

## Apoptosis:

### A Case Where Too Much or Too Little Can Lead to Autoimmunity

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#### Abstract

Apoptosis is a physiological process of cell death that normally occurs when cells are damaged or no longer needed. One of its major roles is the maintenance of peripheral immune tolerance, by eliminating activated T and B cells beyond the course of an infection, and thus terminating immune responses. When apoptosis becomes dysfunctional, either being “too much” or “too little,” a variety of different disease states may be triggered. For example, insufficient apoptosis of activated immune cells is the basis of the Canale Smith Syndrome /ALPS, whereas excessive apoptosis of  $\beta$  islet cells of the pancreas is involved in the pathogenesis of autoimmune diabetes mellitus. In this review, we explain the fundamental aspects and molecular mechanisms of apoptosis and their relevance to several important human autoimmune diseases.

**Key Words:** Apoptosis, autoimmunity, diabetes mellitus, Canale-Smith Syndrome/ALPS, acute lymphoproliferative syndrome, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis.

#### Introduction

APOPTOSIS, OR PROGRAMMED CELL DEATH (PCD), is a normal physiological process occurring in senescent or damaged cells. It is a form of demise that serves to eliminate these cells when they are no longer needed. It plays an important role in embryogenesis and normal tissue homeostasis, and is involved in certain pathological conditions as well, such as malignancy, acquired immunodeficiency syndrome (AIDS), certain neurodegenerative diseases, and in autoimmunity (1). Although apoptosis has become one of the major areas of research today, this naturally occurring form of cell death has

been a subject of great interest since 1885, when Flemming (2) provided the first clear description of the process, then called “chromatolysis.” The term “apoptosis” (from the Greek for the “falling off” of leaves from a tree) was coined by Kerr and Wyllie in 1972 (3). Further developments in the field, particularly in the last decade, paralleled advances in molecular biology, biochemistry, and genetics.

#### General Mechanisms

Although “apoptosis” refers to cell death, it has several features distinguishing it from necrotic cell death (Table). In contrast to necrosis, apoptosis follows a stereotypical pattern of morphological and biochemical changes, independently of the initiating event (Fig. 1). This process is characterized by plasma membrane blebbing, cytoplasmic and organelle contraction and shrinkage, nuclear chromatin condensation, and ultimately non-random DNA fragmentation of multiples of 180–200 base pairs, which is the hallmark of apoptosis (4). The subsequently generated cellular fragments, or apoptotic bodies, rapidly become ingested, via a receptor-mediated mechanism, by neighboring cells and resident tissue

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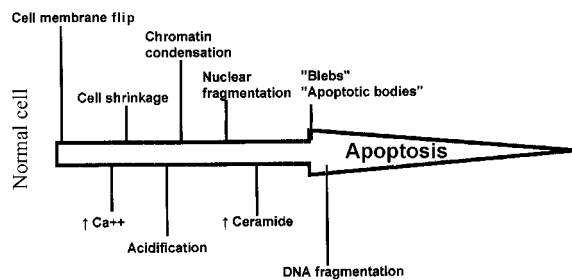
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**TABLE**  
*Cellular Modifications Distinguishing Apoptosis from Necrosis*

	<b>Apoptosis</b>	<b>Necrosis</b>
Cellular role	Active	Passive
Distribution	Dispersed	Contiguous
Morphology	Decreased volume of the cell	Increased volume of the cell
Cellular membrane	Preserved	Loss of integrity
Induction	Slow (hours)	Rapid (seconds – minutes)
Cell removal	Rapid	Slow
Tissue inflammation	Absent	Present



**Fig. 1.** The process of programmed cell death. Apoptosis, or programmed cell death (PCD), follows a stereotypical pattern of cellular modifications at the morphological and biochemical levels, independently of the initiating event. “Cell membrane flip” refers to the exteriorization of phosphatidylserine during the early stage of PCD.

macrophages. This highly efficient elimination of apoptotic cells occurs rapidly and without tissue inflammation or damage (5).

