Mod Rheumatol (2005) 15:383–390
DOI 10.1007/s10165-005-0430-x

REVIEW ARTICLE

**Gerhard E. Grossmayer · Luis E. Munoz · Udo S. Gaipl
Sandra Franz · Ahmed Sheriff · Reinhard E. Voll
Joachim R. Kalden · Martin Herrmann**

Removal of dying cells and systemic lupus erythematosus

Received: July 29, 2005 / Accepted: August 23, 2005

Abstract Systemic lupus erythematosus (SLE) is a very heterogeneous systemic autoimmune disease, in which autoantibody synthesis against nuclear constituents is the main immunological characteristic. These autoantibodies underwent affinity maturation and isotype switching. Additionally, T-cell tolerance against nuclear autoantigens should be affected in these autoimmune patients. Nuclear material derived from apoptotic and/or necrotic cells may serve as an important source of autoantigens. However, dead and dying cells as well as cellular debris are rapidly removed from tissues by phagocytes without eliciting inflammation or immune responses under healthy conditions. During apoptosis nuclear components are strongly modified through enzymatic reactions. If these cells are not timely cleared, those autoantigens may be released, taken up, and presented by dendritic cells in tissues or presented by follicular dendritic cells in lymph nodes to T and B cells, respectively. This could be a mechanism for breaking the peripheral self-tolerance. In this article we focus on the deficient clearance of apoptotic cells in SLE patients and its importance in development of this autoimmune disease.

Key words Apoptosis · Autoimmunity · Clearance · Necrosis · Systemic lupus erythematosus (SLE)

G.E. Grossmayer · L.E. Munoz · U.S. Gaipl · S. Franz · A. Sheriff · J.R. Kalden · M. Herrmann (✉)
Institute for Clinical Immunology, Department of Medicine 3, Friedrich-Alexander University of Erlangen-Nuremberg, Glückstrasse 4a, 91054 Erlangen, Germany
Tel. +49-9131-85-36345; Fax +49-9131-85-35776
e-mail: martin.herrmann@med3.imed.uni-erlangen.de

R.E. Voll
IZKF Research Group N2, Nikolaus-Fiebiger Center of Molecular Medicine, Erlangen, Germany

G.E.G. and L.E.M. contributed equally to this work.

Introduction

The immune mechanisms of the pathogenesis of systemic lupus erythematosus (SLE) are still not fully understood. This disease shows a wide array of clinical manifestations resulting from inflammatory reactions in multiple organs. Immunological abnormalities observed in the autoimmune disease SLE are characterized by autoantibody synthesis against nuclear constituents, immune complex deposits in tissues, and complement activation. Antinuclear autoantibodies are not exclusively generated in SLE, but also in other autoimmune diseases like scleroderma, rheumatoid arthritis, and Sjögren's syndrome. Several genetic and environmental factors as well as infections have been implicated as important elements of the disease (reviewed in Herrmann et al.[1] and Kuenkele et al.[2]). How intracellular proteins become targets of immune responses is important in understanding the etiology of autoimmune diseases.

Anti-double-stranded DNA antibodies (anti-dsDNA) are one of the most important criteria for the diagnosis of the disease. They show high-affinity binding activity in contrast to low-affinity naturally occurring anti-dsDNA antibodies from normal healthy donors (NHD), which did not go through the isotype switch from IgM to IgG or IgA. Affinity maturation of immunoglobulins for the antigen and isotype switching are processes dependent on T-cell help. This takes place in the germinal centers (GCs) of lymphoid tissues. The role of T cells in the development of chronic autoimmunity in SLE is supported by the fact that patients with SLE respond to T-cell specific immunosuppressive drugs like cyclosporin A.[3]

Although human DNA is known to be poorly immunogenic and does not bear T-cell epitopes, DNA–histone complexes and nucleosomes are released in SLE patients in high amounts into the circulation.[4,5] The nucleosomes are targets of anti-dsDNA autoantibodies and of anti-histone autoantibodies, as shown by enzyme-linked immunosorbent assays with autoimmune sera and monoclonal antibodies.[6,7] Those autoantibodies are predominantly of the IgG isotype and molecular analyses revealed a high degree of somatic muta-

tions in their V_H regions. These are unique features of an antigen-driven immune response.[8,9] Nucleosomes can be recognized and internalized by DNA-specific B cells, which in turn process and present DNA-associated proteins and thereby receive T-cell help.[10]

The presence of histone-specific T cells in SLE patients[11,12] reinforces the theory that nuclear material derived from apoptotic and/or necrotic cells may serve as an important source of autoantigens. Therefore, cell death is the most likely phenomenon to provide these autoantigens. Apoptosis and necrosis are the two main forms of cell death in multicellular organisms and they are important for tissue homeostasis. Whether cells die through apoptosis or necrosis is determined by the initial stimulus and the microenvironment. Regardless of the type of cell death, dying cells have to be quickly eliminated from tissues in order to prevent further damage. Usually, neither apoptotic nor necrotic cell material is easily found in tissues because of the quick removal by a highly efficient scavenger system (macrophages, polymorphonuclear cells [PMNs] and immature dendritic cells [DCs]).[13,14] Apoptotic cells are cleared in early stages and therefore elicit neither inflammation nor immune responses.[15] Further more, they can cause immunosuppressive effects after they are phagocytosed.[16] On the other hand, necrotic cells induce inflammation and favor the initiation of immune responses.[17] In this article we discuss the cells, receptors, and danger signals involved in the mechanism of clearance of dying cells, and show how defects in this process may contribute to the etiology and pathogenesis of SLE and other autoimmune diseases.

