Cell Death and Differentiation (1999) 6, 13–21

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Review

Apoptosis in the effector phase of autoimmune diabetes, multiple sclerosis and thyroiditis

Satomi Ohsako[1] and Keith B. Elkon*,[1]

[1] Hospital for Special Surgery – Cornell University Medical College, New York, New York 10021, USA

* corresponding author: Keith Elkon, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, USA. tel: (212) 606-1074; fax: (212) 717-1192; e-mail: elkonk@hss.edu

Received 20.7.98; revised 23.9.98; accepted 28.9.98
Edited by M. Piacentini

Abstract

The immune system is unusual in two respects. It produces billions of new cells daily that traffic throughout the body and cells within the system proliferate rapidly following exposure to an infectious agent. Both of these attributes require that cell production be regulated by cell death. Human diseases characterized by accelerated cell death leading to immunodeficiency disorders or by reduced cell death leading to systemic autoimmune diseases have been identified. In certain autoimmune diseases, the immune system directs its powerful cytotoxic effector mechanisms against specialized cells such as oligodendrocytes in multiple sclerosis, the β cells of the pancreas in diabetes mellitus and thyrocytes in Hashimoto's thyroiditis. In this review, we examine the cytotoxic effector pathways implicated in cell death in organ specific autoimmune disorders.

Keywords: apoptosis; autoimmunity; diabetes mellitus; systemic lupus erythematosus; multiple sclerosis; thyroiditis

Abbreviations: LT, lymphotoxin; IDDM, insulin dependent diabetes mellitus; MS, multiple sclerosis; EAE, experimental allergic encephalomyelitis; OGD, oligodendrocytes; MBP, myelin basic protein; SLE, systemic lupus erythematosus; HT, Hashimoto's thyroiditis

Regulation of cell death in the immune system

The TNF receptor superfamily of proteins now comprises almost 20 members. Receptors in this family include the type 1 and 2 TNF receptors (TNFR-1 and TNFR-2, respectively), CD40, CD30 and the p75 NGFR.[1] These receptors are responsible for diverse biological responses such as inflammation, proliferation, anti-viral activity and cell death. These proteins exert their effects primarily within the immune system, but some members (e.g., the p75 NGFR and TNFRs) subserve important functions in the nervous system and other organs.

At least six receptors, TNFR, Fas (CD95/APO-1), DR3 (APO-3/TRAMP/wsl-1/LARD), DR4 and DR5 (TRAIL receptors) and CD30, are capable of transmitting a death signal to cells of the immune system. All but CD30 contain a homologous region of about 80 amino acids called the 'death domain' within the intracytoplasmic portion of the molecule (Figure 1). The death domain is critical for binding the adaptor molecules, FADD (MORT-1) and/or TRADD, that lead to activation of caspases and programmed cell death. The molecular pathways leading to caspase activation are discussed elsewhere.[2,3] Since Fas and TNF-R are the best studied members of this family, and the roles of other death receptors in the pathogenesis of autoimmune disorders are not known, this review will discuss Fas, TNF and lymphotoxin.

The two TNF-receptors, type 1 and type 2, are ubiquitously expressed whereas the ligands, TNF-α and -β (LT-α), are expressed predominantly by activated macrophages and T cells. On most cells, TNF-α has a pro-inflammatory action, most likely by promoting activation of NF-κB. Translocation of NF-κB to the nucleus leads to transcription of numerous cytokine and adhesion molecule gene products.[4] NF-κB activation is also associated with inhibition of apoptosis. TNF-α is expressed in the thymus, although TNF-α deficient mice have apparently normal thymic selection. The pro-apoptotic effect of TNF-α has been observed in certain tumor cell lines, in murine $CD8^+$ T cells[5] and human $CD4^+$ T cells.[6] TNF-α mediated apoptosis is thought to be induced by the type 1 receptor, although this point remains somewhat contentious. The lymphotoxins (LT) α and β are functionally closely related to TNF-α. LT-α is secreted as a homotrimer that can bind either TNF-R1 or -R2. LT-α may also be cell associated with the type 2 transmembrane protein, LT-β, as the heterotrimers, LT-α 1β2 or LT-α 2β1. LT-α 1β2 binds to the LT-β receptor whereas LT-α 2β1 binds to TNF-R1.