Although apoptosis is a complex and tightly regulated process, it has been described in very simple organisms and was highly conserved during evolution, suggesting that it has a fundamental physiological role in species survival. The genetic control of apoptosis was first dissected in the nematode *Caenorhabditis elegans*. It was determined that 14 genes (CED 1–14) are responsible for the regulation of PCD during the development of *C. elegans*. Two of the gene products (CED-3 and CED-4) are inducers of apoptosis, one (CED-9) inhibits apoptosis, and at least 6 of these genes encode proteins that are required for the engulfment of apoptotic cells (6, 7). The cell death process was further determined to involve four distinct phases: (a) initiation, (b) effector, (c) degradation, and (d) engulfment phase. The initiation phase is triggered by an apoptotic stimulus, and is subject to very intricate regulation. If pro-apoptotic signals override the anti-apoptotic signals, the cell will eventually enter the effector phase, in which

the cell becomes “committed” to die. The degradation phase consists of the typical morphological and biochemical changes of PCD, and is beyond regulation (6). The fourth phase involves the receptor-mediated engulfment of apoptotic bodies by phagocytes (8). Defects in the regulation at any of these phases may ultimately result in disease.

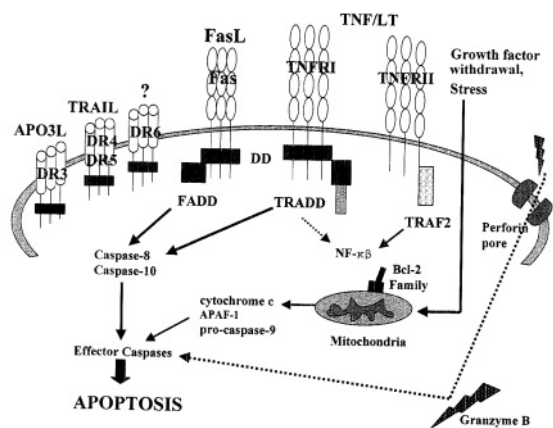
### Specific Molecular Mechanisms

The various biochemical, molecular and genetic factors modulating apoptosis are complex, and are not yet fully defined. Ultimately, PCD is the net result of the balance between pro- and anti-apoptotic stimuli. The most important regulatory mechanisms of PCD in mammalian cells belong to the following categories: death receptors, caspases, mitochondria, the Bcl-2 family of proto-oncogenes, and certain tumor-suppressor genes.

### Death Receptors

Signal transduction after an apoptotic stimulus consists of early events, occurring at the plasma membrane via specific death receptors, and subsequent rapid triggering of a very complex, albeit orderly, intracellular proteolytic cascade of proteases, called “caspases,” ultimately resulting in cellular demise within hours (Fig. 2).

Death receptors are located on the surface of certain cells and act as sensors to extracellular apoptotic signals. Such signals are provided by the receptor’s specific cognate ligand, which may be membrane-bound or in a soluble form. The ligand-receptor interaction rapidly triggers the recruitment of early signal-transducing molecules. The prototypical death receptors are Fas (CD95; APO-1) and tumor necrosis factor receptor I (TNF-RI) and TNF-RII, and their corresponding ligands are Fas ligand (FasL) and TNF- $\alpha$ , respectively (9) (Fig. 2).



**Fig. 2.** Cell death pathways. Interactions between cell death receptors and their respective ligands are illustrated, as well as their major intracellular signal transduction pathways. The initial steps occur at the plasma membrane, by the binding of ligand to its cognate receptor, resulting in clustering and recruitment of upstream adaptor molecules. These, in turn, facilitate enzymatic activity of the caspases. Fas/FasL interactions as well as the perforin-mediated internalization of Granzyme B induce PCD by activating caspases. However, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) may induce two distinct outcomes: it may either trigger a death signal through the TNF receptor I (TNF-RI)-associated death domain (TRADD), or stimulate cell proliferation or cytokine transcription through the nuclear factor  $\kappa\beta$  (NF- $\kappa\beta$ ) pathway. Mitochondria-mediated apoptosis is induced by cellular stress or growth factor withdrawal, and is usually independent of death receptors in most cell types. Cell death pathways are modulated by the Bcl-2 family of proteins and by other downstream signal transduction pathways (see text). FasL = Fas ligand; LT = lymphotoxin; DD = death domain; FADD = Fas-associated death domain; TRAF2 = TNFR2-associated factor 2; APAF-1 = apoptotic protease activating factor-1.

