Reviews

The Fate of the U1 snRNP Autoantigen during Apoptosis: Implications for Systemic Autoimmunity

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Abstract

Recent studies have implicated the dying cell as a potential reservoir of modified autoantigens that may initiate and drive systemic autoimmunity in susceptible hosts. The uridine-rich small nuclear ribonucleoprotein complex is a common target for autoantibodies present in the serum of patients with systemic lupus erythematosus and SLE-overlap syndromes. Four modifications occurring in this complex during apoptosis have been described to date: the caspase-mediated cleavage of the U1-70K protein, the U1 RNA and the Sm-F protein, and the association with hyperphosphorylated SR proteins. In addition, the U1 snRNP complex has been shown to translocate from its normal subcellular localization to apoptotic bodies near the surface of cells undergoing apoptosis. This redistribution might facilitate exposure of the modified components of the U1 snRNP complex to the immune system when the clearance of apoptotic cell remnants is somehow disturbed. The modifications in the U1 snRNP components during apoptosis might represent the initial epitopes to which an immune response is generated and may be the trigger for the production of autoantibodies to this complex in patients with SLE or SLE-overlap syndromes. Therefore, it can be hypothesized that the exposure of elevated levels of apoptotically modified U1 snRNP to the immune system of a genetically susceptible individual might lead to the breaking of immunologic tolerance towards the U1 snRNP complex.

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Systemic autoimmune diseases represent a complex spectrum of disorders that include systemic lupus erythematosus, myositis, Sjögren's syndrome, systemic sclerosis, and mixed connective tissue disease. In these diseases, the immune system is misdirected against a wide range of self-antigens and the resulting immune effector pathways cause specific tissue damage. Unlike tissue-specific autoimmune diseases, the autoantigens targeted in the systemic autoimmune diseases are ubiquitously expressed molecules that function in essential biologic pathways. Examples are nucleosomes and splicing ribonucleoproteins that are autoantigens in SLE and aminoacyl-tRNA synthetases in myositis.

Little is understood about the mechanisms by which the immune system begins to target these antigens, but recent studies have implicated the dying cell as a potential reservoir of modified autoantigens that might initiate and drive systemic autoimmunity in susceptible hosts [1–7]. In the past few years, increasing evidence has shown that autoimmunity may be a consequence of defects occurring in the signaling, execution and/or clearance pathways involved in apoptosis.

The first molecular evidence of a potential link between apoptosis and autoimmunity was the demonstration that in MRL/lpr and glp mice, well-known animal models of autoimmune disease, mutations were present in the Fas gene and the Fas-ligand gene, respectively [8]. The relevance of these findings was recently confirmed by the fact that patients with autoimmune lymphoproliferative disorders were also found to bear mutations of the Fas gene [9]. Since the complex pathogenesis of autoimmune diseases cannot be fully explained by the alteration of one signaling pathway alone, events occurring in the execution phase and/or clearance pathways of apoptosis may also play an important role. The execution phase of apoptosis is characterized by extensive post-translational modifications of cellular proteins, resulting in alterations in the structure of these proteins as well as in their antigenic properties. Interestingly, many proteins and protein complexes that are autoantigenic in autoimmune patients have been shown to be uniquely modified during apoptosis [3]. It has been postulated that these modifications may uncover cryptic epitopes and/or create novel epitopes to which no tolerance exists and could therefore provoke a local autoimmune response in genetically susceptible individuals [1,2].

A combination of proper genetic factors (mutations in genes involved in the apoptotic process), environmental factors (viral infection, ultraviolet light, drugs, toxins) resulting in massive apoptosis, and the apoptotic cells as the source of modified autoantigens is necessary for the bypass of tolerance that is required for autoantibody formation.

