

TRAIL and thymocyte apoptosis: not so deadly?

Dear Editor,

Death receptor/death ligand interactions have been recognized as important triggers of cell death in various cell types. So far, best understood are the signaling events that are activated upon binding of the Fas ligand to the Fas (CD95) receptor, and subsequently lead to apoptosis in the target cell. Fas-mediated apoptosis plays a crucial role in many physiological and pathophysiological events, such as immune homeostasis, immune privilege, tissue turnover, and cell-mediated cytotoxicity.^{1,2} Similar to Fas ligand, TRAIL/Apo-2 ligand (Tumor Necrosis Factor-related apoptosis-inducing ligand) has been proposed to represent an important cytotoxic effector molecule, by which cytotoxic T cells and natural killer cells eliminate their target cells. TRAIL has particularly received strong scientific attention due to its proposed role in tumor immune surveillance and its potential therapeutic application in cancer patients.³

Apart from tumor immunity, however, TRAIL has also been implicated in various other aspects of immune cell regulation. For example, neutralization of TRAIL causes an exacerbation of experimental rheumatoid arthritis, autoimmune encephalomyelitis and diabetes, suggesting that TRAIL-induced apoptosis or effector cell modulation represents an important regulatory process in the pathogenesis of autoimmune diseases. Recently, Lamhamedi-Cherradi *et al.*⁴ have proposed TRAIL/TRAIL receptor-mediated cell death as an underlying mechanism of thymic negative selection. Thymocytes from TRAIL-deficient mice were found to be more resistant to antigen- or CD3/T cell receptor (TCR)-mediated apoptosis, and soluble TRAIL receptor was reported to block thymic cell death. This finding, however, is quite controversial and has therefore been questioned by other investigators.⁵⁻⁷ For example, Simon *et al.*⁵ have not been able to block thymic negative selection by soluble TRAIL receptor, and Cretney *et al.*⁶ have found normal antigen-driven negative selection in the very same TRAIL-deficient mice. Furthermore, CD3/TCR-mediated apoptosis *in vivo* has been reported to require the release of systemic glucocorticoids and may thus not necessarily reflect the biology of negative selection.⁸ Particularly incompatible with this proposed role of TRAIL in thymic negative selection is the observation that the adaptor molecule FADD (Fas-associated death domain) is a crucial element in the TRAIL-induced signaling pathways leading to apoptosis, however, overexpression of dominant-negative FADD does not alter thymic negative selection.^{7,9} Thus, the role of TRAIL–TRAIL receptor interaction in the establishment of central tolerance is far from being well accepted. The different publications describing TRAIL or TRAIL-R

expression in the thymus, or their involvement in thymic negative selection are summarized in Table 1. They demonstrate that TRAIL and TRAIL receptor are expressed in the thymus and thymocytes, and that thymocytes are sensitive to TRAIL, yet come to different conclusions regarding the role of TRAIL–TRAIL receptor interaction in TCR-induced thymocyte apoptosis.

The discrepancy between these different reports is difficult to reconcile and may partially depend on the different experimental systems used. We have thus reinvestigated the role of TRAIL in activation-induced apoptosis in thymocytes using TRAIL-deficient mice, and have extended our experiments to other apoptosis triggers. Both, *in vitro* and *in vivo* activation of thymocytes by CD3/TCR ligation using anti-CD3 ϵ -crosslinking antibodies caused massive induction of apoptosis in CD4⁺CD8⁺ immature thymocytes (Figure 1a and b). Importantly, double-positive thymocytes from wild type and TRAIL-deficient mice expressed low but detectable levels of TRAIL-R (as assessed by flow cytometry), which became upregulated upon stimulation with anti-CD3 (data not shown). In agreement with the finding of Lamhamedi-Cherradi *et al.*,⁴ we found a significant inhibition of anti-CD3-induced apoptosis in TRAIL-deficient thymocytes, *in vitro* as well as *in vivo*. This would indicate that TRAIL might play an important role in CD3/TCR-mediated thymocyte apoptosis and possibly also negative selection.

