The role of apoptosis and removal of apoptotic cells in the genesis of systemic lupus erythematosus

Justin H. Fransen, Luuk B. Hilbrands, Claudia M. Koeter, Jo H.M. Berden, Johan van der Vlag

Nephrology Research Laboratory, Nijmegen Centre for Molecular Life Sciences, Department of Nephrology, Radboud University Nijmegen Medical Centre, Netherlands

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease with an unknown aetiology that is characterized by the production of autoantibodies against nuclear components, such as DNA, histones and nucleosomes. Many studies report a role of aberrant apoptosis in the development of SLE, which may be the result of an increased rate of apoptosis, or apoptosis at the wrong time or place. In addition, insufficient clearance of apoptotic cells and debris may explain the development of SLE. The accumulation of apoptotic cells may result from defects in recognition of apoptotic cells by phagocytes, opsonins or their receptors, or simply from a reduced intrinsic phagocytic capacity of phagocytes. When apoptosis exceeds the clearance capacity, apoptotic blebs will segregate and autoantigens with apoptosis-induced modifications will be released. This released apoptotic cell debris can be taken up by professional antigen presenting cells, such as dendritic cells. These cells will present modified autoantigens in an immunogenic fashion to T cells, which subsequently activate autoantibody producing B cells. In summary, development of SLE may be the result of aberrant apoptosis and/or decreased clearance of apoptotic material by phagocytes.

Key words: apoptosis, apoptotic cell, phagocytosis, autoimmunity, systemic lupus erythematosus.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an immune complex-mediated autoimmune disease, which can lead to inflammation of multiple tissues/organisms, including skin and kidneys. The most characteristic feature of SLE is the presence of antibodies against a variety of nuclear antigens, such as dsDNA, nucleosomes, histones and other DNA/RNA-binding proteins [1-3]. Autoantibodies against nucleosomes and DNA are already found many years before the patient is diagnosed with SLE [4]. Local deposition of anti-nuclear antibodies in a complex with nuclear autoantigens finally induces serious inflammatory conditions such as glomerulonephritis. The levels of anti-nucleosome and anti-dsDNA antibodies seem to correlate with glomerulonephritis [1]. The nucleosome, 146 bp dsDNA wrapped around a histone octamer core, is a major autoantigen in SLE [5, 6]. In patients with SLE, apoptotic cells and immune complexes with nuclear autoantigens, such as nucleosomes, have been observed in several tissues, including the germinal centre of the lymph nodes, the epidermis [7, 8], the kidneys [9, 10], and the circulation [11, 12]. The presence of apoptotic cells and released autoantigens can be explained by deregulated apoptosis or insufficient clearance of the apoptotic cells by phagocytes,
such as macrophages and dendritic cells (DC). Importantly, histone and nucleosome-specific autoreactive T and B cells have been found. Therefore, the formation of nuclear autoantibodies in SLE seems to be T cell-dependent, which strongly suggests the involvement of an immunogenic presentation by DC of nuclear autoantigens to T cells. Most likely, these nuclear autoantigens are modified during apoptosis, making them more immunogenic, because novel T cell epitopes to which no tolerance exists are generated [13-16]. However, the exact aetiology of SLE is still not known and a multitude of factors could be involved, ranging from hormones, such as estrogen [17], virus infections, such as Epstein Barr virus, and molecular mimicry [18-20], to genetic predisposition, as for example in genes encoding HLA, PARP, Fas receptor, Fasl and Fcy receptor, and C1q [21-23]. Nevertheless, many experimental data suggest (defects in) apoptosis or clearance of apoptotic cells and debris as the key determinants in the pathogenesis of lupus [6, 15, 24].

