To the editor:

Is Toso an antiapoptotic protein or an Fc receptor for IgM?

We have read with great interest the publication by Nguyen et al entitled “Toso regulates the balance between apoptotic and nonapoptotic death receptor signaling by facilitating RIP1 ubiquitination.”1 Because there are important discrepancies between their results1 and ours2 with regard to the ligand specificity of the cell-surface Toso protein, we wish to provide comments and some

Figure 1. Effect of FcR/H9262R on Fas-mediated apoptosis in Jurkat cells. (A) Jurkat cells transduced with the bicistronic retroviral construct containing both human FcμR and GFP cDNAs (FcμR/GFP) or only GFP cDNA (GFP) as a control were incubated with biotin-labeled, anti-FcμR/Toso mAbs (HM7 [γ2b], HM14 [γ1x] or 1E4 [γ1x]; Abnoca Corp) or isotype-matched control mAbs (γ2b or γ1x) as described.2,4,5 The bound mAbs were detected by addition of phycoerythrin-labeled streptavidin (PE-SA). For IgM binding, cells were incubated with myeloma or hybridoma-derived IgM of both human and mouse origin at 10 μg/mL, washed, and then incubated with biotin-labeled, rat anti–mouse FcμR mAb (clone 332.12) for mouse IgM or mouse anti–human FcμR mAb (clone SA-DA4.4) for human IgM. Biotin-labeled, control mAbs of the same isotype (rat γ2b or mouse γ1x) were also included. Stained cells were analyzed by Accuri C6 Flow Cytometer. Because all staining profiles with control mAbs were indistinguishable, only the result with biotin-labeled mouse IgG1 control mAb is shown for simplicity. Note the expression of FcμR/Toso on FcμR/GFP transductants as determined by reactivity with 3 different mAbs and by IgM-ligand binding. (Only the binding result with mouse IgM antibody is shown.) (B) Both cell lines were cultured at 37°C for 12 (top panel) and 20 hours (bottom panel) without or with agonistic mouse anti–human Fas mAb of IgM (CH11; 10 ng/mL; Millipore) or IgG3 isotype (2R2; 1 μg/mL; Invitrogen) or with a recombinant human FasL (superFasL; Enzo Life Sciences) at 3 different protein concentrations (1, 10 and 100 ng/mL). Cells were stained with 7-aminoactinomycin D (7-AAD) and allophycocyanin (APC)–labeled annexin V to identify early (annexin V/7-AAD–) and late (annexin V+/7-AAD–) apoptotic and dead (annexin V+/7-AAD–) cells by flow cytometry.2 Note the resistance of the FcμR/GFP transductant to Fas-mediated apoptosis by IgM mAb, but not by IgG3 mAb and FasL. These experiments were performed 3 times with essentially the same results.
Response

Antiapoptotic function of Toso (Faim3) in death receptor signaling

We appreciate the interest in our work. In their comment Honjo et al argue that Toso per se would not have ant apoptotic activity on CD95-induced apoptosis,1 as, in contrast to our findings,2 inhibitory activity would only be observed upon on CD95-ligation by the IgM mAb CH11. Our conclusion that Toso exhibits ant apoptotic effects on death receptor signaling is based on multiple lines of experimental evidence. Knockdown of Toso renders cells more susceptible to CD95L-induced apoptosis, while overexpression of Toso results in decreased apoptosis in response to stimulation with CD95L, the natural ligand for CD95. Toso also provides relative protection from TNFα-induced apoptosis and necrosis and TRAIL-induced cell death, suggesting that Toso serves as a general regulator of death receptor signaling. Furthermore, the inhibitory function of Toso in death receptor-induced apoptosis was not only restricted to the human system, but was also observed in experiments using cells from Toso knockout mice. Thus, while Honjo et al draw their conclusion solely from an overexpression study in a single cell type,1 we have confirmed our findings in several different cell types and different species, including Jurkat cells, BJAB cells and human and mouse primary T cells.2 Importantly, the observation of an ant apoptotic function of Toso is further supported by independent studies that also explored apoptosis induced by physiologic death receptor ligands. Song and Jacob demonstrated that overexpression of Toso protects Jurkat cells from CD95L- and TNFα-induced apoptosis and that primary T cells from Toso transgenic mice are relatively resistant to CD95L-induced apoptosis.3 Also, consistent with our results, Richter et al reported that knock-down of Toso increases CD95L-mediated apoptosis in primary human T cells.4 As an additional note, while the specific Jurkat cell line used by

References

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