The structure of Fas and Fas ligand and the physiochemical basis of the Fas/FasL interaction has recently been reviewed.[7] Fas is expressed at high levels on double positive thymocytes, activated lymphocytes and on some non-lymphoid cells.[8] Fas ligand (FasL) expression is restricted to activated T cells of the CD4Th1 and CD8 subsets, NK cells and specialized cells at immune-privileged sites. The latter include Sertoli cells in the testis and the anterior chamber of the eye.[9] While a function for Fas in the thymus now appears to be emerging,[10] a key role for Fas in the elimination of activated cells in the peripheral immune system [activation induced cell death (AICD)], is now well established. By eliminating activated T cells through suicide and fratricide,[11] activated B cells and

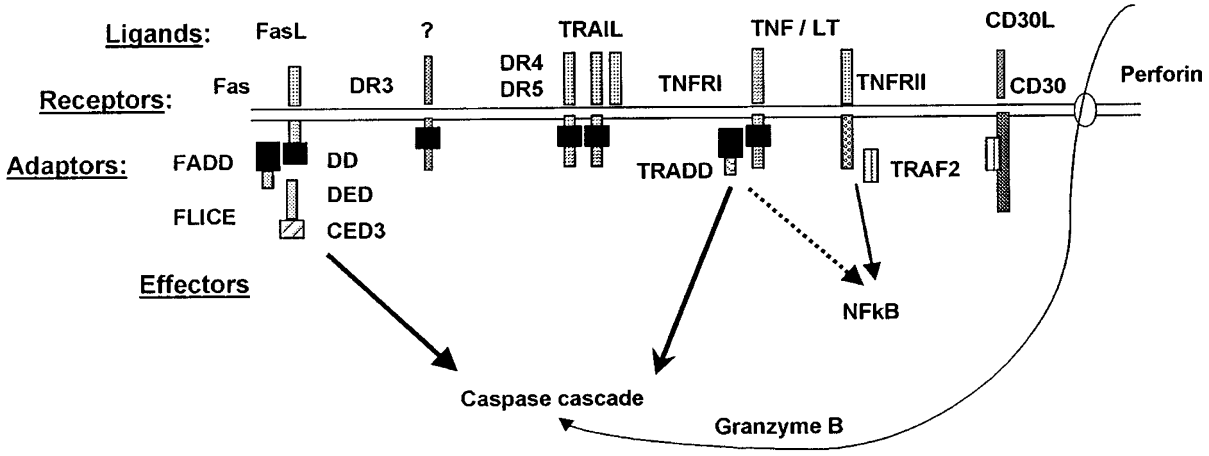

Figure 1 Cell death pathways: Inducers and early signal transduction pathways of the major known apoptosis pathways. Interaction of the ligand with the receptor causes receptor clustering and recruitment of the adaptor molecules. These, in turn, facilitate enzymatic activity of the caspases. Note that TNF-α may induce a death signal through TRADD or stimulate cell activation through the NF-κB pathway. Cell death pathways are modulated by the Bcl-2 family of proteins and by other downstream signal transduction pathways. Dissolution of the cell is orchestrated by caspases and nucleases. DD = death domain, DED = death effector domain, CED3 = *c. elegans* 3 homologous domain. Granzyme B also activates caspases but is inserted into the cell through pores created by perforin

macrophages by 'murder',[12] the peripheral immune system is purged of potentially autoreactive lymphocytes and antigen presenting cells that present self peptides in the context of high levels of co-stimulatory molecules.[13]

The perforin/granzyme pathway is generally considered the major effector pathway of $CD8^+$ T cells and NK cells in the antiviral immune response. However, the role of this pathway in eliminating viruses may be more limited than previously proposed.[14,15] Furthermore, whereas perforin may promote necrosis through osmotic lysis, granzyme B activates caspases resulting in programmed cell death.[16] (Figure 1). Cytotoxicity induced by this pathway may therefore demonstrate a mixed picture.

Cell death and autoimmunity

Autoimmune disease occurs when a sustained adaptive immune response is mounted against self antigens. The importance of apoptosis in this context relates to tolerance induction in the central lymphoid organs, regulation of apoptosis in the peripheral immune system and as a mechanism of cell death in the effector phase of the immune response. In the case of systemic autoimmune disease such as systemic lupus erythematosus (SLE), a humoral immune response to self antigens, predominantly intracellular nucleoproteins is observed.[17] This implies loss of tolerance at the B cell level, although autoantibody production is also T cell driven.[18] A defect in apoptosis leads to the lupus-like diseases in lpr (mutations in Fas) and gld (mutation in FasL) mice, as well as humans with Fas[19–21] and Fas ligand[22] mutations. Systemic autoimmunity in these situations can be explained by failed deletion of potentially autoreactive lymphocytes or antigen presenting cells in the peripheral immune system. Bcl-2 transgenic mice on certain strain backgrounds develop a lupus-like disease[23] and a lupus strain with a normal Fas genotype, NZB/W F1, also has impaired apoptosis of B cells[24] and lower expression of Fas on activated B cells.[25] Of interest, the immune response in a number of autoimmune diseases may be targeted to the products of apoptotic cells[26] and apoptotic cells can, in some experimental circumstances, elicit a transient autoimmune response.[27] Apoptosis in the context of systemic autoimmune diseases has been reviewed recently,[28] so that the current review will focus on apoptosis in organ specific autoimmune diseases.

