The NF-κB Signaling Pathway Is Not Required for Fas Ligand Gene Induction but Mediates Protection from Activation-induced Cell Death*

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Stimulation of T cells by antigens or mitogens triggers multiple signaling pathways leading to activation of genes encoding interleukin-2 and other growth-regulatory cytokines. The same stimuli also activate the gene encoding an apoptosis-inducing molecule, Fas ligand (FasL), which contributes to activation-induced cell death. It has been proposed that the signaling pathways involved in cytokine gene induction also contribute to activation-induced FasL expression; however, genetic evidence for this proposal is lacking. In the present study, the role of the NF-κB signaling pathway in FasL gene expression was examined using a mutant T cell line deficient in an essential NF-κB signaling component, IκB kinase γ. These mutant cells have a blockade in signal-induced activation of NF-κB but remained normal in the activation of NF-AT and AP-1 transcription factors. Interestingly, the NF-κB signaling defect has no effect on mitogen-stimulated FasL gene expression, although it completely blocks the interleukin-2 gene induction. We further demonstrate that NF-κB activation is required for protecting T cells from apoptosis induction by mitogens and an agonistic anti-Fas antibody. These genetic results suggest that the NF-κB signaling pathway is not required for activation-induced FasL expression but rather mediates cell growth and protection from activation-induced cell death.

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The abbreviations used are: TCR, T cell receptor; IL-2, interleukin-2; FasL, Fas ligand; AICD, activation-induced cell death; NF-κB, nuclear factor of activated T cells; AP-1, activator protein 1; NF-κB, nuclear factor κB; IKK, IκB kinase; GFF, green fluorescence protein; FITC, fluorescein isothiocyanate; PI, propidium iodide; RPA, RNase protection assay; ELISA, enzyme-linked immunosorbent assay; PMA, phorbol 12-myristate 13-acetate; RT, reverse transcription; PCR, polymerase chain reaction; HA, hemaglutinin; EMSA, electrophoretic mobility shift assay; NLS, nuclear localization signal.

interleukin-2 (IL-2) and many other cytokines involved in T cell proliferation and differentiation (for reviews see Refs. 1–3). Additionally, the T cell activation program also involves induction of genes mediating activation-induced cell death (AICD), a mechanism required for the maintenance of immune cell homeostasis (4, 5). A major gene mediating AICD is Fas ligand (FasL) (6–8), a member of the tumor necrosis factor family (9). FasL specifically interacts with its cognate receptor, Fas, thereby triggering a cascade of proteolytic events resulting in the death of Fas-expressing cells (10, 11). The importance of Fas-mediated apoptosis is evidenced by studies using the lpr and gld mice that harbor inactivating mutations in the Fas and FasL genes, respectively (12, 13). These mice suffer a severe lymphoproliferative disorder and die shortly after birth because of an excessive accumulation of lymphocytes in addition to autoimmune diseases. In T cells and many other cell types, expression of FasL is subject to strict regulation whereas expression of Fas is largely constitutive (9, 14, 15). Therefore, a large effort has been made to the understanding of how the FasL gene is activated by T cell activation signals.

Sequence analysis of the upstream region of FasL gene has revealed potential binding sites for a number of transcription factors known to participate in IL-2 gene regulation. These include the nuclear factor of activated T cells (NF-AT) (16, 17), activator protein-1 (AP-1) (18, 19), and nuclear factor κB (NF-κB) (20–22). The physiological role of NF-AT in activation-induced FasL gene expression has been demonstrated through both biochemical and genetic studies (16, 17, 23–25). Mutation of the NF-AT binding sites in FasL promoter completely blocks the inducible transcription from this promoter (16, 26). Consistently, germ line inactivation of the NF-ATp gene in mice largely blocked FasL gene induction by TCR signals (24, 25). On the other hand, the role of AP-1 and NF-κB in FasL gene regulation remains elusive. Promoter truncation studies reveal that removal of the promoter distal region of FasL gene, which contains the binding sites for AP-1 and NF-κB, has no effect on the inducibility of the FasL promoter (16, 23). In contrast, other studies using promoter mutation and NF-κB overexpression approaches suggest that NF-κB plays a critical role in FasL promoter induction by T cell activation agents (20, 27). These latter findings suggest a proapoptotic role of NF-κB in AICD, as opposed to its well known antiapoptotic function in many other systems (28–34). However, genetic evidence for a role of NF-κB in the induction of either FasL gene expression or AICD is currently lacking.