Apoptosis

Apoptosis is the process of programmed cell death, and in a healthy individual about $10^{10}$-$10^{11}$ cells go into apoptosis each day. Apoptosis is involved in several biological processes including the formation, shaping and maintenance of tissues and organs, the regulation of the immune response by deletion of B and T cells, and the cellular response after damage of DNA. Apoptosis can be induced by intrinsic factors, such as DNA damage, and by extrinsic factors, such as the binding of a ligand to its receptor, for example the binding of Fas ligand to the Fas receptor [15]. After induction, apoptosis proceeds by following a cascade of complex signal transduction pathways that include the activation of caspases (cysteine-aspartic-acid-proteases) and endonucleases. Characteristic for apoptosis at the molecular level is the cleavage of chromatin in repetitive units of nucleosomes (“DNA laddering”; see Figure 1A). At the cellular level, apoptosis is characterized by the segregation of apoptotic blebs (Figure 1B) that contain autoantigens targeted in SLE [25, 26]. Early in apoptosis, the phospholipid phosphatidylserine (PS) re-orients to the outer side of the lipid bilayer, which allows the binding of annexin V, while at a later stage cells also become permeable for the DNA intercalating compound propidium iodide (PI), shown in Figure 2.

As introduced above, deregulated apoptosis may play a role in the development of SLE. In this paper, we present an updated overview of the association of SLE with factors related to apoptosis (Table I), and factors involved in the clearance of apoptotic material (Table II).

Figure 1. Cleavage of chromatin and formation of apoptotic blebs are characteristic for apoptosis. (A) During apoptosis the linker DNA between nucleosomes is cleaved by endonucleases such as CAD (caspase activated DNase). Extraction of DNA from apoptotic cells, subsequently separated according to size by agarose gel electrophoresis and stained with ethidium bromide, reveals DNA laddering. Lane A: from bottom to top corresponding to mono-, di-, tri-, tetra-, penta-, hexa-nucleosomes, etc., in which repetitive units of about 190 base pairs correspond to the DNA of the mononucleosome. Lane M: Molecular weight marker (size in base pairs). (B) During apoptosis blebs segregate at the cell surface. The apoptotic blebs contain clustered SLE autoantigens. The apoptotic cell and blebs were stained with Annexin V-FITC.
### Table I. Factors related to apoptosis associated with SLE

<table>
<thead>
<tr>
<th>Factor name</th>
<th>Abbreviation</th>
<th>Function</th>
<th>Human/mouse</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas receptor</td>
<td>FasR (CD95)</td>
<td>Induction of apoptosis, deletion autoreactive lymphocytes</td>
<td>Human, mouse</td>
<td>[27-32, 57-59]</td>
</tr>
<tr>
<td>Fas ligand</td>
<td>FasL (CD95L)</td>
<td>Induction of apoptosis, deletion autoreactive lymphocytes</td>
<td>Human, mouse</td>
<td>[27-32]</td>
</tr>
<tr>
<td>Interleukin 2</td>
<td>IL-2</td>
<td>Proliferation of T cells and response to Fas mediated apoptosis</td>
<td>Human, mouse</td>
<td>[33-37], [57–59]</td>
</tr>
<tr>
<td>B-cell activating factor</td>
<td>BAFF-receptor</td>
<td>Survival signal for B cells, maintenance of auto-reactive B cells</td>
<td>Mouse</td>
<td>[45, 46]</td>
</tr>
<tr>
<td>cAMP-responsive element modulator</td>
<td>CREM</td>
<td>Influences IL-2 transcription by binding to the IL-2 promoter</td>
<td>Human</td>
<td>[35, 36]</td>
</tr>
<tr>
<td>Programmed cell death-1</td>
<td>Pdcd1 or PD-1</td>
<td>Regulation of the generation of Foxp3+CD4+ regulatory T cells</td>
<td>Human, mouse</td>
<td>[39-41]</td>
</tr>
<tr>
<td>Programmed cell death-1 ligand</td>
<td>PD-1L (CD274)</td>
<td>Regulation of the generation of Foxp3+CD4+ regulatory T cells</td>
<td>Mouse</td>
<td>[42]</td>
</tr>
<tr>
<td>Src homology 2 domain-containing transforming protein C1</td>
<td>Shc1 or p66Shc</td>
<td>Negative regulator of lymphocyte activation</td>
<td>Mouse</td>
<td>[43]</td>
</tr>
<tr>
<td>Corin-1a</td>
<td>Coro1a</td>
<td>Involved in survival, migration and activation of T cells</td>
<td>Mouse</td>
<td>[44]</td>
</tr>
<tr>
<td>A proliferation-inducting ligand</td>
<td>APRIL</td>
<td>Regulation of B cell proliferation</td>
<td>Human</td>
<td>[53]</td>
</tr>
<tr>
<td>B cell lymphoma protein family</td>
<td>Bcl-2, BFI-1, Bcl-XL</td>
<td>Anti-apoptotic protein. Prevents apoptosis of cells, including autoreactive lymphocytes</td>
<td>Human</td>
<td>[31, 60, 136]</td>
</tr>
<tr>
<td>BCL2-like 1I (apoptosis facilitator)</td>
<td>BCL2L11 or Bim</td>
<td>BH-3 only protein. Induction of apoptosis, deletion of autoreactive immune cells</td>
<td>Mouse</td>
<td>[54-59]</td>
</tr>
<tr>
<td>Deoxyribonuclease I</td>
<td>DNase1</td>
<td>Fragments chromatin</td>
<td>Human, mouse</td>
<td>[61-63]</td>
</tr>
</tbody>
</table>