**Signaling by Fas:** Fas is expressed on many different cell types, whereas FasL is mainly expressed on T lymphocytes. The major role of the Fas pathway of PCD is the termination of immune responses by causing peripheral deletion of activated mature T lymphocytes. However, it is also involved in preventing inflammation in “immune-privileged” sites, such as the eyes, brain and testes, where FasL is also highly expressed. Another important function of this pathway is the killing of virus-infected or transformed cells (10). Mutations in Fas or FasL are associated with peripheral lymphoid tissue expansion and autoimmune disease (11–13). When FasL binding to Fas takes place, it induces trimerization of the receptor and clustering of its intracellular death domain (9). This leads to recruitment of the cytoplasmic protein FADD (Fas-associated death domain), which subsequently triggers the proteolytic cascade of

caspases that ultimately leads to cell death (Fig. 2) (14–16). Each step is under strict regulation by a wide array of molecules (17, 18).

**Signaling by TNF-RI:** TNF- $\alpha$  is a soluble cytokine produced by activated T lymphocytes and macrophages in response to inflammation and infection (19). After it binds to TNF-RI, events such as receptor trimerization and clustering occur similarly to Fas (20), with intracellular signaling via the recruitment of an adaptor protein, TRADD (TNF receptor-associated death domain) (21), and via the action of several intermediary proteins, finally leading to the activation of transcription factors NF- $\kappa\beta$  and AP-1 (22). In contrast to Fas, TNF-R-mediated signaling often results in cell activation and proliferation rather than apoptosis, depending on the cell type, the receptor (TNF-RI or II) engaged, and on the interplay of other regulators.

While Fas and TNF receptors are the best characterized, a number of other death receptors have recently been identified, such as death receptor 3 (DR3; also called APO-3/TRAMP/WSL-1/LARD), and death receptors 4 (DR4), 5 (DR5; also called APO-2/TRAIL-R2/TRICK 2/KILLER) and 6 (DR6) (Fig. 2). The role of these new death pathways in immune regulation and in human diseases remains to be determined. For review, see Ashkenazi and Dixit (23).

## Caspases

The various death receptors, once triggered, converge upon a common proteolytic, “executioner” pathway, known as the caspase cascade, that ultimately results in the disassembly and degradation of the cell. Caspases belong to the family of cysteine proteases (previously known as ICE [interleukin-1 $\beta$ -converting enzyme] family, now renamed caspases), and are homologous to the CED-3 gene product of *C. elegans* (24). These proteins exist in the cytoplasm as proenzymes with little catalytic activity, but once activated, play important roles in the initiation and effector phases of apoptosis (25). Each caspase consists of a structurally related molecule with a prodomain, and a large (~20 kD) and small (~10kD) subunit that combine to form tetramers after activation (26). The catalytic site thus generated has an unusual substrate specificity for cleavage at Asp-X peptide bonds, and possibly for the substrate’s tertiary structure as well. Currently 13 different mammalian caspases have been described, 11 of

which have human counterparts (27, 28). They can be classified into different groups according to their substrate specificity and function (28–30): (a) upstream enzymes [caspases 8, 9, 10], that serve to initiate and amplify the death signal; (b) CED-3-like [caspases 2, 3, 6, 7], that are involved in the rapid cleavage of structural and vital cellular components, such as PARP (poly(ADP-ribose) polymerase), in DNA repair and supervision of genomic integrity, lamins (important constituents of the nuclear membrane), and certain enzymes involved in mRNA processing and DNA fragmentation (31); and (c) ICE-like [caspases 1, 4, 5 and 13], that may be involved in inflammation rather than death.

