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

Submitted: 20 October 2008
Accepted: 5 November 2008

Arch Med Sci 2009; 5, 3A: S 466–S 477

Corresponding author:
Johan van der Vlag, PhD
Nephrology Research Laboratory (279)
Nijmegen Centre for Molecular Life Sciences
Department of Nephrology
Radboud University Nijmegen Medical Centre, P.O. Box 9101
6500 HB Nijmegen,
The Netherlands
Phone: +31 24 361 65 39
Fax: +31 24 354 03 39
E-mail:
J.vanderVlag@NIER.umcn.nl

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/organs, 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 oestrogen [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 Fcγ 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-orientates 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).

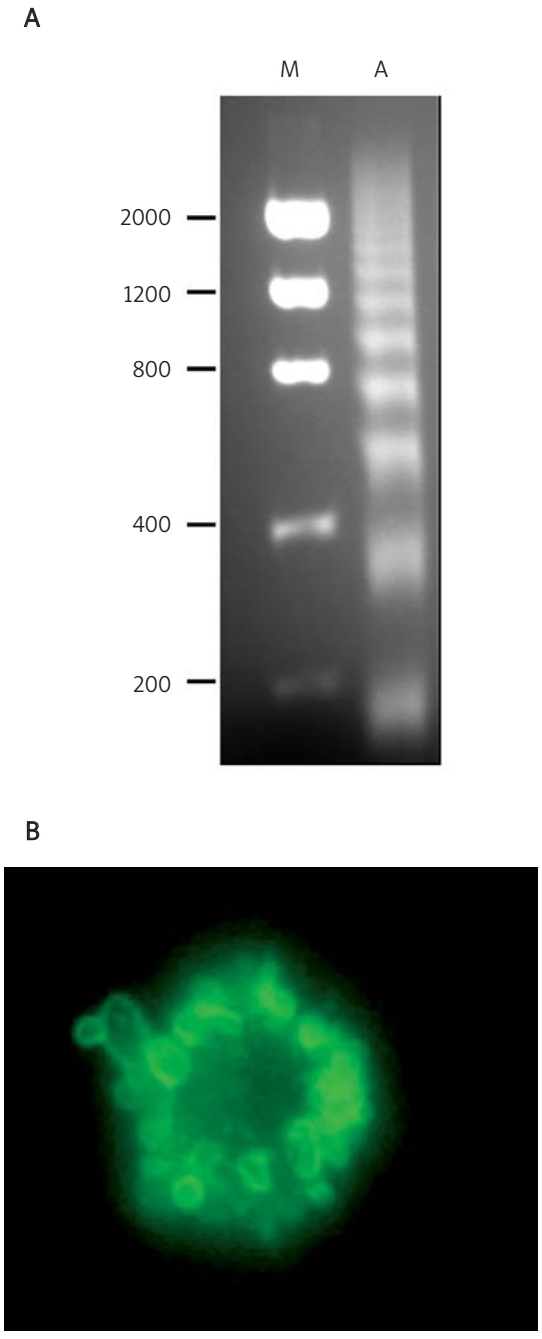

Figure 1. Cleavage of chromatin and formation of apoptotic blebs are characteristic for apoptosis. (A) During apoptosis the linker DNA between nucleosomes is cleaved by endonucleases such as CAD (caspase activated DNase). Extraction of DNA from apoptotic cells, subsequently separated according to size 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

Factor name	Abbreviation	Function	Human/mouse	Reference
Fas receptor	FasR (CD95)	Induction of apoptosis, deletion autoreactive lymphocytes	Human, mouse	[27-32, 57-59]
Fas ligand	FasL (CD95L)	Induction of apoptosis, deletion autoreactive lymphocytes	Human, mouse	[27-32]
Interleukin 2	IL-2	Proliferation of T cells and response to Fas mediated apoptosis	Human, mouse	[33-37], [57–59]
B-cell activating factor receptor	BAFF-receptor	Survival signal for B cells, maintenance of auto-reactive B cells	Mouse	[45, 46]
B-cell activating factor	BAFF or BLyS	Survival signal for B cells, maintenance of auto-reactive B cells	Human, mouse	[45-52]
cAMP-responsive element modulator	CREM	Influences IL-2 transcription by binding to the IL-2 promoter	Human	[35, 36]
Programmed cell death-1	Pdcd1 or PD-1	Regulation of the generation of $Foxp3^+CD4^+$ regulatory T cells	Human, mouse	[39-41]
Programmed cell death-1 ligand	PD-1L (CD274)	Regulation of the generation of $Foxp3^+CD4^+$ regulatory T cells	Mouse	[42]
Src homology 2 domain-containing transforming protein C1	Shc1 or p66Shc	Negative regulator of lymphocyte activation	Mouse	[43]
Coronin-1a	Coro1a	Involved in survival, migration and activation of T cells	Mouse	[44]
A proliferation-inducting ligand	APRIL	Regulation of B cell proliferation	Human	[53]
B cell lymphoma protein family	Bcl-2, Bfl-1, Bcl-X_L	Anti-apoptotic protein. Prevents apoptosis of cells, including autoreactive lymphocytes	Human	[31, 60, 136]
BCL2-like 11 (apoptosis facilitator)	BCL2L11 or Bim	BH-3 only protein. Induction of apoptosis, deletion of autoreactive immune cells	Mouse	[54-59]
Deoxyribonuclease I	DNase1	Fragments chromatin	Human, mouse	[61-63]