Apoptotic cells as a reservoir of autoantigens

Modifications of autoantigens during cell death

Proteolytic cleavage is the best characterized type of modification of autoantigenic proteins that occurs during apoptosis. Cleavage of proteins during apoptosis is largely mediated by caspases [10], a family of cysteinyl aspartate-specific proteases that are specifically

SLE = systemic lupus erythematosus
U snRNP = uridine-rich small nuclear ribonucleoprotein

activated in response to an apoptotic stimulus, resulting in the irreversible changes associated with apoptosis. Some of the caspase substrates are structural proteins essential for maintaining nuclear and cytoplasmic architecture, while others are enzymes essential for repairing damaged cell components. The U1-70K protein [11], which is a component of the U1 snRNP particle, poly(ADP-ribose) polymerase (PARP) [12], and α-fodrin [13] are examples of autoantigenic proteins that are cleaved during apoptosis. Other autoantigens, including fibrillarin, CENP-B, alanyl-tRNA synthetase and Ku-70, are cleaved during cytotoxic T lymphocyte-induced apoptosis by granzyme B, a serine protease that is present in the CTL granules [14,15]. One example of this latter form of apoptosis, which occurs in a pro-inflammatory setting, is the death of virally infected cells induced by CTLs.

In addition to caspase and granzyme B-mediated cleavage, other post-translational modifications of autoantigens occurring during apoptosis, which might have consequences for their antigenicity and lead to the generation of autoantibodies, have been described, such as hyperphosphorylation, dephosphorylation, cross-linking by transglutaminase, (de)ubiquitination, poly(ADP-ribosyl)ation, and citrullination [reviewed in 3,4]. Another mechanism by which auto(antigens) can be fragmented, possibly resulting in the creation of novel epitopes, is via reactive oxygen radicals. Several autoantigens that are targeted in scleroderma (e.g., topoisomerase I, the large subunit of RNA polymerase II, and NOR 90) are uniquely susceptible to cleavage by reactive oxygen species in the presence of metal ions [16].

Morphologic changes in the apoptotic cells

In addition to modifications of proteins and protein complexes, apoptosis also induces major morphologic changes in the apoptotic cell, some of which might be relevant for autoimmunity. The most interesting aspect of these morphologic changes is related to an observation first made more than 15 years ago, namely: nuclear antigens that are present in keratinocytes become available for binding to immunoglobulin G autoantibodies derived from SLE patients' sera upon irradiation of the cells with ultraviolet light [17]. Almost a decade later, it was shown that induction of cell surface expression of autoantigens on keratinocytes by UV light correlated with the induction of apoptosis [18]. The autoantigens were shown to cluster into two discrete cell surface "membrane blebs." The larger blebs, called apoptotic bodies, contained predominantly nuclear autoantigens like nucleosomes, Sm proteins, U1-70K, Ro60, La, PARP, NuMA, Mi-2 and Ku/DNA-PK. The smaller structures, called apoptotic surface blebs, were recognized by autoantibodies specific for endoplasmic reticulum components, as well as Ro52, α-fodrin, Jo-1, SRP and ribosomal components. This observation raised the intriguing possibility that cells undergoing apoptosis are uniquely suited to present modified self-proteins to the immune system in such a way that they escape normal mechanisms of peripheral tolerance.

Another interesting aspect is the fact that the cytoplasmic membrane is drastically rearranged during apoptosis, thereby exposing molecules that in non-apoptotic cells are exclusively present at the inner layer of the cytoplasmic membrane, such as phosphatidylserine [reviewed in 5]. Serum contains relatively high concentrations of proteins that are able to bind specifically to the surface of apoptotic cells, e.g., binding to phosphatidylserine. These proteins do not have a high affinity for non-apoptotic cells. The apoptotic cell-binding proteins include annexin V, β2-glycoprotein I and complement factor C1q, and they are frequently recognized by autoantibodies from patients with systemic autoimmune diseases, suggesting that proteins associated with apoptotic cells can become immunogenic as well [reviewed in 5].

Immunogenicity aspects of apoptotic cells

Recent studies have emphasized that apoptotic cells are not immunologically inert but rather have either positive or negative immune effects, depending on the antigen-presenting cell with which they interact. Under normal conditions, apoptotic cells are quickly and efficiently removed by macrophages and other phagocytic cells without being exposed to the immune system [19]. Phagocytosis of apoptotic cells by macrophages induces the release of interleukin-10 and transforming growth factor-β and inhibits the production of pro-inflammatory cytokines, such as tumor necrosis factor-α, thereby creating an anti-inflammatory environment [5,19]. This rapid response of phagocytosing macrophages prevents the remnants of apoptotic cells being presented to the immune system in a pro-inflammatory context. Any disturbance of this subtle equilibrium between the generation and clearance of apoptotic cells will expose apoptotically modified autoantigens to the immune system.

