Apoptosis in Autoimmune Diseases

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Abstract

roid disease, rheumatoid arthritis, Fas/Fas ligand pathway, immune privileged site

Over the past decade, our understanding of apoptosis, or programmed cell death, has increased greatly, with the identification of some of the major components of the apoptotic program and the processes regulating their activation. Although apoptosis is an intrinsic process present in all cells, it can be regulated by extrinsic factors, including growth factors, cell surface receptors, cellular stress and hormones. Apoptosis plays an important role in autoimmune diseases. Normal thyrocytes could induce apoptosis of infiltrating activated T cells and protect against attack by such cells, *i.e.*, the normal thyroid tissues act as an immune privileged site. In Hashimoto's thyroiditis (HT), Fas-mediated apoptosis of thyrocytes in a section of tissues is due to at least two separate mechanisms, the first by infiltrating activated T cells, and the other by FasL-positive thyrocytes in a suicidal or fratricidal fashion. A common feature of autoimmune diseases such as systemic lupus erythematosus (SLE) is the breakdown of tolerance of self antigens, a consequence of which is the production of autoantibodies reactive with multiple self proteins. Evidence is accumulating that modifications of autoantigens during apoptosis lead to the development of autoantibodies by bypassing the normal mechanisms of tolerance. Tissue homeostasis is maintained through a balance between cell proliferation and apoptotic cell death. Rheumatoid arthritis (RA) is characterized by pronounced hyperplasia of the synovial tissue, cell infiltration and periarticular osteoporosis. Enhanced Bcl-2 expression and NF- κ B nuclear translocation of synovial cells are induced by inflammatory cytokines and/or growth factors. These synovial cells become resistant toward apoptosis triggered by various stimuli. The infiltrated cells which are defect in activation-induced cell death can cause autoimmunity by allowing the survival of autoreactive T and B cells. These data suggest that apoptosis might be implicated with the pathogenesis of autoimmunity, whereas the mechanisms might be distinct in each autoimmune disease.

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Key words: systemic lupus erythematosus, autoimmune thy-

Introduction

Apoptosis or programmed cell death is a normal physiological cell suicide function that is highly conserved among all animals. This regulated process of cell death plays a critical role during embryogenesis, tissue homeostasis and remodeling, and serves to remove unwanted cells such as self-reactive lymphocytes, tumor cells, cells with irreparable DNA damage or those infected with viruses. Insufficient apoptosis thus contributes to the pathogenesis of cancer, autoimmune disorders and sustained viral infection, while excessive apoptosis results in inappropriate cell loss and consequently degenerative disorders (1–4). This review will focus on the role of apoptosis in the pathogenesis of the autoimmune disorders such as autoimmune thyroid diseases, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

Phases of Apoptotic Death

Apoptotic cell death can be divided into a “triggering phase” (*e.g.*, ligation of “dedicated death receptor” such as Fas, or withdrawal of growth/survival factors), a “signaling phase” (*e.g.*, protein kinase cascades that include MAPK family, JNK and p38), an “execution phase” (*e.g.*, activation of caspases and nucleases), and a “burial phase” (*e.g.*, phagocytosis of dying cells by neighboring cells) (5).

Triggering phase

Fas ligand (FasL) and tumor necrosis factor α (TNF- α) are the prototypical inducers of apoptosis. These ligands induce clustering of their cognate receptors (Fas, TNFR I or TNFR II), which leads to recruitment of the early signal-transducing molecules. The Fas/FasL system is the most studied receptor-mediated apoptotic pathway (Fig. 1). Fas/Apo-1/CD95 is a type I transmembrane protein with a cysteine-rich extracellular domain and is a member of the tumor necrosis factor receptor (TNFR) superfamily (6, 7). A variety of cell types express Fas,

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Figure 1. Apoptotic pathways in CD95 type I and CD95 type II cell.

