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What is This?
Apoptosis in the pathogenesis of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a prototype inflammatory autoimmune disease resulting from autoimmune responses against nuclear autoantigens. During apoptosis many lupus autoantigens congregate inside the cells and are susceptible to modifications. Modified nuclear constituents are considered foreign and dangerous. Therefore, apoptotic cells have to have to be efficiently removed to avoid the accumulation of apoptotic debris and the subsequently development of autoimmune responses. Hence, apoptosis and clearance of apoptotic cells/material are considered key processes in the aetiology of SLE. Clearance deficiencies may account for the development of autoimmunity by inducing a loss of tolerance in lymphoid tissues. Furthermore, phagocytosis of apoptotic cells may lead to a pro-inflammatory response in the presence of autoantibodies. This may sustain inflammatory conditions and the pathology found in overt lupus.


Introduction

Systemic lupus erythematosus (SLE) is a prototype inflammatory autoimmune disease resulting from autoimmune responses against nuclear autoantigens. The primary clinical and pathological manifestations are consequences of local inflammation events initiated by widespread immune-complex deposition in various tissues.1 A characteristic hallmark of SLE is the production of autoantibodies against nuclear components. During apoptosis many lupus autoantigens congregate inside the cells and are susceptible to modifications. The fate of not cleared apoptotic cells is to spill out these modified autoantigens constituting endogenous danger signals that are subsequently presented to antigen-presenting cells. Thus, over-riding peripheral tolerance mechanisms and triggering autoimmunity.2 Therefore, apoptotic cell/material removal has to be very efficient to avoid accumulation of apoptotic debris. Actually, both apoptosis and the clearance of apoptotic material have been implicated in the aetiopathogenesis of SLE as depicted in Figure 1. In this review, we summarise the role of apoptosis and the clearance of apoptotic material, in the initiation and maintenance of SLE.

Cell death and clearance

There are two types of cell death such as apoptosis and necrosis. Necrosis is an accidental cell death occurring after acute mechanical, physical or chemical damage to cells, which causes a rapid metabolic collapse of cells, swelling of the cytoplasm (oncosis) and ultimately cell rupture. The chromatin of necrotic cells is initially not fragmented. Cell rupture leads to release of intracellular components causing local inflammation. Necrosis can also be seen as the final end of any cell death process because apoptotic cells undergo secondary necrosis if not cleared properly.3

Apoptosis is a programmed cell death that follows characteristic biochemical and morphological features. Apoptosis can be induced by extrinsic (e.g., Fas ligand) or intrinsic factors (e.g., DNA damage). These stimuli lead to activation of caspases and changes in the plasma membrane.4,5 Accompanied with changes in chromatin structure and composition, cells finally disintegrate into apoptotic blebs.7
Apoptosis occurs during various fundamental biological processes and continuously, dead cells are cleared from tissues by a highly efficient phagocytic system. This ubiquitous process is carried out by professional phagocytes [macrophages, immature dendritic cells (DC)] or by non-professional phagocytes (neighbouring cells, monocytes). The phagocytes have a plethora of receptors to find and engulf early apoptotic cells (reviewed in).

**Apoptosis and apoptosis-induced autoantigen modification in SLE**

The role of apoptosis in the development of SLE is supported by several mouse models with an abnormal function of factors involved in apoptosis. Interference with the expression of Fas (lpr), FasL (gld), Bcl2/Bim, programmed cell death 1, phosphatase and tensin homolog, B lymphocyte stimulator and TACI leads to accumulation of apoptotic cells/blebs/chromatin resulting in breaking of tolerance with ultimately the formation of anti–nuclear antibodies and lupus-like glomerulonephritis (reviewed in).

During apoptosis, proteins, DNA and RNA are modified by cleavage through proteases, caspases and endonucleases. In addition, specific apoptosis-induced post-translational modifications of autoantigens can take place, which include methylation, (de)phosphorylation, (de)ubiquitination, citrullination, ADP-ribosylation and transglutamination (reviewed in). Nuclear autoantigens (chromatin) targeted in SLE are clustered in blebs at the surface of apoptotic cells. Normally, phagocytes will quickly remove apoptotic cells and blebs long before they could have released their modified contents. In SLE, the processes of apoptosis and/or the clearance of apoptotic material may be disturbed (reviewed in). Apoptosis-induced modified nuclear autoantigens are exposed to the immune system and recognised as non-self antigens or in other words act as a danger signal. Dendritic cells, in particular plasmacytoid dendritic cells (pDC), respond to nucleic acid-containing immune complexes by producing the type I interferon IFN-α, a key mediator in the pathogenesis of SLE. Myeloid dendritic cells play an important role in maintaining the balance between immunity and tolerance and can be activated by modified autoantigens. Ultimately, these processes result in an immunogenic response and the formation of autoantibodies to the modified (nuclear) autoantigens (see Figure 1). Importantly, apoptosis-induced modification of autoantigens was reported for several autoimmune diseases like SLE and RA. Recently, we were able to show that apoptosis-induced histone acetylation is a target for autoantibodies in patients with SLE and lupus mice. Histone acetylation appeared to be pathogenic in lupus-prone mice, and hyperacetylated nucleo-

![Figure 1](image-url)
somes could mature dendritic cells that led to T-cell activation. Furthermore, we found that apoptotic blebs were able to mature dendritic cells (unpublished results).

