Apoptosis 2000; **5**: 419–424

CD95/CD95L interactions and their role in autoimmunity

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CD95 (Fas/Apo-1) is a broadly expressed death receptor involved in a variety of physiological and pathological apoptotic processes. Since its discovery, defects in CD95/CD95L system have been proposed as major pathogenic factors responsible for impaired immunological tolerance to self antigens and autoimmunity. Later, analysis of altered sensitivity to CD95-induced apoptosis in cells targeted by the immune response has revealed an unexpected role for CD95 and CD95L in organ-specific autoimmunity. CD95 has been shown to be expressed and functional in virtually all cell types that are target of the organ-specific autoimmune response. Here we review some of the major findings concerning the role of CD95 in autoimmunity, in dysfunctions due to increased or decreased CD95-induced apoptosis.

Keywords: apoptosis; diabetes; Fas; organ-specific autoimmunity; thyroiditis.

Introduction

Apoptosis is a carefully regulated mechanism that plays a key role in normal tissue development and homeostasis. CD95/CD95L interactions are critically involved in this process. CD95 is a 45 kDa type I transmembrane protein with an extracellular cysteine-rich domain and an intracytoplasmatic death domain. It belongs to the tumor necrosis factor (TNF) receptor family and it is expressed at high levels on activated lymphocytes and in several tissues such as spleen, liver, lung, kidney and ovary.[1–3] CD95L is a 40 kDa type II transmembrane protein predominantly expressed on activated lymphocytes (CD8 and CD4 Th1 subsets), NK cells, erythroblasts, immune privileged tissues and certain tumors.[4–7] CD95/CD95L interaction induces recruitment of several signaling molecules, which initiate a cascade of biochemical events that lead to cell death. CD95L is homotrimeric and binds to three CD95 molecules, clustering the receptors and forming a death-inducing signaling complex that causes caspase-8 to enzymatically cleave and activate itself.[8] Active caspase-8 then cleaves and activates other caspases that initiate dismantling of cellular structures thus leading to apoptotic death.[9] CD95L is also released as a soluble form that can induce apoptosis in an autocrine or paracrine CD95 fashion and has been shown to be involved in activation induced death of T cells. While apoptosis plays an important role in physiological conditions, inappropriate activation of this process can lead to disease. Excessive apoptosis has been observed in degenerative diseases and in organs undergoing ischemia and riperfusion. On the other hand, cancer is often the result of decreased apoptosis.

Immune system homeostasis is tightly regulated by apoptosis to eliminate self-reactive lymphocytes and avoid autoimmune reactions. Thus, the first studies on apoptosis in the context of autoimmunity related to the elimination of potentially reactive lymphocytes.[10]

The murine MRL strain carrying the *lpr* (lymphoproliferative)[11] and *gld* (generalized lymphoproliferative disease)[12] mutations, which affect CD95 and CD95L respectively, are the prototype mouse models for lymphoproliferation-associated autoimmune disease. The autoimmune lymphoproliferative syndrome of *lpr* and *gld* homozygous mice is characterized by multiple autoantibodies, hypergammaglobulinemia and circulating immune complexes as well as arthritis and glomerulonephritis, a condition which closely resembles human systemic lupus erythematosus (SLE). In addition, the animals develop a progressive massive lymphoadenopathy due to the accumulation in secondary organs of functionally inert, non-proliferating $CD3^+$ $B220^+$ $CD4^-$ $CD8^-$ (double negative) T cells.[11]

These observations challenged several groups to search for similar alterations in human SLE. However, no consistent defects in expression or function of CD95 and CD95L have been found in SLE patients, probably because the human disease results from the interaction of multiple genetic traits with an abnormal apoptotic program.[13,14]

Recently, several spontaneous CD95 mutations involving its intracellular domain have been characterized in

This work was supported by a grant from AIRC to Ruggero De Maria.

