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REVIEW

POSTTRANSLATIONAL PROTEIN MODIFICATIONS, APOPTOSIS, AND THE BYPASS OF TOLERANCE TO AUTOANTIGENS

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The role of apoptosis or programmed cell death in the pathophysiology of rheumatic diseases has been an active area of research, and advances in the field were reviewed in a recent issue of *Arthritis & Rheumatism* (1). Defects in the regulation of apoptosis have been observed in both hematopoietic and nonhematopoietic tissues derived from patients with systemic lupus erythematosus (SLE), a disease characterized by immune abnormalities that allow the production of antinuclear antibodies directed against self antigens, particularly RNP complexes (2,3). In this review, we discuss the role of cell death in the generation of autoantibodies in patients with SLE and scleroderma, and we present a unifying hypothesis to explain how defective apoptosis or ineffective clearance of apoptotic cells and modified autoantigens might contribute to the bypass of tolerance that is required for autoantibody formation.

SLE, apoptosis, and autoantibodies

A role of in vivo apoptosis in the pathogenesis of anti-DNA antibodies, a serologic hallmark of SLE, was suggested by several important observations (4). DNA purified from the serum of patients with SLE was shown to be present in oligosome-sized bands when separated electrophoretically, implying that these patients had circulating nucleosomes as the source of antigen (5). As discussed below, cleavage of DNA into oligonucleosomes during apoptosis is a hallmark of dying cells, suggesting that apoptotic cells may be the original source of circulating oligosomes that drive the immune response in SLE patients. Similar results have been found in vivo in young MRL-*lpr/lpr* mice (6), and in vitro in cells derived from patients with SLE and from healthy controls (7,8). This phenomenon appears to be SLE-specific since other diseases characterized by proportionately large numbers of apoptotic cells (e.g., acquired immunodeficiency syndrome, systemic vasculitis, and chemotherapy or irradiation-treated malignancy) are generally not associated with high titers of specific autoantibodies (9).

The clinical observation that patients with SLE frequently develop photosensitivity rashes and systemic exacerbations of disease following exposure to sunlight led several groups to investigate the role played by ultraviolet (UV) light in the development of SLE. Dermatologic and mucosal manifestations comprise 4 of the 11 diagnostic criteria for SLE, and autoantibodies are known to be deposited at the dermal–epidermal junction, particularly in specimens derived from sun-exposed areas of lupus patients (10,11). Human keratinocytes derived from healthy neonatal foreskins show enhanced binding of autoantibodies to their surface following UV irradiation (12). Antibodies directed against La, Sm, RNP, and Ro are specifically associated with membrane blebs of UV-damaged cells, a finding of special interest since antibodies to Ro are frequently associated with cutaneous lupus. The ability of UV light to trigger apoptosis in a variety of cell types led to a comparison of the UV sensitivity of cells derived from patients with SLE and from healthy control patients. Remarkably, keratinocytes, fibroblasts, and lymphocytes derived from patients with SLE have heightened sensitivity to UV-induced apoptosis, and UV-irradiated keratinocytes derived from SLE patients demonstrate enhanced binding of autoantibodies to their surface when compared with keratinocytes from healthy control patients (13). The

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ability of UV light to trigger apoptosis, autoantigen expression, and clinical exacerbations suggested a possible role of these events in disease pathogenesis.

Further analysis of keratinocytes undergoing UV-induced apoptosis demonstrates the formation of at least 2 distinct cell surface "blebs," structures bound by plasma membrane that are visible by light microscopy in cells undergoing apoptosis. The large blebs contain nucleosomal DNA, Ro, La, and small nuclear RNP (snRNP) complexes, while the small blebs contain Ro as well as ribosomal and endoplasmic reticulum (ER) components (14). Infection of cells with Sindbis virus also leads to apoptosis and packaging of autoantigens in apoptotic blebs; interestingly, viral particles are colocalized with ribosomal and ER components exclusively in small blebs (15). Other molecules have been observed in association with keratinocyte surface blebs, including complement C1q (complete deficiency of which is almost uniformly associated with SLE) (16), and phosphatidylserine, a procoagulant implicated in antiphospholipid antibody syndrome that is usually restricted to the inner leaf of the plasma membrane but is flipped to the outer leaflet of the plasma membrane of apoptotic blebs (17). Taken together, these reports provide strong evidence that cells undergoing apoptosis act as unique reservoirs of autoantigens, and that clinically relevant, common inducers of apoptosis, such as sun exposure and viral infection, may be important triggers leading to the development of autoantibodies or to the perpetuation of an immune response.

