

## Apoptosis and Autoimmunity

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The association between autoimmunity and apoptotic cell death is under extensive investigation. The process of apoptosis (programmed cell death) defines a series of biochemical and morphologic events that contribute to the normal homeostasis and regulation of immune autoreactivity [1,2]. Upon encounter with antigen in the periphery, T cells can undergo receptor-induced cell death. B cells that bind and present antigenic peptides on major histocompatibility complex molecules to T cells can also undergo receptor-induced apoptosis.

The mechanisms for induction and propagation of programmed cell death, or recently defined AICD (antigen-induced cell death), and homeostasis of immune tolerance include extrinsic stimuli, intracellular signaling, cleavage events, the migration and exposure of intracellular components (as blebs) on the membrane, the clearance of apoptotic debris by disposal proteins and the complement system, and eventually phagocytosis by macrophages. We present here experimental evidence for autoimmune phenomena due to aberrations at each of these checkpoints.

If the preload is excessive, as in massive cell death (for example, upon infection or exposure to sun irradiation), regulatory clearance mechanisms cannot effectively dispose of apoptotic debris and thereby enables the persistence of antigens for stimulation of the immune system. Normal mice injected with syngeneic apoptotic thymocytes developed antinuclear autoantibodies and anticardiolipin and anti-ssDNA antibodies [3]. Casciola-Rosen et al. [4] demonstrated that following exposure to ultraviolet B irradiation of keratinocytes, autoantigens are clustered in two distinct populations of blebs at the surface of apoptotic cells. These autoantigen clusters have in common their proximity to the endoplasmic reticulum and nuclear membranes – sites of increased generation of reactive oxygen species in apoptotic cells. Oxidative modification at these sites may be a mechanism that unites this diverse group of molecules together as autoantigens. Infection of HeLa cells with Sindbis virus causes apoptosis characterized by cell surface blebs containing viral proteins and autoantigens, such as SS-a/Ro and the U1-70-kd protein [5].

During apoptosis, the cellular contents of the nucleus, cytosol and membrane are brought together in close proximity, a mechanism that could lead to epitope spreading. Altered structures of intracellular proteins produced during cleavage events in apoptosis could also be a source of immunogenic antigens. Cleavage by granzyme B is a common property of autoantigens. Schachna et al. [6] suggest that granzyme B cleavage of centromeric

protein-C is responsible for autoantibodies that preferentially recognize cleavage fragments rather than the full protein representing a subgroup of scleroderma patients. In this issue of *IMAJ*, Malmegrim and co-workers [7] present another example where abnormal splicing of U1RNP during apoptosis can lead to autoimmunity and the development of a lupus-like disease by epitope spread.

The mechanisms of abnormal intracellular signaling leading to apoptosis are complex. The key to understanding the physiologic or pathologic regulation of apoptosis is to understand how caspase activation is controlled [see reviews 8–10]. Caspases exist as inactive precursors that can be activated by proteolysis. Some caspases are cleaved and activated by other upstream caspases that are in turn controlled by adaptor proteins (pro-caspase 9/Apaf-1, caspase 8/FADD). The ability of adaptor proteins to activate caspases is also determined by a number of families of regulatory proteins. For example, the anti-apoptotic Bcl-2 family members can prevent activation of caspase 9 by Apaf-1, cellular FLIP can prevent FADD from activating caspase 8, and inhibitor of apoptosis proteins can directly prevent caspase activity. Subject to this regulation, the adaptors connect the caspases to a large number of signal transduction pathways.

Immune system cells can also influence the survival of each other, as well as other types of cells, by liberating cytokines that either promote survival (e.g., interleukin-2 or granulocyte macrophage colony-stimulating factor) or induce cell death (e.g., tumor necrosis factor family members or interferon-gamma). Cytotoxic T cells and natural killer cells can directly kill other cells, such as virally infected cells, by inducing them to undergo apoptosis by the granule-exocytosis pathway or by membrane-bound ligands for TNFR family members. In autoimmune diseases, somatic cells may die by apoptosis that is directly or indirectly caused by cells of the immune system [8].

The afterload, the resistance to clearance by defective disposal proteins, may also lead to autoimmunity. An example would be altered function of C-reactive protein [11], serum amyloid P component [12], and C1q [11]. Experimental knockout mouse models of each of these proteins develop a lupus-like disease. CRP binds to apoptotic cells and augments the classical pathway of

TNFR = tumor necrosis factor receptor  
CRP = C-reactive protein

complement activation, but protects the cells from assembly of the terminal complement components. Furthermore, CRP enhances opsonization and phagocytosis of apoptotic cells by macrophages associated with the expression of the anti-inflammatory cytokine transforming growth factor-beta. The anti-inflammatory effects of CRP require CIq. These observations demonstrate that CRP and the classical complement components act in concert to promote non-inflammatory clearance of apoptotic cells and may help to explain how deficiencies of the classical pathway and CRP lead to impaired handling of apoptotic cells and increased necrosis with the likelihood of immune response to self [11]. Serum amyloid P component binds *in vivo* both to apoptotic cells, the surface blebs of which bear chromatin fragments, and to nuclear debris released by necrosis. Mice with targeted deletion of the SAP gene spontaneously develop antinuclear autoimmunity and severe glomerulonephritis, a phenotype resembling human systemic lupus erythematosus [12].