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Table 1. Involvement of altered CD95-induced apoptosis in human autoimmune conditions

CD95 induced apoptosis	Autoimmune disease	Cell type involved
Excessive	Insulin-dependent diabetes mellitus	Beta cell
	Hashimoto's thyroiditis	Thyrocyte
	Multiple sclerosis	Oligodendrocyte
	Sjogren's syndrome	Salivary and lachrymal cells
	Ulcerative colitis	Colonocyte
	Diamond-blackfan anemia	Erythroblast
Defective	Canale-Smith syndrome	T and B lymphocytes
	(autoimmune lymphoproliferative syndrome)	
	Rheumatoid arthritis	Synoviocyte

humans.[15] These mutant proteins have a dominant -negative effect on CD95-mediated apoptosis of activated T cells and are associated with a chronic disease called Canale-Smith syndrome,[12] also known as autoimmune lymphoproliferative syndrome[16] or human lymphoproliferative syndrome with autoimmunity.[17] Patients present within the first 2 years of life with lymphadenopathy, hepatosplenomegaly, hemolytic anemia, thrombocytopenia and hypergammaglobulinemia.[18] They have an increased number of autoantibodies and circulating double negative T cells. This syndrome closely resembles the *lpr* and *gld* phenotypes. Both humans and animals have a defective CD95 function that implicates an accumulation of lymphocytes in lymph-nodes. In the Canale-Smith patients the lymph-node size is frequently reduced during bacterial infections, probably because the release of cytotoxic cytokines promotes lymphocyte apoptosis through alternative pathways.

Although lymphadenopathy, autoimmune thrombocytopenia and complications of blood transfusion continue into adolescence and adulthood, abnormal CD95 function is often compatible with long-term survival.[19]

The high sensitivity of activated lymphocytes to CD95-induced apoptosis suggested that CD95L might be an important effector in acute Graft-Versus-Host Disease (GVHD). In recent studies the parent-into-F1 model of GVHD was used to investigate the role of CD95 and CD95L in the severe reduction in host lymphocytes and the immunodeficiency characteristic of the acute form of GVHD. Acute GVHD mice exhibit significant upregulation of CD95 and CD95L. Moreover functional studies demonstrated that CD95/CD95L interactions contributed to the anti-host CTL activity in acute GVHD mice together with the perforin-dependent pathway. The observation that CD95/CD95L upregulation could be blocked by anti-IFN-γ Abs suggests that their interaction is critically dependent on Ag-specific $CD8^+$ T cells activation and IFN-γ production.[20,21]

In contrast to systemic autoimmunity, organ-specific autoimmune diseases are characterized by a cell-mediated attack against selected cell types that may result in tissue destruction. Cell targets are pancreatic β cells in insulin-dependent diabetes mellitus (IDDM), thyrocytes in Hashimoto's thyroiditis (HT), oligodendrocytes in multiple sclerosis (MS), synoviocytes in rheumatoid arthritis, salivary and lachrymal glands in Sjogren's syndrome, and crypt epithelial cells in ulcerative colitis.[22]

Both systemic and organ-specific autoimmune diseases may be the result of altered susceptibility to CD95-induced apoptosis (Table 1).

Insulin-dependent diabetes mellitus

IDDM is a chronic autoimmune disease characterized by selective T-cell-mediated destruction of insulin-producing pancreatic β cells.[23]

Most of the knowledge on the pathogenesis of IDDM comes from studies on the nonobese diabetic (NOD) mouse, which spontaneously develops an autoimmune disease that shares many features with human IDDM.[24] The pathogenesis of this multifactorial disease involves both environmental and genetic components. Among the genetic factors, Major Histocompatibility Complex (MHC) class II genotype (I-A and I-E in mice; DR and DQ in humans) is the strongest predisposing condition.[25] Several β-cell autoantigens (such as insulin, GAD65/67, HSP60) have been identified, but little is actually known about their role in the disease process since none of them is sufficient to induce IDDM after immunization and only insulin is specific to the pancreas.[23] Many evidences indicate that both CD4 and CD8 T-cell subsets are required for β-cell destruction in IDDM. Two different cytotoxic mechanisms seem to be involved in this process: (1) the effector cell release of perforin-containing granules on target cells and (2) the CD95/CD95L lytic pathway.[26,27] The former requires TCR-MHC interaction, while CD95L can kill bystander cells in the absence of cell contact. Perforin-deficient NOD mice displayed reduced incidence and delayed onset of diabetes,

suggesting that autoreactive T-cell clones may kill pancreatic β cells in a MHC-dependent CD95 fashion.[28] However, β-cell destruction can occur in the absence of cell contact between effector and target cells, a condition required for MHC-based perforin-mediated cytotoxicity. Indeed, $CD4^+$ cells can transfer diabetes in the absence of $CD8^+$ cells by targeting MHC class II negative β cells, which can be killed by a death pathway that is clearly independent of MHC-TCR interaction.[29] In line with these results, $CD95L^+$ $CD4^+$ cells have been shown to kill $CD95^+$ β cells in human pancreata isolated from newly diagnosed IDDM patients. During the insulitis process, activated islet-infiltrating macrophages produce nitric oxide and secrete IL-1β that in turn induces nitric oxide production in β cells. This results in selective nitric oxide-mediated upregulation of functional CD95 molecules on β cells, which are subsequently killed by CD95L-producing T cells.[30]

