



Review

Involvement of Fas/FasL System in the Pathogenesis of Autoimmune Diseases and Wilson's Disease

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Abstract. The interaction of Fas with FasL has been demonstrated to be implicated in the pathogenesis of several autoimmune and liver diseases. Recently, attention has been focused on the hypothesis that thyrocytes and β cells undergo massive Fas/FasL-mediated apoptosis during autoimmune response. Similarly, hepatocyte cell death occurring following copper accumulation points towards the same mechanism.

Key words: autoimmune diseases; apoptosis; Fas; Hashimoto's thyroiditis; Wilson's disease; insulin-dependent diabetes mellitus.

In recent studies the involvement of Fas-mediated apoptosis has been proposed as a major pathogenetic mechanism underlying several degenerative and autoimmune diseases¹⁴, as insulin-dependent diabetes mellitus (IDDM)³⁷, Hoshimoto's thyroiditis (HT)¹⁷, Wilson's disease (WD)⁴⁰ and multiple sclerosis (MS)¹¹.

Fas (CD95/APO-1) is a 335 amino acid cell surface protein with a molecular weight of 43–44 kDa, belonging to the newly characterized family of death receptors (DRs), which includes the tumor necrosis factor receptor-1 (TNFR-1), DR3, DR4, and DR5^{6, 31, 32, 42}. Fas is a widely expressed cytokine receptor characterized by an extracellular domain composed of three cysteine-rich repeats, a transmembrane domain and an intracellular region, containing the so called "death domain", responsible for engaging the apoptotic pathway upon binding with its ligand (FasL)³¹. FasL is a trimeric type II membrane protein member of the TNF family, which also includes APO-2L/TRAIL, the natural ligand for

DR4 and DR5³⁵. Fas triggering results in a cascade of biochemical events involving the recruitment of the adaptor FADD which links Fas to downstream caspases through FLICE/MACH-1^{4, 28}. Early in this pathway, a caspase-dependent acidic sphingomyelinase activation induces ceramide generation, which is converted to GD3 ganglioside, a killer glycolipid, by the action of GD3 synthase¹⁰. As physiological functions, Fas promotes the deletion of potentially harmful, damaged or unnecessary cells during the immune response^{24, 29}. Moreover, Fas-FasL interaction appears to be critical in tissue remodelling, normal cell turnover, embryonic development, tissue atrophy, and metamorphosis²⁴. Fas is normally expressed in a variety of different tissues such as liver, spleen, lung, kidney, lymph nodes and ovary. However, inappropriate expression or excessive Fas activity, or functional alterations of the Fas/FasL system contribute to the pathogenesis of several human diseases^{11, 14, 24, 27}, including organ-specific autoimmune

diseases such as HT¹⁷ and IDDM³⁷, and an autosomal recessive disorder called WD^{1, 40}.

Hashimoto's Thyroiditis

HT is an autoimmune disease characterized by a destructive process which overcomes the capacity of thyroid replacement^{7, 13}.

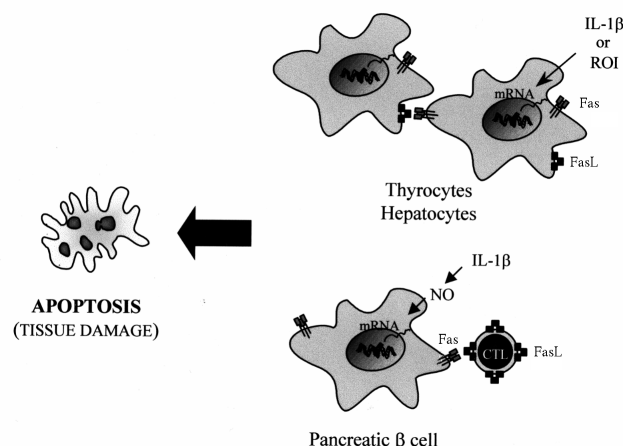


Fig. 1. Proposed mechanisms for Fas-mediated tissue damaging in Hashimoto's thyroiditis (HT), Wilson's disease (WD) and insulin-dependent diabetes mellitus (IDDM). On the top of the diagram it is shown the apoptotic mechanism underlying HT and WD. Local IL-1 β or ROI production induces Fas expression and primes thyrocytes and hepatocytes for FasL-mediated destruction. On the bottom of the diagram it is shown the apoptotic mechanism underlying IDDM. Following an initial β cell damage resulting in inflammation, IL-1 β -induced Fas expression primes β cells for destruction by FasL-producing CTLs. Abbreviations: CTL – cytotoxic T lymphocyte, FasL-Fas ligand, IL-1 β – interleukin 1 β , NO – nitric oxide, ROI – reactive oxygen intermediate