An alternative fate for apoptotic cells might be phagocytosis by dendritic cells. Recent evidence indicates that DCs[1] are able to present antigens derived from apoptotic cells to both class I and class II-restricted T cells, which will give rise to a primary immune response towards apoptotic cells [5,20]. The second stage of autoimmunity involves opsonization of apoptotic cells by autoantibodies and subsequent pro-inflammatory responses of the immune system. Environmental factors seem to control whether the uptake by DCs results in antigen presentation and a pro-inflammatory response or in antigen presentation and establishment of T cell tolerance. The mechanisms that underlie these processes are not well characterized yet, although it seems that the number of apoptotic cells is one of the factors determining the response by phagocytes [5,21]. It has been suggested that low numbers of apoptotic cells are removed predominantly by macrophages and are thus accompanied by an anti-inflammatory response, while large numbers of apoptotic cells ("massive apoptosis") may also encounter DCs, which could lead to an immune response.

Interestingly, impaired phagocytosis of apoptotic cells has been observed in SLE patients [22]. However, there was significant heterogeneity in the level of impairment among patients that did

CTL = cytotoxic T lymphocyte
UV = ultraviolet

DCs = dentritic cells

not appear to correlate with disease activity or therapy. Accordingly, it has been reported that SLE patients have increased levels of circulating apoptotic cells [23]. C1q deficiency is strongly associated with the development of SLE in both humans and mice [24,25]. The recent observation of the presence of high titers of antinuclear antibodies and of increased numbers of uncleared apoptotic cells in the kidneys of an SLE-susceptible C1q-null mouse supports the idea that abnormal clearance of apoptotic cells may play a role in the pathogenesis of SLE [25]. Recently, it was reported that mice carrying mutations in the Mer tyrosine kinase have impairments in phagocytosis and clearance of apoptotic cells and also develop anti-DNA autoantibodies [26]. These findings provide new *in vivo* evidence to support the concept that defects in clearance of apoptotic cells may underlie systemic autoimmunity.

Although there are at present no data that directly address whether, and by what mechanisms, impaired clearance of apoptotic cells may initiate or exacerbate the autoimmune state *in vivo*, delayed clearance may either a) change the compartmentalization of autoantigens (allowing leakage of modified self-autoantigens during secondary necrosis, and access to the cell contents by dendritic cells); or b) provide apoptotic cells and membrane-bound fragments access to different (pro-immune) populations of antigen-presenting cells from which they are normally excluded. In this way, ineffective clearance of apoptotic cells may contribute to the bypass of tolerance that is required for autoantibody formation.

To sum up, the idea that dying cells are the basis of systemic autoimmunity is supported by four important observations: a) autoantigens are specifically modified during cell death (apoptosis and secondary necrosis); b) modified autoantigens translocate to the cell surface in cells undergoing apoptosis and may thus become accessible to the immune system; c) apoptotic cells are specifically bound by serum proteins, which are recognized by autoantibodies in many autoimmune patients; and d) impaired clearance of apoptotic cells, which has been reported in patients with SLE, might result in a temporarily increased exposure of modified self-components to the immune system.

Modifications of the U1 snRNP complex during apoptosis

The U1 snRNP complex as autoantigen in SLE and SLE-overlap syndromes

During splicing, non-coding or intron sequences are removed from precursor mRNA molecules in order to obtain functional, mature mRNA. The first and triggering step in this process is the association of the U1 small nuclear ribonucleoprotein complex with the 5'-splice site. This snRNP complex consists of the U1 snRNA molecule (165 nts) and the U1 snRNP-specific proteins U1A, U1C, and U1-70K plus a set of eight proteins, called Sm proteins (B/B', D1, D2, D3, E, F and G), which are core components of all the snRNPs (U1, U2, U4/U6 and U5) (28) [Figure 1].

