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An IL-2-Dependent Switch Between CD95 Signaling Pathways Sensitizes Primary Human T Cells Toward CD95-Mediated Activation-Induced Cell Death

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The CD95 (APO-1/Fas) system plays a critical role in activation-induced cell death (AICD) of T cells. We previously described two distinct CD95 (APO-1/Fas) signaling pathways: 1) type I cells show strong death-inducing signaling complex (DISC) formation and mitochondria-independent apoptosis and 2) DISC formation is reduced in type II cells, leading to mitochondria-dependent apoptosis. To investigate the relevance of these pathways, we set up an in vitro model that mimics the initiation and the down phase of an immune response, respectively. Freshly activated human T cells (initiation) are resistant toward CD95-mediated AICD despite high expression of CD95. We previously reported that these T cells show reduced DISC formation. In this study, we show that freshly activated T cells are CD95-type II cells that show high expression levels of Bel-2 and display a block in the mitochondrial apoptosis pathway. Furthermore, we show that, upon prolonged culture (down phase), human T cells undergo a switch from type II to type I cells that renders T cells sensitive to CD95-mediated AICD. Finally, we demonstrate that this switch is dependent on the presence of IL-2. Our observations reveal for the first time that the existence of coexisting CD95 signaling pathways is of physiological relevance. The Journal of Immunology, 2003, 171: 2930–2936.

Upon encounter of Ag, T cells proliferate and exert effector functions such as production of cytokines and lysis of target cells. After elimination of the Ag, T cells are removed and only few survive as memory T cells. Removal of T cells occurs by apoptosis and is called activation-induced cell death (AICD). In CD4+ T cells, AICD is mainly mediated by the CD95 system (1). However, other mechanisms like TNFR2 activation, reactive oxygen species, or Bim-mediated mitochondrial cytochrome c release may also contribute to AICD (2).

CD95 belongs to the family of death receptors (3). Triggering of CD95 leads to clustering of the receptor and formation of a death-inducing signaling complex (DISC) comprised of the adaptor molecule Fas-associated death domain (FADD), procaspase-10, and pro-caspase-8 (4). Recruitment of pro-caspase-8 to the DISC leads to autoproteolytic activation of caspase-8 (5). Activation of caspase-8 at the DISC can be counteracted by cellular (c)-FLIP proteins (6). The short splice variant of c-FLIP, c-FLIPS, has been reported to confer resistance to CD95-mediated apoptosis in primary human T cells upon costimulation with CD28 (7) and in germinal center B cells upon costimulation via CD40 (8–10). Moreover, FLIP proteins have been reported to play a role in tumorigenesis (11, 12) and recent reports suggest that they might contribute to the proposed costimulatory potential of CD95 during T cell activation (13–15).

Our laboratory has identified two cell types, termed type I and type II, with respect to signaling pathways triggered by CD95 (16). In type I cells, induction of apoptosis is accompanied by activation of large amounts of caspase-8 at the DISC directly initiating a caspase cascade. In contrast, in type II cells DISC formation is markedly reduced and strong activation of caspases occurs after loss of the mitochondrial transmembrane potential (ΔψM) (16). Moreover, FLIP proteins have been reported to play a role in tumorigenesis (11, 12) and recent reports suggest that they might contribute to the proposed costimulatory potential of CD95 during T cell activation (13–15). Our laboratory has identified two cell types, termed type I and type II, with respect to signaling pathways triggered by CD95 (16). In type I cells, induction of apoptosis is accompanied by activation of large amounts of caspase-8 at the DISC directly initiating a caspase cascade. In contrast, in type II cells DISC formation is markedly reduced and strong activation of caspases occurs after loss of the mitochondrial transmembrane potential (ΔψM) (16). Moreover, FLIP proteins have been reported to play a role in tumorigenesis (11, 12) and recent reports suggest that they might contribute to the proposed costimulatory potential of CD95 during T cell activation (13–15). Our laboratory has identified two cell types, termed type I and type II, with respect to signaling pathways triggered by CD95 (16). In type I cells, induction of apoptosis is accompanied by activation of large amounts of caspase-8 at the DISC directly initiating a caspase cascade. In contrast, in type II cells DISC formation is markedly reduced and strong activation of caspases occurs after loss of the mitochondrial transmembrane potential (ΔψM) (16). Moreover, FLIP proteins have been reported to play a role in tumorigenesis (11, 12) and recent reports suggest that they might contribute to the proposed costimulatory potential of CD95 during T cell activation (13–15).
We have previously reported that short-term activated compared with long-term activated T cells, mimicking T cells at the initiation and at the down phase of the immune response, respectively, show impaired DISC formation (26). This implies that primary human T cells shortly after activation imitate type II cells while they become type I cells upon prolonged stimulation. However, type II cell lines are readily sensitive to CD95-mediated apoptosis while short-term activated T cells are resistant. To resolve the molecular events involved in this switch, we investigated mitochondridial changes during CD95-mediated apoptosis in short-term and long-term activated primary T cells as well as the role of IL-2 in this process.