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

Factor name	Abbreviation	Function	Human/mouse	Reference
Macrophage receptor with collagenous structure	MARCO	Clearance of apoptotic cells in the marginal zone of the spleen	Mouse	[85]
Fcγ receptor IIB	FCGRIIB (CD32)	Binding and uptake of IgG immune complexes and induction of B-cell apoptosis	Mouse	[92-94]
Fcγ receptor IIA	FCGRIIA (CD32)	Clearance of IgG immune complexes and activation of phagocytes	Human, mouse	[89–91, 97-99]
Milk fat globule-EGF factor 8 protein	MFGE8	Binds to PS on apoptotic (B) cells and mediates uptake by phagocytes	Human, mouse	[100, 101]
c-mer proto-oncogene tyrosine kinase	MerTK or c-mer	Mediates uptake of apoptotic cells by phagocytes (via Gas6)	Mouse	[102, 103]
Mannan binding lectin	MBL	Mediates the uptake of apoptotic material by phagocytes	Human, mouse	[107-109]
Complement component 1, q subcomponent	C1q	Mediates the uptake of apoptotic material by phagocytes	Human, mouse	[73, 106, 107, 111, 137]
Pentraxin related gene	PTX3	Binds to apoptotic cells	Human	[124, 125]
Serum amyloid P-component	SAP or Apcs	Binds to apoptotic cells	Human, mouse	[116-123]
C reactive protein, pentraxin-related	CRP	Binds to apoptotic cells	Human, mouse	[115, 138]

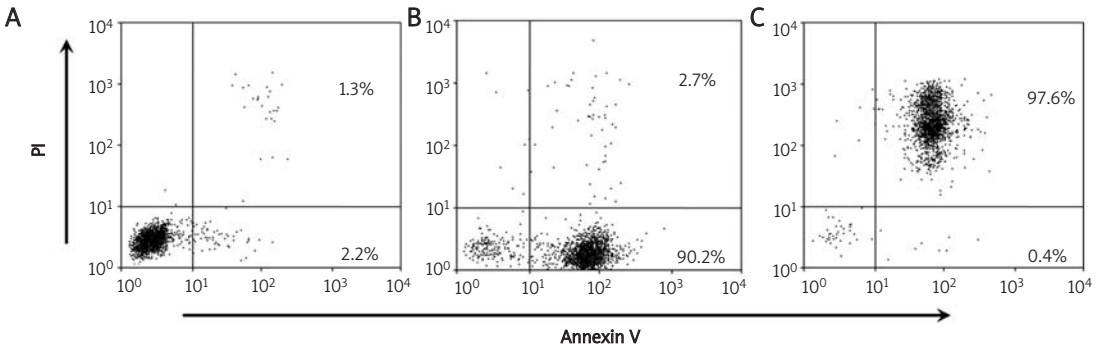

Figure 2. Annexin V binding and propidium iodide staining discriminate between early and late apoptotic cells. (A) Living cells are negative for Annexin V-FITC binding and propidium iodide (PI) staining. Annexin V binds to the phospholipid phosphatidylserine (PS), which is normally present at the cytoplasmic site of the lipid bilayer. Propidium iodide is a fluorescent dye that intercalates between the bases of DNA. When cells go into apoptosis the phospholipid phosphatidylserine (PS) re-orientates within the lipid bilayer from the inside to the outside. Therefore, early apoptotic cells become positive for annexin V binding (B). When apoptosis proceeds, cells lose their membrane integrity, making them permeable for PI, which then stains DNA. Late apoptotic cells, sometimes referred to as secondary necrotic cells, are, therefore, double positive for annexin V binding and PI staining (C)

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 increased 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-X_L), 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-X_L) 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 (Bcl2/11$^{-/-}$) and Fas ($^{lpr/lpr}$) 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 Bcl-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 chromatin, the complex of DNA and associated proteins, into nucleosomes. DNase1 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 II. 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