Recognition and uptake of apoptotic cells

Phagocytosis and the immune response

Phagocytosis plays a central role in the defense of pathogens. Phagocytes ingest infectious agents soon after colonization and represent the first barrier against microbial intruders. The initiation of an adaptive immune response requires specialized cells capable of phagocytosis and presentation of processed antigens on their surfaces. These tasks are performed by antigen-presenting cells (APCs), which are mainly dendritic cells in the skin and tissue-specific macrophages. After ingesting a pathogen APCs travel to secondary lymphoid organs and interact with naive T cells in the T-cell zone of a lymph node. T cells encountering their specific antigen proliferate and differentiate into effector cells. Naive B cells entering the T-cell zone from the circulation need two stimuli for clonal expansion and differentiation: binding of antigen and interaction with helper T cells recognizing the same antigen. These activated B cells migrate into the lymphoid follicles to form GCs consisting of centroblasts and centrocytes. Follicular dendritic cells (FDCs) play an essential role in affinity maturation of B cells by providing survival signals for centrocytes with the optimal antigen-binding receptors.[18] The antigen is opsonized with C3d and fixed to the surface of FDCs via CR2/CD21.[19,20] Positively selected centrocytes migrate into the mantle zone where they specifically interact with helper T cells and receive further signals for proliferation and differentiate into plasma cells or memory cells. Centrocytes having low affinity for the antigen are not provided with the mentioned survival signals and execute apoptosis. Specialized phagocytes named tingible body macrophages (TBMs) engulf apoptotic B cells rapidly, so that virtually no free apoptotic material can be found in GCs (Fig. 1a).[21,22]

The study of lymph node histological sections demonstrated that GCs of some SLE patients contain much unengulfed nuclear material, indicating an insufficient disposal of apoptotic cells. The finding that the number of TBMs containing ingested apoptotic nuclei is significantly reduced in a subgroup of SLE patients leads to the assumption that a phagocytosis defect is causally involved in the clearance deficiency. Such deficiency in phagocytosis leads to accumulation of apoptotic cells that subsequently enter the stage of secondary necrosis, which is critical because of the release of nuclear autoantigens and danger signals. C3d may coat these potential autoantigens and facilitate the binding on the surface of FDCs, which might supply autoreactive B cells with survival signals.[22] This can increase the probability of an encounter with autoreactive T cells, resulting in clonal expansion and autoantibody production (Fig. 1b).

Apoptosis and necrosis

Cell death occurs in two main forms, apoptosis and necrosis, each having different initial triggers. Apoptosis is defined as programmed cell death or cellular suicide, whereas necrosis arises due to a violent external stimulus.[23] The observation that already unicellular organisms are able to perform a kind of programmed suicide, if they are virus infected, might give a hint to the origins of apoptosis.[24] Multiple molecules involved in the death machinery are conserved during evolution. In multicellular organisms apoptosis appears during ontogenesis, development, and in the regulation of tissue homeostasis. Potentially dangerous cells like injured, infected, extraneous, and mutated cells are eliminated quickly and quietly. Specialized macrophages, immature DCs, or neutrophils are responsible for the efficient removal of dying cells.[25]

Apoptosis can be initiated at a certain state of development or by extrinsic signals resulting in the activation of a series of proteolytic reactions. Proteins, enzymes, and chromatin are cleaved – "the cell shreds its entrails." A hallmark of apoptotic cells, in contrast to necrotic cells, is that they maintain their membrane integrity over time. Thus, the release of intracellular components that could damage the surrounding tissue, induce inflammation, or elicit immune responses is prevented.[26] To ensure immediate recognition and uptake by phagocytes, apoptotic cells undergo very early membrane modifications. If apoptotic cells are cleared in time, no inflammation will be induced.[16,27]

The necrotic cell is morphologically characterized by swelling and finally the disruption of the cell membrane.