While the clustering of autoantibodies on the surface of apoptotic cells has been best studied in SLE, this phenomenon has been recently described for two other diseases characterized by the development of specific autoantibodies. Wegener's granulomatosis, a systemic vasculitis characterized by pulmonary, renal, and other clinical manifestations, is associated with serum antineutrophil cytoplasmic autoantibodies (ANCA) in most affected patients. Granules of apoptotic, but not untreated, neutrophils have been shown to bind ANCA in a region just below the intact cell membrane (18). Similarly, components of the translational apparatus, such as signal recognition particle (SRP) and ribosomes, are known targets of the immune response in polymyositis/dermatomyositis, and have been localized to the surface blebs of cells undergoing apoptosis (14,19).

Modifications of autoantigens during cell death

Programmed cell death is characterized by typical morphologic changes in the dying cell (e.g., chromatin condensation and membrane blebbing), that result from biochemical modifications of nuclear and cytoplasmic structural proteins (for review, see ref. 1). In response to an apoptotic stimulus, a cascade of cysteine proteases with aspartic acid substrate specificity, or "caspases" (20), is activated. At least 39 different proteins are known to be cleaved during apoptosis (Tables 1 and 2). Some of these are structural proteins that are essential for maintaining nuclear (e.g., lamins A, B, and C) and cytoplasmic (e.g., fodrin, actin, and gelsolin) architecture, and others are enzymes essential for repairing damaged cells (e.g., DNA-dependent protein kinase [DNA-PK], poly[ADP-ribose] polymerase [PARP], and topoisomerases [topo]). The concerted proteolysis of these key cellular targets is thought to account for the irreversible cellular changes associated with apoptosis. It is remarkable that 17 of the known apoptotic protease substrates have been identified as autoantigens or are constituents of larger complexes (e.g., spliceosomes and nucleosomes) that contain a protein recognized by autoantibodies (Table 1). At least one of these proteins (U1–70 kd) is a constituent of apoptotic blebs (14,21). It is currently unknown whether the 22 remaining proteins (Table 2) are components of apoptotic blebs or targets of the autoimmune response in SLE, scleroderma, or related diseases.

It has been proposed that proteolysis may produce novel epitopes required for the production of autoantibodies (14,22); however, only a subset of known lupus autoantigens are cleaved during apoptosis. In addition to the caspase cascade that is activated during apoptosis, several kinase cascades are activated, including stress-activated protein kinase (SAP kinase) pathways (for review, see ref. 23), suggesting that autoantigen phosphorylation during apoptosis may also be involved in the subsequent development of autoantibodies. Interestingly, many kinases are caspase substrates themselves (Table 2), and their cleavage has been shown to have important effects on their function. For example, cleavage of protein kinase Cδ (PKCδ) activates its kinase activity and contributes to phenotypic changes associated with cell death (24,25), while cleavage of p21-activated kinase 2 (PAK2) generates a constitutively active fragment that influences cell shape, externalization of phosphatidylserine, and the subsequent formation of apoptotic blebs (26). Moreover, cleavage of a mitogen-activated protein kinase kinase (MEKK-1) and protein kinase Cθ (PKCθ) is sufficient to induce apoptosis in some cells, suggesting that kinase cascades that are activated during apoptosis may be critical regulators of cell death (27,28).