Like the clotting and complement cascades, the caspase cascade proceeds in an autocatalytic manner, leading to intense amplification of the initial apoptotic signal. This cascade is regulated by various cofactors (e.g., apoptotic protease activating factor-1; APAF-1, see below), and inhibitors (32–35) at the posttranslational level, by protein-protein interactions (Fig. 2). For review, see Thornberry and Lazebnik (36). Another group of proteases involved in PCD is the family of serine proteases, the most important member being Granzyme B. The Granzyme B-perforin pathway is a critical component of granule exocytosis and the main apoptotic mechanism involved in target-cell PCD induced by cytotoxic T cells and natural killer (NK) cells (Fig. 2). For review, see Greenberg (37).

### Mitochondria

Mitochondria are important cellular organelles that are thought to have arisen from an endosymbiotic relationship between eukaryotes and purple bacteria some 2 billion years ago (38). They play a central role in the orchestration of apoptosis, and are located upstream of the caspase cascade (39). Their mechanism of action includes: (a) release of specific proteins, such as cytochrome c, which activates procaspase-9 by binding to APAF-1, a human homologue of CED-4, and thus triggering the caspase cascade (32, 40) (Fig. 2); (b) the disruption of electron transport, which is an early feature of PCD; and (c) modification of cellular oxidation-reduction potential. The initial event leading to these effects occurs at the level of the mitochondrial membrane, with disruption of its outer leaflet, matrix swelling and finally loss of the mitochondrial transmembrane potential

(MTP) (41). Several members of the Bcl-2 family reside at the level of the mitochondrial membrane (42), and may possibly function as pores after homo- or heterodimerization, thereby regulating the MTP (39, 43, 44). It has been shown that Bcl-2 directly or indirectly prevents the release of cytochrome c (40, 42). Bax, a death-inducing member of the Bcl-2 family, may act in a caspase-independent manner, presumably by causing damage to the mitochondrial membrane (45). For review, see Green and Reed (46).

### Bcl-2 Family of Proto-Oncogenes

Many proto-oncogenes are involved in maintaining cell survival and proliferation. The most important ones are those belonging to the Bcl-2 family. This group of related gene products has survival-promoting (Bcl-2, Bcl-xL, Bag-1, Bik) or death-promoting (Bax, Bak, Bad) capacities (47, 48). Bcl-2 is a functional and structural homolog of CED-9 (6), and encodes a membrane-associated protein located at the mitochondrial and perinuclear membranes (49, 50). Its role is to promote cell survival and proliferation. Translocations of the Bcl-2 gene, leading to overexpression, were first discovered in B cell lymphomas (51–53), and have been associated with poor prognosis of certain cancers (54, 55) as well as with resistance to several chemotherapeutic agents (56–58). For review, see Strasser et al. (1).

### Tumor-Suppressor Genes

One of the most important tumor-suppressor genes is p53. The p53 gene product can induce cell death in response to DNA damage (59). Mutations causing its loss of function are associated with many human cancers (60). When p53 becomes activated, two cellular outcomes are possible: apoptosis or cell cycle arrest (61). The role of p53 in cell cycle arrest involves many regulatory proteins, such as the cyclin-dependent kinase (Cdk) inhibitor p21 (62), and the retinoblastoma gene product Rb (63). Mutations in these regulatory proteins are also reported to result in abnormal cellular growth, for example, the development of retinoblastoma in Rb-mutated individuals. The mechanism by which p53 causes PCD is less well defined, but may proceed through the triggering of transcription of specific genes (64). For review, see Evan and Littlewood (65).