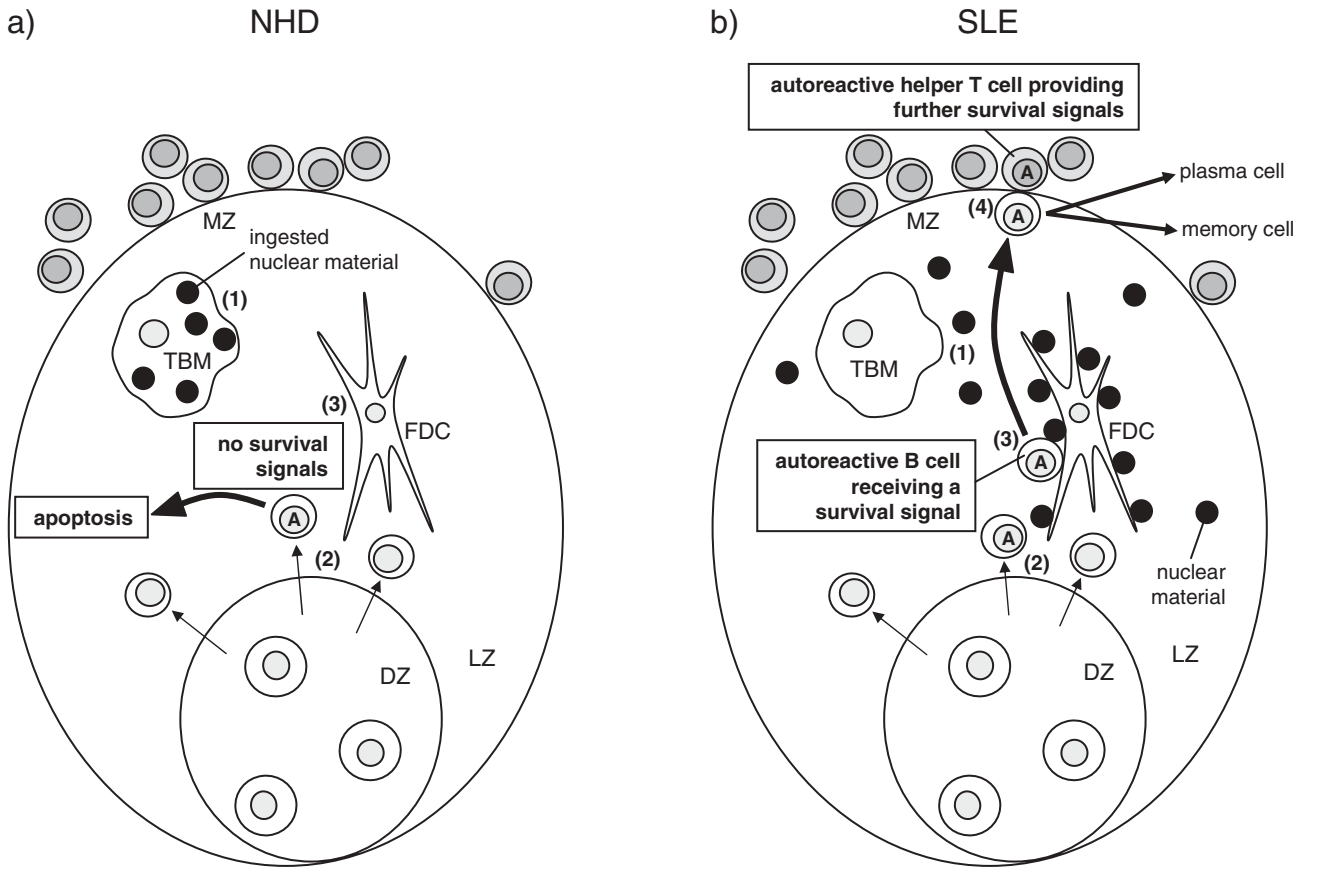

Fig. 1a,b. Events taking place in germinal centers (GCs) of lymph nodes. The description focuses on the differing fate of autoreactive cells (*A*) occurring in systemic lupus erythematosus (*SLE*) patients as well as in normal healthy donors (*NHD*). **a** In the healthy situation apoptotic lymphocytes are efficiently removed (*1*) by tingible body macrophages (*TBM*) and therefore no free apoptotic material bearing autoantigens can be found. During somatic hypermutation of centroblasts in the dark zone (*DZ*) an autoreactive B cell can emerge (*2*) and enters, together with nonautoreactive B cells, the light zone (*LZ*). As there are no autoantigens (*3*) that could be presented on follicular dendritic cells (*FDC*), the autoreactive B cell is not provided with survival signals and executes apoptosis. **b** In SLE, clearance deficiency leads to the accumulation of nuclear material (*1*) in the light zone of the GC. FDCs bind this nuclear material to their surface. During somatic hypermutation an autoreactive B cell can emerge (*2*) and enters, together with nonautoreactive B cells, the light zone. Here it recognizes nuclear autoantigens presented by FDCs and receives a short-term survival signal (*3*). After migration into the mantle zone (*MZ*) it specifically interacts with an autoreactive helper T cell and obtains further survival signals (*4*), submitting the differentiation into plasma cells and memory cells

Loss of physical integrity leads to the release of intracellular fluids, organelles, and proteins bearing proinflammatory capacity, which attract inflammatory cells. The uptake and clearance of necrotic cells is usually combined with inflammation and an immune response.[28] Those cells that have lost their membrane integrity by a violent physical or chemical stimulus still keep an intact nucleus and are called primary necrotic cells. The DNA content of detergent-lysed cells can be measured by flow cytometry after, e.g., staining with propidium iodide. Nuclei from primary necrotic cells, like those from viable ones, show a diploid DNA content.[29]

In contrast, apoptotic cells have nuclei with reduced DNA content. Nuclear condensation and loss of membrane asymmetry, besides DNA fragmentation, are further characteristics of a cell undergoing apoptosis. In the very early phase, membranes of apoptotic cells become acid labile.[30] The exposure of phosphatidylserine (PS) on the outer leaflet of apoptotic cells is an important primary "eat-me signal." During the late phases of apoptotic cell death, there are several further membrane alterations leading to the recognition of the apoptotic cells by additional adaptor molecules (reviewed in Hart et al.[31]). Those late apoptotic cells still preserve their membrane integrity. If the membrane integrity is lost during apoptosis, the dying cells are then called secondary necrotic cells.[32] Due to the disintegration of the membrane, secondary necrotic cells give access to potential intracellular autoantigens. However, the term "late apoptotic cells" is often used instead of "secondary necrotic cells" in the literature.

Molecules, receptors, and consequences of apoptotic cell phagocytosis

During the progression from apoptosis to secondary necrosis dying cells expose various "eat-me" signals. The early apoptotic cell initiates an anti-inflammatory effect while reaching the stage of secondary necrosis induces an increasingly proinflammatory reaction.[33] The recognition by phagocytes, the correct translation of the signals, and phagocytosis are mediated by a diversity of adaptor molecules and receptors. The fine-tuning of the recognition process is ensured by highly redundant and hierarchically ordered receptors.