Most of the individual protein components of the U1 snRNP complex are targeted by autoantibodies present in the serum of patients suffering from SLE or SLE-overlap syndromes [reviewed in 28]. The three specific proteins of the U1 snRNP complex (U1-70K, U1A, and U1C) contain the epitopes that react with anti-U1 RNP

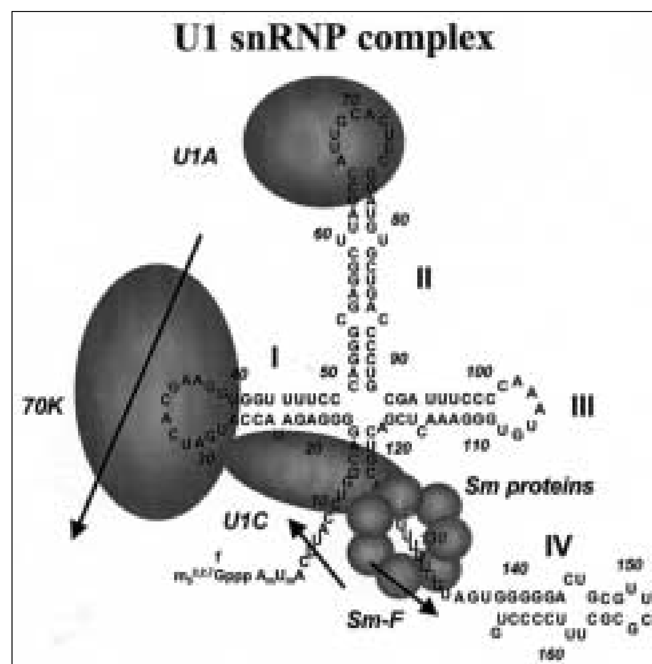

Figure 1. Modifications of the autoantigenic U1 snRNP complex during apoptosis. During apoptosis the U1-70K protein is cleaved by caspase-3 into a 40 kDa fragment that remains associated with the U1 snRNP complex. The U1 snRNA, which is the backbone of the U1 snRNP complex, is modified during apoptosis by specific removal of the first 5–6 nucleotides including the 2,2,7-trimethylguanosine cap. Furthermore, the Sm-F protein is proteolytically cleaved in apoptotic cells, generating a 9 kDa apoptotic fragment that also remains associated with the U snRNP complexes in apoptotic cells. All cleavages are indicated by arrows.

autoantibodies present in sera of patients with the SLE-overlap syndrome, mixed connective tissue disease. However, anti-U1 RNP antibodies are also commonly associated with anti-Sm autoantibodies in SLE patient sera and are therefore not disease-specific. In contrast, anti-Sm autoantibodies are specific for SLE, making the presence of these autoantibody populations in patient sera an important diagnostic criterion for this disease [28]. In immunoblotting, anti-Sm autoantibodies react predominantly with the Sm proteins B/B', D1, D3 and, to a lesser extent, D2 [28]. Only a few sera also react with denatured E, F, or G on immunoblots [29]. However, it has been shown that some anti-Sm SLE patient sera react strongly with one or more of the E, F and G proteins in immunoprecipitation studies (under native conditions) [30]. In addition, the majority of anti-Sm sera efficiently immunoprecipitates the E-F-G complex. The E-F-G complex recognition was detected exclusively in anti-Sm patient sera and not in patient sera with other serotypes. Because most of the anti-Sm patient sera contain antibodies that precipitate the E-F-G complex considerably more strongly than the individual proteins, it was concluded that the E, F and G proteins harbor immunodominant antigenic determinants that are expressed exclusively or predominantly on the assembled E-F-G complex [30].

The U1 snRNA molecule itself is a major target of autoimmunity in SLE-overlap syndromes, and it has been shown that changes in

the titer of the anti-U1 snRNA autoantibodies may correlate with the severity of the disease [reviewed in 28].