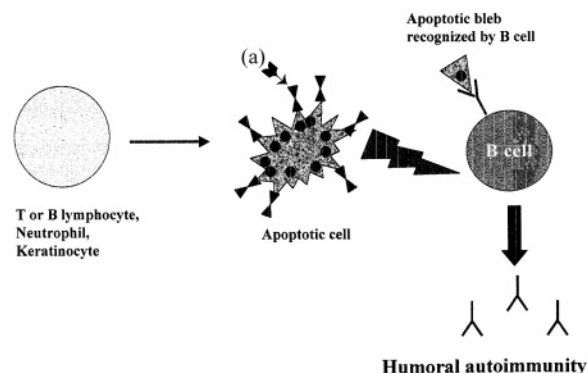
## Other Apoptosis Modifiers

Aside from the genetically mediated regulators previously mentioned, the cell is also subjected to many other apoptosis modifiers, such as various cytokines (e.g., IL-4, IL-2, IL-10) (66–68), circulating or membrane-bound molecules capable of triggering specific ligand-receptor interactions (e.g., Fas ligand/Fas, TNF- $\alpha$ /TNF-RI), and nitric oxide. Nitric oxide is a potent pro-inflammatory molecule and modulator of the immune system (69–73); it has been implicated in various pathological conditions, such as joint damage related to rheumatoid arthritis (74–77) and osteoarthritis (78–80), as well as in diseases associated with vascular dysfunction (81–84). Various reports have suggested that nitric oxide may play a role in PCD (85–88). A mitochondrial-dependent, pro-apoptotic effect has recently been described. Hortelano et al. showed that nitric oxide increased the mitochondrial membrane potential and chemically modifies cytochrome c, thereby altering its structure and promoting its release from the mitochondria, which subsequently activates caspase-3 (89). An anti-apoptotic effect has also been proposed, where nitric oxide induces the S-nitrosylation of caspases, thereby preventing their activation and promoting resistance to Fas-mediated PCD (90).

## Role of Apoptosis in Autoimmune Diseases

### Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the involvement of multiple organs and the presence of serum autoantibodies against various nuclear and cytoplasmic antigens (91). Although the precise immune disturbance in SLE remains obscure, one hypothesis is that the clearance of apoptotic cells is defective (92–94). Apoptosis is associated with the externalization of phosphatidylserine, which may be important in the generation of antiphospholipid antibodies (95), a common feature of SLE (Fig. 3). Exposure of keratinocytes to ultraviolet irradiation has been shown to cause a redistribution of many nuclear antigens (e.g., RO, La, and nucleosomal DNA) to near the surface of the cell in apoptotic blebs (96, 97). Because lymphocytes from lupus patients have an activated phenotype and undergo accelerated PCD compared with normal individuals *in vitro*, it has been suggested that apoptotic blebs contain modified self-antigens that could



**Fig. 3.** Apoptotic cells as a source of immunogens in systemic lupus erythematosus (SLE). The early stages of programmed cell death are associated with the externalization of phosphatidylserine (a) and with the redistribution of various modified nuclear antigens (•) such as Ro, La, and nucleosomal DNA, to the surface of the cell within apoptotic blebs. These apoptotic blebs thus contain potential immunogens, which may drive the humoral autoimmunity seen in human SLE (see text).

be immunogenic. Mevorach et al. recently showed that the immunization of normal mice with a large amount of apoptotic cells resulted in a transient immune response (antiphospholipid and anti-ss-DNA autoantibodies). Taken together, these findings support the indirect evidence that the products of apoptotic cells may drive the humoral autoimmune response in SLE (98) (Fig. 3).

The mechanisms whereby apoptotic cells are efficiently identified, removed and degraded by adjacent phagocytes in mammalian cells are complex and are not yet fully understood. Several ligands and receptors, such as  $\alpha_v\beta_3$  integrin (99) and CD36 (100), have been implicated, and several members of the complement system have recently gained recognition. Deficiencies in the early components of the complement cascade have been shown to predispose humans and mice to lupus-like diseases (101–105), and Mevorach et al. recently reported (106) that serum complement facilitates the uptake of apoptotic cells by phagocytes *in vitro*, in some cases amplified by the presence of C-reactive protein (CRP) (107).