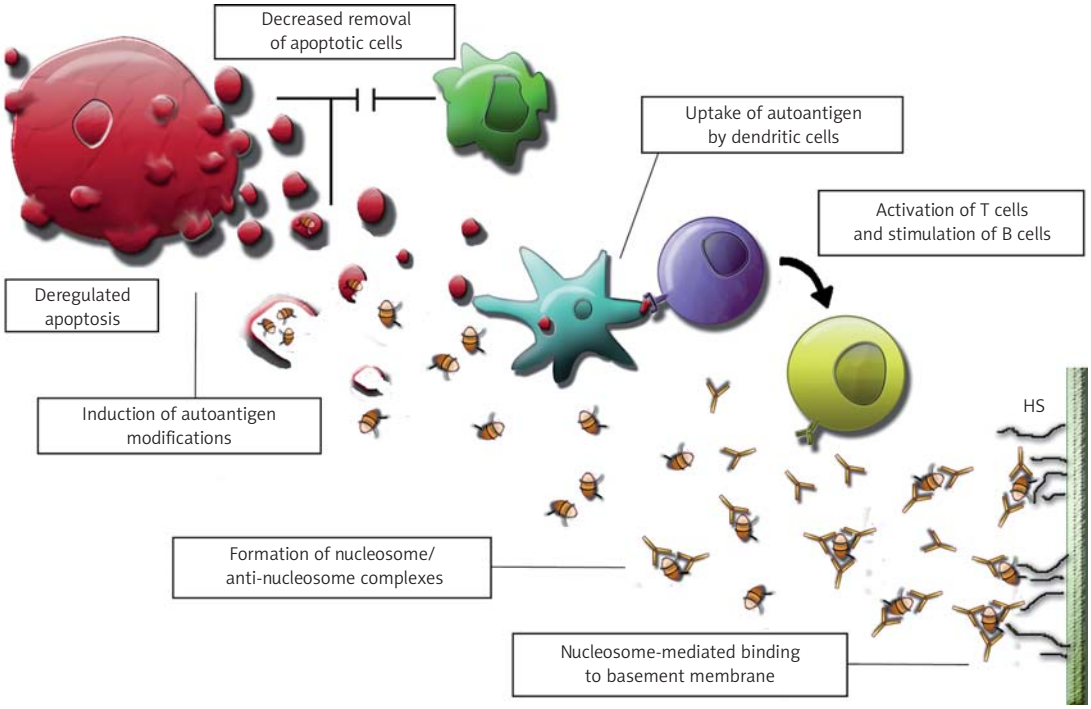

Figure 3. Hypothesis for the genesis of SLE. As a consequence of aberrant apoptosis and/or reduced clearance, apoptotic cells accumulate and apoptotic debris is released. Autoantigens can be modified and cluster in blebs formed at the apoptotic cell surface. Apoptotic blebs containing apoptosis-induced autoantigen modifications [16] can segregate and their contents be released. This apoptotic material can be ingested by DC, which then leads to DC maturation and presentation of (modified) autoantigens in an immunogenic way to autoreactive T cells recognizing the (modified) autoantigens [135]. Activated T cells will stimulate B cells to produce autoantibodies that can form immune complexes with chromatin released from apoptotic cells. Deposition of these immune complexes in basement membranes leads to serious inflammation, such as lupus nephritis. Adapted from [15]

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, α-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 recognises 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 4β.

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 autoantibody 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 autoantibodies 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 FcγRIIA receptor, can subsequently lead to maturation of DC and the production of pro-inflammatory cytokines [88-91]. Defects in the immune inhibitory FcγRIIB receptor have been linked to the development of autoimmunity and immune complex deposition in the kidney [92-95]. Dendritic cells deficient in FcγRIIB produce high amounts of the pro-inflammatory cytokine IL-12 when exposed to apoptotic cells. The amount of expression of FcγRIIB can be modulated by certain cytokines, which varies with the type of cells (macrophage, B cell and DC) [95, 96]. FcγRIIA and FcγRIIIA are also genetically associated with susceptibility to the development of lupus nephritis [97, 98]. FcγRIIA 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 FcγRIIA 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 $\alpha_v\beta_3$ 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 will present the modified autoantigens to T cells, resulting in T cell and subsequent B cell activation. The activated B cells will produce autoantibodies which can form immune complexes with autoantigens released from late apoptotic cells. Deposition of these immune complexes in basement membranes will result in local inflammation, such as glomerulonephritis.

Acknowledgments

The Ph.D. programme of the Radboud University Nijmegen Medical Centre and the Dutch Kidney Foundation (grant C05.2119) supported this work.