Death ligand/death receptor interactions are not only involved in activation-induced apoptosis and cell-mediated cytotoxicity, but have also been implicated in apoptosis induction upon DNA damage.^{1,10} DNA damage may induce the expression of death receptors and also death ligands through p53- and/or Jun kinase/NF κ B-dependent mechanisms. Since thymocytes are exquisitely sensitive to DNA damage, we analyzed thymocyte apoptosis in response to UV and γ -irradiation *in vitro* and *in vivo* as well as other cell death triggers, that is, the glucocorticoid dexamethasone and Fas ligation in TRAIL-deficient and control mice. Surprisingly, we observed that UV- and γ -irradiation as well as glucocorticoid-induced apoptosis was significantly attenuated in TRAIL-deficient thymocytes. In marked contrasts, however, induction of Fas receptor-mediated apoptosis occurred with similar kinetics in wild type and TRAIL-deficient thymocytes (Figure 1a and b).

Apoptosis resistance of TRAIL-deficient thymocytes does thus not appear to be restricted to CD3/TCR-mediated apoptosis, but extends to other apoptosis triggers, with the exception of Fas. While it is conceivable that DNA damage-induced apoptosis in thymocytes may involve TRAIL/TRAIL receptor interaction, there is no evidence so far that glucocorticoids signal via TRAIL. So far, the key mediators of glucocorticoid-induced

Table 1 List of publications describing TRAIL or TRAIL-R expression in the thymus, or investigating TRAIL/TRAIL-R interaction in thymocyte negative selection

Publication	Species	TRAIL expression	TRAIL-R expression	TRAIL-ind. apoptosis	Role in neg. selection	Model system for neg. selection	<i>In vivo</i> / <i>in vitro</i>	Mechanism of TRAIL neutralization
Lamhamedi-Cherradi <i>et al</i> ⁴	Mouse	n.a.	n.a.	n.a.	Yes	Anti-CD3, antigen,	<i>In vivo</i> , <i>In vitro</i>	Soluble rec., TRAIL-KO
Cretney <i>et al</i> ⁶	Mouse	n.a.	n.a.	n.a.	No	Anti-CD3, superantigen	<i>In vivo</i> , <i>In vitro</i>	Anti-TRAIL, TRAIL KO
Simon <i>et al</i> ⁵	Human	n.a.	Yes (protein)	Yes	No	Anti-CD3, superantigen	<i>In vitro</i>	Soluble rec.
Abdalla <i>et al</i> ¹	Chicken	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Camacho <i>et al</i> ²	Mouse	Yes (RNA)	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.
Pan <i>et al</i> ³	Human	n.a.	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.
Wiley <i>et al</i> ⁴	Human	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Newton <i>et al</i> ²⁵	Mouse	Yes (RNA)	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.
Chaudhary <i>et al</i> ²⁶	Human	n.a.	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.
Walczak <i>et al</i> ²⁷	Human	Yes (RNA)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a., not analyzed; sol. rec., soluble TRAIL receptor