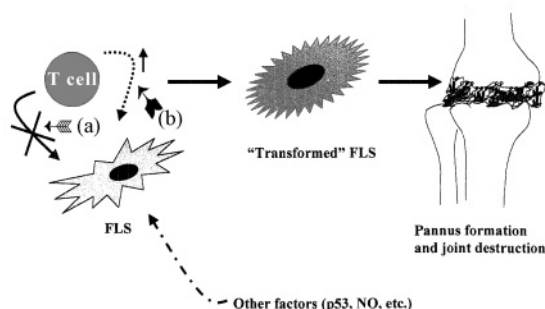
It has also been proposed that defects in certain pro- and anti-apoptotic molecules play a role in lupus-like disease, although most of the evidence is experimental. Most patients with SLE do not have Fas/FasL mutations (108, 109). However, the fact that rare cases with either Fas (110) or FasL (111) mutations exist

suggests that defective function of a related death effector could play a role in the pathogenesis of SLE. The levels of soluble Fas (sFas), a secreted form of Fas capable of inhibiting FasL binding *in vitro*, is elevated in the plasma of many patients with SLE. This finding, however, is not specific to SLE, and its role in disease pathogenesis has not yet been elucidated (112, 113). Other regulators of PCD may also be abnormal in human lupus, such as members of the Bcl-2 family (109, 114, 115), T cell costimulatory mechanisms involving CD28 and CTLA4 (116–119), and CD40 (120, 121).

Despite these important advances, further research is needed to understand the precise role of apoptosis in SLE.

### Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease associated with significant morbidity and increased mortality. The hallmark of RA is the presence of joint swelling and destruction due to the inflammation and proliferation of the synovial membrane. At the cellular level, there is remarkable hyperplasia of synoviocytes, mainly of the “fibroblast-like synoviocytes” (FLS), located in the deeper layer of the synovium, which acquire invasive and damaging potential (122). The infiltration of various inflammatory cells into the sublining area results in synovial thickening and pannus formation, which proceeds to destroy adjacent structures (Fig. 4). The local secretion of a number of pro-inflammatory cytokines by the pannus, such as IL-1, IL-6, and TNF- $\alpha$ , leads



**Fig. 4.** Schematic representation of the role of defective apoptotic mechanisms underlying the pathophysiology of rheumatoid arthritis (RA). Infiltrating activated T cells in the RA joint relay an insufficient pro-apoptotic signal (a), or perhaps an increased anti-apoptotic signal (b) to adjacent fibroblast-like synoviocytes (FLS). The net result leads to persistence and enhanced proliferation of the FLS, pannus formation, and joint destruction.

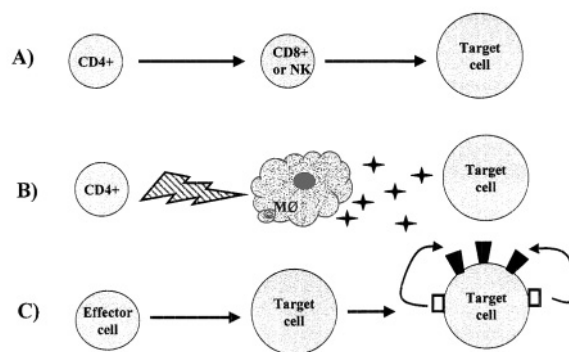
to the damage of soft tissues around the joint as well as to the prototypical bony erosions of RA. Various reports suggest that defects in the regulation of apoptosis of different cell types is implicated in the pathogenesis of RA (Fig. 4). The RA synovium has an abnormally high proliferative rate, and acquires the unusual ability to invade and destroy cartilage and bone. These observations have led several authors to compare it with tumors (122). This “transformed” phenotype may be explained by the altered expression or function of various anti-apoptotic genes, oncogenes, or tumor suppressors. Among the anti-apoptotic genes studied to date, *Bcl-2*, *Bcl-XL*, and *Survivin* were reported to be overexpressed in RA, FLS and T cells; thus, they may contribute to synovial hyperplasia (123–128). Defects in B-cell apoptosis in RA have also been proposed, to explain the local and especially the systemic autoimmunity observed (129). Somatic mutations in the tumor suppressor gene p53 have also been reported in RA synoviocytes and mononuclear cells, but not in RA skin or in normal or osteoarthritis synovium (130). These mutations occur in what are called “hotspots” (regions in the gene that are often mutated in human neoplasias). Most of the p53 mutations seen in RA synovium are characteristic of oxidative deamination by nitric oxide, suggesting that the nitric oxide generation observed in rheumatoid joints resulting from chronic inflammation may contribute to these somatic genetic modifications (131). The Fas/ FasL pathway of PCD may also be implicated in RA. Fas and FasL are both constitutively expressed in the RA synovium. Both are detected on FLS and mononuclear cells, but the death-inducing FasL is mainly expressed on the invading CD4+ (helper) and CD8+ (cytotoxic) lymphocytes. Although FLS have been shown to be sensitive to Fas-induced cell death by anti-Fas antibodies (132), only a small proportion of FLS actually undergo spontaneous apoptosis in RA joint samples (124). This may be due to the deficient ability of infiltrating FasL-bearing T cells to kill their Fas+ target cells (FLS and mononuclear cells) (133–135). Another possible explanation for the deficient Fas-mediated PCD could be the increased nitric oxide found in RA synovium, as mentioned previously (136). In addition to its pro-inflammatory effects, nitric oxide has recently been implicated in playing a role in Fas-mediated apoptosis, by its ability to S-nitrosylate and thus prevent the activation of caspases (136).