NF-κB represents a family of transcription factors that regulate a large variety of genes involved in the immune and inflammatory responses (35). One of the well studied NF-κB target genes in T cells is IL-2 (36), which plays a critical role in
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NF-κB is a transcription factor that regulates the expression of genes involved in immune response and cell growth. It is activated by various stimuli, including pro-inflammatory cytokines. The NF-κB pathway involves the IKK complex, which phosphorylates IκBα, allowing NF-κB to translocate to the nucleus and induce gene expression.

**Materials and Methods**

**Cell Lines and Reagents—SVT35** is a Jurkat cell line stably transfected with a CD14 reporter gene driven by eight copies of SV40 promoter. This cell line is used to study NF-κB activation.

**RT-PCR, RNase Protection, and Northern Blot Analyses—SVT35** parental and derivative cell lines were treated as indicated and harvested 24 h post-transfection. Total RNA was isolated using TRI reagent. Northern blot analysis was performed using a 32P-labeled cDNA probe.

**Immunoblot Assays—** Western blotting was performed using antibodies specific to NF-κB and IκBα. Blots were probed with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies and detected using chemiluminescence.

**Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay—** Nuclear extracts were prepared from treated and untreated cells using the Nuclear Extraction Kit. EMSA was performed using oligonucleotide probes labeled with 32P and run on a native gel.

**Apoptosis Assay—** Apoptosis was analyzed by measuring DNA fragmentation using a commercially available kit. Cells were stained with annexin V and propidium iodide, and analyzed by flow cytometry.

**Discussion**

The NF-κB pathway plays a crucial role in immune response and cell survival. The activation of NF-κB is tightly regulated by various intracellular and extracellular stimuli. The IKK complex is a key regulator of this pathway, and its activation results in the phosphorylation and degradation of IκBα, allowing NF-κB to translocate to the nucleus and induce gene expression. Understanding the regulation of NF-κB activation is crucial for the development of new therapeutic strategies for immune-related diseases.
RESULTS

**JM4.5.2 T Cells Are Defective in NF-κB Signaling, Which Can Be Functionally Rescued by Stable Expression of IKKγ**—Stimulation of TCR with an agonistic anti-CD3 antibody triggers the protein kinase C and calcium signals (61). These two signals can also be elicited by the mitogen PMA and the calcium ionophore ionomycin, respectively (61). In synergy with the CD28 costimulatory signal, the protein kinase C stimulator PMA potently activates NF-κB through enhanced phosphorylation and degradation of the inhibitors IκBα and IκBβ (62, 63). By somatic mutagenesis, we have recently isolated a Jurkat T cell line, JM4.5.2, that is defective in PMA/CD28-mediated NF-κB activation (52). Initial characterization of this cell line revealed that it lacked expression of the essential NF-κB signaling component IKKγ. As shown in Fig. 1, IKKγ was readily detected by immunoblotting in the parental Jurkat cells (SVT35, lane 1) but not in the JM4.5.2 cells (lane 2). To confirm that the lack of IKKγ contributed to the NF-κB signaling defect in the mutant cells, an expression vector encoding HA-tagged IKKγ (HA-IKKγ) was stably introduced to the JM4.5.2 cells by retrovirus-mediated gene transfer. As control, the JM4.5.2 cells were transduced with a GFP expression vector. The exogenous HA-IKKγ was readily detected in two individual IKKγ stable cell clones (JM4.5.2-IKKγ #8 and #10, lanes 4 and 5) but not in the GFP-transduced cell clone (JM4.5.2-GFP, lane 3).

To examine the effect of exogenous IKKγ on NF-κB signaling, the different cell lines were stimulated with mitogens followed by analysis of the inducible degradation of IκBα and activation of NF-κB. As expected, IκBα was completely degraded when the parental SVT35 cells were stimulated with PMA together with anti-CD28 (Fig. 2A, lane 3). A similar result was obtained by cellular stimulation with PMA plus ionomycin (lane 2). However, these mitogenic stimuli failed to induce IκBα degradation in the mutant JM4.5.2 cells (lanes 4–6). More importantly, the defect in IκBα degradation was largely rescued in the mutant cell clones stably expressing exogenous IKKγ (lanes 10–15). This rescuing effect was specific because it was not detected in the JM4.5.2-GFP cells (lanes 7–9). Parallel EMSA revealed that the IκBα degradation was associated with activation of the DNA binding activity of NF-κB (Fig. 2B). Thus, genetic reconstitution of the IKKγ gene in JM4.5.2 cells is sufficient to restore NF-κB signaling.