Table 1. Modifications of autoantigens during apoptosis

Autoantigen	Disease	Reference
Caspase cleavage		
Topoisomerase I	Scleroderma, PM	77
Topoisomerase II	SLE, fibrosing alveolitis	77, 78
UBF/NOR-90	Sjögren's disease, scleroderma	77
PARP	SLE	79
DNA-PK	SLE, scleroderma, overlap syndromes	22
U1–70 kd	SLE, scleroderma, MCTD	21
hnRNP C1 and C2	Scleroderma, psoriasis	80
NuMA	Sjögren's syndrome	77
Lamins A, B, C	SLE-like disease, APS	81
α fodrin	Sjögren's disease	82
SRP72	PM/DM	Utz and Anderson (unpublished)
Actin	Autoimmune hepatitis	83, 84
SP1	UCTD	85, 86
Keratin	GVHD, DLE	87, 88
Phosphorylation	SLE, SLE overlap syndromes	
pp200		29
pp90		30
pp46		29, 30
pp17		29
SR splicing factors (pp54, pp42, pp34, and pp23)		29, 30
DNA cleavage	SLE, SLE overlap syndromes	3, 5
Transglutaminase crosslinking		
Histone H2B	SLE	5, 40
Actin	Autoimmune hepatitis	40, 83, 84
Tubulin	SLE	40, 89
Troponin	Necrobiosis lipoidica	40, 90
Ubiquitin conjugation/ deconjugation		
Histone H2A	SLE	5, 41, 42
Topoisomerase II	SLE, fibrosing alveolitis	77, 78, 91

* PM = polymyositis; SLE = systemic lupus erythematosus; UBF/NOR90 = nucleolar organizing region; PARP = poly(ADP-ribose) polymerase; DNA-PK = DNA-dependent protein kinase; MCTD = mixed connective tissue disease; hnRNP = heterogeneous nuclear ribonucleoprotein; NuMA= nuclear mitotic apparatus protein; APS = antiphospholipid antibody syndrome; SRP72 = signal recognition particle 72; DM = dermatomyositis; SP1 = SP1 transcription factor; UCTD = undifferentiated connective tissue disease; GVHD = graft-versus-host disease; DLE = discoid lupus erythematosus; SR = serine/arginine-rich.

We recently reported that proteins phosphorylated during apoptosis are commonly precipitated by autoantibodies derived from patients with SLE, and that a serine kinase activity was present in immunoprecipitates prepared from apoptotic cell extracts using sera from patients with SLE and SLE overlap syndromes (29). Four of these proteins have been identified as serine/arginine-rich splicing factors (SR proteins), and it has been shown that phosphorylated forms of these proteins are specifically associated with the U1 snRNP autoantigen complex during apoptosis (30). Antibodies recognizing components of the U1 snRNP (including anti-Sm antibodies) are frequently found in the serum of patients with SLE, mixed connective tissue disease (MCTD), and other lupus overlap syndromes (2,3). As discussed in more detail below, the localization of the U1 snRNP particle to cell surface blebs of apoptotic cells, together with modifications of the complex during cell death (e.g., caspase-mediated cleavage of U1–70 kd and association with phosphorylated SR proteins) may be critical determinants in the development of an immune response to the U1 snRNP particle in susceptible individuals.

Several components of RNA polymerase I (RNA Pol I) (i.e., S5 and S6) are specifically recognized by autoantibodies derived from SLE patients only when they are phosphorylated, suggesting that phosphorylation may play a direct role in determining the immunogenicity of some proteins (31). Although T cell recognition of phosphopeptides bound to self–major histocompatibility complex (MHC) molecules has not yet been reported, T cells specific for carbohydrate- or trinitrophenyl-modified peptides are generated as part of the immune response to these compounds (32,33). The ability of T cells to recognize hapten-modified peptide, but not hapten alone or peptide alone, supports the concept that T cells can specifically recognize modified peptides. Even if T cells cannot recognize phos-

Table 2. Other caspase substrates as potential autoantigens

DFF	bcl-2
IL-1β	Gas2
Huntingtin	IκB
Rb	PKC-related kinase 2
PKCδ	β catenin
PKCθ	Replication factor C 140
PAK2 kinase	Focal adhesion kinase
Gelsolin	MEKK-1
D4-GDI	Phospholipase A_2
SREBP1	PITSLRE family kinases
SREBP2	FKBP46

* DFF = DNA fragmentation factor; IL-1β = interleukin-1β; Gas2 = growth arrest–specific protein 2; IκB = inhibitor of nuclear factor κB; Rb = retinoblastoma gene product; PKC = protein kinase C; PAK2 kinase = p21-associated kinase 2; MEKK-1 = mitogen-activated protein kinase kinase; D4-GDI = GDP dissociation inhibitor D4; SREBP = sterol regulatory element binding protein; PITSLRE kinases = a cdc2-like family of kinases containing a PITSLRE motif; FKBP46 = nuclear immunophilin FKBP46.

phopeptide epitopes, phosphorylation might target U1–70 kd or SR proteins for degradation by proteases to which they are not normally exposed. Although SR proteins possess "DXXD" motifs that are potential recognition sites for apoptotic caspases (34), we have not observed site-specific cleavage of SR proteins in cells undergoing apoptosis (unpublished observations). It is also possible that phosphorylation could target SR proteins to the proteasome, a degradative organelle that is essential for the production of selected antigenic peptides (for review, see ref. 35). Modified self peptides (either phosphopeptides or peptides produced by proteolytic pathways selectively activated during apoptosis) produced in this manner could be presented by either class I or class II MHC molecules.

