Review

Activation-Induced Cell Death in T Cells and Autoimmunity

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Activation-induced cell death (AICD), which results from the interaction between Fas and Fas ligand, is responsible for maintaining tolerance to self-antigen. A defect in AICD may lead to development of autoimmunity. During the last several years, much progress has been made in understanding the mechanism(s) of AICD and its potential role in the pathogenesis of autoimmune diseases. In this review, we summarize the most recent progress on the regulation of the susceptibility of T cells to AICD and its possible involvement in autoimmune diseases. *Cellular & Molecular Immunology*. 2004;1(3):186-192.

Key Words: AICD, Fas, T cell, autoimmunity

Introduction

T cell development is regulated not only by proliferation and differentiation but also by apoptosis (1-3). T cell apoptosis is believed to maintain homeostasis and self-tolerance in the immune system. For example, thymocytes that fail to rearrange their T cell receptor (TCR) gene will die of neglect (4) and those that recognize self-antigens will be eliminated by apoptosis, a process called negative selection (5-7). In peripheral T cells, a form of apoptosis induced by repeated TCR stimulation, known as activation-induced cell death (AICD), may be responsible for the peripheral deletion of autoreactive T cells (8, 9). AICD results from the interaction between Fas and Fas ligand (FasL), and activated T cells expressing both Fas and FasL are killed either by themselves or by interacting with each other (9-14). Thus, Fas-mediated AICD is an important mechanism for maintaining tolerance to self-antigen (8). The importance of AICD in the maintenance of self-tolerance is illustrated by the autoimmune diseases that develop in mice and humans with inherited defects in Fas or FasL (15-17). This review summarizes the available present knowledge on the regulation of Fas-mediated T cell AICD and its potential role in autoimmune diseases.

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Signaling pathways of Fas-mediated cell death

Ligation of Fas with FasL induces trimerization of the Fas receptor, wherein the adaptor protein, Fas-associated death domain protein (FADD), binds to the trimerized Fas cytoplasmic region through the interaction of the respective death domains. Pro-caspase-8 [also called FADD-like IL-1β-converting enzyme (FLICE)] is then recruited to FADD through binding of the death effector domains (DEDs), which in turn induces self-activation of caspase-8. The Fas receptor, FADD and pro-caspase-8 form a functional death-inducing signaling complex (DISC). The activated caspase-8 is released into the cytosol where it results in activation of a caspase cascade that initiates apoptosis (1, 2, 14, 18, 19) (Figure 1).

Regulation of T cell susceptibility to AICD

Transcriptional regulation of FasL expression

FasL expression is regulated predominantly at the transcriptional level, which is a major way that FasL-mediated AICD is controlled. The transcription factors involved in this process include: early growth response genes (Egr) and nuclear factor of activated T cells (NF-AT), nuclear factor kappa B (NF-κB), c-Myc, activator protein-1 (AP-1), secretory protein-1 (SP-1), and interferon regulatory factors (IRFs).

NF-AT is activated by TCR stimulation and is a key regulator of FasL expression. The inhibition of FasL induction in T cells by cyclosporin A (CsA) has directed the search to factors that are sensitive to CsA (20, 21). CsA inhibits the calcineurin-dependent dephosphorylation of NF-AT, which is required for nuclear translocation and function of NF-AT in the expression of IL-2 and other cytokine genes (22). NF-AT consensus sequences have been identified in the human and mouse FasL promoters,

Abbreviations: AICD, activation-induced cell death; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa B; AP-1, activator protein-1; SP-1, secretory protein-1; IRFs, interferon regulatory factors; CsA, cyclosporin A.