**JM4.5.2 Cells Are Competent in Signal-induced Activation** of NF-AT and AP-1—We next determined whether the signaling defect observed in JM4.5.2 cells affected the activation of other transcription factors known to respond to T cell activation signals. These included NF-AT and AP-1, both known to be critical for cytokine gene induction upon T cell stimulation (3). Because NF-AT and AP-1 have also been implicated in FasL gene regulation, analysis of these parallel pathways in the IKKγ-deficient cells is important for assessing the role of NF-κB in activation-induced FasL expression. For these studies EMSA was performed to detect the DNA binding activity of NF-AT, AP-1, and NF-κB in the parental, mutant, and IKKγ-reconstituted mutant Jurkat cells (Fig. 3A). As expected, JM4.5.2 cells failed to show inducible NF-κB DNA binding activity, and this defect was rescued in the IKKγ-stable cell clones. In contrast to that seen with NF-κB, induction of neither NF-AT nor AP-1 was inhibited in the JM4.5.2 mutant cells. Furthermore, no significant differences in the DNA binding activity of NF-AT and AP-1 were observed in the IKKγ-stable clones versus the GFP clone (lanes 6, 8, and 10).

To functionally demonstrate the different transcription factor pathways in IKKγ-deficient cells, reporter gene assays were performed using luciferase reporter plasmids driven by specific binding sites for NF-AT (NF-AT-Luc), AP-1 (AP1-Luc), and NF-κB (κB-Luc). As expected, JM4.5.2 cells had a blockade in mitogen-mediated induction of the κB-Luc (Fig. 3B, column 3). No NF-κB activity was detected even when the mutant cells were costimulated with the anti-CD28 antibody (column 4), a treatment greatly enhancing the NF-κB activation signal (Refs. 63 and 64 and Fig. 3B, column 2). In agreement with the results seen in the EMSA experiments, induction of the NF-AT and AP-1 reporters remained normal in the JM4.5.2 cells (Fig. 3, C and D, columns 3 and 4). Further, IKKγ reconstitution in the mutant cells efficiently rescued the κB activation defect (Fig. 3B, columns 7–10) but did not generate an appreciable effect on the induction of NF-AT and AP-1 reporters (Fig. 3, C and D, columns 7–10). These results, along with those obtained with EMSA, strongly suggest that the signaling defect observed in JM4.5.2 cells is specifically located in the NF-κB pathway.

**The NF-κB Signaling Pathway Is Not Required for Mitogen-induced FasL Gene Expression but Is Essential for the Induction of IL-2 Gene**—The role of NF-κB in FasL gene induction has been addressed by biochemical approaches including over-
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Fig. 3. JM4.5.2 cells exhibit defects in signal-induced NF-κB activation but remain functional in activation of NF-AT and AP-1. A, DNA binding analysis by EMSA. The indicated cell lines were either not treated (N7) or stimulated for 2 h with PMA plus ionomycin (P/I). Nuclear extracts were subjected to EMSA using 32P-labeled oligonucleotide probes specific for the indicated transcription factors. The Oct-1 probe was used to monitor the nuclear extract concentration in the different lanes. B–D, luciferase reporter gene assays. Parental SVT35, JM4.5.2, or the indicated JM4.5.2 stable clones were transfected with κB-Luc (B), NF-AT-Luc (C), or AP1-Luc (D) reporters. 40 h post-transfection, the cells were stimulated with either PMA plus ionomycin (P/I) or PMA plus ionomycin and anti-CD28 (P/I/C). Luciferase activity was determined as described under "Materials and Methods" and is presented as fold induction relative to the basal level measured in untreated cells. The values shown in this and subsequent figures are the means ± S.E. from three independent experiments.

expression of the RelA subunit of NF-κB and its inhibition by nonspecific inhibitors (20, 27). The IKKγ-deficient JM4.5.2 T cell line provides a powerful genetic system for investigating the function of NF-κB in signal-induced FasL gene expression. We first examined the effect of the NF-κB signaling deficiency on the induction of FasL promoter by performing reporter gene assays using a luciferase gene driven by 1.2 kilobases of the FasL promoter (FasL-Luc). The inducibility of this promoter construct was lower than a shorter form covering the first 486 nucleotides of FasL promoter (23) (data not shown). This is likely due to the presence of negative regulatory sequences in the upstream region of the FasL promoter (17). However, we chose to use this 1.2-kilobase promoter construct because it covers the potential binding sites for NF-κB, AP-1, as well as NF-AT (18, 20). As expected, the activity of the FasL promoter was induced by mitogens in the parental Jurkat cells (Fig. 4A). Interestingly, the induction of FasL promoter activity was not affected by the NF-κB signaling defect in JM4.5.2 cells. Parallel studies revealed that the signal-induced activation of the IL-2 gene promoter was completely blocked in the IKKγ-deficient cells (Fig. 4B). Furthermore, expression of exogenous IKKγ in the JM4.5.2 cells efficiently restored the IL-2 promoter induction but did not generate a significant change in the induction of the FasL promoter activity.