In addition to caspase cleavage and protein phosphorylation, at least 4 other potential autoantigen modifications during apoptosis or cellular injury may be involved in the subsequent development of autoantibodies in autoimmune disease. First, several scleroderma autoantigens (UBF/NOR90, topo I, and RNA Pol II) are fragmented when incubated with heavy metals (36). The fragmentation is mediated by an oxidation reaction that has been proposed to occur in vivo during episodes of ischemia reperfusion that characterize systemic sclerosis. Metal ions colocalize with scleroderma autoantigens in nucleoli, and it has been proposed that cleavage of these antigens in response to oxidative stress may unmask cryptic epitopes in a manner analogous to caspase cleavage of lupus autoantigens (36).

Second, exposure of humans to mercury is associated with lupus-like autoimmune disease (37), and mice treated with mercury develop autoantibodies directed against the nucleolar protein fibrillarin in a genetically-restricted manner (38). Treatment of cells in vitro with mercury results in cell death and inhibition of cysteine crosslinking of fibrillarin, a modification that has been proposed to change the antigenicity of the molecule (39). Third, at least 4 autoantigens (histone H2B, actin, tubulin, and troponin) are substrates for tissue transglutaminase, an enzyme that is activated during apoptosis and which catalyzes the crosslinking of substrate proteins through the formation of $\epsilon(\lambda$-glutamyl) lysine crosslinks and (N,N-bis[λ-glutamyl] polyamine bonds [for review, see ref. 40]). This modification has been suggested to create neoepitopes or to increase the half-life of proteins present in apoptotic cells, thus increasing the duration of time that these modified antigens are exposed to the immune system.

Finally, ubiquitinated histone H2A (uH2A) is present in normal cells but is absent from cells undergoing apoptosis induced by transforming growth factor β1, suggesting that the ubiquitin-conjugating apparatus responsible for maintaining uH2A is disrupted during apoptosis (41,42). While no other posttranslation modifications have been observed during apoptosis, we speculate that other modifications (e.g., acetylation, methylation, citrullination [43], or dephosphorylation) will be described for those molecules (e.g., Ro, La, Ku, and ribosomal P) which are present in apoptotic blebs but are not known to be modified during apoptosis.

Although this review is focused on posttranslational protein modifications and the development of autoantibodies, it should be noted that proteins are not the only macromolecules that are modified during apoptosis. One of the earliest biochemical events in programmed cell death is the cleavage of DNA into histone-containing internucleosomal fragments, which are detectable as "DNA ladders" when visualized by agarose gel electrophoresis. The double-stranded DNA (dsDNA) breaks produced during this process are potential targets for binding by the Ku autoantigen. Moreover, Ku, histone, and DNA have all been identified as constituents of apoptotic blebs, suggesting that this group of autoantigens, like the U1 snRNP complex described above, may also be modified and packaged in cell surface blebs during apoptosis (14). Similar observations have been made for phospholipid, in which antiphospholipid antibodies derived from patients with antiphospholipid antibody syndrome have been shown to bind with greater affinity to apoptotic cells than to control cells, a phenomenon that correlates with the presentation of the procoagulant phosphatidylserine on the cell surface (17,44).