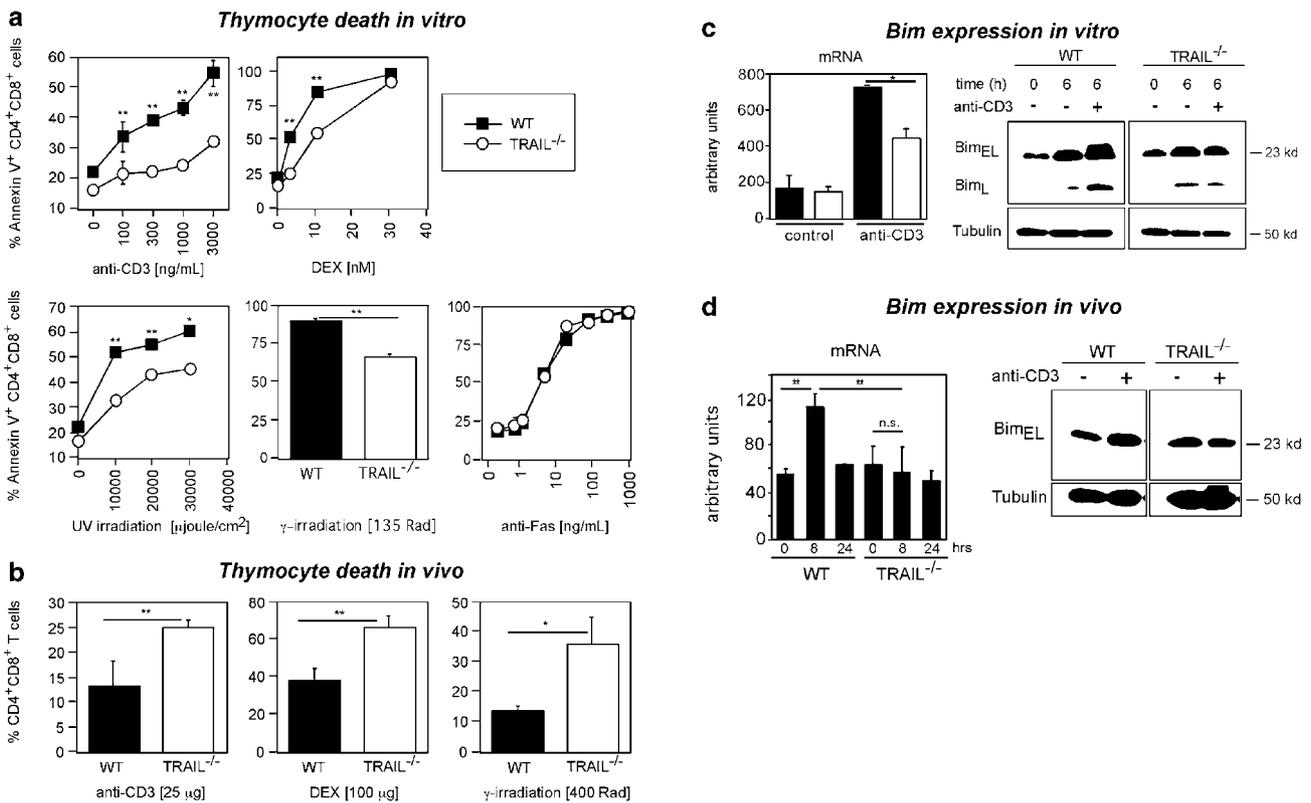


Figure 1 Induction of apoptosis in wild type and TRAIL-deficient thymocytes *in vitro* and *in vivo*. (a) Thymocyte apoptosis *in vitro*. Thymocytes from age- and sex-matched wild-type or TRAIL-deficient Balb/c mice were isolated and exposed to various doses of plate-bound anti-CD3 (0–3 µg/ml) plus soluble anti-CD28 (10 µg/ml), the glucocorticoid dexamethasone (Dex, 0–40 nM), UV-irradiation (0–40 000 µJ/cm²), γ -irradiation (135 rad), or anti-Fas antibody (Jo-2, 0–1000 ng/ml plus 0.1 µM cycloheximide). Apoptosis in CD4⁺ CD8⁺ thymocytes was assessed 18 h later by Annexin V staining and flow cytometry. (b) Thymocyte apoptosis *in vivo*. Age- and sex-matched wild-type or TRAIL-deficient mice were injected with anti-CD3 (25 µg) or dexamethasone (Dex, 100 µg), or irradiated with 400 rad. After 24 (γ -irradiation, Dex) or 48 h (anti-CD3) thymocytes were isolated and the percentage of CD4⁺ CD8⁺ thymocytes was assessed by flow cytometry. Typical experiments out of 3–5 are shown. Statistics was assessed by Student's *t*-test. *indicates $P < 0.05$, ** $P < 0.01$. (c) Bim expression *in vitro*. Thymocytes were left untreated or anti-CD3 stimulated *in vitro* and Bim mRNA expression (after 4 h) was assessed by real-time RT-PCR (filled bars, wild type, WT; unfilled bars, TRAIL-deficient) and protein expression (Bim_{EL} and Bim_L) (after 0 h and 6 h, control and anti-CD3) by Western blot. (d) Bim expression *in vivo*. Wild-type (WT) or TRAIL-deficient mice were injected with 25 µg anti-CD3, and thymocytes were harvested after 0, 8 and 24 h for analysis of Bim mRNA expression by real-time RT-PCR or protein levels (0 and 8 h) by Western blotting

thymocyte apoptosis remain elusive, albeit members of the BH3-only protein family may contribute to this form of apoptosis.^{11–13} It is well established that antigen-, glucocorti-