We next examined the role of NF-κB in the induction of endogenous FasL and IL-2 genes. The steady state level of FasL and IL-2 mRNAs was analyzed by RT-PCR following mitogen stimulation. In this respect, we treated the cells with PMA/IONOMYCIN either in the absence or presence of anti-CD28, because maximal induction of the IL-2 gene requires the CD28 signal (65). As expected, the mRNAs for both FasL and IL-2 were markedly induced upon stimulation of the parental SVT35 Jurkat cells with PMA/IONOMYCIN (Fig. 5A, lane 2). Moreover, the level of IL-2 mRNA was further enhanced when the cells were costimulated with anti-CD28 (Fig. 5A, middle panel, lane 3), a synergistic effect resulting largely from CD28-enhanced NF-κB activation (64, 66, 67). However, we observed that the CD28 signal did not enhance the level of FasL mRNA but even moderately inhibited the induction of this apoptosis-regulatory gene (upper panel, lane 3). These results indicated that NF-κB might not be important for FasL gene induction. Consistently, the inducible FasL mRNA expression was not affected by the IKKγ deficiency in the JM4.5.2 cells (Fig. 5A, top panel, lanes 5 and 6). In sharp contrast, induction of IL-2 gene expression was completely blocked in the IKKγ-deficient cells (middle panel, lanes 5 and 6). The essential role of IKKγ in
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mediating IL-2 gene induction was further demonstrated by the functional restoration of the IL-2 mRNA induction in the IKKγ-stable cell clones (middle panel, lanes 11, 12, 14, and 15). Because RT-PCR is a semi-quantitative method of RNA detection, we performed RPA and Northern blot analyses to further validate the results obtained with RT-PCR. These assays again revealed that induction of the IL-2 mRNA (Fig. 5C), but not the FasL mRNA (Fig. 5B), was dependent on the presence of IKKγ. Together, these results clearly demonstrate that the NF-κB signaling pathway is not required for activation-induced FasL gene expression but plays an essential role for the induction of the IL-2 gene.

The NF-κB Signaling Pathway Mediates Protection of Activation-induced T cell Death—NF-κB is known to function as an antiapoptotic factor in cells treated with tumor necrosis factor-α and certain chemotherapeutic compounds (28–30). Interestingly, recent studies indicate that this transcription factor may also serve as a critical pro-apoptotic factor in AICD (20). Inhibition of NF-κB by a peptide carrying the nuclear localization signal (NLS) of NF-κB p50 blocks TCR-mediated apoptosis (20). However, the specificity of this experiment is questioned by the recent finding that the p50 NF-κB peptide blocks activation of not only NF-κB but also NF-AT, AP-1, and STAT (68). To more definitively determine the role of NF-κB in AICD, we performed apoptosis analysis using our NF-κB-defective T cell lines. The parental SVT35 Jurkat cell line and its derivatives, lacking or expressing the IKKγ, were stimulated with PMA/ionomycin, mitogens that trigger the TCR signals (61). Apoptosis was analyzed by annexin/PI staining (60). The early and late stage apoptotic cells (stained with annexin and annexin/PI, respectively) and surviving cells (double negative) were quantitated by a fluorescence-activated cell sorter (Fig. 6). As expected, stimulation of the parental Jurkat cells with PMA/ionomycin resulted in significant (about 40%) cell death, although a large population (57.4%) of the stimulated cells remained alive (Fig. 6A, panel b). Further, consistent with the survival promoting function of the CD28 signal (69, 70), the percentage of cell death was reduced to approximately 30% when the cells were costimulated with the anti-CD28 antibody (PI/C) (panel c). More importantly, the IKKγ-deficient JM4.5.2 cells were remarkably more sensitive to apoptosis induction by mitogens. Up to 86% of the mutant cells were killed upon stimulation with PMA/ionomycin (panel c), and this killing activity could not be inhibited by the CD28 signal (panel f). These results demonstrate that the NF-κB signaling pathway is not required for activation-induced T cell death. Additionally, these data also suggest that NF-κB may protect T cells from undergoing activation-induced apoptosis. This notion was further supported by a similar experiment performed with the IKKγ-reconstituted JM4.5.2 cell clones. In both cell clones, the level of cell death following mitogen stimulation was significantly lowered (Fig. 6B, panels d and f). Together, the results presented above suggest that NF-κB is not required for activation-induced FasL gene expression or apoptosis but rather mediates protection from T cell death.