Tolerance bypass and the U1 snRNP complex

A common feature of autoimmune diseases such as SLE, systemic sclerosis, Sjögren's disease, and MCTD is the breakdown of tolerance to self antigens, a consequence of which is the production of antibodies reactive with multiple self proteins (3). Anti-Sm antibodies reactive with components of the U snRNP complexes are specific markers of SLE (2,3). The immune response to the U1 snRNP complex has been extensively studied both in humans with SLE and in murine models of this disease (2,45–50). MRL/Mp-*lpr*/*lpr* (MRL/*lpr*) mice develop a systemic autoimmune disease that closely resembles human SLE (for review, see ref. 51). These animals exhibit hypergammaglobulinemia, autoantibody production (including anti-dsDNA and anti–U1 snRNP), and immune complex glomerulonephritis, features that are shared by patients with SLE. The *lpr* mutation has been localized to the structural gene for Fas, a cell surface

molecule that is important for the elimination of lymphocytes (for review, see refs. 52 and 53). The persistence of these lymphocytes results in profound lymphadenopathy, and appears to potentiate the expression of an autoimmune predisposition inherent in the MRL strain. Thus, C57Bl/6J-*lpr*/*lpr* (B6-*lpr*) mice develop neither disease-specific autoantibodies reactive with U1 snRNP nor immune complex glomerulonephritis (51). Although B6-*lpr* mice can develop autoantibodies reactive with IgG (rheumatoid factors) and chromatin, this autoimmune response does not produce the lupus-like syndrome observed in the MRL/*lpr* strain (51).

The production of disease-specific anti-Sm antibodies appears to be determined by a single recessive MRL gene (54). The specific expression of anti-Sm antibodies in human lupus and in the MRL/*lpr* mouse suggests that this gene might contribute to the bypass of self tolerance that is central to the development of autoimmunity in both humans and mice. Although the identity of this disease susceptibility gene is not known, several genes essential for autoantibody production have been identified by breeding immune receptor–deficient mice onto the MRL/*lpr* background. These experiments have established that class II MHC and CD4 are required for autoantibody formation and immune complex glomerulonephritis (55,56). These results suggest that the antigen(s) responsible for triggering CD4+ helper T cells required for autoantibody formation are presented by class II MHC. Surprisingly, MRL/*lpr* mice lacking T cell receptor α produce autoantibodies that form immune complexes leading to glomerulonephritis (57). This result implies that other cell types (e.g., γ/δ T cells and/or natural killer cells) can provide help for autoantibody production in mice lacking α/β T cells. The relative contribution of α/β T cell–independent autoantibody production in the MRL/*lpr* mouse is not known.

The self antigen that drives the production of autoantibodies reactive with U1 snRNP is also unknown. Although the composition of the U1 snRNP complex is not known to be altered in patients with SLE, the results described above have demonstrated profound alterations in the structure and localization of this complex in apoptotic cells (14,17,21,29,30). Because individual components of antigenic particles can influence the immune response to other components in the particle (a process known as "epitope spreading" [47]), cleavage of U1–70 kd or the recruitment of phosphorylated SR proteins to the U1 snRNP particle could initiate autoantibody formation. This is particularly true in the case of modified self peptides to which T cells might not be tolerized. The resulting T cell response could drive the maturation of potentially self-reactive B cells (including B cells reactive with components of snRNPs) that circulate in normal individuals (58–65). These antigens might be rendered accessible to B cells when they are transferred to apoptotic blebs at the plasma membrane.

Peripheral tolerance to self antigens is maintained by sequestration of the self antigen (U1 snRNP normally resides in the nucleus) and lack of T cell help. Although self peptides derived from snRNP proteins are probably expressed on the surface of antigen-presenting cells (APC), potentially self-reactive T cells capable of recognizing these ubiquitous self peptides are subject to deletion during thymic development. The phosphorylation of these self peptides during apoptosis has the potential to produce neoepitopes to which T cells have not been rendered tolerant. This could result from phosphorylation-induced cleavage of SR proteins (including the U1–70 kd protein, which contains 2 SR domains and is phosphorylated in vivo by an unidentified serine kinase [21,34,66]) to produce peptides that are only presented by cells undergoing apoptosis (analogous to the ubiquitin-dependent degradation of IκB following phosphorylation [67]). Alternatively, phosphorylated peptides could be presented by self-MHC and directly activate T cells capable of driving autoantibody production.