HT has been classified in a number of ways, based on clinical, serological, and histological findings, but the relationship among these variants remains unclear⁹. Chronic autoimmune thyroiditis has two clinical forms: a goitrous form often referred to as Hashimoto's disease, and an atrophic form called atrophic thyroiditis. Both forms are characterized by the presence of thyroid autoantibodies and by varying degrees of thyroid dysfunction; they seem to differ only for the absence or presence of goiter. Progressive disease appears to be signaled by a high titer of thyroid antibodies and elevated serum thyrotropin concentrations, especially in association with thyroid atrophy.

The pathogenesis of HT is a complex interaction of several mechanisms, in which the thyrocyte may participate more actively than previously thought⁴⁴. The pathogenetic mechanism is believed to involve the activation of T lymphocytes specific for thyroid antigens

and the subsequent generation of auto-antibody response, but the mechanisms responsible for initiating thyroid autoimmunity and promoting the progression to the disease remain a matter of debate. The direct killing of thyrocytes by cytotoxic T cells has been classically regarded as the major pathogenic mechanism in HT. This concept has been raised following the observation that a large number of T cells contribute to the lymphocytic infiltration observed in HT. Moreover, several studies on animal models have shown that T cells are required for the development of both spontaneous and immunization-induced experimental autoimmune thyroiditis. However, although T cells are likely to play a major role in the initiation and amplification of the autoimmune response against thyroid cells, there is no evidence for a direct involvement of cytotoxic effector T cells in autoimmune thyrocyte destruction. Indeed, our recent studies support the possibility that the destructive process is due to thyrocyte suicide through Fas-mediated apoptosis.

Apoptosis has been occasionally revealed in histological section of normal thyroid¹². However, several reports have shown that apoptotic cell death is abnormally induced during the pathologic phases leading to clinical hypothyroidism²³. IL-1 β , an inflammatory cytokine found in HT glands, induces Fas expression and apoptosis of normal thyroid cells, in a Fas-dependent manner. Both normal thyroid cells and cells from patients with HT express FasL, thus providing the basis for selective elimination of HT thyrocytes, which following IL-1 β exposure coexpress Fas and FasL and undergo apoptosis. Hence, IL-1 β -induced Fas expression may be considered as a critical limiting factor in the acceleration of thyrocyte destruction during the course of the inflammatory process¹⁷. Accordingly, we recently found that a considerable number of infiltrating T lymphocytes (ITL) in HT, particularly those approaching the thyroid follicles, undergo apoptosis through the interaction with FasL-producing thyrocytes. In fact, virtually all lymphocytes located in proximity to thyrocytes are pre-apoptotic and accumulate GD3, a killer ganglioside required for induction of apoptosis following Fas crosslinking³⁸. It is therefore unlikely that ITL are directly involved in the autoimmune destruction, suggesting a prevalent role for auto-crine/paracrine Fas-FasL interaction among thyrocytes in the execution phase leading to thyrocyte depletion.

Insulin-Dependent Diabetes Mellitus

IDDM is a T cell mediated autoimmune disease resulting from a selective destruction of pancreatic

β cells³. Several β cell antigens recognized by autoreactive T cells has been suggested as candidate targets in the disease process: insulin, glutamic acid decarboxylase (GAD) 65/67, 38 kDa autoantigen, HDP60, ICA69, and IA-2. Recently, several reports in rodent models and in humans have shown that β cell damage may be prevented or delayed by immunization with some of these antigens, including GAD and insulin²⁰.

A number of data support the autoimmune pathogenesis of IDDM: 1) the abundant accumulation of macrophages, dendritic cells, B and T lymphocytes at the periphery of islets; 2) the important role of several cytokines in the disease process, including IFN- γ , IL-4, IL-10 and IL-1 β ; 3) the massive infiltration of both CD8 and CD4 positive T cells into the islets.