Cleavage of the U1-70K protein during cell death

In 1994, Casciola-Rosen and collaborators [11] reported that the U1-70K protein was specifically cleaved during apoptosis, and they subsequently demonstrated that the U1-70K protein was cleaved at position ^{338}DGPD^{341} by caspase-3, resulting in an N-terminal 40 kDa (aa 1-341) and a C-terminal 22 kDa (aa 342-437) protein fragment. Recently, we demonstrated that it is likely that the U1-70K protein is cleaved while it is associated with the intact U1 snRNP complex in apoptotic cells, and that the N-terminal 40 kDa fragment remains associated with the U1 snRNP complex [31].

The U1-70K protein was also shown to be cleaved during necrotic cell death [32], CTL-induced apoptosis [14,15] and metal-catalyzed oxidative reaction [16], each leading to the appearance of distinct fragments. In a recent study, the preferential recognition of apoptotically and oxidatively modified forms of the U1-70K autoantigen appeared to be associated with distinct clinical rheumatic disease manifestations [33]. When an unselected group of individuals with antibodies to U1-70K was examined, the patients with lupus skin disease showed increased reactivity to apoptotic U1-70K fragments as compared to patients without lupus skin disease, and subjects with Raynaud's phenomenon had increased immune reactivity to metal-catalyzed oxidative fragments compared to patients without Raynaud's phenomenon. This finding provides *in vivo* evidence for the hypothesis that immune recognition of modified forms of self-antigens may be relevant for the pathogenesis of systemic rheumatic diseases [33].

Cleavage of the U1 snRNA molecule during apoptosis

Recently, the U1 snRNA, which is the backbone of the U1 snRNP complex and an autoantigen in SLE-overlap syndromes, was also found to be modified during apoptosis by the specific removal of the first 5–6 nucleotides including the 2,2,7-trimethylguanosine (TMG) cap [31]. Cleavage of U1 snRNA appeared to be a universal apoptotic phenomenon, as this snRNA was cleaved in various cell types (Jurkat, human T cell leukemia; HeLa, human cervix carcinoma; HL-60, human promyelocytic leukemia; 4B1, mouse cells overexpressing the human Fas-receptor) rendered apoptotic with a variety of stimuli (anti-Fas mAb, murine Fas-ligand, anisomycin, staurosporine, cycloheximide). It was observed that U1 snRNA cleavage is specific (no such modifications were detected for the other non-autoantigenic U snRNAs tested) and that U1 snRNA cleavage is markedly inhibited in the presence of caspase inhibitors, indicating that an apoptotically activated ribonuclease is responsible for the specific modification of the U1 snRNA during apoptosis [31].

Cleavage of the Sm-F protein during apoptosis

The fate of the autoantigenic Sm proteins has been studied by many investigators in recent years. However, no modification specifically occurring during apoptosis had been found so far for the Sm proteins. Recently, we investigated the effects of apoptosis on the Sm proteins and for the first time demonstrated that one of the Sm proteins, the Sm-F protein, is proteolytically cleaved in apoptotic cells (Malmegrim KCR, Saelens X, Pruijn GJM, Vandenabeele P, van Venrooij WJ. Submitted). The Sm-F protein is one of the eight Sm proteins, core components of the U snRNPs that are essential for the splicing of pre-mRNAs in eukaryotes. Sm-F is also a component of the autoantigenic E-F-G complex, that is recognized by anti-Sm patient sera [30]. Cleavage of the Sm-F protein generates a 9 kDa apoptotic fragment that remains associated with the U snRNP complexes in apoptotic cells. Sm-F cleavage is prevented by peptide caspase inhibitors and by overexpression of Bcl-2. Recombinant caspase-1 and caspase-8 cleave the Sm-F protein *in vitro*. The cleavage site, identified by mutagenesis, is located near the C-terminus and appeared to be an unconventional caspase cleavage site, EEED^{81}↓G. A C-terminally truncated mutant of the Sm-F protein, representing the largest apoptotic cleavage fragment, is capable of forming the E-F-G complex *in vitro* and is recognized by many anti-Sm patient sera. This finding suggests a possible role for the apoptotic modification of the Sm-F protein in triggering the autoimmune response against the Sm proteins, and consequently anti-Sm autoantibody formation in patients.