### Table II. Factors related to clearance of apoptotic material associated with SLE

<table>
<thead>
<tr>
<th>Factor name</th>
<th>Abbreviation</th>
<th>Function</th>
<th>Human/mouse</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage receptor with collagenous structure</td>
<td>MARCO</td>
<td>Clearance of apoptotic cells in the marginal zone of the spleen</td>
<td>Mouse</td>
<td>[85]</td>
</tr>
<tr>
<td>Fcy receptor IIIB</td>
<td>FCGRIIB (CD32)</td>
<td>Binding and uptake of IgG immune complexes and induction of B-cell apoptosis</td>
<td>Mouse</td>
<td>[92-94]</td>
</tr>
<tr>
<td>Fcy receptor IIA</td>
<td>FCGRIIA (CD32)</td>
<td>Clearance of IgG immune complexes and activation of phagocytes</td>
<td>Human, mouse</td>
<td>[89–91, 97-99]</td>
</tr>
<tr>
<td>Milk fat globule-EGF factor B protein</td>
<td>MFGE8</td>
<td>Binds to PS on apoptotic (B) cells and mediates uptake by phagocytes</td>
<td>Human, mouse</td>
<td>[100, 101]</td>
</tr>
<tr>
<td>c-mer proto-oncogene tyrosine kinase</td>
<td>MerTK or c-mer</td>
<td>Mediates uptake of apoptotic cells by phagocytes (via Gas6)</td>
<td>Mouse</td>
<td>[102, 103]</td>
</tr>
<tr>
<td>Mannan binding lectin</td>
<td>MBL</td>
<td>Mediates the uptake of apoptotic material by phagocytes</td>
<td>Human, mouse</td>
<td>[107-109]</td>
</tr>
<tr>
<td>Complement component 1, q subcomponent</td>
<td>C1q</td>
<td>Mediates the uptake of apoptotic material by phagocytes</td>
<td>Human, mouse</td>
<td>[73, 106, 107, 111, 137]</td>
</tr>
<tr>
<td>Pentraxin related gene</td>
<td>PTX3</td>
<td>Binds to apoptotic cells</td>
<td>Human</td>
<td>[124, 125]</td>
</tr>
<tr>
<td>Serum amyloid P-component</td>
<td>SAP or Apcs</td>
<td>Binds to apoptotic cells</td>
<td>Human, mouse</td>
<td>[116-123]</td>
</tr>
<tr>
<td>C reactive protein, pentraxin-related</td>
<td>CRP</td>
<td>Binds to apoptotic cells</td>
<td>Human, mouse</td>
<td>[115, 138]</td>
</tr>
</tbody>
</table>
The role of apoptosis and removal of apoptotic cells in the genesis of systemic lupus erythematosus

Aberrant apoptosis in systemic lupus erythematosus: extrinsic apoptotic pathways

Apoptosis in SLE may be deregulated due to changes in expression or function of proteins involved in the recognition of death signals, such as Fas, or survival signals, such as IL-2 and BAFF. This may lead to increased rates of apoptosis, or apoptosis at the wrong moment and/or place, and thereby to the accumulation of apoptotic cells and debris. Furthermore, a disturbed apoptotic process caused by, for example, mutations in the Fas system or increased expression of anti-apoptotic proteins may lead to the persistence of autoreactive T and B cells.