but the expression levels differ between tissues. Its cognate ligand, FasL, is a type II transmembrane protein that can also exist as a soluble factor in a stable trimer configuration (8). On ligation of FasL, Fas trimerizes and recruits an adaptor protein known as Fas-associated protein with death domain (FADD, also called MORT-1) through its intracellular death domain (9). The cytotoxic signal is further propagated as FADD recruits and interacts with another adapter protein as FADD-like interleukin-1 β converting enzyme (FLICE), also known as MACH or caspase-8 (10). Formation of the Fas-FADD-FLICE/caspase-8 complex, known as death-inducing signal complex (DISC), facilitates the autocleavage and activation of caspase-8 (11). A protein known as inhibitor of FLICE (I-FLICE) and FLICE-inhibitory protein (FLIP) can prevent the formation of DISC required for further apoptotic signaling (12, 13).

Other death receptors can trigger similar pathways and often use the same cellular machinery. TNFR-I can be found by either TNF- α or lymphotoxin- α , and will trimerize and recruit the adaptor protein TNFR-associated death domain (TRADD) (14). TRADD is then able to recruit and interact with several molecules, including FADD, which activates the same apoptotic machinery as in the Fas signaling pathway. Another member

of the TNFR superfamily, death receptor 3 (DR3 or Apo3), similarly recruits TRADD following ligation by Apo3L (15, 16). The TNF-related, apoptosis-inducing ligand (TRAIL, also called Apo2L) can induce death pathways by binding one or two death receptors, DR4 and DR5, which appear to induce apoptosis in a FADD-independent manner (17, 18). TRAIL also has two decoy receptors, DcR1 and DcR2, which do not induce apoptosis. They compete for TRAIL with DR4 and DR5, and inhibit the signaling of apoptosis.

Signaling phase

Apoptosis is a multistep process and protein kinases have been implicated both in the upstream induction phase of apoptosis and in the downstream execution stage, as the direct targets for caspases. The serine/threonine protein kinases that have been suggested to play a role in apoptosis are the mitogen-activated protein kinase (MAPK) family, specifically, p42/44 ERK, p38 MAPK and c-Jun N-terminal kinase (JNK), cyclic AMP-dependent protein kinase A (PKA), protein kinase B (PKB), or Akt and protein kinase C (PKC). The activation of JNK/SAPK and p38 MAP kinases is generally associated with the promotion of apoptosis, while p42/44 ERK activity inhib-

its apoptosis (19, 20).

Execution phase

Cell death proteases known as “caspases” are integral components of apoptotic programs in diverse species (21, 22). Initially synthesized as inactive precursors (zymogens), caspases are activated by proteolytic processing that yields large and small subunits forming the active enzyme. In some cases, an N-terminal “pro-domain” is subsequently removed by autocatalysis. The functional conservation of caspases in apoptotic programs throughout the animal kingdom make them likely targets for influencing the cell death decision. In mammalian cells, activation of caspases is achieved through at least two independent mechanisms which are initiated by distinct caspases, but results in activation of common executioner caspases (Fig. 1). For example, several members of the tumor necrosis factor (TNF) family group of cytokine receptors recruit caspase-8 to their cytosolic domains upon binding cytokine ligands, resulting in proteolytic activation of this proximal caspase (23). Once activated, caspase-8 can induce either directly or indirectly the activation of a number of distal caspases such as caspase-3, -6 and -7 (CD95 type I cells) (24). Another pathway for caspase activation involves cytochrome c, which in mammalian cells is often released from the mitochondria into the cytosol during apoptosis (CD95 type II cells) (25). In “type I” cells, death receptor signaling is not blocked by Bcl-2 and in “type II” cells it is blocked (26, 27). The mechanisms linking death receptor signaling to a Bcl-2 inhibitable pathway come with the finding that the Bcl-2 homology, BH3-only subfamily protein Bid is cleaved by active caspase-8, and the activated truncated Bid targets mitochondria to trigger Bax oligomerization and cytochrome c release. The released cytochrome c binds to Apaf-1, a cytosolic protein. This induces Apaf-1 oligomerization, and the Apaf-1 “apoptosome” recruits and activates procaspase-9 (28). The active caspase-9 then recruits and activates the executioner procaspase-3. The active caspase-3, and other executioner caspases, such as caspase-7, act on key substrates in the cell to orchestrate apoptotic death.