In contrast to systemic autoimmune diseases that are characterized by B lymphocyte stimulation leading to antibody and immune complex mediated tissue injury, organ-specific autoimmune diseases are characterized by a cell mediated attack on specific cell types within the organ. Cell targets are β cells of the islets of Langerhans of the pancreas in insulin dependent diabetes mellitus (IDDM), oligodendrocytes in the brain in multiple sclerosis (MS) and thyrocytes in Hashimoto's thyroiditis (HT). Accompanying the infiltration of cells of the immune system, is significant cell death, usually demonstrated by DNA fragmentation (TUNEL staining) *in situ*. While TUNEL staining cannot be considered to be specific for programmed cell death, additional evidence suggests that apoptotic cell death is at least in part responsible for loss of cell function in these diseases. Apoptosis is usually considered non-inflammatory, but cell fate may be determined as much by energy resources within the cell as by the effector of death[29] and, as discussed below, several effector pathways may induce features of both apoptosis and necrosis.

Insulin dependent diabetes mellitus (IDDM)

IDDM is characterized by selective destruction of the insulin-producing β cells of the islets of Langerhans of the pancreas. Much of what is known about the pathogenesis of IDDM comes from studies of the nonobese diabetic (NOD) mouse[30] that shares many features with human IDDM. The NOD

mouse spontaneously develops IDDM and it has been suggested that disease progression is regulated at two checkpoints: (1) a peri-insulitis comprising T cells, B cells macrophages and dendritic cells is observed at 3–5 weeks of age and (2) the active destruction of the β cells at 4–6 months of age.[31] The major clinical manifestations of diabetes begin at the second checkpoint, when the destruction of β islet cells becomes widespread.

The immuno-pathogenesis of IDDM is complex and will only be briefly summarized here.[32,33] Several mechanisms have been suggested to be related to the initiation of IDDM at 4–6 weeks of age. The diabetogenic major histocompatibility complex (MHC) class II allelic products [I-A^{97} in mice and HLADQβ in humans[32]] are reported to bind peptides with low affinity and might enable self-reactive T cells with low thresholds for activation to be exported from the thymus to the periphery. Many of the self antigens have been identified (insulin, GAD65/67, HSP70), although none is sufficient to induce IDDM following immunization and only insulin is unique to the pancreas. Deficient activation of regulatory CD4$^+$ T cells of the Th2 type (produce IL-2 and IL-4) may facilitate continued immune attack against the target cell.[33] In the effector phase, most evidence indicates that both CD4 and CD8 T cells work in concert to destroy the pancreatic β cells. The CD4$^+$ T cells are Th1 (produce γ interferon and TNF-α) that are capable of killing directly through Fas[34] or may promote effector functions of CD8 and/or NK cells. In at least one transgenic model (NOD.*scid* expressing a diabetogenic CD4$^+$ T cell), apoptosis of the pancreatic β cells has been clearly demonstrated.[35] In this review, we will focus on mechanisms responsible for islet cell death.

Many models, many different results

Perforin[36] C57BL/6 mice that are transgenic for the lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) in the pancreas are tolerant to this antigen. When infected by the LCMV, there is a rapid T cell mediated immune destruction of the pancreas. Since perforin deficient LCMV-GP transgenic mice are protected from diabetes and antigen specific T cells from perforin deficient mice were incapable of adoptively transferring IDDM, these results suggested that perforin dependent cytotoxicity was necessary for β cell destruction (Figure 2A).

TNF-α Administration of TNF-α to NOD mice has opposite effects depending upon the age of the mice. In neonatal mice, low dose TNF-α accelerated the onset and increased the incidence of diabetes whereas TNF-α administered to adult NOD mice prevented IDDM.[37,38] Consistent with these results, NOD mice that expressed a transgene encoding TNF-α relatively late (7 weeks of age) in the pancreas, were protected from diabetes.[39]

TNF-α therefore plays apparently contradictory roles in IDDM at different stages of the disease and emphasizes the pleiotropic nature of this cytokine. While the precise mechanisms responsible for these findings have not yet been elucidated, speculations include the following.[40] In the neonatal period, TNF-α is likely to have an effect on thymic selection such that self-reactive thymocytes are not deleted