Most significantly, the local enhancement of Fas-mediated cell death has proven to be beneficial, at least in murine models of inflammatory arthritis. The intra-articular administration of anti-Fas monoclonal antibody to transgenic mice resulted in marked and rapid clinical improvement of joint inflammation, with significant increase of apoptosis of synovial fibroblasts and mononuclear cells (137). Similarly, the intraperitoneal injection of anti-Fas into mice with severe combined immune deficiency (SCID; lacking mature T and B lymphocytes) with engrafted human RA synovial tissue was equally beneficial, although more toxic (138, 139). Intra-articular transfer of the FasL gene also succeeded in increasing the rate of apoptosis in FLS by cell-to-cell interaction via the Fas/FasL pathway (140). Similar observations were found with the intra-articular transfer of the FADD gene but with far less damage to chondrocytes (141, 142). Further advances in gene-therapy by selectively targeting proliferating cells in the pannus may prove to be a useful adjunct to the treatment of RA in humans in the future.

### Autoimmune Diabetes

Insulin-dependent diabetes mellitus (IDDM) is characterized by the selective destruction of the insulin-producing  $\beta$  cells of the islets of Langerhans of the pancreas. A murine model of IDDM, the nonobese diabetic (NOD) mouse, has permitted the elucidation of many aspects of the pathogenesis underlying human autoimmune diabetes (143). The NOD mouse spontaneously develops IDDM, and the disease progresses through two distinct phases: (a) initially, infiltration of T and B cells, macrophages and dendritic cells occurs, resulting in a peri-insulinitis; and (b) at 4–6 months of age, active widespread destruction of the  $\beta$  cells with insulin-resistant hyperglycemia and the development of major clinical manifestations.

The immunopathogenesis of IDDM is complex, and involves the presence of T cells reactive to peptides derived from various self-antigens (insulin, GAD65/67, HSP70), presented by the diabetogenic major histocompatibility complex (MHC) class II allelic products (I-A<sup>g7</sup> in mice and HLADQ $\beta$  in humans) (144). In the effector phase, it is thought that both CD4+ and CD8+ T cells act in concert to destroy the pancreatic  $\beta$  cells (Fig. 5). The CD4+ cells are capable of killing target cells directly via the Fas receptor (145) or by promoting effector func-