Materials and Methods

Abs and reagents

The mAbs against FADD, extracellular signal-regulated kinase (Erk) 1, cytochrome c, and the polyclonal Ab against Bcl-xL were purchased from BD Biosciences (Heidelberg, Germany). The anti-caspase-8 mAb C15, the anti-c-FLIP mAb NF6, the agonistic anti-CD95 mAb anti-APO-1 and leucine zipper tagged (LZ)-CD95L were described previously (26–29). The HRP-conjugated goat anti-rabbit IgG and the polyclonal anti-CD95 Ab (C-20) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The HRP-conjugated goat anti-mouse IgGl and IgG2b were purchased from Southern Biotechnology Associates (Birmingham, AL). All other chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO).

Preparation of primary T cells

Human peripheral T cells were prepared as described previously (30) and were >90% CD3 positive and CD95 low or negative. For activation, resting T cells (day 0) were cultured at 2 × 10⁶ cells/ml with 1 µg/ml PHA for 16 h (day 1). Day 1 T cells (≥95% CD3 positive) were then washed three times and cultured for an additional 5 days in the absence or presence of 25 U/ml IL-2 (day 2). On day 6, dead cells (∼30% or <5% in the absence or presence of IL-2, respectively) were removed by density centrifugation. Cells were washed and resuspended in fresh medium.

Immunoprecipitation and Western blot

Immunoprecipitation of the CD95 DISC was conducted as described elsewhere (31). Briefly, 2 × 10⁶ cells either unstimulated or treated with 2 µg/ml anti-APO-1 or 2 µg/ml LZ-CD95L for 5 min were lysed in lysis buffer (20 mM Tris-HCl (pH 7.4), 1% Triton X-100, 10% glycerol, 150 mM NaCl, 1 mM PMSF, and protease inhibitors (complete; Roche, Mannheim, Germany)) for 15 min on ice and centrifuged (15 min, 14,000 × g). Subsequently, the DISC was precipitated with protein A beads (Sigma-Aldrich) for 4 h at 4°C. For Western blot analysis, postnuclear supernatant equivalents of 10⁶ cells or 30 µg of protein were used.

In vitro caspase-8 cleavage assay

Pro-caspase-8/a was in vitro translated using a T7 polymerase-directed reticulate lysate system (TNT; Promega, Madison, WI). In vitro cleavage assays were performed as described previously (5).

Surface staining

Briefly, 5 × 10⁶ cells were incubated with 1 µg/ml anti-APO-1 for 15 min at 4°C, washed with PBS, incubated for another 15 min with PE-labeled goat anti-mouse Abs (Dianova, Hamburg, Germany), and analyzed by FACSscan (BD Biosciences, Heidelberg, Germany).

Cytotoxicity assay

Briefly, 10⁶ cells were stimulated with 1 µg/ml anti-APO-1 plus 10 ng/ml protein A, 1 µg/ml LZ-CD95L, or left untreated for 18 h at 37°C. Apoptosis was analyzed by FACSscan according to the method of Nicoletti et al. (32). Specific apoptosis was calculated as follows: (percent experimental apoptosis − percent spontaneous apoptosis)/100 (100 − percent spontaneous apoptosis) × 100.

Determination of mitochondrial membrane potential

Anti-CD95 (1 µg/ml)-treated or untreated cells (5 × 10⁷) were incubated with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl carbocyanine iodide (JC-1, 5 µg/ml; Molecular Probes, Eugene, OR) for 20 min at room temperature in the dark followed by FACSscan analysis.