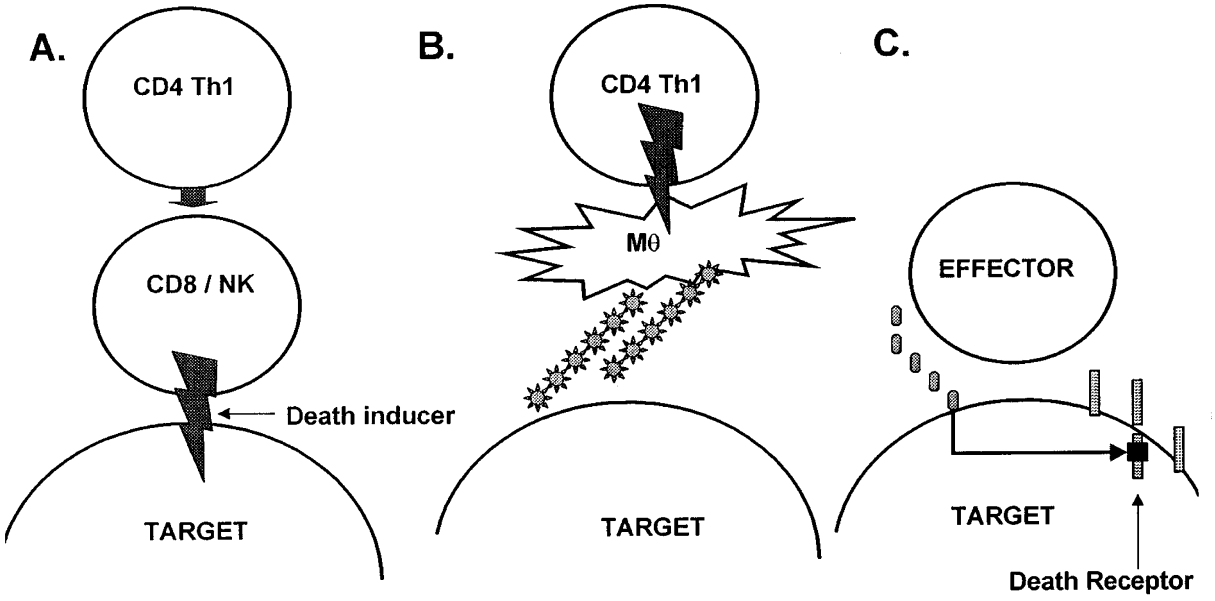

Figure 2 Cellular effectors of death. In IDDM and EAE, Th1 CD4$^+$ T cells are required for the induction of disease. Since CD4$^+$ cells are restricted by MHC class II, which is not usually expressed on the target cell, other cells or mechanisms of cell death are likely to be responsible for tissue destruction. Three non-exclusive models are presented: (**A**) CD4$^+$ cells arm CD8$^+$ T cells or NK cells, most likely through the release of γ-interferon, to kill the target. (**B**) CD4$^+$ T cells respond to a target peptide presented by MHC class II on an activated macrophage. The T cell kills the macrophage thereby releasing reactive oxygen intermediates and lytic enzymes. (**C**) Effector cells induce upregulation of a death receptor which engages the ligand expressed on the same cell

or it could promote increased apoptosis of 'regulatory' $CD4^+$ T cells. In older mice, increased TNF-α is, paradoxically, protective despite its ability to recruit inflammatory cells through upregulation of adhesion molecules and other cytokines. Since chronic exposure to TNF-α downmodulated calcium fluxes following T cell receptor ligation,[41] TNF-α may directly increase the threshold for activation of pathogenic T cells. Alternatively, TNF-α may exert its effect indirectly by influencing antigen presenting cell function. TNF-α promotes B cell activation and could lead to apoptosis of autoreactive cells by enhancing antigen presentation by B cells. TNF-α also promotes the maturation of dendritic cells but impairs antigen presentation.[42] Excess TNF-α may therefore reduce priming of potentially autoreactive T cells in the pancreas. Other possibilities such as reduced sensitivity to apoptosis of the target cells due to upregulation of NF-κB should also be considered. These different possibilities are testable and it will be important to determine whether TNF-α is acting at the level of the antigen presenting cell, T cell or target cell.

Fas NOD/*lpr/lpr* mice were protected from the development of both insulitis and diabetes.[43] Failure to develop diabetes most likely occurred at the level of the target organ since adoptive transfer of diabetic NOD splenocytes to NOD/*lpr/lpr* mice did not induce disease. These findings indicate that Fas/FasL plays a key effector role in the pathogenesis of IDDM in NOD mice.