References

1. Burlingame RW, Cervera R. Anti-chromatin (anti-nucleosome) autoantibodies. Autoimmun Rev 2002; 1: 321-8.
2. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford) 2007; 46: 1052-6.
3. Su Y, Jia RL, Han L, Li ZG. Role of anti-nucleosome antibody in the diagnosis of systemic lupus erythematosus. Clin Immunol 2007; 122: 115-20.
4. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003; 349: 1526-33.
5. Decker P, Singh-Jasuja H, Haager S, Kotter I, Rammensee HG. Nucleosome, the main autoantigen in systemic lupus erythematosus, induces direct dendritic cell activation via a MyD88-independent pathway: consequences on inflammation. J Immunol 2005; 174: 3326-34.
6. Dieker JW, van der Vlag J, Berden JH. Triggers for anti-chromatin autoantibody production in SLE. Lupus 2002; 11: 856-64.
7. Gaipl US, Voll RE, Sheriff A, Franz S, Kalden JR, Herrmann M. Impaired clearance of dying cells in systemic lupus erythematosus. Autoimmun Rev 2005; 4: 189-94.
8. Gaipl US, Munoz LE, Grossmayer G, et al. Clearance deficiency and systemic lupus erythematosus (SLE). J Autoimmun 2007; 28: 114-21.
9. van Bruggen MC, Berden JH. Central role for nucleosomes in lupus. Nephrol Dial Transplant 1996; 11: 1219-22.
10. van Bavel CC, Fenton KA, Rekvig OP, van der Vlag J, Berden JH. Glomerular targets of nephritogenic autoantibodies in systemic lupus erythematosus. Arthritis Rheum 2008; 58: 1892-9.
11. Licht R, van Bruggen MC, Oppers-Walgreen B, Rijke TP, Berden JH. Plasma levels of nucleosomes and nucleosome-autoantibody complexes in murine lupus: effects of disease progression and lipopolyssacharide administration. Arthritis Rheum 2001; 44: 1320-30.
12. Rumore PM, Steinman CR. Endogenous circulating DNA in systemic lupus erythematosus. Occurrence as multimeric complexes bound to histone. J Clin Invest 1990; 86: 69-74.
13. Dieker J, Cisterna B, Monneaux F, et al. Apoptosis-linked changes in the phosphorylation status and subcellular localization of the spliceosomal autoantigen U1-70K. Cell Death Differ 2008; 15: 793-804.
14. Utz PJ, Gensler TJ, Anderson P. Death, autoantigen modifications, and tolerance. Arthritis Res 2000; 2: 101-14.
15. Munoz LE, van Bavel C, Franz S, Berden J, Herrmann M, van der Vlag J. Apoptosis in the pathogenesis of systemic lupus erythematosus. Lupus 2008; 17: 371-5.
16. Dieker JW, Fransen JH, van Bavel CC, et al. Apoptosis-induced acetylation of histones is pathogenic in systemic lupus erythematosus. Arthritis Rheum 2007; 56: 1921-33.
17. Costenbader KH, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. Arthritis Rheum 2007; 56: 1251-62.
18. Albert LJ, Inman RD. Molecular mimicry and autoimmunity. N Engl J Med 1999; 341: 2068-74.
19. James JA, Kaufman KM, Farris AD, Taylor-Albert E, Lehman TJ, Harley JB. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. J Clin Invest 1997; 100: 3019-26.
20. McClain MT, Heinlen LD, Dennis GJ, Roebuck J, Harley JB, James JA. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. Nat Med 2005; 11: 85-9.
21. Wakeland EK, Liu K, Graham RR, Behrens TW. Delineating the genetic basis of systemic lupus erythematosus. Immunity 2001; 15: 397-408.
22. Tsao BP. The genetics of human systemic lupus erythematosus. Trends Immunol 2003; 24: 595-602.
23. Martens HA, Zuurman MW, de Lange AH, et al. Analysis of C1q polymorphisms suggests association with SLE, serum C1q and CH50 levels and disease severity. Ann Rheum Dis 2009; 68: 715-20.
24. Dieker JW, van der Vlag J, Berden JH. Deranged removal of apoptotic cells: its role in the genesis of lupus. Nephrol Dial Transplant 2004; 19: 282-5.
25. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med 1994; 179: 1317-30.
26. Radic M, Marion T, Monestier M. Nucleosomes are exposed at the cell surface in apoptosis. J Immunol 2004; 172: 6692-700.
27. Nagata S, Suda T. Fas and Fas ligand: lpr and gld mutations. Immunol Today 1995; 16: 39-43.
28. Wu J, Wilson J, He J, Xiang L, Schur PH, Mountz JD. Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. J Clin Invest 1996; 98: 1107-13.
29. Fisher GH, Rosenberg FJ, Straus SE, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 1995; 81: 935-46.