Results

Short-term activated T cells are resistant CD95-type II cells

To investigate the molecular mechanisms of AICD during an immune response, we have set up an in vitro model using primary human T cells (30). T cells were purified from peripheral blood (resting T cells, day 0), activated overnight with PHA (short-term activated T cells, day 1), and kept in culture with IL-2-containing medium for an additional 5 days (long-term activated T cells, day 6). Resting and short-term activated T cells are resistant toward CD95-mediated apoptosis, whereas long-term activated day 6 T cells are sensitive (26, 30, 33). Similar results were obtained using agonistic anti-CD95 Abs (anti-APO-1) or trimerized CD95L (CD95L/CD178) (Fig. 1A). Both short-term and long-term acti-
vated T cells express high amounts of CD95 (30). However, DISC formation is reduced in short-term activated day 1 T cells as compared with long-term activated day 6 T cells (26). This suggests that, with respect to DISC formation, day 1 T cells behave like type II cells (low levels of DISC formation), whereas day 6 T cells behave like type I cells (high levels of DISC formation). Type II cell lines are readily sensitive toward CD95-mediated apoptosis and, despite reduced DISC formation, DISC activity can be detected (34). However, only in type II, but not in type I cells, CD95-mediated apoptosis can be blocked by high expression of antiapoptotic Bcl-2 family members (16). To test whether day 1 T cells can be characterized as type II cells, we 1) performed an in vitro caspase-8 cleavage assay to detect DISC activity and 2) analyzed the mitochondrial integrity of day 1 T cells upon CD95 triggering. Day 1 T cells were stimulated with LZ-CD95L or left untreated. T cells were lysed, CD95 was immunoprecipitated, and immunoprecipitates were incubated with radioactively labeled pro-caspase-8. The DISC of day 1 T cells can cleave in vitro-translated caspase-8 as shown by detection of its cleavage products p43 (representing the initial cleavage step between the caspase homology domains), p26 (representing the prodomain), p18, p12, and p10 (representing the mature caspase-8 subunits), indicating its activity (Fig. 1B). Thus, day 1 T cells resemble CD95-type II cells in terms of both DISC formation and DISC activity.

Resistance of short-term activated T cells is associated with Bcl-xL expression and mitochondrial integrity

The mitochondrial integrity of day 1 as compared with day 6 T cells upon CD95 triggering was assessed by analysis of the ΔψM and release of cytochrome c. Day 1 and day 6 T cells were stimulated for different time periods with agonistic mAbs against CD95 (anti-APO-1) and stained with JC-1 to test the integrity of ΔψM. After 4 h of stimulation with anti-APO-1, day 1 T cells did not show a significant decrease in ΔψM (Fig. 2A). In contrast, in day 6 T cells ΔψM dropped already after 1 h. Cytochrome c release into the cytosol was investigated as a second indicator for mitochondrial changes during CD95-mediated apoptosis of primary T cells. After stimulation with anti-APO-1 for 2 h, day 1 and day 6 T cells were subjected to limited lysis with digitonin and resulting cytosolic extracts were analyzed by Western blotting. Cytochrome c could only be detected in stimulated day 6 T cells while stimulated day 1 T cells or unstimulated samples showed no translocation of cytochrome c into the cytosol (Fig. 2B). Equal protein loading was controlled by reprobing the blot for the cytosolic kinase Erk-1. Western blotting for cytochrome c oxidase (subunit IV) showed that the samples were not contaminated with mitochondrial fractions (data not shown). Bcl-xL has been shown to be up-regulated in day 1 T cells while Bcl-2 and Bax expression are not modulated (33). Therefore, T cells were lysed on each day of the culture period and Western blot analysis for Bcl-xL was performed (Fig. 2C). Bcl-xL was expressed at a very low level in resting T cells (day 0) but was highly up-regulated upon activation (day 1). Protein expression of Bcl-xL was barely detectable at day 6 of culture. Thus, Bcl-xL expression correlated with resistance to CD95-mediated apoptosis. In conclusion, short-term activated day 1 T cells form small amounts of an active DISC, but show inhibition of CD95-mediated apoptosis at the level of mitochondria. Therefore, they can be characterized as CD95-type II cells.