Constitutive expression of FasL in the testis and eye contribute to immune privilege at those sites, presumably by inducing apoptosis of activated lymphocytes. Using this principle, allogeneic transplantation of the testis[44] or co-transplantation islets of Langerhans with myoblasts engineered to express FasL[45] promoted survival of the allograft in mice. Investigators have therefore attempted to 'arm' the pancreas with FasL[46] thereby protecting it from immune mediated injury. Unexpectedly, FasL transgenic mice (NOD-RIP-FasL) showed higher rates of spontaneous diabetes and acceleration of disease compared to non-transgenic NOD mice.[46,47] Furthermore, when islet-specific $CD8^+$ T cells were injected to irradiated NOD or NOD-RIP-FasL transgenic mice, FasL transgenic mice demonstrated severe and accelerated diabetes. It was suggested that β cells, which normally do not express Fas, were included to express Fas by the infiltrating inflammatory cells and that the engagement of FasL resulted in β cell apoptosis (Figure 2C). To test this hypothesis, $CD8^+$ islet-specific T cells were adoptively transferred to NOD/*lpr/lpr* mice. As in the experiments cited above, NOD/*lpr/lpr* mice were protected from diabetes supporting the hypothesis that Fas mediated destruction is important in this model. Since the source of FasL was not identified, these experiments do not distinguish between death mediated directly by FasL on $CD8^+$ T cells *versus* FasL on the non-transgenic islet β cell.

Is some cell death in NOD mice caused by intrinsic target cell defects?

In addition to IDDM, NOD mice develop infiltration of salivary glands resembling Sjogren's syndrome in humans. Intriguingly, NOD.*scid* mice (that lack mature T and B lymphocytes) also develop loss of submandibular acinar cells indicating that lymphocytes are not required to induce submandibular acinar cell injury.[48] The submandibular glands also demonstrated increased levels of caspases 1, 2 and 3 and, at 18 weeks of age, both Fas and FasL[49] consistent with the assisted suicide model also proposed for the pancreas (Figure 2C).[50] These findings suggest that defects within the glands may promote apoptosis followed, in immune competent animals, by an immunological attack. Further studies will be required to verify whether the glandular defect is truly intrinsic (NOD.*scid* mice contain cells of the innate immune system), whether the cellular defects extend to the pancreas, and to define more precisely the sequence of events in the apoptotic cascade.

In summary, experimental models illustrate that multiple effector pathways may be utilized by the immune system to destroy the insulin producing β cells of the pancreas. The different results obtained in these models are, in part, explained by the mouse strains used, different models (spontaneous *versus* induced), age at which the experimental variable is introduced, whether the effector molecule is applied at a 'physiological' dose. While manipulation of murine models of IDDM offer powerful ways to explore the pathogenesis of the disease, the manipulation may itself modify immune function or produce results that are not generalizable. The challenge that remains is how to translate these findings into elucidating the mechanisms of spontaneous diabetes in mice and humans. In this regard, the abrogation of IDDM in Fas deficient NOD mice argues strongly that this pathway is involved in tissue injury in spontaneous murine disease. How is Fas induced and why?

Multiple sclerosis (MS) and EAE

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system in which myelin and myelin-producing cells [oligodendrocytes (OGD)] become the target of an inflammatory response resulting in fibrosis (plaque formation). Only a small component of the infiltrating cells comprise autoreactive T cells. The self antigens and the molecular mechanisms responsible for tissue injury are incompletely understood.[51]

Since destruction of OGD results in the loss of myelin and neuronal function, most efforts have been directed toward identifying the mechanisms responsible for the death of these cells. *In vitro* studies have shown that OGD are susceptible to TNF-α and lymphotoxin cytotoxicity.[52] In addition, FasL and anti-Fas antibodies induce death of OGD, although the changes observed were not typical of apoptosis. Death was delayed in onset and the cells did not demonstrate nuclear fragmentation as tested by TUNEL staining.[53] These findings are intriguing, suggesting a novel pathway of Fas mediated cytotoxicity in OGD.

Studies of human MS are limited by the availability of tissue and the inherent problems of attempting to define mechanism by association. Nevertheless, several investigators have addressed the role of apoptosis in the pathogenesis of this disease. Immunohistochemical analy-

sis of normal or control brain sections indicate that low levels of Fas and TNFR-2 were constitutively expressed on OGD. Neither TNFR-1 nor TNF-α were detected whereas low level expression of FasL was observed on microglia (CNS macrophages).[54] In MS, markedly elevated Fas expression was detected on OGD in chronic active and chronic silent MS tissue whereas microglia and infiltrating lymphocytes stained strongly for Fas ligand.[53,55] These observations would be compatible with a straightforward role for either lymphocyte or microglial FasL mediated killing of OGD in MS (Figure 2A). However, the situation is more complex. In contrast to other reports, Bonetti and Raine[54] have failed to find evidence of apoptosis of OGD in tissue lesions freshly obtained from patients. These authors emphasize that OGD may actually proliferate at the site of the lesion. In view of the *in vitro* observations,[53] cell death effectors such as FasL are not excluded by these findings.