30. Rieux-Laucat F, Le Deist F, Hivroz C, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 1995; 268: 1347-9.
31. Liphaus BL, Kiss MH, Carrasco S, Goldenstein-Schainberg C. Increased Fas and Bcl-2 expression on peripheral mononuclear cells from patients with active juvenile-onset systemic lupus erythematosus. J Rheumatol 2007; 34: 1580-4.
32. Xue C, Lan-Lan W, Bei C, Jie C, Wei-Hua F. Abnormal Fas/FasL and caspase-3-mediated apoptotic signaling pathways of T lymphocyte subset in patients with systemic lupus erythematosus. Cell Immunol 2006; 239: 121-8.
33. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. Nat Rev Immunol 2004; 4: 665-74.
34. Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3 (+) CD25 (+) CD4 (+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. J Exp Med 2005; 201: 723-35.
35. Solomou EE, Juang YT, Gourley MF, Kammer GM, Tsokos GC. Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. J Immunol 2001; 166: 4216-22.
36. Kyttaris VC, Wang Y, Juang YT, Weinstein A, Tsokos GC. CAMP response element modulator a expression in patients with systemic lupus erythematosus. Lupus 2006; 15: 840-4.
37. Xiao S, Sung SS, Fu SM, Ju ST. Combining Fas mutation with interleukin-2 deficiency prevents Colitis and Lupus: implicating interleukin-2 for auto-reactive T cell expansion and Fas ligand for colon epithelial cell death. J Biol Chem 2003; 278: 52730-8.
38. Zheng SG, Wang J, Wang P, Gray JD, Horwitz DA. IL-2 is essential for TGF-beta to convert naive CD4+CD25- cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. J Immunal 2007; 178: 2018-27.
39. Prokunina L, Castillejo-Lopez C, Oberg F, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 2002; 32: 666-9.
40. Thorburn CM, Prokunina-Olsson L, Sterba KA, et al. Association of PDCD1 genetic variation with risk and clinical manifestations of systemic lupus erythematosus in a multiethnic cohort. Genes Immun 2007; 8: 279-87.
41. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 1999; 11: 141-51.
42. Wang L, Pino-Lagos K, de Vries VC, Guleria I, Sayegh MH, Noelle RJ. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. Proc Natl Acad Sci U S A 2008; 105: 9331-6.
43. Finetti F, Pellegrini M, Ulivieri C, et al. The proapoptotic and antimitogenic protein p66SHC acts as a negative regulator of lymphocyte activation and autoimmunity. Blood 2008; 111: 5017-27.
44. Haraldsson MK, Louis-Dit-Sully CA, Lawson BR, et al. The lupus-related Lmb3 locus contains a disease-suppressing Coronin-1A gene mutation. Immunity 2008; 28: 40-51.
45. Miller DJ, Hayes CE. Phenotypic and genetic characterization of a unique B lymphocyte deficiency in strain A/WySnJ mice. Eur J Immunol 1991; 21: 1123-30.
46. Enzler T, Bonizzi G, Silverman GJ, et al. Alternative and classical NF-kappa B signaling retain autoreactive B cells in the splenic marginal zone and result in lupus-like disease. Immunity 2006; 25: 403-15.
47. Gross JA, Johnston J, Mudri S, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 2000; 404: 995-9.
48. Mackay F, Woodcock SA, Lawton P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J Exp Med 1999; 190: 1697-710.
49. Khare SD, Sarosi I, Xia XZ, et al. Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. Proc Natl Acad Sci U S A 2000; 97: 3370-5.
50. Stohl W. Systemic lupus erythematosus: a blissless disease of too much BLyS (B lymphocyte stimulator) protein. Curr Opin Rheumatol 2002; 14: 522-8.
51. Zhang J, Roschke V, Baker KP, et al. Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. J Immunol 2001; 166: 6-10.
52. Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. Arthritis Rheum 2001; 44: 1313-9.
53. Lee YH, Ota F, Kim-Howard X, Kaufman KM, Nath SK. APRIL polymorphism and systemic lupus erythematosus (SLE) susceptibility. Rheumatology (Oxford) 2007; 46: 1274-6.
54. Bouillet P, Purton JF, Godfrey DI, et al. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. Nature 2002; 415: 922-6.
55. Chen M, Huang L, Wang J. Deficiency of Bim in dendritic cells contributes to overactivation of lymphocytes and autoimmunity. Blood 2007; 109: 4360-7.
56. Oliver PM, Vass T, Kappler J, Marrack P. Loss of the proapoptotic protein, Bim, breaks B cell anergy. J Exp Med 2006; 203: 731-41.
57. Hughes PD, Belz GT, Fortner KA, Budd RC, Strasser A, Bouillet P. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. Immunity 2008; 28: 197-205.
58. Hutcheson J, Scatizzi JC, Siddiqui AM, et al. Combined deficiency of proapoptotic regulators Bim and Fas results in the early onset of systemic autoimmunity. Immunity 2008; 28: 206-17.
59. Weant AE, Michalek RD, Khan IU, Holbrook BC, Willingham MC, Grayson JM. Apoptosis regulators Bim and Fas function concurrently to control autoimmunity and CD8+ T cell contraction. Immunity 2008; 28: 218-30.
60. Graninger WB, Steiner CW, Graninger MT, Aringer M, Smolen JS. Cytokine regulation of apoptosis and Bcl-2 expression in lymphocytes of patients with systemic lupus erythematosus. Cell Death Differ 2000; 7: 966-72.
61. Martinez Valle F, Balada E, Ordi-Ros J, Vilardell-Tarres M. DNase 1 and systemic lupus erythematosus. Autoimmun Rev 2008; 7: 359-63.
62. Yasutomo K, Horiuchi T, Kagami S, et al. Mutation of DNASE1 in people with systemic lupus erythematosus. Nat Genet 2001; 28: 313-4.
63. Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, Moroy T. Features of systemic lupus erythematosus in Dnase1-deficient mice. Nat Genet 2000; 25: 177-81.
64. Ravichandran KS, Lorenz U. Engulfment of apoptotic cells: signals for a good meal. Nat Rev Immunol 2007; 7: 964-74.
65. Albert ML. Death-defying immunity: do apoptotic cells influence antigen processing and presentation? Nat Rev Immunol 2004; 4: 223-31.
66. Trouw LA, Blom AM, Gasque P. Role of complement and complement regulators in the removal of apoptotic cells. Mol Immunol 2008; 45: 1199-207.
67. Walport MJ. Complement. Second of two parts. N Engl J Med 2001; 344: 1140-4.