coid-, UV and γ -irradiation-induced apoptosis in thymocytes proceeds predominantly via the activation of proapoptotic members of the Bcl-2 family associated with changes in the

mitochondrial membrane permeability, causing the release of proapoptotic factors (e.g. cytochrome *C* or Smac/DIABLO) and subsequent caspase activation. Thus, apoptosis induced by these triggers is effectively antagonized by the antiapoptotic molecule Bcl-2 or its prosurvival homologues. In contrast, Fas ligation in thymocytes leads to a direct activation of caspase-8 and -3 rather than amplification of the signal through Bid cleavage and cytochrome *C* release, as proposed for certain cell types (e.g. hepatocytes¹⁴). Consistently, Fas ligation-induced apoptosis of thymocytes is not modified by overexpression of a Bcl-2 transgene.^{15,16}

Our observations suggest that TRAIL may sensitize thymocytes to mitochondria-dependent but not mitochondria-independent apoptosis pathways. Interestingly, death receptor ligation does not necessarily cause apoptosis, but may also lead to Jun kinase and NF κ B activation enhancing cell survival or activation. These nonapoptotic pathways do not necessarily require FADD, but can proceed via the adaptor molecules RIP-kinase.¹⁷ It is therefore conceivable that TRAIL receptor ligation of immature thymocytes may cause activation of MAP kinases and/or NF κ B, which in turn may alter apoptosis sensitivity of the cells in a FADD-independent manner, for example, by modulating expression levels of IAP family members. Jun kinase signals, on the other hand, have been shown to modulate expression levels and or function of various pro- and antiapoptotic Bcl-2 homologues, including Bim. Jun kinase regulates Bim transcription in neurons¹⁸ and phosphorylation of Bim on Ser 69, which enhances its proapoptotic activity by inhibiting proteosomal degradation.¹⁹

We have analyzed the expression pattern of pro- and antiapoptotic Bcl-2 members in thymocytes of wild type and TRAIL-deficient mice (i.e. Bim, Bmf, Bid, Bad, Bcl-w, Bcl-x and Bcl-2). No significant differences in protein expression were found in untreated thymocytes from TRAIL-deficient and control mice. However, upon TCR stimulation *in vitro* and *in vivo* wild-type thymocytes showed a more pronounced increase in both, Bim mRNA expression and protein levels than TRAIL-deficient thymocytes (Figure 1c and d). Bim therefore represents an attractive target of TRAIL-mediated sensitization since it is well established to be crucial in thymic negative selection.²⁰ Bim-deficient thymocytes show a similar or even stronger resistance to apoptosis induction by anti-CD3, UV, γ -irradiation and dexamethasone than TRAIL-deficient mice (N Corazza, T Brunner, unpublished results). In addition, the proapoptotic activity of Bim is not only reflected in its expression levels *per se*, and TRAIL-mediated signals may possibly modulate its subcellular localization and/or its release from microtubules that is an upstream signaling event in CD3/TCR-mediated apoptosis *in vivo*.²⁰ However, it remains to be further determined how TRAIL modulates thymocyte sensitivity. Most likely, sensitization is a process, which requires a certain amount of time, since, in contrast to Lamhamedi-Cherradi *et al.*,⁴ we were unable to specifically block apoptosis in wild-type thymocytes by the addition of soluble TRAIL receptor (data not shown). Similarly, injection of recombinant TRAIL into TRAIL-deficient mice for 12 h did not sensitize thymocytes to subsequent *ex vivo* apoptosis induction, nor could we detect TRAIL-induced apoptosis *in vitro* (data not shown).

In conclusion, TRAIL appears to have a significant apoptosis-modulating activity in thymocytes *in vivo* since

TRAIL-deficient thymocytes show a marked decrease in apoptosis sensitivity to mitochondria-dependent pathways but not Fas-induced apoptosis. However, our data also suggest that this apoptosis-modulating activity is not restricted to antigen-driven thymic negative selection and is thus likely not a direct mechanism of thymic central deletion. Bim has been implicated in various forms of thymocyte apoptosis, including negative selection. Here, we support the notion that TRAIL ligation may regulate thymocyte apoptosis through the control of the mitochondrial apoptosis pathways, possibly through the control of Bim expression.

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