Hashimoto's thyroiditis

HT is a chronic autoimmune disease characterized by a progressive destruction of thyroid epithelial cells and reduced production of thyroid hormones. Thyroid gland presents a marked T and B lymphocyte infiltration, while a diffuse fibrosis tends to replace the parenchyma.[31,32] In normal thyroid there is an extremely low level of apoptosis that counteracts the slow physiological thyrocyte proliferation, contributing to tissue homeostasis.[33] By contrast, in glands from HT patients apoptotic cell death is strongly accelerated, leading to thyrocyte depletion and hypothyroidism.[34] Normal thyrocytes constitutively express functional CD95L, which is further upregulated during autoimmune thyroiditis.[35] However, such expression does not result in thyroid immune privilege and immune evasion. CD95 is weakly or not expressed in normal thyrocytes, but it is strongly upregulated in thyrocytes from HT glands, possibly as a consequence of the intense inflammatory process.[36,37] In vitro studies have demonstrated that IL-1β and IFN-γ induce CD95 expression in normal thyrocytes. During the autoimmune inflammation, these cytokines promote massive CD95 upregulation in thyrocytes. Simultaneous expression of CD95 and CD95L in thyroid cells has been shown to result in apoptotic cell death by autocrine or paracrine mechanisms.[35] Differently from IDDM, in this unconventional autoimmune disease infiltrating T cells are killed by $CD95L^+$ thyrocytes and do not seem to play an executive role.Grave's disease is another autoimmune thyroid disorder caused by stimulating autoantibodies that activate thyroid hyperplasia. Little or no apoptosis has been detected in Grave's disease glands and this may contribute to thyroid hyperplasia.[38,39] These studies have suggested the possibility that thyroid glands from Grave's disease patients may produce high levels of antiapoptotic factors.[40]

Multiple sclerosis

MS is a progressive demyelinating disease of the central nervous system (CNS) characterized by the loss of neuronal functions. It is considered a T cell-mediated autoimmune disease in which myelin and oligodendrocytes become the target of an inflammatory injury, resulting in the formation of typical lesion, called plaques. These are multifocal areas of inflammation and demyelination comprising macrophages, lymphocytes and plasmacells.[41,42] Like IDDM, MS is associated with genes of the human MHC. $CD4^+$ T cells specific for myelin antigens are believed to initiate and perpetuate the autoimmune process. Nevertheless, oligodendrocytes do not express MHC class II molecules and can not be killed by $CD4^+$ T cells through an antigen dependent mechanism.[43,44]

Due to the difficult access of human CNS, many advances on the pathogenesis of MS derive from studies on the murine experimental allergic encephalomyelitis (EAE) model. EAE is induced in several rodent species by immunization with myelin antigens or by passive transfer of myelin-reactive $CD4^+$ T cells.[41,45,46] The observation that *lpr* or *gld* mutations protect mice from tissue damage in EAE has clearly evidenced the involvement of CD95/CD95L interactions in the disease process.[47–49] Immunohistochemical analysis of normal brain sections indicate that low levels of CD95 are constitutively expressed on oligodendrocytes whereas high levels were detected in oligodendrocytes from active and silent MS lesions. Moreover, the same analysis revealed a high CD95L expression on infiltrating lymphocytes during active and chronic silent MS.[43,50]

Different hypotheses stem from these data. Since macrophages are required for induction of EAE and their death has been observed in tissue lesions, they are believed to play an important role in the pathogenesis of MS.[51,52] During the inflammation phase the production of cytokines, such as IFN-γ or TNF-α, activates CNS macrophages that are killed by T cells in an antigen-dependent MHC-restricted manner via the CD95 pathway.[53] In this model, oligodendrocyte destruction might be mediated by macrophages through the release of cytokines, proteolytic enzymes and other toxic products. Alternatively, oligodendrocytes may be directly killed through the CD95 pathway. This has been suggested because MS oligodendrocytes express CD95, while microglial cells and infiltrating $CD4^+$ T cells express CD95L. Moreover, oligodendrocytes are extremely sensitive to CD95-induced cell death. Thus, although direct demonstration is still missing, it is likely that the interaction of CD95

and CD95L plays a major role in the pathogenesis of MS.