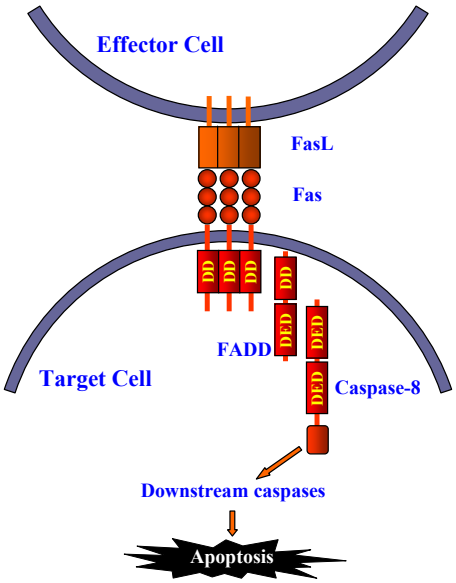

Figure 1. Fas-mediated signaling pathway. (See text)

and NF-AT has been shown to enhance the expression of FasL in several studies (23-26). Accordingly, mice bearing mutations in NF-ATp (NF-ATc2) or in both NF-ATp and NF-AT4 (NF-ATc3) have impaired ability to express FasL and a lymphoproliferative disorder (9, 27, 28). NF-AT exerts its effect *via* at least two footprinted sites (between -487 and -271). NF-AT-mediated induction of FasL requires cooperation of AP-1 (Fos/Jun) (9, 29) and may also work in concert with SP-1 during IL-2-dependent FasL transcription in response to TCR stimulation. Promoter studies and gel shift assays indicate a site between -253 and -244 in the FasL promoter found to bind NF-AT and SP-1 (9) (Figure 2).

Egr family transcription factors appear to be important for FasL expression in T cells, even though Egr-1-deficient mice do not display lymphoproliferative disorder, possibly due to redundancy in the activity among the Egr family members (9). Induction of the Egr2 and Egr3 following TCR ligation is also inhibited by CsA (30, 31). Egr and NF-AT may form composite sites within the FasL promoter and can cooperatively regulate transcription (32). However, recent evidence points to NF-AT as a regulator of the expression of Egr2 and Egr3, implying that the effect of NF-AT on the transcription of FasL is indirect (33, 34) (Figure 2). The NF-AT-mediated FasL induction (*via* Egr2 and Egr3) is preferentially exercised in Th1 *vs* Th2 cells (9). Differential usage of these transcription factors by Th1 and Th2 cells suggests a potential mechanism underlying the differential expression of FasL by distinct T cell lineages and explains the corresponding differences in susceptibility to Fas-mediated AICD (9, 35). Of note, the conflicting data concerning the elements most responsible for the regulation of FasL may be attributable to the variety of cell lines and hybridomas that have been used for its study.

NF-κB also plays a role in T cell activation-induced FasL expression. NF-κB proteins are present in the cytoplasm in association with inhibitors of NF-κB (IκBs). Phosphorylation of IκB results in proteasomal degradation of IκB thereby releases NF-κB from the cytoplasmic NF-κB-IκB complex allowing them to translocate to the nucleus (9). Transfection of Jurkat T cells with the NF-κB subunits p50 and p65 confers resistance against Fas-mediated apoptosis. Reciprocally, inhibition of NF-κB by a soluble peptide inhibitor or the NF-κB inhibitor, IκB, makes the cells more susceptible to Fas-mediated apoptosis (36). Consistent with this report, three NF-κB sites in the FasL promoter have been identified (-1086 to -1076, -138 to -128, and -440 to -428) (9) (Figure 2). Co-expression of RelA p65 with FasL promoter enhanced its activity (37, 38), and co-expression IκB dramatically inhibited the inducible promoter activity (37). In support of these findings, apoptosis-resistant T cells have a deficiency in NF-κB-mediated induction of FasL transcription (39). These findings suggest a role for NF-κB in mediating FasL expression during AICD. However, using a mutant T cell line deficient in an essential NF-κB component, IκB kinase γ, it was found that the NF-κB signaling pathway is not required for FasL gene induction but mediates protection from AICD (40). Since the behavior of a T cell line might be different from that of primary T cells, it would be interesting to examine the FasL expression in T cells from mice deficient for NF-κB to further clarify whether NF-κB is involved in the regulation of FasL expression.