Apoptotic death can be divided into a "triggering phase" (e.g., ligation of "dedicated death receptors" such as Fas, or withdrawal of growth/survival factors), a "signaling phase" (e.g., protein kinase cascades that include JNK and p38), an "execution phase" (e.g., activation of caspases and nucleases), and a "burial phase" (e.g., phagocytosis of dying cells by their neighbors). A defect at any level, particularly the burial phase, could result in increased expression of potential self antigens. Increased numbers of apoptotic lymphocytes and macrophages have been reported in patients with SLE (8,68,69). Although this could result from increased triggering of apoptosis, it could also result from defective signaling, execution, or burial phases of apoptosis, thus delaying completion of the death program. In the MRL/*lpr* mouse, the triggering phase of apoptosis is decreased due to the functional inactivation of Fas. In this case, however, the propensity for autoantibody production lies clearly in the MRL background. It appears that the Fas defect potentiates an intrinsic predisposition to autoantibody production, possibly by preventing the elimination of self-reactive T cells and B cells once they have been expanded by other mechanisms. It is clear that more work needs to be done in defining possible defects in the apoptotic death of hematopoietic cells in patients with SLE.

The mechanism described above is unlikely to

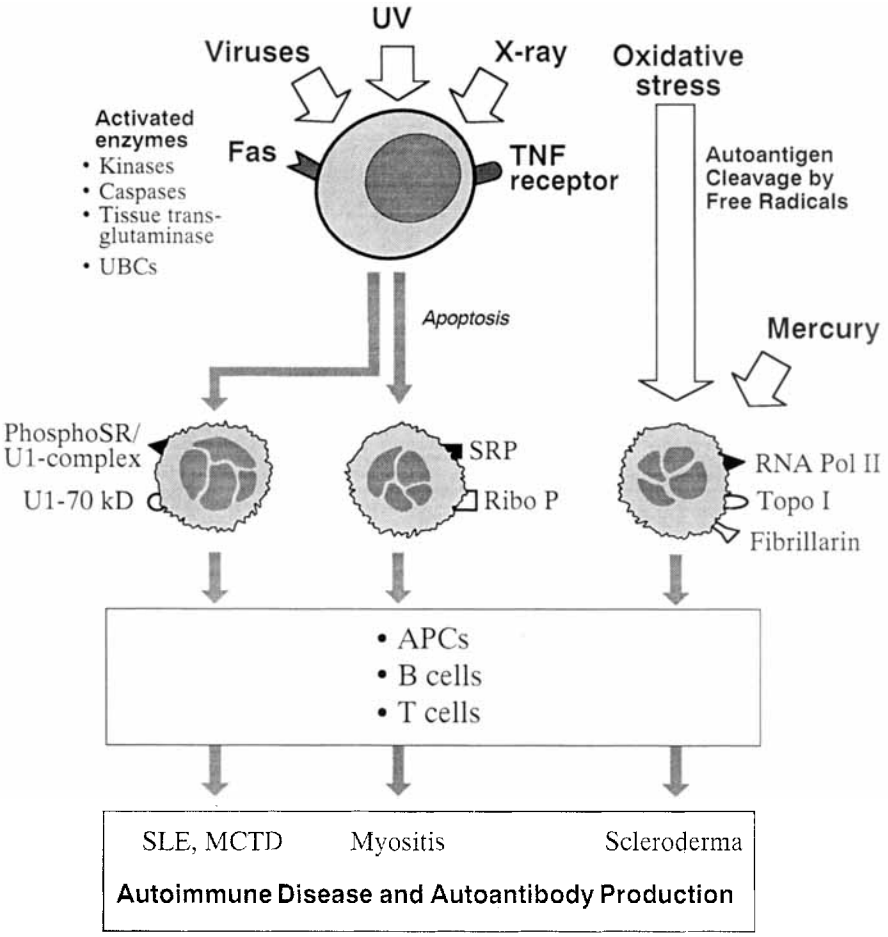

Figure 1. Hypothetical model of autoantibody genesis. Although depicted separately, autoantigens (e.g., RNA polymerase II [RNA Pol II] and topoisomerase I [Topo I]) frequently coexist in the same apoptotic bleb. Details of the model are discussed in the text. UV = ultraviolet (light); X-ray = gamma irradiation; TNF = tumor necrosis factor; UBCs = ubiquitin-conjugating enzymes; PhosphoSR = phosphorylated serine arginine family splicing factors; SRP = signal recognition particle; Ribo P = ribosomal P proteins; APCs = antigen-presenting cells; SLE = systemic lupus erythematosus; MCTD = mixed connective tissue disease.