Regulation of Apoptosis

The regulation of apoptosis is complex and can occur at multiple levels in the signaling pathway, including death receptor or ligand expression levels, the modulation of intracellular signaling components, and the expression of either proapoptotic proteins.

Bcl-2 family

The mitochondrial pathway, is triggered by proapoptotic members of the Bcl-2 family, initially by a subset of these called the “BH3-only subfamily” proteins because they possess only one of the BH domains. These include Bid, Bim, Harikari, Noxa and a number of others. In response to environmental cues these proteins engage another set of proapoptotic Bcl-2 members, the Bax subfamily [which includes Bax, Bcl-x_s, Nbk (also called Bik), Bak and Bad], loosely residing on the mitochondrial outer

membranes or in the cytosol. The interaction causes the latter to oligomerize and insert into the mitochondrial membrane (29). Here the complex acts to trigger the sudden and complete release of cytochrome c and other proteins from all of the mitochondria in the cell. Bcl-2 and its mammalian homologs Bcl-x_L, Bfl-1 (also called A1) and Mcl-1 block death by preventing the mitochondrial release of the intermembrane proteins, including cytochrome c (30).

IAP family

The functions of the “executioner’s scissors”, the caspases, are modulated by another set of proteins, the IAPs (inhibitor of apoptosis proteins, cIAP-1, cIAP-2 and X-IAP). One of these, X-linked IAP (XIAP) binds to and inhibits the proteinase activity of caspase-9, and thereby can block the apoptotic process at this point (31). It may also bind to and inhibit caspase-3. Caspase binding and inhibition are mediated by its BIR domains (baculovirus IAP repeats), which are necessary and sufficient for IAP function. Another region, the RING domain, acts as an ubiquitin ligase, promoting the degradation of XIAP and possibly any caspase it is bound to. XIAP, and probably other IAP molecules, put slow the apoptotic process by binding, inhibiting and perhaps degrading caspases (4).

Smac/DIABLO

A protein with the dual name of Smac/DIABLO is released from the mitochondria along with cytochrome c during apoptosis, and this protein functions to promote caspase activation by associating with the Apaf-1 apoptosome and inhibiting XIAP. Cytochrome c and Smac/DIABLO are released coordinately, Apaf-1 is activated, caspase-9 is inhibited, Smac/DIABLO relieves the inhibition, and apoptosis proceeds (32, 33).

Apoptosis in Autoimmune Thyroid Diseases

Fas and FasL expression

The Fas and TRAIL pathways are present and functional in the thyroid, and there is evidence suggesting their involvement in autoimmune thyroid diseases (34–37). The Fas/FasL system is the first death pathway demonstrated in the thyroid. Considerable debate continues regarding FasL expression levels in both normal and diseased thyrocytes and regarding whether or not this activity is a means of regulating the Fas pathway in the thyroid. Giordano et al reported the constitutive expression of FasL on normal and Hashimoto’s thyroiditis (HT) thyrocytes using immunohistochemistry, flow cytometry and RT-PCR (38). We have also demonstrated by an immunohistochemical method that FasL is constitutively expressed *in situ* on normal thyrocytes (39). The percentage of FasL-positive thyrocytes in Graves’ thyroid was less than in normal thyroids. In contrast, Xerri et al were unable to find FasL in thyrocytes (40). FasL is also expressed on infiltrating mononuclear cells in HT and Graves’ thyroid sections.