IL-2 renders T cells sensitive toward CD95-mediated apoptosis

IL-2 has been described as critical for sensitization of primary T cells toward apoptosis (22). Furthermore, in a mouse model IL-2 down-regulates c-FLIP L and up-regulates CD95L (25). Therefore, we wanted to characterize the influence of IL-2 on resistance and sensitivity of primary human T cells toward CD95-mediated apoptosis. To address the role of IL-2 in the T cell response in our in vitro system, we prepared short-term activated T cells (day 1) as before and cultured them subsequently in the absence or presence of IL-2 (no significant amounts of endogenous IL-2 could be detected in the supernatant of T cell cultures without exogenous IL-2, data not shown). As reported before (Fig. 1B and Refs. 26, 30, and 33), day 0 and day 1 T cells were resistant toward CD95-mediated apoptosis, while day 6 T cells cultured in the presence of IL-2 were sensitive (Fig. 3A). Apoptosis was significantly reduced in day 6 T cells when exogenous IL-2 was omitted from the culture (Fig. 3A). However, the degree of resistance varied among >45 donors from virtually complete inhibition to a residual sensitivity of 15–20% (3A, cf. donors 1 and 2). Analysis of CD95 expression on the cell surface showed comparable levels of CD95 on day 6 T cells cultured with or without IL-2. Judged by mean fluorescence intensity, day 6 T cells cultured without IL-2 showed slightly less (~1.5- to 2-fold) CD95 expression than day 6 T cells cultured with IL-2 (Fig. 3B). Like CD95, expression of the activation marker CD69 was comparable in cultures with or without IL-2 (data not shown), suggesting that T cell activation was not dependent on IL-2. However, T cell proliferation was dependent on IL-2 as judged by CFSE labeling (data not shown). The major fraction of

![FIGURE 2.](http://www.jimmunol.org/)

**FIGURE 2.** Mitochondria from short-term activated T cells are protected from apoptotic changes. A, Loss of ΔψM in long-term but not short-term activated T cells. Day (d) 1 and day 6 T cells were incubated for the indicated time periods with 1 μg/ml anti-APO-1 and 10 ng/ml protein A, stained with JC-1, and subsequently analyzed by FACS. B, Cytochrome c (Cyt c) release in long-term but not short-term activated T cells. Day 1 and day 6 T cells were incubated for 2 h with 1 μg/ml anti-APO-1 and 10 ng/ml protein A or left untreated. Cytosolic extracts were prepared by limited lysis with digitonin, resolved by 15% SDS-PAGE, and analyzed by Western blotting using anti-cytochrome c Abs. The blot was reprobed with anti-Erk-1 Abs to check for equal protein loading. C, Expression of Bcl-xL in primary human T cells. Postnuclear supernatants were prepared from T cells on days 0, 1, and 6 of the culture period, resolved by 12% SDS-PAGE, and analyzed by Western blotting using anti-Bcl-xL Abs. The blot was reprobed with anti-Erk-1 Abs to control equal protein loading.
day 6 T cells cultured in the presence of IL-2 divided approximately three times, whereas a smaller fraction (10–20%) only underwent one or two cell divisions. In the absence of IL-2, virtually no cell division and no inhomogeneity of cell populations could be observed.

**IL-2 does not influence expression of DISC components**

To investigate whether the presence of IL-2 has any influence on the expression of known DISC components, we prepared lysates of day 0, day 1, and day 6 T cells cultured with or without IL-2. Western blot analysis revealed that c-FLIPL was equally expressed in all T cells tested (Fig. 4A). Interestingly, c-FLIPS was expressed in day 1 T cells. This is in agreement with our previous results that c-FLIPS is up-regulated in T cells stimulated via the TCR and CD28 (7). Reprobing the blot with Abs against caspase-8 and FADD showed that these proteins were also expressed at comparable levels (Fig. 4A). Therefore, the key DISC components are expressed equally in resistant day 6 and sensitive IL-2-treated day 6 T cells.