NF-κB Activation Also Inhibits Apoptosis Induction by Anti-Fas—Because AICD is known to be largely mediated by Fas/FasL pathway, we examined whether the mitogens induced expression of FasL protein in the different cell lines. As shown in Fig. 7, both the JM4.5.2 and JM4.5.2-IKKγ cells expressed FasL protein upon mitogen stimulation. We then directly tested whether the NF-κB signaling pathway inhibits Fas-mediated apoptosis. For these studies, the IKKγ-expressing and IKKγ-defective cell lines were pretreated with PMA/Ionomycin/anti-CD28 followed by apoptosis induction by anti-Fas. As shown in Figs. 2 and 3, the mitogen treatment potently induced NF-κB activity in both parental Jurkat cells and the IKKγ-reconstituted JM4.5.2 cells but not in the uninfected JM4.5.2 cells. Thus, if NF-κB indeed inhibits Fas-mediated apoptosis, pretreatment of the IKKγ-expressing cells, but not the IKKγ-deficient cells, would diminish anti-Fas-induced cell death. As expected, anti-Fas efficiently (>90%) killed both the IKKγ-expressing and IKKγ-deficient cell lines when they were not pretreated (Fig. 8, panels b, c, and h). More importantly, pretreatment of the parental Jurkat cells with mitogens markedly inhibited the Fas-mediated apoptosis (panel c). Furthermore, this death-inhibitory activity was hardly detectable in the mitogen-stimulated JM4.5.2 cells (panel f), which was correlated with the lack of NF-κB activation in these IKKγ-deficient cells (Fig. 3). Consistently, activation of the IKKγ-reconstituted JM4.5.2 cell clone #8 resulted in a significant protection from anti-Fas-mediated apoptosis induction (Fig. 8, panel i). Similar results were obtained with clone #10 (data not shown). These findings strongly suggest that NF-κB functions as a potent inhibitor of Fas-mediated apoptosis.

DISCUSSION

The Fas/FasL apoptosis pathway plays a major role in activation-induced cell death or AICD. This pathway is regulated through transcriptional induction of FasL. Thus, a central issue in the study of AICD is how the FasL gene is activated by T cell activation signals. Because the induction kinetics of FasL are similar to that of IL-2, it has been proposed that the same signaling pathways and transcription factors may be involved in the regulation of these two functionally distinct genes. To undertake a molecular dissection of the signaling pathways mediating transcriptional induction of IL-2 and FasL, we have...
generated mutant T cell lines by somatic mutagenesis. One of the mutant cell lines lacks expression of an essential NF-κB signaling component, IKKγ, and is defective specifically in the activation of NF-κB. Using this genetic system, we have shown that the NF-κB signaling pathway is essential for IL-2 gene induction by mitogens and anti-Fas. A, cell death induction in the parental and IKKγ-deficient Jurkat T cells. The parental SVT35 cells and the JM4.5.2 mutant cells were stimulated for 24 h with PMA plus ionomycin (P/I) or PMA plus ionomycin and anti-CD28 (P/I/C) followed by staining the apoptotic cells with annexin V-FITC and PI. The cells stained with annexin V single positive (lower right) and PI/annexin V double positive (upper right) are early and late phase apoptotic cells, respectively. The double negative cells (lower left) are the surviving cells, the percentage of which in the total cell population is indicated. The rest of the cells are largely the apoptotic cells (percentage not indicated). B, JM4.5.2 and its two individual IKKγ stable clones were either not treated (NT) or stimulated for 24 h with PMA/ionomycin/anti-CD28 (P/I/C) followed by apoptosis analysis as described in A.