Rodent models of MS exist where the role of specific death molecules can be experimentally manipulated. Experimental allergic encephalomyelitis (EAE) is characterized by central nervous system (CNS) inflammation and demyelination. The pathology of EAE is similar to that of MS and consists of a perivascular inflammatory infiltrate composed primarily of T cells and macrophages in the spinal cord and brain. EAE can be induced in several rodent species either by active immunization with myelin components or by passive transfer of activated, myelin basic protein (MBP) specific, $CD4^+$ T cells of the Th1, but not the Th2, subset into naive mice.[56] Susceptibility to EAE induced by MBP is determined, in part, by the MHC so that SJL ($H\text{-}2^q$) and PLJ ($H\text{-}2^u$) mice are highly susceptible. Clinical responses to immunization with another antigen, myelin oligodendrocyte glycoprotein (MOG), is less strain dependent.

Early studies suggested a prominent role for TNF-α in the pathogenesis of EAE in that TNF-α is present in the lesions, neutralizing antibodies or soluble receptors suppress disease, MBP specific T cells produce TNF-α and lymphotoxin-α, and overexpression of TNF-α in the CNS leads to demyelination.[57] Despite these compelling data, different conclusions regarding the requirement for TNF-α and/or lymphotoxin-α in the pathogenesis of EAE have been obtained in knockout mice. Suen *et al*[58] found that deficiency of LT-α, but not LT-β, substantially abrogated MOG induced EAE in C57BL/6 mice. This effect could be localized to the T cell since LT-α deficient mice were susceptible to passive transfer of EAE by wild-type T cells. In contrast, Frei *et al*[57] observed that SJL mice deficient in both LT-α and TNF-α were fully susceptible to EAE induced by spinal cord homogenate or proteolipid antigen and TNF-α deficient mice were also fully susceptible to MOG-induced EAE.[59] While the differences in the outcome of these studies could be explained by different mouse strains, antigens, gene deletions or other factors, they emphasize the potential redundancy of any one cytokine as well as the need to assess the role of LT-α more closely in pathogenesis of MS. The protective role of TNF-α[59] is similar to observations in IDDM and the possible mechanisms responsible have been discussed above.

To investigate the role of Fas and FasL in EAE, EAE was induced by MBP or MOG in susceptible mouse strains carrying the lpr or gld mutations.[60,61] In both of these studies, lpr and gld mice showed less severity of disease compared to wild-type controls. These findings could not be explained by the failure to generate $CD4^+$ Th1 cells nor by the failure of entry of inflammatory cells into the CNS. However, apoptotic cell death was diminished in EAE lesions of *lpr* mice as determined by the TUNEL assay. These results indicate that Fas/FasL are important for the progression of clinical disease, most likely by the induction of apoptosis at the site of inflammation. Unfortunately the nature of the cells undergoing apoptosis were not identified in these studies. The authors concluded that Fas or FasL deficiencies offered protection from tissue injury due to diminished 'bystander killing' of oligodendrocytes.

Since Th1 $CD4^+$ T cells are necessary for induction of EAE and it is known that Th1, but only rarely Th2, T cells express FasL, the simplest hypothesis to explain the above findings is that FasL bearing Th1 T cells induce apoptosis of Fas^+ target cells. What are the target cells? CD4 T cells recognize antigen in the context of MHC class II, yet oligodendrocytes and other resident cells in the CNS rarely express class II. We have previously shown that $CD4^+$ Th1 cells kill macrophages that have been activated by γIFN and/or TNF-α in an antigen dependent, MHC restricted mode exclusively by the Fas pathway.[13] Since macrophages are required for expression of EAE[62] and macrophage apoptosis has been observed in tissue lesions,[63] we hypothesize that CD4 Th1 T cells kill MHC class II bearing macrophages that present MBP, MOG and proteolipid antigens i.e. in an antigen specific fashion (Figure 2B). This hypothesis could explain why γIFN and/or TNF-α are implicated in the pathogenesis of this syndrome since they prime the macrophage for Fas mediated apoptosis.[13] Release of proteolytic enzymes and other toxic products from macrophages (including TNF-α itself), would destroy the OGD.