The transcription factor c-Myc is important for the control of cell cycle progression**,** neoplasia, and apoptotic cell death. c-Myc dimerizes with its partner Max to form an active transcription factor complex. T cell activation-induced expression of FasL is regulated by c-Myc. Down-regulation of c-Myc protein *via* antisense oligonucleotides blocks activation-induced expression and function of FasL in activated T cells. Further, FasL promoter activity in T cells is driven by overexpression of c-Myc and inhibited by expression of dominant-negative (DN) mutants of c-Myc

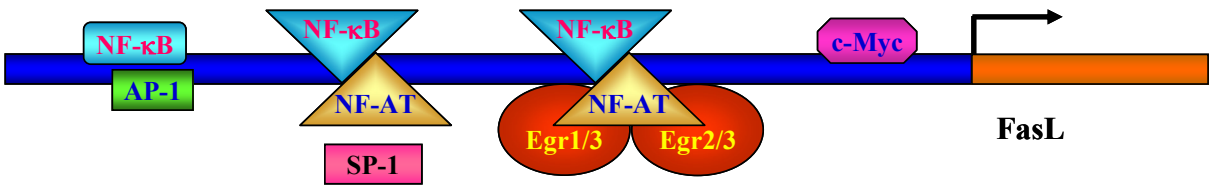

Figure 2. The responsive elements for transcription factors responsible for FasL expression within the proximal promoter of the FasL gene. Different transcription factors work together to regulate FasL gene activity.

and Max (41). This study indicates that c-Myc also controls apoptotic cell death in T cells through regulation of FasL expression. A site at -127 to -121 in the FasL promoter can bind to Myc-Max heterodimers and is necessary and sufficient for the response of this promoter to c-Myc (9) (Figure 2). It has been shown that transforming growth factor β (TGFβ) inhibits FasL expression and subsequent AICD *via* down-regulation of c-Myc expression (42).

TCR-inducible FasL expression is also under the direct influence of the IRF transcription factor family. Deletion and mutagenesis of a putative IRF-1 binding site in the FasL promoter result in deficient expression of FasL. Gel shift assays demonstrate specific FasL promoter binding by IRF-1 and IRF-2. Forced expression of either IRF-1 or IRF-2 leads to FasL promoter activation in T cells and FasL expression in heterologous cells; suppression of IRF-1 expression in T cells results in deficient TCR-induced FasL expression. These data confirm that the IRF family participates in the regulation of FasL gene expression (43).

Regulation of FasL expression by protein kinases

Protein tyrosine kinases (PTKs) not only play a critical role in T cell activation, but are also required for T cell apoptosis (44-47). TCR-induced FasL expression is dependent on Lck src-homology 2 (SH2) domain and kinase activity (44). Lck is necessary and sufficient for FasL expression and apoptosis in mature cycling T cells (45). Furthermore, ZAP-70 is also involved in the regulation of FasL expression. T cells lacking ZAP-70 are unable to upregulate FasL expression and undergo AICD, whereas transfection of wild-type ZAP-70 into the ZAP-70-deficient T cells restores their sensitivity to TCR-induced AICD (47, 48). FasL expression can be induced by stimulating T cells with a combination of phorbol ester and Ca^{2+} ionophore, implicating a role for protein kinase C (PKC) in this process. Further study shows that PKC-θ, a Ca^{2+}-independent PKC isoform that is selectively expressed in T cells, cooperates with calcineurin to induce FasL expression during AICD (49). Protein tyrosine phosphatases (PTPases) also play an important role in the regulation of FasL expression. Peripheral T cells from SH2 domain-containing protein tyrosine phosphatase-1 (SHP-1) -deficient (motheaten; mev) mice are markedly more sensitive than wild-type T cells to AICD, and enhanced AICD of SHP-1-deficient T cells correlates with increased expression of FasL, suggesting a role of SHP-1 in the regulation of FasL expression (50). However, the precise mechanism by which SHP-1 regulates FasL expression remains to be further elucidated.