Impaired clearance of apoptotic cells in SLE

In addition to an aberrant apoptosis, an impaired removal of apoptotic cells and debris may lead to the accumulation of apoptotic cells. Phagocytosis of apoptotic cells is a well-coordinated sequence of events that starts with the attraction of phagocytes by apoptotic cells secreting chemoattractants, the opsonisation of apoptotic cells and the binding of these cells to phagocytes, their internalisation and digestion. Depending on the context, phagocytosis of apoptotic cells may result in either a pro-inflammatory or an anti-inflammatory response. The recognition and removal of apoptotic cells and debris by macrophages and dendritic cells are very complex and involve many factors. Mice deficient in factors such as DNaseI, SAP, C1q, IgM and Mer, all required for a proper opsonisation and clearance of apoptotic cells, spontaneously develops anti–nucleosome auto-antibodies and clinical signs of autoimmune disease, such as glomerulonephritis (reviewed in10,16). Most importantly, it was reported that the clearance of apoptotic material by phagocytes is impaired in both lupus mice and patients.17,18

We showed the accumulation of apoptotic debris in germinal centres (GC) of patients with SLE.19 In normal lymph nodes, apoptotic nuclei can almost exclusively be detected inside tingible body macrophages (TBM), which often displayed an altered morphology in patients with SLE. Not ingested apoptotic nuclei were often found outside these cells although the overall count of apoptotic nuclei was not increased in SLE. Sometimes nuclear debris was observed to be attached to the surfaces of follicular dendritic cells (FDC), which normally retain complement-opsonised immune complexes on their surfaces, promoting affinity maturation. Almost no free apoptotic material could be found in GC of controls. In healthy persons, autoreactive B cells that are generated by somatic mutations do not receive survival signals and execute apoptosis. In SLE, clearance deficiency leads to the accumulation of apoptotic material on the FDC. The latter may now provide short-term survival signals for autoreactive B cells. In the mantle zone, they may obtain further survival signals permitting the differentiation into autoantibodies secreting plasma cells and memory B cells.20 In 2007, Hepburn, et al.21 reported that a high proportion of bone marrow from patients with SLE contained apoptotic debris. The presence of not cleared apoptotic material at those sites of B-cell selection may explain the loss of B-cell tolerance and the induction of T-cell help against autoantigens (See Figure 2).22

We observed an accumulation of apoptotic cells and chromatin in the epidermis of patients with cutaneous lupus erythematosus (CLE).23 After skin irradiation with UVB, we observed no differences in the apoptotic cells count between controls and CLE after 24 h. After 72 h, the skin of patients with CLE often contained even higher amounts of apoptotic cells, whereas they had already been cleared in the controls. However, other groups found that inflammatory lesions in the vicinity of apoptotic cells are present in patients with SLE, whereas they did not observe significant differences in the clearance rate of apoptotic cells.24 The sensitivity of patients with SLE to UVB was not related to increased apoptosis.25 This discrepancy may be explained by the differences in the patients’ cohorts: SLE versus CLE. Nevertheless, sun burn cells were identified as keratinocytes undergoing apoptosis,26 and photosensitivity constitutes one criterion of the American College of Rheumatology for the diagnosis of SLE.27

![Figure 2](image-url)

Figure 2: Specific model for the aetopathogenesis of SLE. Clearance deficiency in the germinal centre may initiate autoimmunity by making modified nuclear autoantigens available to antigen-presenting cells in an inflammatory context. This leads to the loss of B-cell tolerance and the synthesis of anti-nuclear autoantibodies. Additionally, the clearance deficiency in the periphery may supply continuously apoptotic remnants that build immune complexes with anti-nuclear antibodies. Deposition of these immune complexes causes tissue damage and inflammation.
Autoantibody-mediated clearance of nuclear fragments

Nucleosomes, most likely derived from apoptotic cells, can be detected in the circulation of patients with SLE and lupus mice; this may lead to the formation and deposition of immune complexes containing nuclear autoantibodies. Frisoni, et al. postulated that autoantibodies augment the engulfment by DC of dying cells, shifting the immune response toward inflammation and autoimmunity. Autoantibodies promoting phagocytosis of apoptotic and necrotic cells were already reported to be present in SLE sera. These reports show that autoantibodies are able to opsonise dead cells facilitating their ingestion by professional phagocytes.

Recently, we established a whole blood phagocytosis assay, enabling us to monitor the ex-vivo uptake of chromatin by granulocytes and monocytes, which resemble the in-vivo situation. Whereas nuclear fragments were ignored by all controls, many patients with SLE were able to take up this particular ‘prey’. This process correlated with the dsDNA autoantibodies and was transferable to healthy blood-borne phagocytes by antibodies prepared from SLE serum. Importantly, only those patients whose monocytes and granulocytes were able to ingest the nuclear fragments produced high amounts of inflammatory cytokines (unpublished data).

Conclusion

Taken together, apoptosis and clearance of apoptotic cells/material are key processes in the aetiology of SLE. Depending on the context, phagocytosis of apoptotic cells may lead to a pro-inflammatory response or an anti-inflammatory response, which is decisive in the development of autoimmunity and may sustain inflammatory conditions.

References