Autoimmune thyroid disease

Grave's thyrotoxicosis is caused by stimulating autoantibodies to the TSH receptor whereas lymphoid infiltration and destruction of thyrocytes causes myxedema in Hashimoto's thyroiditis (HT). Reports that TSH has an anti-apoptotic effect by downmodulating Fas expression and that this effect is mimicked by IgG from patients with Graves' disease[64,65] is appealing. However, other investigators have failed to confirm changes in Fas expression following exposure of thyrocytes to TSH *in vitro*.[66] Further clarification is required especially with regard to signal transduction through cAMP and protein kinase A (the TSH signal transduction pathway) and the effect on Fas expression and function.

The ready availability of human tissue has allowed a more detailed investigation of apoptosis in thyroiditis.[67,68] Bcl-2 expression was decreased and Fas expression was detected in thyrocytes as well as within the germinal centers of intensely infiltrated areas. These results are consistent with apoptotic death as a mechanism for tissue

injury in Hashimoto's thyroiditis but did not suggest a clearcut mechanism. Giordano *et al*[69] reported that FasL was constitutively expressed on both non-autoimmune and HT thyrocytes. However, Fas was only expressed in thyroid glands obtained from active HT and from nontoxic goiters. As the expression of Fas in HT thyrocytes was thought to be a consequence of the intense inflammatory process, several cytokines were tested for their ability to induce Fas expression on normal thyrocytes. IL-1β was found to be the only cytokine able to induce Fas expression in normal thyrocytes and was abundantly expressed in the thyroid glands obtained from HT. Since FasL expressed on thyrocytes was functionally active, the authors suggested that IL-1β-induced Fas expression, may represent a critical limiting factor for the acceleration of thyrocyte destruction (Figure 2C). These observations remain controversial as another study reported constitutive expression of Fas on thyrocytes and that neither IL-1β nor IFN-γ promoted cell death.[66] While the addition of cycloheximide facilitated anti-Fas antibody mediated apoptosis, the addition of cycloheximide alone failed to do so, suggesting that Fas ligand was not constitutively active in these thyrocytes.

Summary and future perspectives

Review of three organ specific autoimmune diseases indicates that at least some target cell death is due to apoptosis and that effectors of apoptosis, such as TNF-α and FasL, are implicated in tissue destruction. While it is possible that the immune system eventually harnesses its entire armamentarium for tissue destruction, the protection afforded by abrogation of specific effectors in animal models of IDDM and EAE suggests rather that there is sequential employment of different effectors (which might explain why elimination of one effector prevents activation of others). The disease promoting $CD4^+$ Th1 T cells are restricted by MHC class II and therefore unlikely to exert a direct cytotoxic action on the Class I bearing target cell. CD4 cells may arm other effectors and act as an accomplice, induce tissue injury through a 'bystander pathway' involving macrophages or induce receptors for cell death on the target cell 'assisted suicide pathway' (Figure 2).