The general consensus is that thyrocytes can express the death receptor Fas, but little is known about how this expres-

sion is regulated. Giordano et al found that Fas was not expressed on thyrocytes unless interleukin 1 β (IL-1 β) was present (38), whereas we reported constitutive expression of Fas but no induction of apoptosis unless IL-1 β or interferon γ (IFN- γ) were added (34). Bretz et al also reported constitutive expression of Fas on normal thyrocytes and massive induction of Fas-mediated apoptosis following treatment with IFN- γ in combination with either IL-1 β or TNF- α (41). In studies of Fas expression on HT thyrocytes, Hammond et al observed increased Fas expression in patients with HT compared with normals (42). Giordano et al reported constitutive Fas expression on HT thyrocytes and proposed that the thyroid destruction observed in HT is a consequence of simultaneous expression of Fas and FasL by thyrocytes (38). In Graves' disease (GD), we found an increased expression of Fas on thyrocytes as compared with normal thyrocytes, and an increased Fas expression on infiltrating mononuclear cells by immunohistochemical methods (39). Furthermore, a recent study has revealed that human thyrocytes express functional TRAIL (37).

Apoptosis

Although it was initially believed that FasL expression was restricted to activated T cells, it has also been detected in sites normally associated with immune privilege, such as the epithelial cells of the cornea, and Sertoli cells of the testes and neurons. Our results using immunohistochemical analysis showed that normal thyrocytes constitutively express FasL, but express only a small amount of Fas (39). We have previously reported that FasL is also expressed on thyrocytes of normal rats (43). Normal thyrocytes which expressed FasL were able to kill activated T cells *in vitro* in a Fas/FasL-dependent manner. Furthermore, normal thyrocytes were resistant to apoptosis mediated by anti-Fas IgM monoclonal antibodies and by activated T cells expressing FasL molecules (36). These results strongly suggest that normal thyrocytes induce apoptosis of infiltrating activated T cells and protect against attack by such cells, *i.e.*, the normal thyroid tissues act as an immune-privileged site (Fig. 2A).

HT manifests as hypothyroidism that is accomplished by massive infiltration of lymphoid cells. Giordano et al (38) claim that the increased apoptosis observed in the thyroid of patients with HT is a consequence of simultaneous expression of Fas and FasL by thyrocytes, resulting in suicide or fratricide among neighboring cells (Fig. 2A). This model is based on the observation that FasL is constitutively expressed on thyrocytes and that IL-1 β produced by infiltrating immune cells, induces Fas expression on thyrocytes (38). We also examined whether or not thyrocytes are committed to apoptosis in a fratricidal fashion. Cultured normal thyrocytes with or without IL-1 β did not undergo spontaneous apoptosis (39). Differences between the two studies may be due to differences in culture conditions or the subjects used in each study. We showed that unstimulated thyrocytes were able to kill the IL-1 β -stimulated thyrocytes through Fas/FasL interaction (39). These data suggest that Fas-mediated apoptosis of thyrocytes in sections of tissue affected by HT is due to at least two separate mechanisms, the first by

infiltrating activated T cells, and the other by FasL-positive thyrocytes in a fratricidal fashion (Fig. 2A).

The thyroid gland of GD patients contains TUNEL-positive thyrocytes and abundant PCNA-positive thyrocytes, together with mononuclear cell infiltration (39). These results indicate that apoptotic cell death and proliferation of thyrocytes may be abnormally accelerated, however, the proliferation of thyrocytes may outweigh the apoptosis of these cells during the pathologic phase, leading to thyrocyte hyperplasia. Immunohistochemical analysis of sections from GD thyroid glands shows overexpression of Fas, diminished expression of FasL in thyrocytes, and increased production of IL-1 β by infiltrating mononuclear cells. Interestingly, IL-1 β up-regulates Fas expression and down-regulates FasL expression on thyrocytes (34). IL-1 β -treated thyrocytes become sensitive to apoptosis by anti-Fas IgM and activated T cells. Furthermore, IL-1 β -stimulated thyrocytes show reduced cytotoxic activity toward activated T cells. These results suggest that the IL-1 β produced in GD thyroid gland may act on the thyrocytes to reduce their resistance to Fas-mediated apoptosis and lose their cytotoxic activity against activated T cells, thus abolishing the immune-privilege status of the thyroid gland, which may explain an accumulation of activated T cells in thyroid tissue affected by GD (Fig. 2B).