Lastly, abnormal macrophage phagocytic function can lead to autoimmunity. The rapid clearance of apoptotic cells by macrophages is important to inhibit inflammation and autoimmune responses against intracellular antigens. Mice deficient in receptor tyrosine kinases, such as Tyro 3, Axl, and Mer, have defective clearance of apoptotic cells, lymphadenopathy, and features of autoimmunity. The Mer receptor tyrosine kinase seems to be critical for the engulfment and efficient clearance of apoptotic cells. Mer(kd) mice with a cytoplasmic truncation of Mer harbor macrophages deficient in the clearance of apoptotic thymocytes and develop a lupus-like disease [13].

Direct evidence exists associating faulty apoptotic machinery with the development of autoimmune disease in experimental models and in human disease. Genetic evidence has shown that defects in individual cell-death genes can lead to autoimmune disease. In humans, direct evidence is found in deficient Fas leading to the development of the autoimmune lymphoproliferative syndrome, manifested by lymphadenopathy, renal disease and hemolytic anemia [14]. This syndrome parallels the autoimmune phenomena found in *mrl/lpr* mice that lack the Fas protein [14]. Patients with an ALPS-like syndrome but with normal Fas genes (i.e., ALPSII patients) are found to carry alterations to the gene for caspase 10 [15]. Mutations in the extracellular domain of TNFR1 have been found in the rare, dominantly inherited TNFR-associated periodic syndrome (TRAPS) [16]. The inability to down-modulate TNFR1 causes an episodic inflammatory disease in humans. These mutations prevent the efficient removal of TNFR1 from cells and hence increase signalling by TNF. A number of other syndromes with recessive Mendelian inheritance patterns have been described that, like TRAPS, feature recurrent episodes of inflammation [17].

Deletion of genes controlling the other cell-death pathways can cause lupus-like syndromes. The pro-apoptotic BH3-only protein, Bim, promotes apoptosis by binding to and antagonizing anti-apoptotic Bcl-2 family members such as Bcl-2 and Bcl-xl. In

Bim<sup>-/-</sup> mice, autoimmune kidney disease develops [18]. Importantly, this is the first description of a knockout of a 'BH3-only' Bcl-2-family member with a clear phenotype. These mice develop a lupus-like syndrome, like some of the Bcl-2 transgenic mice. T cells in mice lacking IL-2R $\beta$  spontaneously become activated, causing increased production of plasma cells and autoantibodies that cause hemolytic anemia. IL-2 knockout mice exhibit a syndrome resembling inflammatory bowel disease, suggesting that this process is deficient in the gut of these animals [19]. Mice with deleted *Dnase1* develop a lupus-like autoimmune disease [20]. Knockout mice were utilized to demonstrate that merely increasing the abundance of autoantigens can be sufficient to cause autoimmunity in otherwise normal mice. This suggests that DNase1 is needed to promote the clearance of DNA from nuclear antigens that would otherwise accumulate.

Several transgenic mouse models have been created in which expression of a cell death gene leads to autoimmunity. Bcl-2 transgenic mice provided the first experimental proof that inhibition of cell death could lead to autoimmunity [21]. Expression of Bcl-2 in B cells causes a disease resembling SLE, but only in mice with a specific genetic (SJLxC57BL/6) background.

Mice that over-express TNF- $\alpha$  in their beta cells are predisposed towards diabetes when the genes derived from the non-obese diabetic background are present. TNF accelerates diabetes by the induction of islet cell apoptosis, the recruitment of antigen-presenting cells to the islets, and the presentation of islet cell fragments to autoreactive T lymphocytes [22].

BAFF is a TNF-family member that binds to B cells and stimulates their proliferation via its receptors, BCMA and TACI. Increased serum levels of BAFF have been found in (NZBW)<sub>F1</sub> and MRL/*lpr* mice with SLE. Furthermore, transgenic mice expressing BAFF display B and T cell hyperplasia and a lupus-like autoimmune disease [23]. Transgenic models support the reasoning that the systemic multi-organ autoimmune diseases develop as a result of multiple hits: a genetic background, a deleted gene (or genes), and an environmental exposure.

Investigation of the mechanisms of SLE, as the prototypic systemic autoimmune disease, reveals many altered checkpoints in the induction and propagation of the apoptotic process. Lupus autoantigens may be structures that are chemically modified in apoptosis, either through direct caspase proteolysis or due to downstream effects. If not first removed by non-inflammatory processes, apoptotic material is capable of being presented by specialized antigen-presenting cells to induce an immune response. Animals inoculated with apoptotic material develop lupus autoantibodies. In lupus patients, immune responses that specifically target the apoptotically modified form of lupus autoantigen can be identified [24]. Abnormal clearance mechanisms can allow the persistence of antigenic stimulation. Experimental models and human diseases portray the link of apoptosis to autoimmunity.

SAP = serum amyloid P component

ALPS = autoimmune lymphoproliferative syndrome

TRAPS = TNFR-associated periodic syndrome

IL = interleukin

SLE = systemic lupus erythematosus

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