**IL-2 affects DISC formation and mitochondrial apoptotic signaling**

Next, we tested DISC formation in day 6 T cells cultured with or without IL-2. Because cultures without IL-2 contained many dead cells due to background apoptosis, dead cells were removed by density centrifugation. After washing, cells were rested for at least 2 h and subsequently stimulated with LZ-CD95L or left untreated. T cells were lysed, CD95 was immunoprecipitated, and immunoprecipitates were analyzed by Western blotting. Probing with Abs against FADD, caspase-8, and c-FLIP showed stimulation-dependent DISC formation in IL-2-treated day 6 cells (Fig. 4B). In contrast to IL-2-treated day 6 T cells, day 6 T cells cultured without IL-2 showed strongly reduced DISC formation. FADD and caspase-8 were hardly detectable in immunoprecipitates from anti-CD95-stimulated cells. Interestingly, the p43 cleavage intermediate of c-FLIPL was detectable although its recruitment was also reduced when compared with IL-2-treated cells (Fig. 4B). Therefore, the ratio of caspase-8:c-FLIP seems to change in an IL-2-dependent manner, suggesting that c-FLIPL might contribute to resistance observed in day 6 T cells cultured without IL-2. Alternatively, the observed differences might be due to the high sensitivity of the NF6 Ab (26). Day 6 T cells cultured without IL-2 are similar to day 1 T cells with respect to the amount of DISC formed upon stimulation (26). We therefore wanted to know whether DISC activity, despite reduced DISC formation, was detectable as in type II cells and day 1 T cells. We performed an in vitro caspase-8 cleavage assay revealing DISC activity also in day 6 T cells cultured without IL-2 (Fig. 4C). The observation of DISC activity in day 6 T cells in the absence of IL-2 implies that 1) these cells impose like type II cells and 2) that c-FLIP, which acts directly at the DISC (6), plays only a minor role in regulating sensitivity or resistance of the T cells in this study.

Our finding that long-term activated T cells resemble type I cells is in agreement with a report showing that Bcl-2 transgene does not influence expression of DISC components, rendering mitochondria susceptible to apoptotic stimuli in long-term activated (day 6) T cells.

**Discussion**

Using an in vitro human T cell culture model, we have previously shown that short-term activated T cells are resistant to CD95-mediated apoptosis while long-term activated T cells, representing the down phase of an immune response, are sensitive (26, 30, 33, 35). Thus, we could show that the resistance of short-term activated T cells to AICD is in part due to a reduction in DISC formation (26). In this study, we show that these short-term activated T cells can be characterized as CD95-type II cells, as 1) the DISC, despite formation of low amounts, is active and 2) inhibition of the mitochondrial apoptosis pathway, presumably due to high expression of Bcl-xL, leads to resistance toward CD95-mediated apoptosis. Long-term activated T cells form high amounts of the DISC (26) and impose, therefore, as type I cells. In addition, Bcl-xL is down-regulated in these cells (Fig. 2C and Ref. 33), resulting in a loss of $\Delta$ψM and cytochrome c release. Thus, primary T cells switch from type II to type I cells during prolonged activation. Furthermore, we show that the switch from CD95 type II to CD95 type I is dependent on IL-2, providing insight as to how this cytokine might act as a sensitizer toward AICD. Our observation that primary human T cells undergo a switch between the two CD95 signaling pathways reveals for the first time that the existence of coexisting CD95 signaling pathways is of physiological relevance.

Our finding that long-term activated T cells resemble type I cells is in agreement with a report showing that a Bcl-2 transgene does...
not protect long-term activated T cells from AICD (36). After activation for 5 days with Con A and IL-2, peripheral T cells from Bcl-2-transgenic mice died to the same extent as T cells from control mice. Unfortunately, resting or short-term activated T cells were not included in that study.

It should be noted that, although the purity of T cells was between 90 and 95% in all experiments analyzed, we observed differences between donors in several parameters. Sensitivity of day 6 T cells toward CD95-mediated apoptosis in the absence of IL-2 varied from 0 to 20%. Nevertheless, we consistently observed IL-2-dependent increases in apoptosis sensitivity of generally ≥60%. These differences correlate with the biochemical parameters of DISC formation observed in the T cells. We also observed differences in the number of cell divisions assessed by CFSE labeling. The major fraction of day 6 T cells cultured in the presence of IL-2 divided approximately three times, whereas a smaller fraction (10–20%) only underwent one or two cell divisions. In the absence of IL-2, virtually no cell division and no inhomogeneity of cell populations could be observed (data not shown). These data agree with the role of IL-2 as a major cytokine for T cell proliferation during activation. Thus, we assume that our biochemical data largely represent cells having undergone three divisions when cultured with IL-2 and no division when cultured without IL-2. As we observed an inhomogeneity only under conditions of maximal DISC formation,