Changes of the phospholipid distribution in the plasma membrane during apoptosis

The cell membrane of an intact cell shows an asymmetric distribution of phospholipids due to an adenosine triphosphate (ATP) dependent mechanism: the outer leaflet primarily contains sphingomyelin and phosphatidylcholine, whereas the inner leaflet harbors most of the PS and phosphatidylethanolamine (PE). In the early phase of apoptosis and in the absence of ATP, as in necrosis, PS and PE are exposed on the outer leaflet of the membrane.[34,35] Phosphatidylserine can be recognized by various receptors expressed on phagocytes: the vitronectin receptor ($\alpha_v\beta_3$ integrin),[36] the β_2-glycoprotein-1 receptor,[37] scavenger receptors (SR), CD68[38] and class A scavenger receptors (SR-A),[39] the ATP-binding cassette transporter 1 (ABC1),[40] the lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1),[41] and the lipopolysaccharide receptor CD14.[42] The anti-inflammatory and immunosuppressive effects generated by PS exposure can be explained by several mechanisms. Inflammatory signals like interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and nitric oxide are suppressed in PS-mediated phagocytosis of apoptotic cells whereas the production of TGF-β, an anti-inflammatory cytokine, is increased.[16,43] In contrast, necrotic cells often exert proinflammatory effects although they also expose PS.[44–46] Necrotic cells lose their membrane integrity and released intracellular PS-binding proteins, such as annexins, that could interact with PS, resulting in a masking of its immunosuppressive effects.[47]

The role of pentraxins in the uptake of dying cells

The family of pentraxins includes, besides other molecules, the C-reactive protein (CRP) and serum amyloid P (SAP). Pentraxins consist of highly conserved glycoproteins composed of 10 identical subunits arranged in two cyclic pentamers. Their binding to ligands is calcium dependent and, as acute-phase reactants, they are involved in the regulation of inflammation.[48]

The freely in human serum circulating secretory phospholipase A2 IIA (sPLA2 IIA) needs the binding to PS to hydrolyze phospholipids on the outer membrane leaflet. Therefore, sPLA2 IIA is only able to lyse phospholipids on the surface of membranes if the cell exposes PS on the outer leaflet, as in apoptosis and necrosis. The products of sPLA2 IIA are high amounts of lysophospholipids on cell surfaces like lysophosphatidylcholine, which bears binding sites for CRP.[49,50] Binding of CRP activates complement via the classical pathway, permitting opsonization of the dying cells. Additionally, CRP itself represents an opsonin as it interacts with Fc receptors of phagocytes, favoring the phagocytosis process. Furthermore, CRP owns affinity to membranes and nuclear constituents, like histones and small nuclear ribonucleoproteins of necrotic cells. As CRP binds to nuclei of necrotic cells, but is unable to pass the plasma membrane of apoptotic cells, this selective binding might be a fine-tuning mechanism in differentiating apoptosis and necrosis.[33]

Serum amyloid P shows binding activity toward a multitude of molecules, reflecting its manifold functions. It binds to extracellular matrix components, to carbohydrates, to microbial polysaccharides, to PE, to C1q, and C4 binding protein. Moreover, SAP specifically binds to DNA, chromatin, and nucleic compounds released during necrosis, and its interaction with nuclei leads to the solubilization of nuclear chromatin.[51]

Phosphatidylethanolamine is found on early as well as on late apoptotic cells, so SAP should bind to cells in many phases of cell death. However, relevant binding was observed only to cells in the late stages of apoptosis.[52] The exact targets of SAP binding on apoptotic cells still remain elusive. Serum amyloid P is able to bind to the Fcγ receptors (CD16, CD32, CD64) on phagocytes and might have an opsonizing function for bound material.[53]

High mobility group B1 (HMGB1) protein distinguishes apoptotic from primary necrotic cells

The high mobility group B1 (HMGB1) protein is a potent inflammatory mediator released by primary necrotic cells. In apoptotic cells, HMGB1 is "frozen" on the chromatin and remains immobilized even under conditions of secondary necrosis.[54,55] This is an important distinctive feature between primary and secondary necrotic cells, representing a further mechanism of fine-tuning the response against dying cells.[56]

Complement opsonizes dying cells

Dying cells activate the complement system through several mechanisms and C1q, C3, and C4 have been implicated in the clearance process of dying cells, since the withdrawal of such components deeply affects the phagocytosis of apoptotic as well as necrotic cells in vitro and in vivo.[19,57] We observed that complement binding is an early event in necrosis and a rather late event in the case of apoptosis. Therefore, we think that at least C3 and C4 act as a backup mechanism to clear apoptotic cells before they enter the dangerous stage of secondary necrosis.[32] Recently, it has been suggested that previous deposition of IgM on apoptotic cells is required for complement activation through the classical pathway and for the further opsonization of these cells by the C3 component.[58] The

hereditary C1q deficiency is the strongest proof of the importance of this complement protein in the pathogenesis of autoimmunity against nuclear structures, because it illustrates many typical characteristics of human SLE disease. For example, in C1q knockout mice, glomerulonephritis and accumulation of apoptotic material in renal tissues has been observed. However, only mild forms of SLE-like syndromes are found in such mice.[59,60] Accumulation of late apoptotic cells, debris, or chromatin seems to be associated with antinuclear autoimmunity in mice and humans. However, the pathways involved seem to differ considerably. We have also found that C1q is necessary for an efficient uptake of necrotic cell-derived degraded chromatin by monocytes and human monocyte-derived macrophages.[29]