The involvement of mitogen-activated protein kinase (MAPK) family members in AICD has been the subject of much investigation and some confusion. There are three major subgroups of MAPKs in mammalian cells: extracellular signal-regulated kinase (ERK), c-Jun-NH_2-terminal kinase (JNK) and p38 MAPK. p38 MAPK may be an upstream regulator of caspases, whereas JNK functions downstream of the caspases. Interestingly, FasL expression is down-regulated by caspase inhibitors and a DN JNK. These data indicate that p38 MAPK and downstream JNK converge to regulate FasL expression at different times after TCR stimulation to elicit maximum T cell AICD (12). Consistent with these findings, inhibition of p38 MAPK by the specific inhibitor SB203580 prevents AICD. The inhibition of AICD is achieved by suppression of FasL promoter activation (51). Most importantly, AICD in T cells is significantly impaired in JNK-1/JNK-2-deficient mice or mice deficient in GADD45-γ, an upstream regulator of JNK (52-54), suggesting that JNK is actively involved in the regulation of AICD in primary T cells. In addition, ERK pathway is required for AICD and appears to regulate the induction of the orphan nuclear steroid receptor Nur77 and FasL expression during AICD (55). The roles of p38 MAPK and ERK in AICD have been difficult to assess owing to the lethality of the mice deficient for these MAPKs, but T cells lacking MAPK kinase 3 (MKK3), an upstream activator of p38 MAPK, are relatively resistant to AICD. This function contrasts the deficiency of MKK6, another upstream activator of p38 MAPK, which does afford protection (52). T cell-specific p38 MAPK- or ERK-deficient mice may be helpful for clarifying this issue.

IL-2 is not only a T cell growth factor, but also sensitizes T cells to AICD. One important role of IL-2 in mediating the susceptibility of T cells to AICD is upregulating FasL expression (56). IL-2-inducible T cell kinase (Itk) may play a crucial role in this process. Decreased FasL expression and AICD were observed in T cells from mice lacking Itk (57). Itk is critical for the activation of phospholipase C-γ1 (PLC-γ1), leading to calcium mobilization in response to TCR stimulation. Itk-deficient T cells are defective in the activation of ERK and JNK pathways, the expression of Egr2 and Egr3, and consequently FasL expression (57). However, the transcriptional mechanism(s) by which IL-2 controls FasL expression may differ from TCR-induced FasL expression. The IL-2-treated T cells have high nuclear expression of SP-1 and NF-AT but lack the Egr2 and Egr3 that could be induced by anti-CD3 stimulation and have been implicated in FasL gene activation (58). The role of cytokines in AICD may not only be mediated through the regulation of FasL expression, but also *via* additional mechanisms. Recent data have shown that T cells lacking interferon (IFN-γ) are also resistant to AICD, and this resistance is abrogated by the addition of exogenous IFN-γ (59). The IFN-γ-potentiated AICD may be mediated by the Th1-specific signal transducers and activators of transcription-1 (STAT-1), which assists control IFN-γ production, resulting in reduced caspase-8 expression (59).

Regulation of c-FLIP expression in Fas-mediated AICD

The expression of Fas and FasL does not always correlate with the level of apoptosis, suggesting that some inhibitors exist in Fas-mediated signaling pathway. Indeed, a new family of viral FLICE inhibitory proteins (v-FLIP) has recently been described, which interfere with caspase-8 recruitment to the DED of FADD (60). Subsequently, a cellular homologue of v-FLIP has been identified by several groups and variously named c-FLIP, Casper, I-FLICE, CASH, FLAME-1, MRIT, CLARP, or usurpin (61-68). On the mRNA level, several c-FLIP splice variants exist. On the protein level, however, only two endogenous forms: c-$FLIP_L$ and c-$FLIP_S$. c-$FLIP_L$ is structurally similar to caspase-8, since it contains two DEDs and a caspase-like