Since we previously reported that IL-1 β stimulates the proliferation of cultured thyrocytes, the increase in the number of PCNA-positive thyrocytes may also be due to the action of IL-1 β *in vivo* on thyrocytes (44). Taken together with the data that IL-1 β -primed thyrocytes become sensitive to Fas-mediated apoptosis, it seems that both the proliferation and apoptosis of thyrocytes could be regulated by IL-1 β , which may account for the presence of both PCNA-positive and TUNEL-positive thyrocytes. We have also reported that Graves' serum IgG containing thyroid stimulating antibody (TSAb) markedly inhibits Fas-mediated apoptosis of IL-1 β -treated thyrocytes (35). A recent study by Mihara et al suggested that thyroid hormones, which are present at higher levels in patients with GD, can induce apoptosis in lymphocytes (45). These data suggest a mechanism for thyrocyte survival against active killing of infiltrating lymphocytes (Fig. 2B). Further studies are necessary to fully characterize the significance of thyrocyte apoptosis found in GD thyroid glands.

Apoptosis in Systemic Lupus Erythematosus

A common feature of autoimmune diseases such as SLE, systemic sclerosis, Sjögren's syndrome (SS) and mixed connective tissue disease (MCTD) is the breakdown of tolerance of self antigens, a consequence of which is the production of antibodies reactive with multiple self proteins (46). Evidence is accumulating that modifications of autoantigens during apoptosis lead to the development of autoantibodies by bypassing normal mechanisms of tolerance (5, 47). Clinically relevant, common inducers of apoptosis, such as sun exposure, viral infection, gamma irradiation, or stimulation of a dedicated death receptor such as TNFR or Fas, may be important triggers lead-

A

B

Figure 2. Role of Fas/Fas ligand in normal thyroid and autoimmune thyroid diseases. A) Normal thyroid and Hashimoto's thyroiditis. B) Graves' disease.

ing to the development of autoantibodies or to the perpetuation of an immune response. In patients with SLE, increased numbers of apoptotic lymphocytes and macrophage have been reported (48). 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. Sustained apoptosis by repeated or persistent 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 phosphorylation, deubiquitination, or caspase-mediated cleavage of different cadres of autoantigens. Modified autoantigens would ultimately drive a T and B cell response to those molecules, and epitope spreading would lead to the development of autoantibodies directed against other, more abundant, components of macromolecular complexes. These autoantibodies exert pathologic effects.

Apoptosis in Rheumatoid Arthritis

Pronounced hyperplasia of synovial tissue

Tissue homeostasis is maintained through a balance between cell proliferation and apoptotic cell death (1, 2). RA is characterized by pronounced hyperplasia of the synovial tissue; however, apoptotic cell death of synovial cells is also identified in histologic sections (49–51), suggesting that the relative rate of apoptotic cells to proliferating cells is suppressed in proliferating tissues such as the synovium of RA patients. We (52) and other investigators (50, 53) demonstrated that both synovial cells and infiltrating lymphocytes in rheumatoid synovium express functional Fas and that these cells undergo Fas-mediated apoptosis *in vivo* (50) and *in vitro* (52, 53). However, intractable synovial proliferation is often observed in patients with RA, which may lead to cartilage and bone destruction. The T cells in the rheumatoid synovium are activated and activated T cells *in vitro* are reported to express FasL (50). Therefore, in the rheumatoid synovium, the interaction between Fas antigen on synovial cells and FasL on activated T cells may cause apoptosis of synovial cells and induce regression of proliferation of the synovium which can be seen in patients with RA. However, the function of the Fas/FasL system seems to be incapable of eliminating the cells in the proliferating RA synovium. Bcl-2 is highly expressed on synovial fibroblasts in the synovial lining and the sublining region from RA (54). Increased NF- κ B binding activity in synovial tissue extracts by electrophoretic mobility shift assays and enhanced nuclear localization in the synovial lining have been demonstrated in RA compared with osteoarthritis (OA) (55–57). Collectively, these data suggest that Bcl-2 as well as NF- κ B may be regulated by the inflammatory cytokine milieu.