Altered carbohydrates as recognition system

Lectins are carbohydrate-binding proteins considered as players of the innate immune system, because they are usually involved in the recognition of characteristic carbohydrate patterns.[61] Lectins facilitate microbial removal and also play a role in apoptotic cell clearance. For example, the mannose binding lectin (MBL), the C1q complement component, and the surfactant proteins A (SPA) and D (SPD) are collagen-specific lectins (collectins) that efficiently bind apoptotic and necrotic cells and contribute to their removal thanks to the recognition of atypical carbohydrate structures.[57,62,63] We have found that the *Narcissus pseudonarcissus* lectin, the *Griffonia simplificolia lectin II*, and *Ulex europeaus agglutinin I* lectins have an increased binding activity for apoptotic cells in comparison to viable cells. This binding activity was specific for mannose, fucose, and *N*-acetylgalactosamine and was mainly found in late phases of the apoptotic process when compared with the binding of AxV. Interestingly, those lectins were able to bind with higher intensity to necrotic cells than to apoptotic ones.[64] Therefore, we can state that the exposure of those special sugar structures is a feature of late apoptotic cells and may serve as an additional removal mechanism for cells that have escaped earlier clearance mechanisms. Such changes may suggest a major event before secondary necrosis.

Clearance deficiency in SLE

Dead cells need to be removed from tissues as soon as possible. Although every minute thousands of cells are dying in our organism, apoptotic cells are rarely seen in healthy tissues and in peripheral blood. Therefore, there has to exist a very efficient "garbage disposal system" in order to avoid the accumulation of dead cells. Apoptotic cell removal is ensured mainly by the respective mononuclear phagocyte system in every tissue, namely, macrophages, dendritic cells, Kupffer cells, microglia, alveolar macrophages, and many others. Evidence from gene-targeted mouse models illustrates deficiencies in the clearance of apoptotic cells together with a very heterogeneous picture of autoimmunity. For example, the C1q-deficient mouse shows high numbers of apoptotic bodies in glomerular structures and spontaneous autoantibody response.[60] Mice that lack secreted IgM have more immune complex deposits in the glomeruli and develop more anti-dsDNA antibodies in comparison to wild-type mice.[65] As stated above, IgM is also required for the recognition and opsonization through the complement system of apoptotic cells, and both C1q and IgM are thought to act synergistically in the removal of apoptotic cells from tissues.[58] It was proved that these molecules mediate the clearance of apoptotic cells and immune complexes also in humans.[66]

Serum amyloid P is the major acute-phase reactant in mice, while in humans CRP fulfils this duty.[48] When the SAP gene is targeted in mice, they spontaneously develop autoimmunity against nuclear structures.[67] The interaction of SAP with nuclei also leads to the solubilization of nuclear chromatin.[51] It has long been known that CRP levels are low in patients with SLE. The low level of CRP may therefore also contribute to the impaired phagocytosis of dying cells in SLE. Furthermore, CRP is able to bind to membranes and several nuclear constituents of necrotic cells like histones and small nuclear ribonucleoproteins.[33] However, the exact role of human CRP in the clearance process remains to be clarified.

The DNaseI-deficient mouse displays glomerulonephritis and antinuclear autoantibodies.[68] In humans, serum DNAseI is responsible for the degradation of nuclear material, which is, e.g., accidentally released by secondary necrotic cells. Furthermore, DNaseI acts together with C1q in humans to efficiently degrade necrotic cell-derived chromatin.[29]

A deficiency in the engulfment of yeast and bacteria in patients with SLE was found several years ago.[69–71] We demonstrated that macrophages from SLE patients are also impaired in the phagocytosis of autologous apoptotic material in vitro.[72] This impaired clearance ability might be one important reason for the accumulation of dead and dying cells found in tissues of a subgroup of patients with SLE. We also observed that the number of TBMs which usually ingest apoptotic material in the GCs of lymph nodes was strongly reduced in some SLE patients. In contrast to all controls, apoptotic material was associated with the surfaces of FDCs.[22] The defects seen in macrophages from SLE patients seem also to be an intrinsic problem of this cell type. We have quantitatively measured the adhesion of these macrophages to plastic, and have found that the adhesion index in SLE patients is significantly lower than that in NHD after 1 week of in vitro differentiation in the presence of GM-CSF (our own unpublished data). Focal adhesions are critical events in the differentiation of monocytes to macrophages. They are actively formed after a ligand–receptor interaction and are also important in migration and perhaps in the engulfment process, since both are actin-dependent phenomena.[73,74] We also investigated the proliferation and differentiation capabilities of CD34-positive hematopoietic stem cells obtained from the peripheral blood of SLE patients and healthy donors. While the pro-

liferation rates of the stem cells in patients and controls were similar, the differentiation into macrophages was diminished in some SLE stem-cell cultures. Macrophages derived from the blood of SLE patients were different in morphology, died earlier, showed reduced adherence, and had in general a lower phagocytic activity.[75]

Phagocytosis by PMNs was also found to be deeply depressed in SLE patients.[76] Furthermore, phagocytosis by PMNs under inflammatory conditions is impaired in the autoimmune mouse strain MRL/lpr.[77] We are currently investigating the phagocytic function of PMNs from healthy donors, from RA patients, and from SLE patients under noninflammatory conditions. We found that the phagocytosis defects in some SLE patients are heterogeneous.[75] Phagocytosis by PMNs of yeast and *Escherichia coli* was also depressed in our examined blood from patients with SLE.