At present, no other changes specifically occurring during cell death have been reported for Sm proteins. However, it has been demonstrated that the C-terminal arginine-glycine (RG) dipeptide repeats of the human Sm-D1, Sm-D3 and Sm-B/B' contain symmetric dimethylarginines (sDMAs) in normal cell cultures. The sDMA-modified C terminus of Sm-D1 was shown to constitute a major linear epitope for anti-Sm autoantibodies, which suggested that also non-apoptotic post-translational modifications of Sm proteins may play a role in the etiology of autoantibody formation in SLE [4,34,35].

Association of phosphorylated SR proteins with the U1 snRNP complex in apoptotic cells

Some proteins are recognized by autoantibodies although they are not substrates for apoptotic proteases such as caspases and granzymes. In an attempt to identify other post-translational modifications, Utz and collaborators [36,37] screened a large number of human and mouse autoimmune sera for the ability to precipitate novel phosphoproteins from radiolabeled apoptotic Jurkat cell lysates. Almost all lupus sera were capable of precipitating new phosphoproteins in such an assay, suggesting that this autoantigen modification may also be important [36]. It was also shown that a serine kinase activity is present in immunoprecipitates prepared from apoptotic Jurkat cell extracts using sera from patients with SLE and SLE-overlap syndromes. Of the seven phosphoproteins initially discovered in this way, four were definitively identified as members of the serine-arginine (SR) family of RNA splicing factors [36,37]. SR proteins are critical regulators of constitutive messenger RNA splicing. Sera known to recognize the U1 snRNP complex (confirmed by their ability to precipitate U1 snRNA) selectively precipitated a phosphoprotein complex (pp54, pp42, pp34, and pp23) from apoptotic cells. Monoclonal antibodies reactive with U1 snRNP proteins precipitated the same phosphoprotein complex from apoptotic lysates. The phosphorylation and/or recruitment of these proteins to the U1

snRNP complex was induced by multiple apoptotic stimuli (e.g., Fas ligation, gamma irradiation, or UV irradiation), and was blocked by overexpression of Bcl-2.

Phosphorylated SR proteins were also shown to associate with the U3 snoRNP complex, which is a major autoantigenic complex in scleroderma [38]. Although no study has yet addressed the question of whether SR proteins are themselves targets of an immune response in SLE, mixed connective tissue disease or scleroderma, it has been proposed that their association with the U1 snRNP and U3 snoRNP contributes to the immunogenicity of other components of these important autoantigen particles [37].

U1 snRNP complex and bypass of immunologic tolerance

Molecular immunity and epitope spreading

Several studies have shown that an initial immune response against a single component of a multi-component complex (e.g., U1 snRNP) can lead to a response against other components of the same complex [reviewed in 6,9]. For example, mice immunized with human U1A, a specific protein of the U1 snRNP complex, develop autoimmune responses against various components of murine U1 snRNP. By contrast, the same mice strain immunized with murine U1 snRNP components failed to develop an immune response. This shows that initiation of an autoimmune response can be triggered by molecules similar but not identical to the autologous proteins. Similar observations were made with rabbits immunized with peptides derived from the Sm-B/B' autoantigen, which resulted in an initial response against the Sm B/B' derived peptides and other Sm-B/B' epitopes [6,39]. Subsequently, the immune response spread to other U1 snRNP components, such as U1-70K, U1A and U1C. These observations can be extrapolated to the possible role of apoptotic modifications in the initiation of an autoimmune response. As described above, apoptosis results in cleavage of the U1-70K protein, producing a relatively stable 40 kDa product that remains associated with the U1 snRNP complex [11,31]. The U1-70K 40 kDa fragment is similar but not identical to the native U1-70K protein and may induce a primary immune response. Subsequently, the response could spread both intra- and intermolecularly to the other components of the complex, leading to autoantibodies directed to U1A, U1C, U1 snRNA and the Sm proteins. In principle, this idea could also be applied to modifications found in other components of the U1 snRNP complex, e.g., the cleavage of the Sm-F protein.