Multiple death signals and receptors can be involved in the induction of apoptosis. The Fas receptor (FasR; CD95) and Fas ligand (Fasl; CD95L) system induces apoptosis in activated lymphocytes. Mice deficient in FasR (lpr) or Fasl (gld) show lymphoproliferation and development of SLE-like features [27]. Mutations in the genes encoding FasR or Fasl in humans lead to familial autoimmune lymphoproliferative syndrome [28-30]. In patients with juvenile onset of SLE, increased expression of FasR on T cells has been detected [31]. However, mutations in the FasR-FasL system are not common in patients with SLE [28]. Nevertheless, when all available data are taken together, a malfunctioning FasR-FasL system may lead to the development of SLE [32].

In addition to death signals, the lack of survival signals also can lead to the induction of apoptosis. Binding of the cytokine IL-2 to the IL-2 receptor is an important survival signal for T cells leading to proliferation, but, paradoxically, IL-2 signalling seems also important for sensitization of T cells to Fas-mediated apoptosis. For the development of autoimmunity, especially the role of IL-2 as a promoter of tolerance, and not immunity, appears to be important [33]. Decreased expression of IL-2 or IL-2 receptor can be involved in the development of autoimmunity in mice and man [34, 35]. Reduced IL-2 levels can be caused by increased expression of the cAMP-responsive element modulators (CREM), p-CREM and CREM-α that inhibit the transcription of IL-2 [35, 36]. Interestingly, mice deficient for both IL-2 and Fas do not develop lupus-like disease, as found in mice with single gene defects in Fas or IL-2. These double-deficient mice developed inflammation in the colon, but had a life expectancy comparable to control mice [37]. This observation stresses the complexity of the pathogenesis of SLE.

Reduced IL-2 levels also could lead to impaired development of regulatory T cells (Tregs), which are important in maintaining the tolerance to self by inhibition of autoreactive T cells. Within Tregs, IL-2 promotes the expression of the transcription factor forkhead box protein P3 (Foxp3), which is required for the proper development of these cells [34, 38].

Programmed cell death-1 (PD-1 or Pdcd1) is an inhibitory immunoreceptor that belongs to the same family as CTLA-4, and has been linked to the development of SLE [39, 40]. Disruption of PD-1 induces lupus-like autoimmune disease in mice [41]. Interestingly, PD-1 ligand-induced signalling was shown to regulate the generation of Foxp3+ regulatory T cells [42]. Shc or p66Shc is another inhibitory regulator of lymphocytes, which in particular inhibits TCR coupling to the Ras/MAPK pathway priming T cell apoptosis. Mice deficient in p66Shc develop lupus-like disease, including autoantibody production, and immune complex
deposition in skin and kidney [43]. Finally, it was shown that mice with a nonsense mutation in the Coronin-1A encoding gene (Coro1A) developed lupus. The Coronin-1A protein appeared to be important for the survival, migration and activation of T cells [44].

Similarly to T cells, B cells are sensitive to survival signals, such as B cell activating factor (BAFF), also known as BLYS, which binds to the BAFF receptor [45]. BAFF signalling is required for the maintenance of autoreactive B cells in the marginal zone, and increases BAFF expression in mice results in the development of autoimmunity and SLE-like manifestations [46-49]. Interestingly, commonly used mice models for lupus, MRL/lpr and (NZBxNZW) F1 are characterized by high expression of BAFF [50], whereas treatment of these lupus mice with a soluble receptor for BAFF, TACI, inhibited the development of proteinuria and improved survival [47]. In some patients with SLE, elevated BAFF levels are found, which correlate with anti-dsDNA titres [51, 52]. A polymorphism in a proliferation-inducing ligand (APRIL), related to the BAFF protein, has recently been linked to SLE susceptibility [53].