Various cytokines such as IL-1 β , platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor β (TGF- β) and TNF- α which are present in synovial tissues from RA patients, have been shown to stimulate the proliferation of human synovial cells. Stimulation of TGF- β becomes markedly resistant to Fas-mediated and

proteasome inhibitor-mediated apoptosis (52, 58). Fas antigen expression on synovial cells is inhibited by the addition of TGF- β 1 with up-regulation of Bcl-2, Bcl-x_L and XIAP. Kobayashi et al (59) reported that bFGF-treated synovial cells are also resistant to Fas-mediated apoptosis. The expression of FLIP, an inhibitory molecule of Fas-mediated apoptosis, is augmented in bFGF-treated synovial cells. These results indicate that bFGF treatment increased the expression of FLIP, resulting in resistance toward Fas-mediated apoptosis.

We demonstrated that IL-1 β inhibits the expression of Fas antigen on synovial cells and suppresses Fas mediated apoptosis of synovial cells without autoregulation of Bcl-2 (60). TNF- α and IL-1 β increase nuclear NF- κ B activity (57). TNF- α may induce a death signal through a TNFR1-associated death domain (TRADD) or stimulate cell activation through the NF- κ B pathway, and the coincident inhibition of apoptosis (61, 62). Whereas the activation of NF- κ B blocks cell killing, its inhibition enhances the cytotoxicity of TNF- α and promotes apoptosis in synovial cells (57, 58).

Infiltrated cells in synovial tissues

The inflammatory infiltrate in RA comprises T, B cells, macrophages and neutrophils. Despite the increased expression of Fas and FasL on infiltrating T cells (63), *in situ* observations of synovial lymphoid aggregates suggest low levels of apoptosis (49, 64). This may be due to the high expression of Bcl-2 (49, 65), the production of an antiapoptotic factor by stromal cells (63), or cell-to-cell interaction between lymphocytes and synovial cells (66).

Previous studies suggested that an increased production of cytokines and cell proliferation mediated by activation of NF- κ B through Tax protein may have a major pathologic role in the induction of autoimmune disorders described in humans such as HTLV-I associated myelopathy (HAM), SS and RA (67–71). We showed that the activation of NF- κ B via Tax protein in HTLV-I infected cells renders the cells resistant to apoptosis (72). The expression of anti-apoptotic gene products such as XIAP to suppress caspase cascade, results in resistance toward apoptosis (72). Transgenic mice carrying the env-pX region of HTLV-I develop autoimmune arthropathy, an animal model of human RA (73). Splenic T cells derived from the transgenic mice are more resistant to apoptosis induced by anti-Fas monoclonal antibody (74). The lack of apoptotic cell death in *lpr* and in *gld* mice causes various autoimmune disorders by the inability to eliminate autoreactive T cells (75, 76). Various kinds of viruses encode antiapoptotic proteins such as E1B for an adenovirus (77) and BHRF1 for an Epstein-Barr virus (78) and transgenic mice overexpressing Tax protein develop diseases quite similar to SS (79) and RA (73). Although the precise molecular mechanisms in the process are not fully understood at present, these data may imply new insight to why autoimmune disorders are developed in HTLV-I seropositive subjects. Perhaps, viral protein-mediated inhibition of apoptosis could also be involved in autoimmune disorder induced by virus other than HTLV-I.

Periarticular osteoporosis

Periarticular osteoporosis is a clinical common features in RA (Fig. 3). Osteoporosis is characterized by a decrease in the absolute amount of bone caused by an imbalance between bone resorption and bone formation. The volume of bone is determined by a balance between two opposing processes, osteoblastic bone formation and osteoclastic bone resorption. Although the precise mechanisms that control the number of bone cells are not known at present, the evidence is increased that apoptosis of bone cells may involve the regulation of bone metabolism (80, 81). For example, it is reported that glucocorticoid treatment has a profound effect on osteoblast production and promotes apoptosis of osteoblasts (82).