Recently, Greidinger and Hoffman [40] reported a study that analyzed the appearance of U1 RNP antibody specificities in sequential autoimmune antisera. In patients from whom multiple samples were drawn, they determined the order in which IgG antibodies to the U1 RNP proteins U1A, Sm-B/B', U1C, Sm-D and U1-70K appeared. It was found that orderly patterns of the emergence of anti-U1 RNP protein antibodies seem to exist in humans, and that two proteins, U1-70K and Sm-B/B', show characteristics of early immunogens in the development of human U1 snRNP immunity [40]. Because not all the U1 components were analyzed in this study, no conclusions could be drawn for the other components of the U1 snRNP complex (Sm-E, Sm-F, Sm-G and the U1-RNA).

Based on the model that modifications in autoantigens during cell death (apoptosis and secondary necrosis) represent the initial epitopes to which an immune response is generated in the early stages of the autoimmune process, the patient antibodies that recognize these modifications should be present in relatively large quantities compared to antibodies recognizing the unmodified autoantigen [6]. Therefore, a very important issue to investigate is whether selective targeting of the modified autoantigen can be observed in the very early onset of autoimmunity. However, for such studies, the availability of appropriate patient material (serum samples of patients in the early phases of the disease) is a major difficulty since these antibody specificities may only be prominently expressed in patient sera during the early stages of the disease, i.e., before the clinician has seen the patient and diagnosed the disease.

Initiation of the anti-U1 snRNP autoimmune response

Since apoptosis has been implicated as a trigger of autoimmunity, one of the most striking findings is that at least one apoptosis-associated autoantigen modification, and often several modifications, affect at least one component of every major disease-specific autoantigen particle that has been identified to date [3]. In the case of the U1 snRNP particle, four apoptosis-associated modifications have been identified so far. Based on recent advances in the research on apoptosis and autoimmunity discussed in this review, a comprehensive model for the etiology of autoimmunity has been suggested [1,2,6]. The U1 snRNP autoantigen represents a prototypic example for this model.

An essential prerequisite for the development of autoimmunity is a proper genetic background (e.g., mutations in the CD95, CD95L, C1q, Mer, caspases, Bcl-2 genes and other undefined genetic defects; human leukocyte antigen haplotype). Individuals carrying susceptibility for autoimmunity once exposed to certain environmental factors may encounter settings conducive to the onset of an autoimmune response. Locally sustained apoptosis by exposure to a stimulus – whether repeated (e.g., UV light, drugs, toxins) or persistent (e.g., viral or microbial infection) – may lead to uncontrolled or 'massive' apoptosis that represents a continuous source of modified autoantigens. The type of tissue in which massive apoptosis is induced plays a role in determining the specificity of systemic autoimmunity. For example, a viral infection of muscle tissue may lead to massive apoptosis and an influx of CTLs, which in turn might result in myositis. Subsequently, the overdose of apoptotic cells overloads the normal route for the disposal of apoptotic cells, which involves phagocytosis by macrophages, in an anti-inflammatory environment. Instead, apoptotic fragments become available to DCs, which process and present fragments of apoptotic cells containing the modification(s) that were induced during cell death to the immune system (autoreactive lymphocytes) in a pro-inflammatory context. These might represent the unique epitopes to which no effective tolerance exists and thus a specific immune response is initiated. For instance, for the U1 snRNP particle this initial immune response