We further observed that the phagocytosis of primary necrotic cells by macrophages is also highly dependent on complement and other thermolabile serum components. Additionally, in experimental settings with macrophages derived from NHD we found that sera from some SLE patients decreased the phagocytosis activity of those macrophages and other SLE sera highly increased it (our own unpublished data). This again highlights that intrinsic as well as extrinsic factors are important for an efficient clearance of apoptotic and necrotic cells.

Immunological outcome of a deficient clearance

The isolation of autoreactive T cells from patients with SLE and their ability to induce the production of anti-dsDNA autoantibodies in vitro suggests that somehow the tolerance mechanisms against self-antigens have been broken in these patients.[11,78,79] The apoptotic process implies a very intense enzymatic degradation of nuclear proteins and DNA,[80] leading to the exposure of novel autoantigens that are recognized by autoimmune sera.[81] These modifications may also render cryptic epitopes into T-cell-dependent dominant autoantigens.[82] The presence of high amounts of such T-cell-dependent autoantigens in tissues, as it occurs in clearance deficient circumstances, may break the peripheral tolerance. For example, late apoptotic cells and apoptotic cell-derived "blebs" can activate complement without the binding of a specific antibody. They become mainly coated with the C3d component of complement.[19,83] In lymphoid tissues, FDCs bind C3d opsonized particles via the CD21 receptor and eventually present modified autoantigens to autoreactive B cells.[84,85] These positively selected B cells can now migrate to the mantle zone of the GC where they receive appropriate signals from T helper cells. They proliferate and differentiate thereafter into memory or plasma cells. Thereby, in some SLE patients the retention of autoantigens on the surface of FDCs as well as the presentation of nuclear autoantigens by DCs may override the highly important mechanism of B- and T-cell tolerance, respectively. In Fig. 1 we describe the events that take place in the peripheral lymphoid tissues, and suggest that an ineffective scavenger system may be responsible for many of the pathological alterations found in SLE patients (Fig. 1b).

The evidence favoring a clearance deficiency of apoptotic cells as the core mechanism in the pathogenesis of SLE is growing rapidly. New therapeutic interventions should be considered on the basis of this pathogenic mechanism.

Acknowledgements This work was supported by the Interdisciplinary Center for Clinical Research (IZKF) (project numbers A4 and N2) at the University Hospital of the University of Erlangen-Nuremberg, by "Deutsche Forschungsgemeinschaft" SFB 643 (project B5), by the research training grant GRK 592 from the German Research Society (DFG) to S.F., by the Programme Alban, the European Union Programme of High Level Scholarships for Latin America, scholarship no. E04D047956VE to L.M., and by the European Commissions [E.U. (QLK3-CT-2002-02017_APOCLEAR)].

References

1. Herrmann M, Voll RE, Kalden JR. Etiopathogenesis of systemic lupus erythematosus. Immunol Today 2000;21:424–6.
2. Kuenkele S, Beyer TD, Voll RE, Kalden JR, Herrmann M. Impaired clearance of apoptotic cells in systemic lupus erythematosus: challenge of T, B cell tolerance. Curr Rheumatol Rep 2003;5:175–7.
3. Beyer TD, Kuenkele S, Gaipl U, Kolowos W, Voll R, Baumann I, et al. Systemic lupus erythematosus. In: Kalden JR, Herrmann M, editors. Apoptosis and autoimmunity. Weinheim: Wiley-VCH; 2003. p. 187–204.
4. Steinman CR. Circulating DNA in systemic lupus erythematosus. Isolation and characterization. J Clin Invest 1984;73:832–41.
5. 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.
6. Burlingame RW, Rubin RL. Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. J Immunol Methods 1990;134:187–99.
7. Amoura Z, Chabre H, Koutouzov S, Lotton C, Cabrespines A, Bach JF, et al. Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. Arthritis Rheum 1994;37:1684–8.
8. Radic MZ, Weigert M. Genetic and structural evidence for antigen selection of anti-dsDNA antibodies. Annu Rev Immunol 1994;12:487–520.
9. Winkler TH, Fehr H, Kalden JR. Analysis of immunoglobulin variable region genes from human IgG anti-dsDNA hybridomas. Eur J Immunol 1992;22:1719–28.
10. Amoura Z, Piette JC, Bach JF, Koutouzov S. The key role of nucleosomes in lupus. Arthritis Rheum 1999;42:833–43.
11. Voll RE, Roth EA, Girkontaite I, Fehr H, Herrmann M, Lorenz HM, et al. Histone-specific Th0 and Th1 clones derived from systemic lupus erythematosus patients induce double-stranded DNA antibody production. Arthritis Rheum 1997;40:2162–71.
12. Desai-Mehta A, Mao C, Rajagopalan S, Robinson T, Datta SK. Structure and specificity of T cell receptors expressed by potentially pathogenic anti-dsDNA autoantibody-inducing T cells in human lupus. J Clin Invest 1995;95:531–41.
13. Surh CD, Sprent J. T-cell apoptosis detected in situ during positive and negative selection in the thymus (see comments). Nature 1994;372:100–3.
14. Janeway CA. Immunobiology: the immune system in health and disease. New York: Current Biology Publications; 1999. p. 490–509.
15. Savill J. Apoptosis in resolution of inflammation. J Leukocyte Biol 1997;61:375–80.

16. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature 1997;390: 350–1.
17. Fadok VA, Bratton DL, Guthrie L, Henson PM. Differential effects of apoptotic versus lysed cells on macrophage production of cytokines: role of proteases. J Immunol 2001;166:6847–54.
18. Janeway CA. Immunobiology: the immune system in health and disease. New York: Current Biology Publications; 1999. p. 236–92.
19. Mevorach D, Mascarenhas JO, Gershov D, Elkon KB. Complement-dependent clearance of apoptotic cells by human macrophages. J Exp Med 1998;188:2313–20.
20. Navratil JS, Korb LC, Ahearn JM. Systemic lupus erythematosus and complement deficiency: clues to a novel role for the classical complement pathway in the maintenance of immune tolerance. Immunopharmacology 1999;42:47–52.
21. Janeway CA. Immunobiology: the immune system in health and disease. New York: Current Biology Publications; 1999. p. 318–21.
22. Baumann I, Kolowos W, Voll RE, Manger B, Gaipl U, Neuhuber WL, et al. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. Arthritis Rheum 2002;46:191–201.
23. Cohen JJ. Apoptosis. Immunol Today 1993;14:126–30.
24. Ameisen JC. The origin of programmed cell death. Science 1996; 272:1278–9.
25. Savill J, Haslett C. Granulocyte clearance by apoptosis in the resolution of inflammation. Semin Cell Biol 1995;6:385–93.
26. Ha HJ, Kubagawa H, Burrows PD. Molecular cloning and expression pattern of a human gene homologous to the murine mb-1 gene. J Immunol 1992;148:1526–31.
27. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 1998;101:890–8.
28. 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.
29. Gaipl US, Beyer TD, Heyder P, Kuenkele S, Bottcher A, Voll RE, et al. Cooperation between C1q and DNase I in the clearance of necrotic cell-derived chromatin. Arthritis Rheum 2004;50:640–9.
30. Heyder P, Gaipl US, Beyer TD, Voll RE, Kern PM, Stach C, et al. Early detection of apoptosis by staining of acid-treated apoptotic cells with FITC-labeled lectin from *Narcissus pseudonarcissus*. Cytometry 2003;55A:86–93.
31. Hart SP, Smith JR, Dransfield I. Phagocytosis of opsonized apoptotic cells: roles for "old-fashioned" receptors for antibody and complement. Clin Exp Immunol 2004;135:181–5.
32. Gaipl US, Kuenkele S, Voll RE, Beyer TD, Kolowos W, Heyder P, et al. Complement binding is an early feature of necrotic and a rather late event during apoptotic cell death. Cell Death Differ 2001;8:327–34.
33. Sheriff A, Gaipl US, Voll RE, Kalden JR, Herrmann M. Apoptosis and systemic lupus erythematosus. Rheum Dis Clin North Am 2004;30:505–27, viii–ix.
34. Brouckaert G, Kalai M, Krysko DV, Saelens X, Vercammen D, Ndlovu, et al. Phagocytosis of necrotic cells by macrophages is phosphatidylserine-dependent and does not induce inflammatory cytokine production. Mol Biol Cell 2003;10:10.
35. Bratton DL, Fadok VA, Richter DA, Kailey JM, Frasch SC, Nakamura T, et al. Polyamine regulation of plasma membrane phospholipid flip-flop during apoptosis. J Biol Chem 1999;274: 28113–20.
36. Savill J, Dransfield I, Hogg N, Haslett C. Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. Nature 1990; 343:170–3.
37. Cheng HM. Apoptotic antigens, B2-glycoprotein I, and anti-phospholipid autoantibodies (letter; comment). Blood 1996;88: 4396.
38. Ramprasad MP, Fischer W, Witztum JL, Sambrano GR, Quehenberger O, Steinberg D. The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68. Proc Natl Acad Sci USA 1995;92:9580–4.
39. Platt N, da Silva RP, Gordon S. Class A scavenger receptors and the phagocytosis of apoptotic cells. Biochem Soc Trans 1998;26: 639–44.
40. Luciani MF, Chimini G. The ATP binding cassette transporter ABC1, is required for the engulfment of corpses generated by apoptotic cell death. EMBO J 1996;15:226–35.
41. Oka K, Sawamura T, Kikuta K, Itokawa S, Kume N, Kita T, et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. Proc Natl Acad Sci USA 1998;95:9535–40.
42. Devitt A, Moffatt OD, Raykundalia C, Capra JD, Simmons DL, Gregory CD. Human CD14 mediates recognition and phagocytosis of apoptotic cells. Nature 1998;392:505–9.
43. Fadok VA, Savill JS, Haslett C, Bratton DL, Doherty DE, Campbell PA, et al. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. J Immunol 1992;149: 4029–35.
44. Binder RJ, Anderson KM, Basu S, Srivastava PK. Cutting edge: heat shock protein gp96 induces maturation and migration of CD11c+ cells in vivo. J Immunol 2000;165:6029–35.
45. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int Immunol 2000;12:1539–46.
46. Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: endogenous activators of dendritic cells. Nat Med 1999;5:1249–55.
47. Stach CM, Turnay X, Voll RE, Kern PM, Kolowos W, Beyer TD, et al. Treatment with annexin V increases immunogenicity of apoptotic human T-cells in Balb/c mice. Cell Death Differ 2000;7: 911–5.
48. Pereira Da Silva JA, Elkon KB, Hughes GR, Dyck RF, Pepys MB. C-reactive protein levels in systemic lupus erythematosus: a classification criterion? Arthritis Rheum 1980;23:770–1.
49. Hack CE, Wolbink GJ, Schalkwijk C, Speijer H, Hermens WT, van den Bosch H. A role for secretory phospholipase A2 and C-reactive protein in the removal of injured cells. Immunol Today 1997;18:111–5.
50. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189–97.
51. Pepys MB, Dash AC, Markham RE, Thomas HC, Williams BD, Petrie A. Comparative clinical study of protein SAP (amyloid P component) and C-reactive protein in serum. Clin Exp Immunol 1978;32:119–24.
52. 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.
53. 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.
54. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418: 191–5.
55. Rovere-Querini P, Capobianco A, Scaffidi P, Valentinis B, Catalanotti F, Giazzon M, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO Rep 2004;5:825–30.
56. Gaipl US, Brunner J, Beyer TD, Voll RE, Kalden JR, Herrmann M. Disposal of dying cells: a balancing act between infection and autoimmunity. Arthritis Rheum 2003;48:6–11.
57. Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, et al. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo (In Process Citation). J Exp Med 2000;192:359–66.
58. Quartier P, Potter PK, Ehrenstein MR, Walport MJ, Botto M. Predominant role of IgM-dependent activation of the classical pathway in the clearance of dying cells by murine bone marrow-derived macrophages in vitro. Eur J Immunol 2004;35:252–60.
59. Berkel AI, Petry F, Sanal O, Tinaztepe K, Ersoy F, Bakkaloglu A, et al. Development of systemic lupus erythematosus in a patient with selective complete C1q deficiency. Eur J Pediatr 1997;156: 113–5.
60. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, et al. Homozygous C1q deficiency causes glomerulone-