68. Grimsley C, Ravichandran KS. Cues for apoptotic cell engulfment: eat-me, don't eat-me and come-get-me signals. Trends Cell Biol 2003; 13: 648-56.
69. Kim SJ, Gershov D, Ma X, Brot N, Elkon KB. Opsonization of apoptotic cells and its effect on macrophage and T cell immune responses. Ann NY Acad Sci 2003; 987: 68-78.
70. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis Rheum 1998; 41: 1241-50.
71. Munoz LE, Frey B, Pausch F, et al. The role of annexin A5 in the modulation of the immune response against dying and dead cells. Curr Med Chem 2007; 14: 271-7.
72. Clarke C, Smyth MJ. Calreticulin exposure increases cancer immunogenicity. Nat Biotechnol 2007; 25: 192-3.
73. Donnelly S, Roake W, Brown S, et al. Impaired recognition of apoptotic neutrophils by the C1q/calreticulin and CD91 pathway in systemic lupus erythematosus. Arthritis Rheum 2006; 54: 1543-56.
74. Honore C, Hummelshoj T, Hansen BE, Madsen HO, Eggleton P, Garred P. The innate immune component ficolin 3 (Hakata antigen) mediates the clearance of late apoptotic cells. Arthritis Rheum 2007; 56: 1598-607.
75. Bondanza A, Zimmermann VS, Rovere-Querini P, et al. Inhibition of phosphatidylserine recognition heightens the immunogenicity of irradiated lymphoma cells in vivo. J Exp Med 2004; 200: 1157-65.
76. Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J. Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. Nature 2002; 418: 200-3.
77. Nauta AJ, Daha MR, van Kooten C, Roos A. Recognition and clearance of apoptotic cells: a role for complement and pentraxins. Trends Immunol 2003; 24: 148-54.
78. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity 2007; 40: 560-6.
79. Bratton DL, Henson PM. Apoptotic cell recognition: will the real phosphatidylserine receptor (s) please stand up? Curr Biol 2008; 18: R76-9.
80. Kobayashi N, Karisola P, Pena-Cruz V, et al. TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. Immunity 2007; 27: 927-40.
81. Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. Nature 2007; 450: 435-9.
82. Park SY, Jung MY, Kim HJ, et al. Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. Cell Death Differ 2008; 15: 192-201.
83. Park SY, Kang KB, Thapa N, Kim SY, Lee SJ, Kim IS. Requirement of adaptor protein GULP during stabilin-2-mediated cell corpse engulfment. J Biol Chem 2008; 283: 10593-600.
84. Lee SJ, So IS, Park SY, Kim IS. Thymosin beta4 is involved in stabilin-2-mediated apoptotic cell engulfment. FEBS Lett 2008; 582: 2161-6.
85. Wermeling F, Chen Y, Pikkarainen T, et al. Class A scavenger receptors regulate tolerance against apoptotic cells, and autoantibodies against these receptors are predictive of systemic lupus. J Exp Med 2007; 204: 2259-65.
86. Devitt A, Parker KG, Ogden CA, et al. Persistence of apoptotic cells without autoimmune disease or inflammation in CD14-/- mice. J Cell Biol 2004; 167: 1161-70.
87. Frisoni L, McPhie L, Colonna L, et al. Nuclear autoantigen translocation and autoantibody opsonization lead to increased dendritic cell phagocytosis and presentation of nuclear antigens: a novel pathogenic pathway for autoimmunity? J Immunol 2005; 175: 2692-701.