**Fig. 5.** Models proposed to explain the death of target cells in IDDM and EAE. Th1 CD4+ T cells are responsible for the induction of disease. MHC class II surface expression is required for cells to interact and thereby restrict CD4+ cells. However, MHC class II molecules are not usually expressed on the target cells of these diseases. Three non-exclusive models are presented, by which other cells or mechanisms of cell death may cause tissue destruction. **A)** CD4+ T cells prime CD8+ T cells or NK cells to kill the target cells. **B)** CD4+ T cells interact with the MHC class II-peptide complex of activated macrophages (M $\phi$ ). This causes killing of the macrophage, thereby releasing substances toxic to the adjacent target cells. **C)** Effector cells induce the upregulation of a death receptor which engages its ligand (expressed on the same cell).

tions in CD8+ and/or NK cells. Apoptosis of the  $\beta$  cells has been clearly demonstrated in at least one transgenic murine model of IDDM, the NOD-*scid* mouse (146). Many different mechanisms have been proposed to explain the observed PCD in the  $\beta$  pancreatic cells; these mechanisms vary with the different models studied. The major mechanisms implicated include perforin-dependent cytotoxicity, TNF- $\alpha$  (in neonatal, but not in adult NOD mice), and the Fas/FasL pathway of PCD (147–158).

### Multiple Sclerosis

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), in which myelin and myelin-producing cells (oligodendrocytes; OGD) become the target of an inflammatory response, resulting in fibrosis and ensuing plaque formation. The infiltrating cells responsible for the tissue damage are diverse, and only a small proportion of these cells are autoreactive T cells. Although interest in this entity has increased in the past few years, the identity of the self-antigens and precise molecular mechanisms responsible for tissue injury remain incompletely understood (159). Destruction and cell death of OGD is thought to be the central event in MS, leading to the loss

of myelin and to neuronal dysfunction. Although apoptosis is not the sole mechanism involved (160), several studies highlight its role in disease pathogenesis (161, 162). Immunohistochemical analysis of normal brain sections demonstrates that Fas is constitutively expressed on OGD, and low levels of FasL are present on microglia (CNS macrophages) (161). Upregulation of Fas and FasL was noted in samples obtained from chronic active and chronic silent MS disease (160, 162). These observations would be compatible with a straightforward role for either lymphocyte or microglial FasL-mediated killing of OGD in MS. Furthermore, the Fas/FasL pathway of cell death was shown to be involved in the pathogenesis in rodent models of MS. The rodent counterpart of MS, experimental allergic encephalomyelitis (EAE), is also characterized by CNS inflammation and demyelination, with perivascular inflammatory infiltrates composed mainly of T cells and macrophages in the spinal cord and brain (163). Induction of EAE in susceptible mouse strains carrying the *lpr* or *gld* mutations demonstrated a decrease in severity of disease compared to wild-type controls (164, 165). The amelioration of disease was accompanied by a decrease in apoptotic cell death in EAE lesions of *lpr* mice. These results indicate that the Fas/FasL pathway is important for the progression of clinical disease, most likely by the induction of apoptosis at the site of tissue inflammation (Fig. 5). Although the nature of the cells undergoing apoptosis was not established, these studies suggest that deficiencies in Fas or FasL offer protection from tissue injury, possibly due to decreased killing of OGD. The simplest explanation may be that FasL-bearing T cells are the apoptosis inducers. The target cells remain unknown, but may be MHC class II-bearing macrophages, which have been shown to be required for the expression of EAE (166) and are often apoptotic in tissue lesions of EAE (167). The subsequent release of various proteolytic enzymes and other toxic products (such as TNF- $\alpha$ ) from the apoptotic tissue macrophages would thus lead to the destruction of OGD and disease expression (Fig. 5).

### Conclusions

Apoptosis, or programmed cell death, is a normal physiological process whereby useless and potentially harmful cells are eliminated in a highly conserved and tightly regulated fashion. The dysfunction of an apoptotic pathway, re-

sulting in either “too much” or “too little” apoptosis, may lead to the development of many different disease states, such as cancer, AIDS, various neurodegenerative and cardiovascular diseases, and autoimmunity, for which the therapeutic options have until now remained limited. Further research into the specific mechanisms involved in the defective apoptosis underlying these and other diseases will ultimately serve to improve therapeutic options.

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