Symposium on Apoptosis and Medical Diseases*

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1. Fas-induced Apoptosis

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Introduction

Homeostasis in vertebrates is tightly regulated by cell death as well as by cell proliferation. The death of cells during embryogenesis, metamorphosis, and normal tissue turnover is “programmed cell death”, mediated by a process called “apoptosis”. Cytotoxic T cells and natural killer (NK) cells kill the target cells by inducing apoptosis. Apoptosis can be distinguished from necrosis, which occurs as a result of injury, complement attack, severe hypoxia and hyperthermia. Morphological and biochemical analysis of apoptotic cell death indicated that apoptosis is accompanied by condensation of cytoplasm, loss of plasma membrane microvilli, segmentation of nucleus, and extensive degradation of chromosomal DNA into oligomers of 180 bp. Cellular proliferation and differentiation are mediated by cytokines. Our studies on Fas and Fas ligand have indicated that apoptosis is also mediated by a cytokine and its receptor in some cases. Here, I summarize the current status of the Fas death factor system.

Fas and Fas ligand, and Fas-mediated apoptosis

Fas is a type I membrane protein belonging to the tumor necrosis factor (TNF)/receptor family (1). The Fas ligand (FasL) is a member of the TNF family, and synthesized as a type II membrane protein (2). Similar to TNF, matrix metalloprotease cleaves the membrane-bound FasL to make its soluble form, which is a trimer (3).

Binding of FasL seems to cause trimerization of Fas, and rapidly induces apoptosis (4, 5). A domain of about 80 amino acids in the Fas cytoplasmic region (a death domain) is responsible for the death signal. Fas-mediated apoptosis occurs in enucleated cells, and is independent from RNA or protein synthesis. Similar to the apoptosis in C. elegans, the Fas-mediated apoptosis is executed by members of the ICE (interleukin-1β-converting enzyme) family (6) and can be inhibited by an oncogene product Bcl-2.

The members of the ICE superfamily were recently designated caspase (cysteine aspase), and are divided into three subgroups, caspase 1-like, caspase 3-like and caspase 2-like proteases. The Fas engagement sequentially activates caspase family proteases to proceed apoptosis (7) (Fig. 1). In fact, the recent identification of signaling molecules in the Fas-mediated apoptosis indicated that a caspase family protease is recruited to the Fas cytoplasmic region through an adaptor (5).

Physiological and pathological roles of the Fas system

Genetic and molecular analyses of the Fas and FasL genes

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have revealed that mouse mutations of lymphoproliferation (lpr) and generalized lymphoproliferative disease (gld) are loss-of-function mutations in the Fas and FasL genes, respectively (8, 9). Mice carrying the lpr or gld mutation develop

Figure 1. Models for apoptosis signaling by FasL. Binding of FasL to Fas induces trimerization of the Fas receptor, which recruits caspase-8 via an adaptor, FADD/MORT1. The oligomerization of caspase 8 may result in self-activation of proteolytic activity, and trigger the caspase cascade. The activated caspase members can cleave various substrates such as poly(ADP-ribose) polymerase (PARP), lamin, rho-GDI, and actin, and cause morphological changes of the cells and nuclei.

Figure 2. Three types of killing by the Fas and FasL system. A) Activation-induced suicide of T cells. Mature T lymphocytes are activated by T-cell receptor interaction with antigen-presenting cells. The activated T cells express FasL, which binds to the Fas-expressing activated T cells to induce apoptosis. B) CTL-mediated killing of target cells. Virally infected cells present viral antigen as a complex with MHC. The cytotoxic T cells recognize the antigen and become activated, leading to the expression of FasL. FasL then binds to Fas on the target cells to induce apoptosis. C) Killing of inflammatory cells in immune privilege sites and killing of CTL by tumor cells. Stromal cells in the immune privilege sites such as the eye and testis, and some tumor cells constitutively express FasL. When activated T cells or neutrophils enter an immune privileged site, FasL binds to Fas on these cells and kills them to prevent inflammation. Similarly, when CTL or NK cells approach tumor cells, the tumor cells counterattack these cells to escape from the immune destruction.
lymphadenopathy and splenomegaly by accumulating CD4⁻/CD8⁻ cells of the T cell origin, and develop autoimmune disease in some strains of mice. Human patients carrying a defect in the Fas gene have been also identified. Immunological and biochemical analysis of these mutant mice indicated that the Fas system is involved in peripheral clonal deletion, and the activation-induced suicide of T cells to down-regulate the immune reaction (Fig. 2A) (4).

FasL is expressed in activated T cells and NK cells (2, 3), and works as an effector of cytotoxic T cells and NK cells to remove the cells infected by virus, or cancerous cells (Fig. 2B) (4, 10). When agonistic anti-Fas antibody or the recombinant FasL was administered into mice, mice were killed by acute liver failure (11), suggesting an involvement of the Fas-induced apoptosis in CTL-mediated tissue distraction. In fact, we and others could show that CTL-mediated autoimmune diseases such as hepatitis, thyroiditis and insulitis are caused by overfunction of the Fas-mediated apoptosis (12–14). In addition, stroma cells of the eye and testis constitutively express FasL (15, 16), which is believed to kill the inflammatory cells infiltrating into these tissues (Fig. 2C). This mechanism may explain the immune privilege nature of these tissues. Furthermore, some non-lymphoid carcinoma cells were found to express FasL constitutively, which may explain the immune evasion of tumor cells (17).

Perspectives

Many growth and differentiation factors regulate proliferation and differentiation of mammalian cells during its development. So far, three death factors (TNF, FasL and TRAIL) have been identified. Loss-of-function mutations of the Fas system in lpr and gld mice pointed out the importance of this death factor system in mammalian homeostasis, specifically in the life and death of lymphocytes. It is possible that many more death factor and receptor systems that regulate apoptosis in a tissue-specific manner, will be found in future. Signals of growth and differentiation are mediated by phosphorylation and dephosphorylation of proteins, and by small second messenger molecules such as cAMP and phosphatidyl inositol. These signals are reversible in most cases. On the other hand, the apoptosis signal triggered by death factors is irreversible, that is, a protease cascade is activated by the death signal, and they cleave various cellular components, which lead to morphological changes of the cells and nuclei that is typical for apoptosis.

As described above, the Fas death factor system is a double-edged sword. If this system is properly regulated, it is useful to down-regulate the immune reaction, and to remove the virally infected cells as well as cancerous cells. But, if this system is exaggerated, it is deleterious to cause tissue destruction. It is now possible to consider various applications of this system to human diseases. The first obvious application is the killing of tumor cells with the Fas system, since many cancer cells express the functional Fas. However, since the systemic treatment of the patients with FasL causes a strong side effect, methods of local administration and/or proper targeting of FasL to the tumor should be devised. The other application of this system is to block the FasL-induced tissue destruction. If the involvement of the Fas system in human diseases such as fulminant hepatitis, acquired immunodeficiency syndrome (AIDS) and other CTL-induced tissue destruction are proved, neutralizing antibodies against Fas or FasL, or inhibitors of the Fas-mediated apoptosis would have potential use for clinical application.

References