88. Boule MW, Broughton C, Mackay F, Akira S, Marshak-Rothstein A, Rifkin IR. Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. J Exp Med 2004; 199: 1631-40.
89. Means TK, Latz E, Hayashi F, Murali MR, Golenbock DT, Luster AD. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. J Clin Invest 2005; 115: 407-17.
90. Bave U, Magnusson M, Eloranta ML, Perers A, Alm GV, Ronnblom L. Fc gamma RIIa is expressed on natural IFN-alpha-producing cells (plasmacytoid dendritic cells) and is required for the IFN-alpha production induced by apoptotic cells combined with lupus IgG. J Immunol 2003; 171: 3296-302.
91. Bave U, Vallin H, Alm GV, Ronnblom L. Activation of natural interferon-alpha producing cells by apoptotic U937 cells combined with lupus IgG and its regulation by cytokines. J Autoimmun 2001; 17: 71-80.
92. McGaha TL, Karlsson MC, Ravetch JV. FcgammaRIIB deficiency leads to autoimmunity and a defective response to apoptosis in Mrl-MpJ mice. J Immunol 2008; 180: 5670-9.
93. Rahman ZS, Manser T. Failed up-regulation of the inhibitory IgG Fc receptor Fc gamma RIIB on germinal center B cells in autoimmune-prone mice is not associated with deletion polymorphisms in the promoter region of the Fc gamma RIIB gene. J Immunol 2005; 175: 1440-9.
94. Brownlie RJ, Lawlor KE, Niederer HA, et al. Distinct cell-specific control of autoimmunity and infection by FcgammaRIIb. J Exp Med 2008; 205: 883-95.
95. Liu Y, Masuda E, Blank MC, et al. Cytokine-mediated regulation of activating and inhibitory Fc gamma receptors in human monocytes. J Leukoc Biol 2005; 77: 767-76.
96. Rudge EU, Cutler AJ, Pritchard NR, Smith KG. Interleukin 4 reduces expression of inhibitory receptors on B cells and abolishes CD22 and Fc gamma RII-mediated B cell suppression. J Exp Med 2002; 195: 1079-85.
97. Tsao BP. Update on human systemic lupus erythematosus genetics. Curr Opin Rheumatol 2004; 16: 513-21.
98. Salmon JE, Millard S, Schachter LA, et al. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. J Clin Invest 1996; 97: 1348-54.
99. Harley JB, Alarcon-Riquelme ME, Criswell LA, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet 2008; 40: 204-10.
100. Hanayama R, Tanaka M, Miyasaka K, et al. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science 2004; 304: 1147-50.
101. Yamaguchi H, Takagi J, Miyamae T, et al. Milk fat globule EGF factor 8 in the serum of human patients of systemic lupus erythematosus. J Leukoc Biol 2008; 83: 1300-7.
102. Cohen PL, Caricchio R, Abraham V, et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. J Exp Med 2002; 196: 135-40.
103. Scott RS, McMahon EJ, Pop SM, et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 2001; 411: 207-11.
104. Wallet MA, Sen P, Flores RR, et al. MerTK is required for apoptotic cell-induced T cell tolerance. J Exp Med 2008; 205: 219-32.
105. Kuraya M, Ming Z, Liu X, Matsushita M, Fujita T. Specific binding of L-ficolin and H-ficolin to apoptotic cells leads to complement activation. Immunobiology 2005; 209: 689-97.