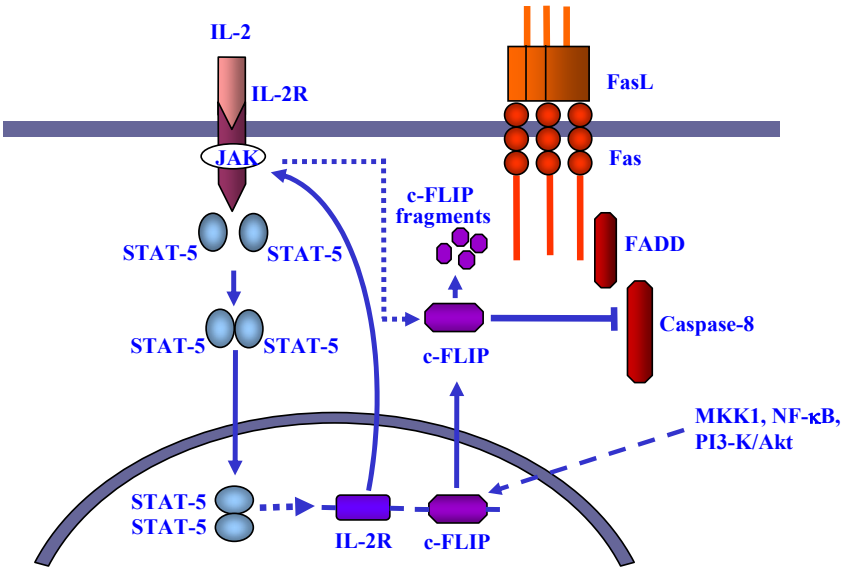

Figure 3. A model of AICD regulation by IL-2. In this model, IL-2 induces STAT-5 phosphorylation which dimerizes and translocates to the nucleus, activates IL-2 receptor genes and leads to the upregulation of IL-2 receptor expression on the cell surface. Upregulation of IL-2 receptor expression enhances the sensitivity of T cells to IL-2, and favors the degradation of c-FLIP which facilitates the development of AICD. MKK1, NF-κB and PI3-K/Akt have been shown to regulate c-FLIP expression.

domain. However, this domain lacks residues that are important for the catalytic activity of caspase-8, most notably the cysteine with the active site. c-FLIP$_S$ structurally resembles v-FLIP (61). c-FLIP expression is primarily regulated at the posttranslational level. IL-2 may sensitize T cells to AICD *via* promoting c-FLIP degradation (69) (Figure 3). The role of c-FLIP in Fas-mediated cell death is still controversial. Studies using c-FLIP$_L$ transgenic mice revealed that c-FLIP$_L$ suppresses FasL-induced T cell apoptosis but not AICD (70). Several possibilities may explain why AICD is not impaired in c-FLIP$_L$ transgenic mice. First, c-FLIP$_S$ may be the major isoform responsible for regulating the susceptibility of T cells to AICD (71-73). In support of this notion, c-FLIP$_S$ but not c-FLIP$_L$, completely blocks the cleavage of pro-caspase-8 at the DISC (52, 73, 74). Indeed, c-FLIP$_L$ transgenic T cells are not completely resistant to FasL-induced T cell death (69). Second, the levels of transgenic c-FLIP$_L$ in T cells may not be sufficient to block caspase-8 activation required for AICD. It has been shown that c-FLIP$_L$ expression at low levels enhances pro-caspase-8 processing at the DISC, whereas at high levels inhibits Fas-mediated apoptosis (75). The role of c-FLIP in the regulation of AICD *in vitro* and *in vivo* has also been strongly supported by the following evidence. Retrovirus-mediated overexpression of c-FLIP blocks Fas-induced apoptosis of activated T and B cells, which leads to the production of autoantibodies and to the development of autoimmune diseases (76). In keeping with this finding, c-FLIP may be involved in controlling lymphoadenopathy in IL-2-deficient mice (77). These observations suggest that the modulation of c-FLIP is necessary to maintain self-tolerance. Most importantly, c-FLIP-deficient cells are specifically sensitive to death receptor-induced apoptosis (78). T cells overexpressing c-FLIP$_L$ are partially or completely resistant to Fas-mediated T cell death, supporting the involvement of c-FLIP in Fas-mediated cell death (69, 79). Taken together, these findings suggest that c-FLIP does regulate T cell susceptibility to Fas-mediated AICD (Figure 3). The discrepancy on the role of c-FLIP in AICD in c-FLIP transgenic mice might be due to the levels of c-FLIP$_L$ overexpression or different genetic backgrounds. It should be noted that T cell-specific c-FLIP-deficient mice would provide a very useful tool for solving this discrepancy.