Other CD95-mediated organ specific autoimmune diseases?

An opposite mechanism, involving defective CD95-induced apoptosis has been implicated in uncontrolled synovial cell proliferation leading to rheumatoid arthritis. An inadequate apoptosis in this autoimmune disease may promote extended survival of synoviocytes and explain the hyperplastic nature of the pannus and the consequent destruction of cartilage and bone.[54,55] Several studies suggest that the production of cytokines, such as TGF-β, IL-1β and TNF-α, during the joint inflammatory process may stimulate synoviocyte proliferation and reduce their sensitivity to CD95-mediated apoptosis.[56,57]

High levels of soluble CD95 have been detected in synovial fluid during acute rheumatoid arthritis, contributing to inhibition of synoviocyte and inflammatory cell apoptosis.[58] Despite the simultaneous expression of CD95 and CD95L, low levels of apoptosis have been also described in infiltrating T lymphocytes, which are likely to maintain and amplify inflammation in joint lesions.[54,59,60]

Sjogren's syndrome is a lymphoproliferative disease characterized by a destructive mononuclear cell infiltration in salivary and lachrymal glands. In the first phase of disease gland lesions are limited to periductal areas. Later the lymphocytic infiltrate enlarges and destroys acinar epithelium decreasing glandular secretion. The observation that MRL-*lpr*/*lpr* mice develop a salivary gland lymphoid infiltrate similar to human Sjogren's syndrome suggested that defects in CD95/CD95L mediated apoptosis might be involved in the pathogenesis of this disorder.[61,62] Infiltrating T cells in focal lesions of salivary glands from Sjogren's syndrome patients express CD95 and very low levels of CD95L, but they are blocked in their ability to commit to apoptosis. In contrast to ITL, acinar cells are CD95 and CD95L positive and may undergo CD95-mediated apoptosis.[63] Again, murine experimental models increased the understanding of pathogenetic mechanisms. In addition to autoimmune diabetes, NOD mice develop loss of salivary glands resembling human Sjogren's syndrome. Surprisingly, immunodeficient NOD/*scid* mice maintain loss of submandibular acinar cells, suggesting that lymphocytes are not necessary to cause salivary gland damage. Similarly to thyrocytes in HT, in both human and experimental Sjogren's syndrome, submandibular gland cells show high levels of CD95 and CD95L, suggesting a mechanism of suicide or fraticide.

Ulcerative colitis is a chronic inflammatory gut disease in which exogenous and endogenous factors lead to mucosal alterations resulting in the loss of colonic epithelium.[64] In normal colonic crypts, columnar epithelial cells proliferate, differentiate, migrate in the upper portion and are removed. It has been hypothesized that before being removed these cells undergo CD95-mediated apoptosis, which contributes to the physiological regulation of the colonic epithelium homeostasis.[65–67] Human colonic epithelial cells constitutively express high levels of CD95 throughout the crypts, while the expression of CD95L is still controversial. The observation that *lpr* or *gld* mice do not develop UC suggests that the interaction between CD95 and CD95L may have a role in the pathogenesis of the disease. The level of CD95 expression on epithelial cells in ulcerative colitis is comparable to that of normal enterocytes or slightly increased. The presence of apoptotic bodies in the crypt epithelium of ulcerative colitis patients suggests that the gradual loss of the crypt is due to apoptosis.[68] It has been proposed that under inflammatory conditions colonocytes acquire an increased sensitivity to CD95 mediated apoptosis and are killed by CD95L-expressing lamina propria lymphocytes.[69,70]

Conclusions

Excessive or defective susceptibilities to CD95-induced apoptosis have been proposed as major pathogenetic mechanisms in a variety of autoimmune diseases. While rare inactivating mutations of CD95 or CD95L are clearly responsible for several human and mouse lymphoproliferative diseases, conclusive data on target destruction in organ-specific autoimmunity are still missing. Although it is likely that CD95-induced apoptosis contributes to target cell destruction in organ-specific autoimmunity, further studies are required to find out whether multiple cytotoxic mechanisms may contribute to tissue destruction in a single individual or whether a single autoimmune disease may be heterogeneous and result from different pathogenetic mechanisms in different individuals.

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