106. Nauta AJ, Trouw LA, Daha MR, et al. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. Eur J Immunol 2002; 32: 1726-36.
107. Nauta AJ, Castellano G, Xu W, et al. Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. J Immunol 2004; 173: 3044-50.
108. Kristjansdottir H, Saevarsdottir S, Grondal G, et al. Association of three systemic lupus erythematosus susceptibility factors, PD-1.3A, C4AQ0, and low levels of mannan-binding lectin, with autoimmune manifestations in icelandic multicase systemic lupus erythematosus families. Arthritis Rheum 2008; 58: 3865-72.
109. Monticielo OA, Mucenic T, Xavier RM, Brenol JC, Chies JA. The role of mannose-binding lectin in systemic lupus erythematosus. Clin Rheumatol 2008; 27: 413-9.
110. Gaipl US, Beyer TD, Heyder P, et al. Cooperation between C1q and DNase I in the clearance of necrotic cell-derived chromatin. Arthritis Rheum 2004; 50: 640-9.
111. Botto M, Dell'Agnola C, Bygrave AE, et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. Nat Genet 1998; 19: 56-9.
112. Daha MR. Pathogenic role of auto-antibodies against complement components in systemic lupus erythematosus. Lupus 2008; 17: 385-8.
113. Gershov D, Kim S, Brot N, Elkon KB. C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. J Exp Med 2000; 192: 1353-64.
114. Marnell L, Mold C, Du Clos TW. C-reactive protein: ligands, receptors and role in inflammation. Clin Immunol 2005; 117: 104-11.
115. Russell AI, Cunninghame Graham DS, Shepherd C, et al. Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. Hum Mol Genet 2004; 13: 137-47.
116. Bijl M, Horst G, Bijzet J, Bootsma H, Limburg PC, Kallenberg CG. Serum amyloid P component binds to late apoptotic cells and mediates their uptake by monocyte-derived macrophages. Arthritis Rheum 2003; 48: 248-54.
117. Mold C, Gresham HD, Du Clos TW. Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc gamma Rs. J Immunol 2001; 166: 1200-5.
118. Bharadwaj D, Mold C, Markham E, Du Clos TW. Serum amyloid P component binds to Fc gamma receptors and opsonizes particles for phagocytosis. J Immunol 2001; 166: 6735-41.
119. Breathnach SM, Kofler H, Sepp N, et al. Serum amyloid P component binds to cell nuclei in vitro and to in vivo deposits of extracellular chromatin in systemic lupus erythematosus. J Exp Med 1989; 170: 1433-8.
120. Zandman-Goddard G, Blank M, Langevitz P, et al. Anti-serum amyloid component P antibodies in patients with systemic lupus erythematosus correlate with disease activity. Ann Rheum Dis 2005; 64: 1698-702.
121. Voss A, Nielsen EH, Svehag SE, Junker P. Serum amyloid P component-DNA complexes are decreased in systemic lupus erythematosus. inverse association with anti-dsDNA antibodies. J Rheumatol 2008; 35: 625-30.
122. Bijl M, Bootsma H, Van Der Geld Y, Limburg PC, Kallenberg CG, Van Rijswijk MH. Serum amyloid P component levels are not decreased in patients with systemic lupus erythematosus and do not rise during an acute phase reaction. Ann Rheum Dis 2004; 63: 831-5.
123. Bickerstaff MC, Botto M, Hutchinson WL, et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nat Med 1999; 5: 694-7.
124. Rovere P, Peri G, Fazzini F, et al. The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. Blood 2000; 96: 4300-6.
125. Baruah P, Propato A, Dumitriu IE, et al. The pattern recognition receptor PTX3 is recruited at the synapse between dying and dendritic cells, and edits the cross-presentation of self, viral, and tumor antigens. Blood 2006; 107: 151-8.
126. Mevorach D, Zhou JL, Song X, Elkon KB. Systemic exposure to irradiated apoptotic cells induces autoantibody production. J Exp Med 1998; 188: 387-92.
127. Shoshan Y, Mevorach D. Accelerated autoimmune disease in MRL/MpJ-Fas (lpr) but not in MRL/MpJ following immunization with high load of syngeneic late apoptotic cells. Autoimmunity 2004; 37: 103-9.
128. Bondanza A, Zimmermann VS, Dell'Antonio G, et al. Cutting edge: dissociation between autoimmune response and clinical disease after vaccination with dendritic cells. J Immunol 2003; 170: 24-7.
129. Bondanza A, Zimmermann VS, Dell'Antonio G, et al. Requirement of dying cells and environmental adjuvants for the induction of autoimmunity. Arthritis Rheum 2004; 50: 1549-60.
130. Georgiev M, Agle LM, Chu JL, Elkon KB, Ashany D. Mature dendritic cells readily break tolerance in normal mice but do not lead to disease expression. Arthritis Rheum 2005; 52: 225-38.
131. Ma L, Chan KW, Trendell-Smith NJ, et al. Systemic autoimmune disease induced by dendritic cells that have captured necrotic but not apoptotic cells in susceptible mouse strains. Eur J Immunol 2005; 35: 3364-75.
132. Tzeng TC, Suen JL, Chiang BL. Dendritic cells pulsed with apoptotic cells activate self-reactive T-cells of lupus mice both in vitro and in vivo. Rheumatology (Oxford) 2006; 45: 1230-7.
133. Wen ZK, Xu W, Xu L, et al. DNA hypomethylation is crucial for apoptotic DNA to induce systemic lupus erythematosus-like autoimmune disease in SLE-non-susceptible mice. Rheumatology (Oxford) 2007; 46: 1796-803.
134. Zhang QH, Link H, Xiao BG. Efficacy of peripheral tolerance induced by dendritic cells is dependent on route of delivery. J Autoimmun 2004; 23: 37-43.
135. Bellone M, Iezzi G, Rovere P, et al. Processing of engulfed apoptotic bodies yields T cell epitopes. J Immunol 1997; 159: 5391-9.
136. Andre J, Cimaz R, Ranchin B, et al. Overexpression of the antiapoptotic gene Bfl-1 in B cells from patients with familial systemic lupus erythematosus. Lupus 2007; 16: 95-100.
137. Bijl M, Reefman E, Horst G, Limburg PC, Kallenberg CG. Reduced uptake of apoptotic cells by macrophages in systemic lupus erythematosus: correlates with decreased serum levels of complement. Ann Rheum Dis 2006; 65: 57-63.
138. Du Clos TW, Zlock LT, Hicks PS, Mold C. Decreased autoantibody levels and enhanced survival of (NZB x NZW) F1 mice treated with C-reactive protein. Clin Immunol Immunopathol 1994; 70: 22-7.