contribute to the pathogenesis of SLE unless there is a defect in the function of APC. However, if lupus APC have a genetic defect that results in aberrant apoptosis, this defect cannot, by itself, produce autoimmune disease. A 50% concordance rate for SLE among identical twins suggests a requirement for unknown environmental factors in disease expression. Although microbial infections have been proposed to contribute to the initiation of disease, definitive evidence of such a mechanism is lacking. It is of interest, however, that intracellular pathogens, particularly viruses, must inactivate the apoptotic program of their host cell in order to allow productive infection (for review, see refs. 70 and 71). Examples of viral proteins that target the apoptotic machinery include 1) SV40 T antigen, which binds to p53, preventing apoptosis; 2) human parvovirus E6, which facilitates the degradation of p53, preventing apoptosis; 3) Epstein-Barr virus BHRF1, a CED-9/*bcl*-2 family member that inhibits apoptosis; 4) herpesvirus simariri ORF16, a CED-9/*bcl*-2 family member that inhibits apoptosis; 5) adenovirus E1B, a CED-9/*bcl*-2 family member that inhibits apoptosis; and 6) vFLIPs (viral FLICE-inhibitory proteins), a family of viral proteins that interfere with the CED-3 homolog FLICE (Fas-associated death domain–like interleukin-1β–converting enzyme) to inhibit apoptosis (for review, see ref. 72).

The nearly universal requirement for intracell-

ular pathogens to inhibit apoptosis points to the importance of this process in host defense. It is possible that aberrant apoptosis in lupus hematopoietic cells can be potentiated by viral infection. Indeed, peculiarities in the expression of SLE in West Africans (who rarely get SLE in their native environment, but commonly get SLE following immigration to North America) has been proposed to be related to differences in the prevalence of infectious pathogens in the different environments (73).

The genesis of autoantibodies: a model

The data presented above have led us to expand on the hypothesis presented by others, that modifications of autoantigens during apoptosis lead to the development of autoantibodies by bypassing normal mechanisms of tolerance (12–14,21,74). In this model (Figure 1), an apoptotic stimulus (e.g., UV light, gamma irradiation, viral infection, or stimulation of a dedicated death receptor such as the receptor for tumor necrosis factor α or Fas) leads to activation of the apoptotic machinery. Susceptibility to disease may be conferred by a variety of genetic and environmental factors, such as expression of a particular MHC molecule (a mechanism that may be important in viral infection) or death receptor or localization of the affected cell type such that it is accessible to an apoptotic stimulus (e.g., keratinocytes and UV exposure). Sustained apoptosis by repeated (e.g., UV light) or persistent (e.g., viral infection) exposure to a stimulus may lead to a continuous source of autoantigens. Individual cell types may preferentially activate different kinase or caspase cascades, leading to the phosphorylation, deubiquitination, or caspase-mediated cleavage of different cadres of autoantigens. In addition, packaging of individual autoantigens (e.g., RNA Pol II and topo I) in the same apoptotic bleb may partly explain the coexistence in the same patient of antibodies that recognize different molecules (so-called "linked autoantibody sets"). Modified apoptotic cell products may be ineffectively cleared in susceptible patients by deficient complement production or by protein crosslinking by transglutaminase, both of which would lead to persistent presentation of autoantigens in apoptotic blebs. A primary defect in cells involved in "burial" (i.e., phagocytes) may also play a role (75). Modified autoantigens would ultimately drive a T and B cell response to these molecules, and epitope spreading would lead to the development of autoantibodies directed against other, more abundant, components of macromolecular complexes.

Although many questions remain unanswered, there are several predictions of this model that should be actively pursued. First, unidentified posttranslational modifications (i.e., other than caspase cleavage, phosphorylation, deubiquitination, and transglutamination) of proteins during apoptosis should be sought for proteins such as Ro, La, Ku, and ribosomal P, which are currently not known to be modified during cell death. Second, it should be possible to identify T cell clones from patients with SLE that are capable of specifically recognizing modified antigens such as phosphorylated SR proteins and caspase cleavage products. Third, immunization of laboratory animals with apoptotic cells or purified apoptotic blebs should lead to the production of autoantibodies specific for the contents of cell surface blebs, and perhaps to the development of autoimmune disease in strains that are normally resistant to disease (76). It is clear that identification of clinically relevant apoptotic triggers such as UV irradiation and viral infection, and definition of the mechanisms leading to autoantigen modifications will undoubtedly lead to a better understanding of autoimmune disease and to the development of novel therapies for SLE and other autoimmune diseases that target these pathways.

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