IL-1 β is considered one of the principal mediators of the β cell inflammatory lesion, since it has been reported to be abundantly present during the insulinitis process and selectively toxic for pancreatic β cells. IL-1 β , secreted by the activated mononuclear and dendritic cells that enter the periductal areas of islets, inhibits insulin synthesis and stimulates nitric oxide (NO) production in pancreatic β cells^{21, 34}. Nitric oxide is an important messenger molecule that plays a critical role in a variety of physiological functions, including immunomodulation and cytotoxicity. During the insulinitis process, inducible NO synthase (NOS) is highly expressed in β cells and islet-infiltrating macrophages³³. Since high doses of both IL-1 β and NO are toxic for islet-cells, it has been hypothesized that the specificity of the autoimmune process leading to IDDM might depend on the preferential vulnerability of β cells to the cytotoxic action of IL-1 β and NO. However, it seems unlikely that this direct cytotoxic effect may be responsible for the massive and selective β cell destruction occurring in the disease process²².

T cell cytotoxicity results from the exocytosis of perforin-containing granules on cognate target cells, and the engagement of Fas on cognate or neighboring target cells by membrane-bound FasL^{19, 26}.

Normal pancreatic β cells do not constitutively express Fas²⁵. However, Fas is rapidly upregulated in β cells upon exposure to physiological amounts of IL-1 β , suggesting Fas-mediated apoptosis as a possible new mechanism involved in β cell destruction³⁹. Importantly, β cells from *pancreata* of newly diagnosed IDDM patients express Fas and show extensive apoptosis among those cells located in proximity to FasL-expressing T lymphocytes infiltrating the IDDM islets. Moreover, *in vitro* studies have shown that L-NMMA, an inhibitor of NOS, prevented IL-1 β -induced Fas expression, while NO donors like sodium nitroprusside

and NOC-18 induced functional Fas expression in normal pancreatic β cell³⁷. Altogether, these results suggested that NO primes pancreatic β cells for Fas-mediated destruction.

The non-obese diabetic (NOD) mouse is the most reliable experimental model for IDDM. In these mice β cells are destroyed by autoreactive T cells probably as a result of existing autoimmunity¹⁶ or inability to regulate autoaggressive T cell responses. Direct demonstration for the role of FasL-based cytotoxicity in the pathogenesis of autoimmune diabetes was obtained in recent studies on fas-negative NOD^{1pr/1pr} mice, which show protection from both spontaneous and T cell-transferred diabetes⁵.

Wilson's Disease

WD is an autosomal recessive disorder, characterized by a defective gene that codes for a copper transporting P-type ATP-ase. This mutation leads to accumulation of copper in a number of organs and particularly in the liver and brain^{8, 36, 41}. The phenotype of this disease is an acute form of fulminant hepatic failure (FHF) and hemolytic anemia.

Livers are regularly cirrhotic at the time of presentation, indicating a previously chronic period of liver disease with acute deterioration. Strand and co-workers hypothesized that apoptosis could be a mechanism of hepatocyte cell death during the course of FHF and provided *in vitro* experimental evidence for copper as a possible apoptosis inducer in hepatocytes⁴⁰.

Initial studies hypothesized a role for copper in initiating free radical generation^{2, 18} and subsequent oxidative change in hepatocyte organelle lipids or thiol proteins⁴³. Later, intracellular accumulation of copper(II)-ion has been shown to dramatically increase the production of ROI, resulting in DNA damage and subsequent wild-type p53 accumulation and transcriptional transactivation, which are associated with Fas upregulation.

Injection of agonistic anti-Fas antibodies in mice results in FHF within hours³⁰. Since in alcoholic cirrhosis, chronic viral hepatitis B and C, and in FHF of different etiology, it has been described an upregulation of the Fas system¹⁵, it is likely that the interaction of Fas with FasL may play an important role in a number of disease resulting in liver damage.

STRAND et al.⁴⁰ found a strong increase of Fas expression in distinct areas of livers from patients with WD and very high *de novo* expression of FasL mRNA in WD livers. Interestingly, copper(II)-ion was able to upregulate FasL expression in hepatoma cells *in vitro*,

suggesting that copper ion may trigger Fas/FasL-mediated hepatocyte suicide in an autocrine or paracrine fashion. Accordingly, the same authors observed reduced rates of copper-induced hepatocyte apoptosis after treatment with a blocking antibody directed against FasL, supporting again the active role played by Fas and FasL in the hepatocyte destruction occurring in WD⁴⁰.

Further studies concerning the mechanisms responsible for tissue destruction in other autoimmune or degenerative diseases are likely to dramatically increase the number of disorders known to be due to hyperfunction of the Fas/FasL system. In this view, targeting Fas-FasL function or expression in specific sites may provide a novel therapeutic strategy to prevent or decrease the susceptibility to pathological Fas-mediated destructive processes.

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