**Aberrant apoptosis in systemic lupus erythematosus: intrinsic apoptotic pathways**

In addition to deregulated extrinsic apoptotic pathways, the aberrant expression or activity of molecules involved in the intrinsic or intracellular apoptotic pathways can lead to deregulated apoptosis and autoimmunity. Proteins of the Bcl-2 family can be anti-apoptotic (e.g. Bcl-2, Bfl-1 and Bcl-xL) or pro-apoptotic (e.g. Bax and Bak) or both (BH3-only proteins). The BH3-only protein Bim (Bcl2-like 11, apoptosis facilitator) induces apoptosis by binding to an anti-apoptotic protein (e.g. Bcl-xL) of the Bcl-2 family [54]. Defects in Bim lead to the persistence of autoreactive B and T cells, the survival of antigen-presenting cells, such as DC, and the induction of autoimmunity [54-56]. Therefore, Bim seems to play an important role in maintaining tolerance and preventing autoimmune reactions. Interestingly, mice defective in both Bim (Bcl2/1–/–) and Fas (Fas/(null)) develop more progressive and severe forms of SLE-like disease compared to mice with the respective single gene defects. Double-deficient mice showed a pronounced increase in the number of activated antigen presenting cells, increased expression of Fc-γ receptors, increased titres of anti-chromatin autoantibodies, and the presence of apoptotic cells in the glomeruli [57-59].

Independent of Bim, direct alterations of members of the Bcl2-family can also be involved in the development of SLE [31]. For example, increased expression of B-cl 2 leads to apoptosis-resistant (autoreactive) lymphocytes [60].

Finally, Dnase1-deficiency may be an important mediator leading to the accumulation of apoptotic chromatin. DNase I is involved in the breakdown of chromatins, the complex of DNA and associated proteins, into nucleosomes. DNaseI deficiencies in mice and man have been related to anti-chromatin autoantibody production and the development of SLE or SLE-like disease [61-63].

**Apoptosis-induced autoantigen modifications in systemic lupus erythematosus**

Apoptosis can lead to modified autoantigens for which no tolerance exists. During apoptosis autoantigens associated with SLE can be modified by cleavage through proteases and endonucleases. Furthermore, autoantigens may be post-translationally modified through addition of acetyl, phosphoryl, methyl, ubiquitin, citrulline, ADP or glutamine residues [13-16, 24]. We have recently identified apoptosis induced hyperacetylation of histones as a pathogenic factor in SLE [16]. Moreover, we found that hyperacetylated nucleosomes led to maturation of DC from lupus-prone mice, as measured by enhanced CD40 and CD86 expression, and IL-6 and TNF-α secretion. Finally, we showed activation of syngeneic T cells by DC matured with hyperacetylated nucleosomes, while normal nucleosomes had no effect.

In summary, many studies show that deregulation of apoptosis plays a central role in the development of SLE, which can be mediated by several factors (Table I). Deregulated apoptosis in SLE may result in the persistence of autoreactive B or T cells, and in the accumulation of apoptotic cells and apoptosis-induced modified autoantigens, which then leads to activation of the immune system and the development of autoimmunity as depicted in Figure 3. Accumulation of apoptotic cells and apoptotic debris in SLE may also be the consequence of an insufficient clearance capacity, which may involve various factors as listed in Table I. We will discuss the contribution of insufficient clearance to the development of SLE in the next paragraphs.

**Deranged removal of apoptotic cells in systemic lupus erythematosus**

Under normal conditions apoptotic cells are removed rapidly via phagocytosis by professional phagocytes, such as macrophages and DC. Apoptotic cells are normally cleared in a non-inflammatory or anti-inflammatory manner, which implies that phagocytosis does not lead to activation of the immune system initiated by maturation of antigen-presenting cells, such as DC, and subsequent activation of T and B cells. Normally, apoptotic cells are removed swiftly in an early phase, thereby preventing potentially harmful molecules being released. However, when apoptosis proceeds...
Apoptotic blebs will be formed at the surface of the apoptotic cells. These blebs contain clustered SLE autoantigens, such as chromatin, and segregate from the dying cell, whereas an apoptotic cell body will remain after the process of blebbing has finished. In later phases of apoptosis membrane integrity is completely lost and autoantigens, most likely modified during apoptosis, will be spilled into the environment. Systemic lupus erythematosus is a prototype immune complex mediated autoimmune disease. Anti-chromatin autoantibodies form immune complexes with circulating chromatin, and deposition of these complexes in basement membranes of the skin and kidneys causes inflammation and tissue destruction.