phritis associated with multiple apoptotic bodies (see comments). Nat Genet 1998;19:56–9.
61. van de Wetering JK, van Golde LM, Batenburg JJ. Collectins: players of the innate immune system. Eur J Biochem 2004;271: 1229–49.
62. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 2001; 194:781–95.
63. Schagat TL, Wofford JA, Wright JR. Surfactant protein A enhances alveolar macrophage phagocytosis of apoptotic neutrophils. J Immunol 2001;166:2727–33.
64. Franz S, Gaipl U, Sheriff A, Voll R, Kalden JR, Herrmann M. The role of carbohydrates in the clearance process of apoptotic cells. Immunobiology 2004;209:449.
65. Boes M, Schmidt T, Linkemann K, Beaudette BC, Marshak-Rothstein A, Chen J. Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. Proc Natl Acad Sci USA 2000;97:1184–9.
66. Botto M, Walport MJ. C1q, autoimmunity and apoptosis. Immunobiology 2002;205:395–406.
67. Bickerstaff MC, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nat Med 1999;5:694–7.
68. 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.
69. Svensson B. Monocyte in vitro function in systemic lupus erythematosus (SLE). I. A clinical and immunological study. Scand J Rheumatol Suppl 1980;31:29–41.
70. Hurst NP, Nuki G, Wallington T. Evidence for intrinsic cellular defects of "complement" receptor-mediated phagocytosis in patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 1984;55:303–12.
71. Salmon JE, Kimberly RP, Gibofsky A, Fotino M. Defective mononuclear phagocyte function in systemic lupus erythematosus: dissociation of Fc receptor-ligand binding and internalization. J Immunol 1984;133:2525–31.
72. 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.
73. McCutcheon JC, Hart SP, Canning M, Ross K, Humphries MJ, Dransfield I. Regulation of macrophage phagocytosis of apoptotic neutrophils by adhesion to fibronectin. J Leukocyte Biol 1998;64: 600–7.
74. Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 1999;17:593–623.
75. Franz S, Gaipl U, Appelt U, Heyder P, Voll R, Kalden JR, et al. The role of a defective clearance in the pathogenesis of systemic lupus erythematosus. Arthritis Res Ther 2004;6:S34.
76. Landry M. Phagocyte function and cell-mediated immunity in systemic lupus erythematosus. Arch Dermatol 1977;113:147–54.
77. Gresham HD, Ray CJ, O'Sullivan FX, Yu CL, Chang KL, Chiu CC, et al. Defective neutrophil function in the autoimmune mouse strain MRL/lpr. Potential role of transforming growth factor-beta defective phagocytosis, decreased tumour necrosis factor-alpha production, and lymphocyte hyporesponsiveness predispose patients with systemic lupus erythematosus to infectionsC3b receptor (CR1) on phagocytic cells from SLE patients: analysis of the defect and familial study. J Immunol 1991;146:3911–21.
78. Theocharis S, Sfikakis PP, Lipnick RN, Klipple GL, Steinberg AD, Tsokos GC. Characterization of in vivo mutated T cell clones from patients with systemic lupus erythematosus. Clin Immunol Immunopathol 1995;74:135–42.
79. Shivakumar S, Tsokos GC, Datta SK. T cell receptor alpha/beta expressing double-negative (CD4–/CD8–) and CD4+ T helper cells in humans augment the production of pathogenic anti-dsDNA autoantibodies associated with lupus nephritis. J Immunol 1989; 143:103–12.
80. Rosen A, Casciola-Rosen L, Ahearn J. Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med 1995; 181:1557–61.
81. Casiano CA, Ochs RL, Tan EM. Distinct cleavage products of nuclear proteins in apoptosis and necrosis revealed by autoantibody probes. Cell Death Differ 1998;5:183–90.
82. Deng H, Apple R, Clare-Salzler M, Trembleau S, Mathis D, Adorini L, et al. Determinant capture as a possible mechanism of protection afforded by major histocompatibility complex class II molecules in autoimmune disease. J Exp Med 1993;178:1675–80.
83. Korb LC, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. J Immunol 1997;158: 4525–8.
84. Carroll MC. The role of complement and complement receptors in induction and regulation of immunity. Annu Rev Immunol 1998; 16:545–68.
85. Camacho SA, Kosco-Vilbois MH, Berek C. The dynamic structure of the germinal center. Immunol Today 1998;19:511–4.