The inflamed synovium in RA, from which pannus develops, is rich in inflammatory cells including activated lymphocytes (83) (Fig. 3). Osteoblasts are reported to adhere to activated T cells through adhesion molecules, and these cellular interactions increase the cytokine production of osteoblasts (84). Apoptosis of human osteoblasts is observed *in vivo* in humans near joints affected by RA. Human osteoblasts express Fas functionally (85), and both the membrane-type and soluble form of FasL from activated peripheral blood mononuclear cells induce apoptosis of these cells (86). Furthermore, human osteoblasts are committed to apoptosis with anti-Fas IgM, and the treatment of both TNF- α and IL-1 β significantly augments Fas expression and Fas-mediated apoptosis (87). These data suggest that activated T cells in the synovium or synovial fluid in RA patients express membranous FasL and produce soluble FasL, and induce apoptosis of osteoblasts, providing one possible mechanism inducing periarticular osteoporosis in patients

with RA.

Another mechanism is excess differentiation and activation of osteoclasts in RA. Osteoclastogenesis is maintained by cellular interaction between osteoblast/stromal lineage cells and hematopoietic osteoclastic progenitor cells (88). Recent studies have identified the essential role of receptor activator of NF- κ B ligand (RANKL) and RANK in the development of osteoclasts. RANKL is expressed on osteoblast/stromal lineage cells, whereas its receptor RANK is preferentially expressed on osteoclast lineage cells (89–91). Binding of RANKL to RANK induces the differentiation, activation and survival of osteoclasts (92). The cytokines such as IL-1 β , -6, -11, -17 and TNF- α which are abundant in synovial tissues from RA, increase the expression of RANKL with a decrease in OPG expression on osteoblasts/stromal cells, resulting in differentiation and activation of osteoclasts (93).

Several drugs used for the management of osteoporosis exert at least part of their beneficial effects by altering the frequency of apoptosis and thus turning the fate of osteoclasts and osteoblasts. Estrogen (94) and aminobisphosphonates (95) promote osteoclast apoptosis. Instead, bisphosphonates and calcitonin (96) protect the apoptosis of osteocytes and osteoblasts. We demonstrated that vitamin K₂ inhibits the Fas-induced apoptosis of osteoblasts through the suppression of Bax expression (97).

Conclusion

There is accumulating evidence that apoptosis, programmed cell death, is a pivotal role in the pathogenesis of autoimmune diseases. A common feature of autoimmune diseases is the breakdown of tolerance of self antigens, a consequence of which is the production of autoantibodies reactive with multiple self proteins. Apoptosis acts as a source of immunogens. It has been speculated that highly accelerated rates and/or abnormal sites or abnormal processing of apoptotic cells could lead to autoantibody production. Defects of apoptotic pathways in T cells promote the survival of potentially autoreactive, proinflammatory cells. Failure to eliminate activated cells can result in prolonged effector functions, such as CD40 ligand “help” for B cells, inappropriate survival of primed autoantibody-producing B cells, or cytokine release by macrophages. Many organ-specific autoimmune diseases are characterized by immune cell infiltration that induce the apoptosis of local specialized cells, resulting in tissue destruction. Increasing evidences suggest that many sites are protected from bystander injury by “arming themselves” with cytotoxic molecules, which induce apoptosis in infiltrating mononuclear cells. Normal thyrocytes constitutively express FasL, and IL-1 β induces the expression of Fas but decreases the expression of FasL, resulting in apoptotic cell death of the glands by suicide or fratricide.

The elucidation of the biochemical pathways and specific proteins that regulate apoptosis provide a remarkable opportunity to manipulate the “death or life” decisions of the cell. For example, FK506 markedly enhances apoptosis of antigen-stimulated peripheral T cells by down-regulation of Bcl-x_L (98,

Figure 3. The inflamed and hyperplastic synovium invaded and destroyed the cartilage and bone. C: cartilage, B: bone, BM: bone marrow, S: synovium, IMC: infiltrated mononuclear cell aggregates.

99). C2-ceramide inhibits the activation of Akt, MEK and ERK1/2 in PDGF-stimulated synovial cells, resulting in the induction of apoptosis in synovial cells (100). The basic understanding and therapeutic manipulation of apoptosis will have far-reaching implications for the future health of autoimmune disease patients.

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