In summary, the inadequate clearance of apoptotic cells seems to represent an important step in the genesis of autoimmunity and inflammation in SLE [6, 24].

Apoptotic cell signals for clearance

Cells undergoing apoptosis display ‘come and get me’ signals, such as the lipid phosphatidylcholine (PC) or protein thrombospondin, and ‘eat me’ signals, such as the lipid phosphatidylserine (PS), complement factor C1q or adhesion molecules, such as ICAM3. These signals on the outside of the cell membrane serve to attract phagocytes and to mediate phagocytosis. In contrast, living cells display on the outside of the cell ‘don’t eat me’ signals, such as CD31, that prevent uptake. All these signals are recognized and bound by receptors expressed on phagocytes. In addition, in some cases ‘bridging’ molecules, for example opsonins, serve as a link between the signals on the surface of the apoptotic cell and the receptors on the phagocyte. Deficiencies in these components can lead to decreased clearance of apoptotic cells and to the development of SLE in humans or SLE-like features in mice [15, 64-68].

One well known ‘eat me’ signal is phosphatidylserine (PS), which is displayed at the outer cell membrane rapidly after the induction of apoptosis (see Figure 2). During the process of apoptosis phospholipases remodel the cell membranes, leading to the reorientation of phospholipids, such as PS, lysophosphatidylcholine and phosphorylcholine, from the inner lipid layer to the outer lipid layer [68, 69]. Factors such as MFG-E8, IgM or CRP can bind ligands such as PS, and are involved in the clearance of early apoptotic cells [6, 7, 64-69]. Phosphatidylserine can be bound directly by recep-
tors on the phagocyte or indirectly via ‘bridging’ molecules [64, 65]. The importance of the PS signal for ingestion of apoptotic cells by phagocytes was demonstrated by the inhibitory effect of annexin V on phagocytosis of apoptotic cells [70, 71].

Another important ‘eat me signal’ is calreticulin, which is present on the surface of apoptotic cells and blebs, and which can be recognized by phagocytes. Calreticulin can be bound by C1q, MBL and ficolin3 and is thereby indirectly recognized by CD91 (LDL-related receptor protein, \(\alpha\)-2-macroglobulin) on phagocytes [66, 72-74], as detailed below.

Receptors on phagocytes facilitating phagocytosis

A myriad of receptors on the surface of phagocytes are involved in the uptake of apoptotic cells and have been associated with SLE. These receptors include, for example, T cell immunoglobulin and mucin-domain-containing molecule (Tim) 1, Tim4, Fc receptors, MerTK, scavenger receptors, lectin-like receptors, CD31 and complement receptors [72-79], as will be discussed below.

Several studies have shown a major role of phosphatidylserine receptor (PSR) on phagocytes in the recognition and clearance of apoptotic cells, which can be modulated by other receptors, such as CD31 [64, 68, 76, 79].

Tim1 and Tim4 receptors are expressed on macrophages and DC and recognize structural features of PS, in particular anionic residues, via their immunoglobulin domain, thereby facilitating phagocytosis [80, 81]. Tim4 is highly expressed on human macrophages and immature DC, and is expressed at a low level on mature DC. This finding correlates with the greater capacity of phagocytosis of immature DC compared with mature DC.

Stabilin 2 is another receptor that recognizes PS and mediates the uptake of apoptotic cells and the subsequent production of anti-inflammatory molecules [64, 82-84]. Recognition of PS by stabilin 2 is calcium dependent and requires interaction with GULP1 (engulfment adaptor PTB domain containing 1) or thymosin 4B.

In the marginal zone of the spleen, scavenger receptor A and macrophage scavenger receptor can bind to apoptotic cells and are involved in their clearance. In the marginal zone, apoptotic cells are mainly ingested by macrophages and not by DC, which normally is an anti-inflammatory process. However, when the apoptotic load exceeds the uptake capacity of macrophages, this can result in uptake and immunogenic presentation by DC, and finally an autoimmune response. Indeed, mice deficient for the class A scavenger receptors (MARCO) develop autoimmune body production, suggesting that absence of these receptors on marginal zone macrophages leads to an immune response [85].

