvement of vascular endothelial growth factor (VEGF) as a relevant cellular response factor. A reasonable possibility is that VEGF has a key role in the cytoprotective mechanisms against CsA-related cell damage and induction of cell resistance against injury. VEGF exerts its effect after binding to membrane tyrosine kinase receptors (VEGFR1, VEGFR2 and VEGFR3, and a complementary receptor, neuropilin-1). Each of these receptors has different signal transduction properties and functions, but VEGFR2 is more important in functional terms [7].

Evidence has shown that circumstances as varied as hypoxia, exposure to reactive oxygen species (ROS), fibroblast growth factor-2 or rupture of inter-endothelial VE cadherin junctions can all stimulate ECs to produce VEGF [8]. As mentioned above, this autocrine VEGF expression appears to be related to a protective mechanism.

*In vitro*, blockade of autocrine VEGF using a specific VEGF blocking antibody ( $\alpha$ -VEGF) significantly