Our data are inconsistent with some recent biochemical studies indicating that NF-κB has a critical role in activation-induced FasL gene expression and AICD (20, 27). It was shown in these studies that incubation of T cells with NF-κB-inhibitory agents, such as cAMP and the NLS peptide of NF-κB p50, markedly diminished TCR-mediated FasL gene induction and apoptosis in T cells. These findings raised the notion that NF-κB serves as a proapoptotic factor in AICD, as opposed to its anti-apoptotic function revealed in the current study, as well as in many other studies (28–34). The discrepancy between our genetic study and the previous biochemical studies likely results from the different experimental systems used. It appears that the NF-κB inhibitors used in the previous studies may lack sufficient specificity. For example, the major cellular target of cAMP is PKA, which is not a specific component of the NF-κB signaling pathway. Inhibition of NF-κB by this agent may also affect other cellular pathways. Although the p50-NLS
peptide was designed to specifically block NF-κB nuclear translocation (71), this peptide has recently been shown to nonspecifically inhibit the activation of multiple transcription factors, including NF-AT, AP-1, STAT, as well as NF-κB (68). Thus, the inhibitory effect of this peptide on FasL induction could be due to the blockade of other transcription factors, especially NF-AT, which is known to be a critical regulator of the FasL gene (24, 25). Our data are based on somatic cell mutagenesis and genetic reconstitution analysis, a technique that has been used for elucidating the physiological function of a large number of signaling molecules, such as Lck (72, 73), RIP (53), SLP-76 (74), LAT (75), and FADD (76). The results obtained with the somatic mutagenesis technique are highly consistent with those obtained from gene knockout studies (53, 75–80). The specificity of our experimental system was further demonstrated by the parallel experiments on the expression of the IL-2 gene. Of course, our data do not exclude the involvement of some NF-κB members, such as p50 and p52, in FasL gene regulation because these members are constitutively expressed in the nucleus. Nevertheless, our study demonstrates, for the first time, that the NF-κB signaling pathway is not required for FasL gene induction in T cells.

The nonessential role of NF-κB in FasL gene expression is in accord with the molecular events known to occur during T cell activation. For example, it has been demonstrated that optimal induction of IL-2 gene requires not only the TCR but also the CD28 signals (81, 82), which is largely due to the potent costimulatory effect of the CD28 signal on NF-κB activation (64, 67, 83, 84). However, the TCR signal is optimal for induction of the FasL gene expression (70), even though this signal only leads to a low level of NF-κB activation (63, 64). Moreover, CD28 costimulation does not enhance but rather inhibits TCR-mediated FasL gene induction (70) and AICD (70, 85). These findings are consistent with the notion that NF-κB is not required for FasL gene induction in T cells. More recently, it has been shown that the FasL promoter can be activated by ionomycin (17), a calcium ionophore that activates primarily NF-AT but is insufficient for activation of NF-κB (67).

Our finding that NF-κB activation prevents Fas-mediated apoptosis provides an important insight into the regulatory mechanism of AICD. We have shown that pretreatment of Jurkat T cells with mitogens potently inhibits anti-Fas-induced cell death. This protective effect is lost in the IKKγ-deficient cells but can be recovered through stable expression of IKKγ. Thus, it is clear that the presence of an intact NF-κB signaling pathway is critical for protecting T cells from activation-induced apoptosis. Newly activated T cells, which contain high levels of NF-κB activity, may be more resistant to Fas-mediated apoptosis induction. However, because activation of NF-κB by physiological stimuli occurs transiently (54, 86), the activated T cells likely lose their anti-apoptotic activity over time and eventually become sensitive to the killing signal. As such, deregulated activation of NF-κB may alter the fate of the activated T cells, causing abnormal T cell growth. One example is the T cells infected with human T cell leukemia virus. Because human T cell leukemia virus induces constitutive NF-κB activity (87), the virus-infected T cells are remarkably insensitive to Fas-induced apoptosis (88), a property associated with their transformation (89). How exactly NF-κB prevents Fas-mediated apoptosis remains unclear; however, induction of
apoptosis-inhibitory genes by this transcription factor may play a primary role. A large number of anti-apoptotic genes have been shown to be under NF-κB control; these include A20 (90), IAP-1 and IAP-2 (91, 92), TRAF1 and TRAF2 (92), Bcl-xL (93), and IEX-IL (94). Most of these genes have been shown to prevent tumor necrosis factor-α-induced cell death. Bcl-xL has also been shown to inhibit AICD (95). Given the potent anti-apoptosis-inhibitory function of NF-κB in diverse cell systems, it is anticipated that more apoptosis-inhibitory genes are regulated by this transcription factor. The IKK-deficient and genetically reconstituted cell lines provide a powerful system for isolating novel NF-κB target genes that may mediate apoptosis inhibition in T cells.

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