CD14 is involved in the recognition of apoptotic cells by phagocytes (macrophages). Mice deficient in CD14 are less effective in clearance of apoptotic cells, resulting in the persistence of apoptotic cells in vivo. However, this does not lead to the production of autoantibodies or the development of autoimmunity, which might be explained by the use of mice non-susceptible for development of SLE, and the retained ability to generate anti-inflammatory signals by macrophages in response to apoptotic cells [86].

In SLE, insufficient clearance of immune complexes, for example existing of apoptotic chromatin and anti-chromatin autoantibodies, leads to their deposition in basement membranes and local inflammation [6]. Immune complexes are normally removed by phagocytosis through binding to Fc receptors or via binding to complement factors. In SLE, binding of opsonins to apoptotic cells opsonizes these cells, thus facilitating their phagocytosis [87]. This engulfment of apoptotic cells or immune complexes containing lupus autoantigens via Fc-receptors, predominantly the immune activating Fc\(\gamma\)RIIA receptor, can subsequently lead to maturation of DC and the production of pro-inflammatory cytokines [88-91]. Defects in the immune inhibitory Fc\(\gamma\)RIIB receptor have been linked to the development of autoimmunity and immune complex deposition in the kidney [92-95]. Dendritic cells deficient in Fc\(\gamma\)RIIB produce high amounts of the pro-inflammatory cytokine IL-12 when exposed to apoptotic cells. The amount of expression of Fc\(\gamma\)RIIB can be modulated by certain cytokines, which varies with the type of cells (macrophage, B cell and DC) [95, 96]. Fc\(\gamma\)RIIA and Fc\(\gamma\)RIIIA are also genetically associated with susceptibility to the development of lupus nephritis [97, 98]. Fc\(\gamma\)RIIA expression on DC seems important for activation and production of cytokines induced by autoantibody-coated apoptotic cells or immune complexes from patients with SLE or lupus mice [88-91]. Interestingly, in a recent genome-wide screen in women with SLE, polymorphisms in the gene encoding Fc\(\gamma\)RIIA were associated with a predisposition to develop SLE [99].

Opsonins and systemic lupus erythematosus

Bridging molecules or opsonins between apoptotic cells and phagocytes are crucial in the process of phagocytosis. MFG-E8 bound to phospholipids, such as PS, on apoptotic cells is recognized by \(\alpha\)\(\beta\)3 integrins on phagocytes [68, 69]. MFG-E8 plays in this way an important role in the phagocytosis of apoptotic B cells in the germinal centres. MFG-E8-deficient mice develop SLE-like features, and produce autoantibodies against dsDNA and other nuclear components; they have an enlarged spleen, renal IgG
deposits and develop proteinuria [100]. Altered levels of MFG-E8 have been found in patients with SLE [101], suggesting that MFG-E8 can play a role in the pathogenesis of SLE.

MerTK−/− mice exhibit reduced clearance of apoptotic cells and manifest SLE-like autoimmunity [102, 103]. The inhibitory effect of apoptotic cells on IL-12 production by DC and inhibition of T-cell activation was absent in MerTK-deficient mice and when MerTK signalling was specifically blocked with an antibody. In addition, antibodies against the MerTK ligand Gas6 were also able to block the inhibitory effect of apoptotic cells on IL-12 production by DC [104].

Complement factors bind to apoptotic cells. The complement initiation molecules C1q, MBL and ficolins can bind to late apoptotic cells in particular, thereby activating the complement system and facilitating clearance through binding of CD91 on phagocytes or via the complex with calreticulin. These factors facilitate the phagocytosis of apoptotic cells by DC and macrophages; however, in SLE the clearance of apoptotic cells via this pathway was shown to be disturbed [66, 73, 74, 105-107]. The classical and lectin complement activating pathways seem to be predominantly involved in clearance of apoptotic cells. The classical pathway can be activated by binding of C1q and the lectin pathway by the binding of MBL. C1q can directly bind to apoptotic cells, but also indirectly via binding of pentraxins or MBL [66, 67, 77]. Polymorphisms in the MBL gene and low serum levels of MBL have been associated with SLE [108, 109]. IgM and CRP bind to lysophospholipids, including phosphorylcholine, and can mediate uptake of apoptotic cells via recruitment of components of the complement system [69]. Furthermore, C1q is needed for uptake of degraded chromatin [7, 110]. Mice lacking C1q develop SLE, indicating the importance in the removal of apoptotic material and prevention of SLE development [111]. Activation of the complement system leads to cleavage of C3 by C3 convertases, resulting in the formation of the complement factor C3b, which can function as an opsonin by binding to CR3 or CR4 [69] on phagocytes. In SLE autoantibodies against complement components are found, with the highest frequency for anti-C1q autoantibodies, especially in patients with renal involvement [112].