**FIGURE 4.** Influence of IL-2 on DISC formation in day (d) 6 T cells. A, Postnuclear supernatants of day 0, day 1, and day 6 T cells cultured with or without 25 U/ml IL-2 were prepared, resolved by 12% SDS-PAGE, and analyzed by Western blotting using anti-c-FLIP, anti-caspase-8 and anti-FADD Abs. B, T cells were cultured until day 6 with or without 25 U/ml IL-2. After removing dead cells, T cells were stimulated with 2 μg/ml LZ-CD95L for 5 min at 37°C or left untreated, lysed, and CD95 was immunoprecipitated with protein A-Sepharose. Subsequently, immunoprecipitates were resolved on 12% SDS-PAGE and analyzed by Western blotting using anti-CD95 (C-20), anti-FADD, anti-caspase-8, and anti-c-FLIP Abs. C, Activity of the CD95 DISC in day 6 T cells cultured with or without IL-2. Day 6 T cells were stimulated with LZ-CD95L for 5 min at 37°C or left untreated. CD95 was precipitated as described above. 35S-Labeled in vitro-translated pro-caspase-8/a was incubated with unstimulated (−) or stimulated (+) CD95 precipitates or without precipitates (input) for 24 h and subsequently subjected to 15% SDS-PAGE.

**FIGURE 5.** Influence of IL-2 on mitochondria in day 6 T cells. Loss of ΔΨm in day 6 T cells cultured in the absence or presence of IL-2. T cells were incubated for the indicated time periods with 1 μg/ml anti-APO-1 and 10 ng/ml protein A, stained with JC-1, and subsequently analyzed by FACS.
inclusion of the minor fraction having undergone fewer divisions would rather lead to an underestimation of the effects observed. It would be nevertheless of interest to investigate the importance of proliferation for AICD in greater detail, a study envisaged in the future.

In our model system, the key DISC components are equally expressed in resistant day 6 and sensitive IL-2-treated day 6 T cells. Previous data with variable CD95 expression on normal and malignant cells make it unlikely that, although day 6 T cells expressed 1.5- to 2-fold more CD95 on the cell surface when cultured with IL-2, this slight increase accounts for the approximately 8-fold difference in apoptosis sensitivity and the strong difference in DISC formation. However, others observed down-regulation of c-FLIP upon T cell activation (25, 37, 38). These differences may be due to different detection methods as protein and mRNA expression of c-FLIP do not necessarily correlate (38, 39). Alternatively, different conditions of T cell stimulation may account for these differences since it was previously shown by DNA microarray analysis that activated T cells express different sets of mRNAs depending on the activation stimulus (40). Another possibility is that c-FLIP expression is differentially regulated in different lymphoid tissues. Whereas we analyzed peripheral blood T cells, Refaeli et al. (25) investigated lymph node and spleen cells. In line with this hypothesis is the finding that c-FLIP is highly expressed in lymph nodes of IL-2-deficient mice but not in splenocytes or thymocytes (41). These three possibilities are not mutually exclusive.

It has been reported previously that induction of cell cycle progression, especially at the G1-S transition or at a late G1 phase checkpoint, induces sensitivity toward AICD (42–45). In addition, it was shown that expression levels of c-FLIP are reduced during the S phase as compared with the G1 phase of the cell cycle (38). We cannot exclude that T cells express different amounts of c-FLIP proteins depending on the stage of the cell cycle. However, we have not detected any down-regulation of c-FLIP in long-term activated T cells although these cells have divided several times during the culture period (26). In addition, consistent with our model, DISC activity was readily detectable in short-term activated as well as in long-term activated T cells cultured in the absence of IL-2, suggesting that c-FLIPN, which exerts its function directly at the DISC level, plays only a minor role in resistance of short-term activated T cells (26, 46).

This study focused on the proapoptotic effect of IL-2 on peripheral human T cells as IL-2 seems to be the major cytokine involved in priming T cells for AICD. The IL-2R belongs to the family of common γ-chain containing cytokine receptors. It might be interesting to explore the effect of other cytokines binding to common γ chain receptors, like IL-4, IL-7, IL-9, and IL-15. The latter is probably the most interesting as it shares also the IL-2Rβ subunit with the IL-2R. Moreover, it was shown that IL-15 mediates resistance toward AICD in T cells (47). Thus, a possible role of IL-15 in our in vitro system awaits further investigation.

The pathways by which IL-2 contributes to survival and proliferation of T cells are well characterized (48–50). In contrast, the pathways that contribute to the proapoptotic function of IL-2 and those that, in particular, influence DISC formation remain elusive. A direct effect of IL-2 signaling on the DISC seems unlikely because T cells need to be cultured for several days in IL-2-containing medium to become sensitive to CD95-mediated apoptosis. Therefore, gene transcription or IL-2-driven differentiation processes are likely to be involved.

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