The signaling pathway involved in the regulation of c-FLIP expression is not completely understood. MKK, NF-κB and phosphatidylinositol 3-kinase (PI3-K) have been implicated in the regulatory process of c-FLIP expression. MKK1 is an upstream activator of ERK. Activation of MKK1 has been shown to lead to expression of c-FLIP. MKK1 inhibition of FADD-induced cell death can be abrogated if induction of c-FLIP is prevented, indicating that c-FLIP mediates MKK1 suppression of FADD-mediated apoptosis (80). It has recently been shown that NF-κB activation upregulates c-FLIP, resulting in increased resistance to FasL or TNF. Restoration of either the c-FLIP$_L$ or alternatively spliced c-FLIP$_S$ in NF-κB-deficient cells inhibits TNF- and FasL-induced cell death efficiently, whereas the expression of inhibitor of apoptosis (IAP) or TNFR-associated factor (TRAF) family members only partially rescues cells from death. Resistance to either FasL- or TNF-induced apoptosis is overcome when cells are incubated in the presence of the protein synthesis inhibitor cycloheximide. This treatment leads to the rapid down-regulation of c-FLIP but not to that of TRAF2. These findings suggest that c-FLIP is an important mediator of NF-κB-controlled anti-apoptotic signals (81). In addition to MKK1 and NF-κB, it has been reported that PI3-K/Akt signaling pathway regulates c-FLIP expression in tumor cells and T cells (82, 83).

Survivin and AICD

Survivin is a novel member of the IAP family that also functions during mitosis. Survivin contains a single baculovirus IAP repeat with 142 amino acids; it is the smallest IAP member. In contrast to most IAPs, survivin lacks a carboxyl-terminal RING finger but instead contains

a coil-coiled region that is presumably required for subcellular localization (84). Survivin is expressed abundantly in the thymus, testis, and proliferating cells (85). In Jurkat T cells, it has been shown that survivin can inhibit AICD in G1 phase (86). However, the role of survivin in T cell apoptosis has been challenged by a recent study using T cell-specific survivin-deficient mice in which survivin has been shown to be not essential for T cell apoptosis but is crucial for T cell maturation and proliferation (84).

Fas-mediated AICD and autoimmunity

Fas- or FasL-deficient mice spontaneously develop lympho-proliferative disorder (15-17), suggesting a critical role of Fas-mediated cell death in the development of autoimmune disease. Multiple sclerosis (MS) and its mouse model, experimental autoimmune encephalomyelitis (EAE), are Th1-mediated autoimmune diseases. T cell-mediated Fas-FasL interactions could directly contribute to the pathogenesis and regulation of MS and EAE (87). In support of this idea, impaired apoptotic deletion of myelin basic protein (MBP)-reactive T cells has been observed in patients with MS (88). Indeed, Fas-mediated AICD has been shown to play a major role in the spontaneous remission in EAE, suggesting an important intrinsic mechanism of autoimmune response (89). Fas and FasL expression in immune cells but not on the target tissue also controls induction of experimental autoimmune uveitis (90).

Since c-FLIP is a mostly upstream inhibitor of Fas-mediated signaling pathway, it is predicted that over-expression of c-FLIP may lead to the development of auto-immunity. Consistent with this idea, elevated expression of c-FLIP in T cells correlates with disease activity in patients with MS (91), and interferon-β therapy has been shown to down-regulate c-FLIP expression in T cells from MS patients (92). Moreover, the defective tolerance of nonobese diabetic thymocytes correlates with the strong TCR-mediated upregulation of c-FLIP (93). The most direct evidence in support of a role for c-FLIP in auto-immunity comes from a recent report in which retrovirus-mediated overexpression of c-FLIP blocks Fas-induced apoptosis of activated T and B cells, thus resulting in the development of autoimmunity (76). Taken together, these findings suggest that increased expression of c-FLIP may favor the escape of autoreactive T cells from central and peripheral deletion. In support of this notion, in a murine model of rheumatoid arthritis, hyper-proliferation of peripheral $CD4^+$ T cells correlates with defective AICD. The impaired AICD might be ascribed to an aberrant expression of c-FLIP which precludes caspase-8 activation at the DISC, and subsequently suppresses the caspase cascade initiated by Fas-FasL interaction. This defect in AICD in autoimmune murine arthritis may lead to the accumulation of autoreactive Th1 cells in the periphery (94).

Concluding remark

Fas-mediated AICD in T cells represents one of the major mechanisms for peripheral tolerance. Regulation of Fas, FasL, and c-FLIP expression by different factors controls T cell susceptibility to AICD. Understanding of the regulation of AICD may lead to the development of potential therapeutic approaches for autoimmune diseases.

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