Other opsonins that are involved in uptake of apoptotic cells through the complement system include the pentraxins, such as pentraxin 3 (PTX3), serum amyloid P component (SAP) and C-reactive protein (CRP), which all seem to be associated with the development of lupus. Binding of CRP to apoptotic cells protects against the assembly of the terminal complement components and induces an anti-inflammatory response [113, 114]. A polymorphism in CRP is associated with reduced basal levels of CRP and with the development of SLE, probably due to improper clearance of apoptotic cells [113-115]. Similar findings have been reported for the pentraxin SAP [116-118]. Serum amyloid P component binds to chromatin in vitro and in vivo in SLE patients and a correlation between anti-SAP antibodies with disease activity has been found [119, 120]. Complexes of SAP and DNA are decreased in patients with SLE [121, 122]. Deletion of the SAP gene in mice results in the development of antinuclear autoimmunity and severe lupus nephritis [123]. The pentraxin PTX3 also binds to apoptotic cells, particularly sequestering cell remnants, thereby regulating the clearance by antigen presenting cells [124, 125].

In summary, apoptotic cells expose several signals that are recognized by receptors on phagocytes or bound by opsonins, thus facilitating their phagocytosis. In SLE, disturbances in these apoptotic signals, phagocytic receptors and/or opsonins may be responsible for reduced clearance of apoptotic cells and debris, and ultimately lead to the development of autoimmunity due to immunogenic presentation by DC of modified autoantigens.

**Immunogenicity of apoptotic cells and debris**

Several studies have examined the effect of injection of apoptotic cells, with or without DC, in mice non-susceptible to lupus and in lupus-susceptible mice strains, i.e. NZBWF1 and MRL/lpr. However, the results of these studies are not uniform. In some studies disease manifestations, such as autoantibody production and proteinuria, were observed after injection of apoptotic cells in lupus-prone mice, or only autoantibody production in normal mice [126, 127]. In other studies effects in lupus and normal mice were only observed when DC were co-injected with apoptotic or necrotic cells [128-132]. In normal, not autoimmune-prone mice, the autoantibody production declined with time after the injections [126, 128], suggesting that chronic exposure or other factors are needed to induce full-blown SLE. When apoptotic DNA was used instead of apoptotic cells, similar effects were observed [133], but the effects can vary with the route of administration [134]. Finally, as outlined in a previous paragraph, apoptosis-induced modifications of autoantigens may be a key event in maturation of DC and subsequent immunogenic presentation by DC to autoreactive T cells.

In summary, high amounts of apoptotic material, such as apoptotic cells and DNA, are able to break tolerance in mice. However, the development of lupus depends on several other factors, including the persistence of autoreactive lymphocytes and deranged clearance of apoptotic material, which is exemplified by the dependence on the lupus-prone background.
Concluding remarks

The genesis of SLE remains elusive; however, aberrant apoptosis and/or reduced clearance of apoptotic cells and debris can be considered key contributing factors, as summarized in Figure 3. Normally, apoptotic cells are rapidly removed by phagocytes, and this takes place in an anti-inflammatory way, i.e. the immune system is not activated. Deregulated apoptosis can increase the number of autoreactive T and B cells. Furthermore, an increased rate of apoptosis and/or reduced clearance of apoptotic material can both lead to increased presence of apoptotic cells and debris. This apoptotic debris may contain apoptosis-induced autoantigen modifications that lead to activation of DC. Dendritic cell populations released from late apoptotic cells. Deposition of these immune complexes in basement membranes will result in local inflammation, such as glomerulonephritis.

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