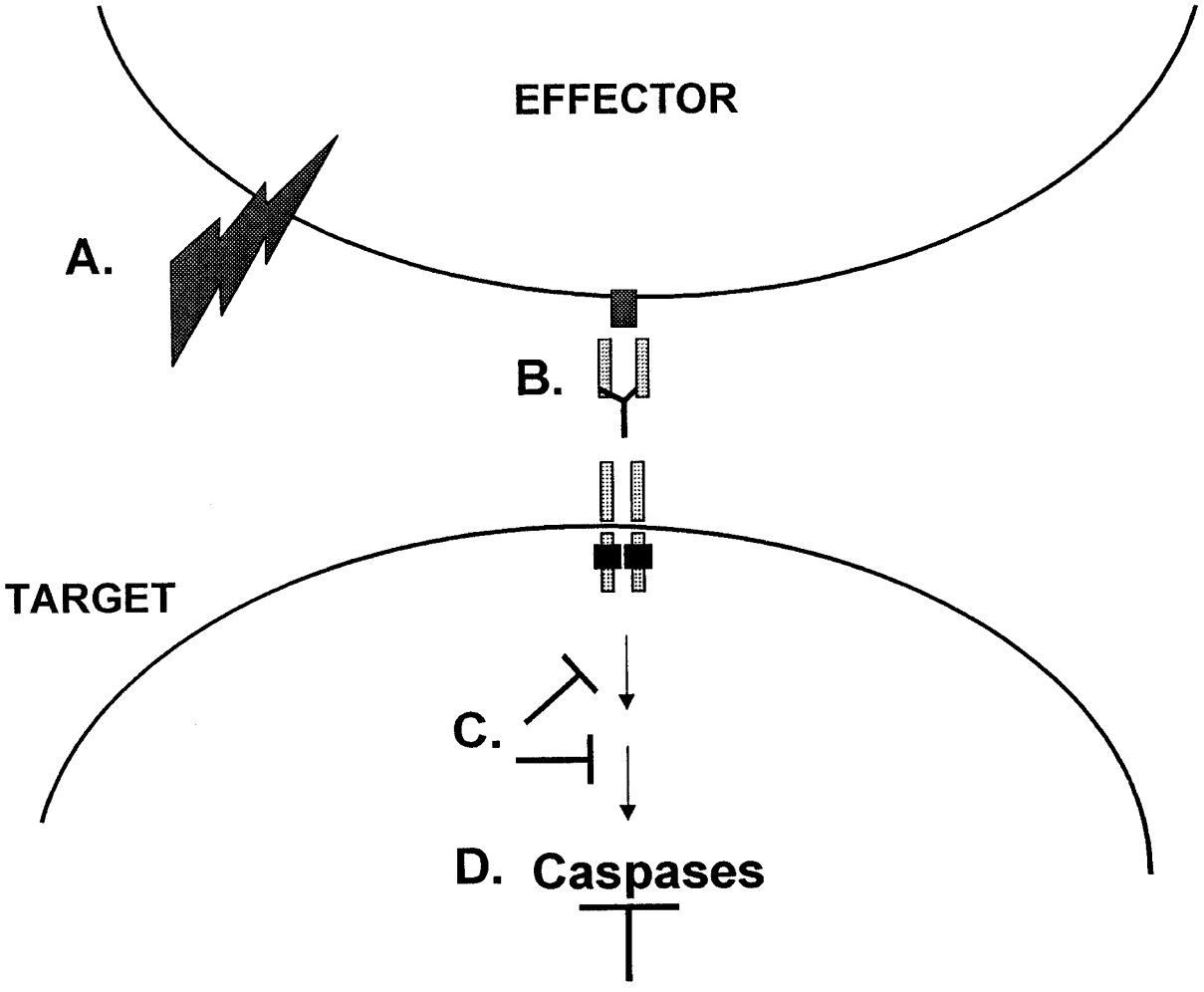

Figure 3 Therapeutic modulation of apoptosis in autoimmune disease. Death of the target cell could be prevented by: (**A**) killing the effector cell; (**B**) blocking a death ligand with a soluble receptor (**C**) Introducing an anti-apoptotic molecule into the target cell and (**D**) inhibiting caspase function

The protection afforded by spontaneous and engineered mutations of effector pathways offers hope that these diseases can be prevented or treated by blocking these pathways *in vivo*. As outlined in Figure 3, there are several levels at which therapy could be administered. If a death receptor was expressed selectively on the effector but not the target cell, a death ligand could be used to delete the effector (A). Soluble receptors or antibodies could be used to neutralize the ligand expressed or secreted by an effector cell (B). Such an approach has been used to block TNF-α in human rheumatoid arthritis[70] although it is likely that the beneficial effect is exerted by neutralizing the pro-inflammatory effect of this cytokine. Anti-apoptotic genes with limited (e.g. the protein FLIP blocks the Fas pathway) or broad (Bcl-2 family) specificity can be introduced into the cell (C). Finally, cell permeable caspase inhibitors can block the execution phase of apoptosis *in vivo* as illustrated experimentally.[71] While all of these approaches are feasible, they are limited by their potential adverse effects. Therapy would need to be relatively specific for the target cell since widespread prevention of cell death for sustained periods of time would lead to neoplasia.

While the emphasis of this review has been on effector pathways leading to cell death of the target cell in organ specific autoimmunity, consideration should also be given to the regulation of apoptosis in lymphocytes and antigen presenting cells in these diseases. Can the emergence of 'rogue' antigen-specific cells with a heightened resistance to apoptosis contribute to their pathogenesis (72–74)? As discussed, in IDDM and EAE there is evidence demonstrating alteration of more global aspects of immune function that influence survival of potentially autoreactive lymphocytes. In IDDM, the diabetogenic MHC may affect T cell selection in the thymus and TNF-α levels may influence survival and function of autoreactive T cells in the periphery. NOD mice develop a rheumatoid arthritis-like disease rather than IDDM when the T cell repertoire is altered.[75] The EAE susceptible strains of mice, SJL and PLJ, are susceptible to other immunological disorders (mercuric chloride induced nephritis, B cell lymphoma, collagen induced arthritis). All of these findings suggest that further understanding of the regulation of apoptosis will have widespread implications for pathogenesis and potential therapy of autoimmune disorders.

Acknowledgements

Supported by grants from the National Institutes of Health, USA and the SLE Foundation, NY. We thank Akshay Vaishnaw and Dalit Ashany for discussion.

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