Ig = immunoglobulin

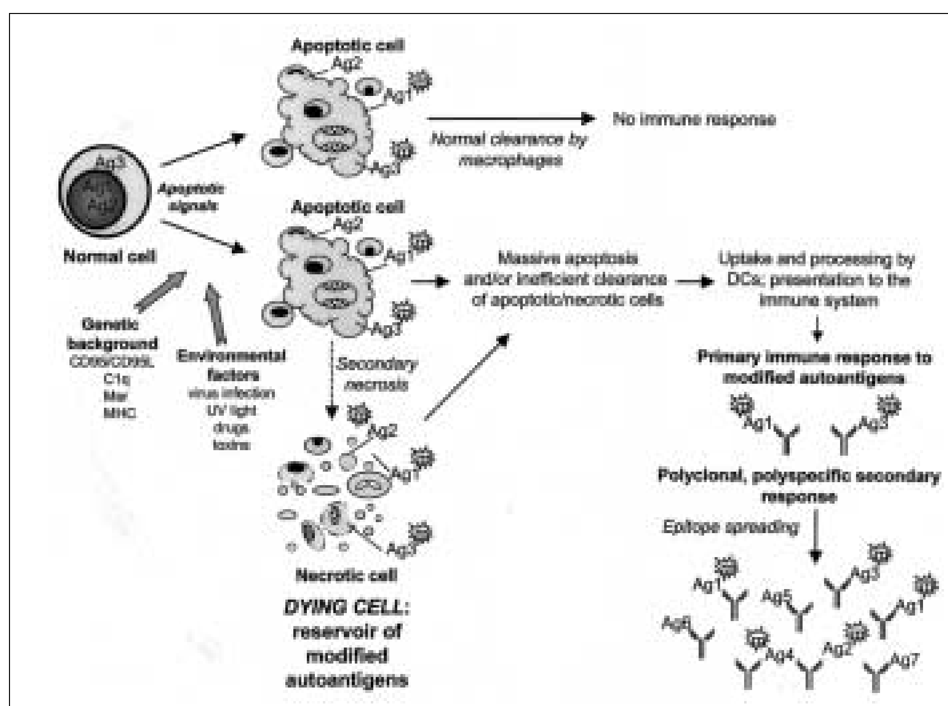

Figure 2. Schematic representation of the hypothesis that dying cells are involved in the initiation and maintenance of systemic autoimmunity. Under normal conditions, apoptotic cells are quickly and efficiently removed by macrophages and other phagocytic cells without being exposed to the immune system. However, in susceptible individuals, a combination of proper genetic and environmental factors leads to massive apoptosis and/or inefficient clearance of apoptotic cells containing modified proteins. Elevated levels of apoptotic and post-apoptotic (cells that underwent secondary necrosis) material become available to professional antigen-presenting cells (DCs), which then process and present the remnants of the dead cells to the immune system in a pro-inflammatory context. Subsequently, a primary immune response against the modified epitopes of the self-antigens is initiated, followed by secondary responses and epitope spreading to unmodified antigens and antigens associated with the initially modified antigen.

could be directed to the modified components of the complex (e.g., cleaved U1-70K, Sm-F and/or U1 snRNA, or phosphorylated SR proteins). Once a primary immunization has occurred, the locally repeated and persistent generation of apoptotic material might efficiently rechallenge the primed immune system (the stringency of this secondary response being significantly lower than that of the primary response). Secondary responses are then characterized by epitope spreading to unmodified antigens and antigens associated with the initially modified antigen. In this way, an initial immune response directed against the modified components of the U1 snRNP complex may promote the formation of autoantibodies reactive with other components of the complex [Figure 2].

The consequent opsonization of apoptotic material by autoantibodies – by antiphospholipid, anti-β2-glycoprotein I and anti-Cq1 antibodies – may both increase the efficiency of apoptotic antigen capture as well as induce the production of pro-inflammatory cytokines. This capacity for immune-driven auto-amplification may be one of the critical principles underlying severe systemic autoimmune diseases.

Conclusion

The (self)-antigen that drives the production of autoantibodies reactive with the U1 snRNP complex, a major autoantigenic complex in SLE and SLE-overlap syndromes, is still unknown. Although the composition of the U1 snRNP complex is not altered in patients with SLE, recent observations have demonstrated profound alterations in the structure and localization of this complex in apoptotic cells. As discussed in detail in this review, the altered localization of the U1 snRNP complex in apoptotic cells, together with modifications of the complex during apoptosis (i.e., the caspase-mediated cleavage of the U1-70K protein, the U1 RNA and the Sm-F protein and the association with hyperphosphorylated SR proteins), may be critical determinants in the development of an autoimmune response to the U1 snRNP complex in susceptible individuals.

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