new findings related to this issue. Toso, named after a Japanese liquor drunk on New Year's day to celebrate long life and eternal youth, was originally designated as an inhibitor of Fas/CD95-induced apoptosis and was also called the Fas apoptotic inhibitory molecule 3.[3] In this originally described apoptosis assay, a mouse monoclonal antibody (mAb) of the IgM isotype (CH11) was used for ligation of Fas. Independently, we cloned a cDNA encoding an IgM Fc receptor (FcμR) based on an IgM binding strategy from cDNA libraries generated from human B-lineage cells including chronic lymphocytic leukemia (CLL) cells.[2] The nucleotide sequence of this FcμR was identical to that of Toso. To determine whether the FcμR inhibits Fas-mediated apoptosis, the apoptosis-prone Jurkat human T-cell line was transduced with bicistronic retroviral constructs containing both FcμR and green fluorescent protein (GFP) cDNAs or the GFP cDNA alone as a control. Cells expressing comparable levels of GFP were enriched from each transductant by FACS and were used in apoptosis assays. The recombinant Fas-ligand (FasL) used by Nguyen et al[1] as well as 2 agonistic Fas-specific mAbs (CH11 [μκ] and 2R2 [γ3κ]) were then used to induce apoptosis of the transductants. The results are shown in Figure 1 and are clearly quite distinct from those reported by Nguyen et al. First, $FcμR^+/GFP^+$, but not GFP^+, cells display IgM binding and are reactive with 3 different mAbs (our HM7 [γ2bκ] and HM14 [γ1κ] anti-FcμR mAbs[2] and 1E4 [γ1κ] anti-Toso mAb[4]; Figure 1A), confirming our previous results.[2,5] Furthermore, Vire et al[6] have also recently described a high level of Toso expression on CLL-B cells together with IgM binding and subsequent internalization; results also consistent with our recent findings.[5] Thus, the lack of IgM binding to Toso reported by the Nguyen group apparently resulted from their usage of transient Toso-transductants, which may not have expressed sufficient levels of Toso to detect IgM binding (see supplemental Figure 12 in Nguyen et al[1]). (By contrast, their functional studies were conducted with stable Toso-transductants.) Second, antiapoptotic activity of Toso/FcμR is observed only when the Fas receptor is ligated by the IgM mAb (CH11), but not when ligated by an IgG3 mAb (2R2) or the native FasL, indicating that Toso/FcμR per se has no inhibitory activity in Fas-mediated apoptosis (Figure 1B). Our results are thus quite different from those of Nguyen, et al who showed that Toso expression on Jurkat cells inhibits FasL-induced apoptosis (see their supplemental Figure 4). The basis for these discrepancies remains unclear and there is a possibility that Toso/FcμR may interact with an additional protein/ligand. An exchange of reagents including Toso/FcμR-transductants would facilitate the resolution of these conflicting data.

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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 antiapoptotic 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 antiapoptotic 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 antiapoptotic 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

Honjo et al[1] seems to be unusually sensitive to CD95L-induced apoptosis (≥ 95% apoptotic cells after only 6 hours of stimulation with 10 ng/mL CD95L), careful analysis of their data presented in Figure 1B still reveals that in response to CD95L-stimulation Toso expressing cells are relatively protected from late apoptosis (there are considerably less late apoptotic cells [= annexinV^+7-AAD^+ cells] in CD95L-stimulated Toso overexpressing cells compared with control cells). Finally, there is not only ample experimental evidence for an antiapoptotic function of Toso in death-receptor biology, but, by the identification of the Toso-RIP1 interaction and the dependence on RIP1 ubiquitination, we also provide a mechanistic basis for the observed antiapoptotic phenotype. In their letter, Honjo et al also provide additional data to support their earlier conclusion[5] that Toso serves as a receptor for IgM. A potential binding of IgM to Toso is of high interest to us, however, despite of using several different experimental models with human and mouse Toso, so far we have not been able to experimentally confirm such an interaction (see supplemental Figures 11-12 in Nguyen et al[2]). The reason for these divergent results is currently unclear, but we agree with Honjo et al that additional ligands for Toso may exist. The identification and characterization of novel physiologic Toso ligands would certainly help to better understand Toso biology.

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To the editor:

The FLT3 and Pim kinases inhibitor SGI-1776 preferentially target FLT3-ITD AML cells

The Pim (for provirus integration site for Moloney murine leukemia virus) family of serine/threonine kinases are strongly involved in oncogenic processes and efforts are ongoing to develop specific Pim kinase inhibitors as anti-cancer therapy.[1] Interestingly, recent reports from Gandhi's group emphasized the SGI-1776 compound (Astex Pharmaceuticals) as a potent Pim inhibitor with potential interest in chronic lymphocytic leukemia[2] and in acute myeloid leukemia (AML).[3] In this latter work, the SGI-1776 compound was showed to induce the apoptosis of both AML cell lines and primary AML samples, and to transiently reduce the size of tumors generated by subcutaneous injection of the MV4-11 human leukemic cell line without evidence for toxicity in mice.[3] To explain the anti-leukemic activity of SGI-1776, the authors demonstrated an inhibition of Pim target phosphorylation such as c-Myc S^{62} and BAD S^{112}. However, as SGI-1776 also inhibits the FLT3 kinase in vitro,[2] major attention should be given to the FLT3 mutational status of AML cells, that is, the presence or not of FLT3 alleles harbouring internal tandem duplication (ITD). Chen and coworkers considered that SGI-1776 was effective against primary AML cells and AML cell lines regardless of their FLT3 mutational status; although a superior effect of this molecule was found in FLT3-ITD AML cells in some experiments.[3] In contrast, we made slightly different observations. Although we also found an inhibition of Pim substrate phosphorylation in SGI-1776 treated AML cells (data not shown), we observed an inhibition of FLT3 autophosphorylation on tyrosine (Tyr) residues in the MOLM-14 FLT3-ITD AML cell line, which was similar to that observed after treatment with the specific FLT3 inhibitor AC-220[4,5] (Figure 1A). Moreover, SGI-1776 inhibited the phosphorylation of well-known signaling relays downstream of FLT3, such as Akt S^{473}, ERK T^{202}/Y^{204} and STAT5 Y^{694}, similarly to AC-220 (Figure 1A). Our results highly suggest that SGI-1776 directly inhibits the FLT3 kinase activity in AML. This conclusion could also explain the observation made by Chen and colleagues that SGI-1776 treatment results in an increased maturation of the FLT3-ITD in MV4-11 cells, assessed by an increased accumulation of the mature 150 kDa form compared with the 130 kDa endoplasmic reticulum form in SGI-1776–treated cells.[3] Indeed, it has been previously demonstrated by Schmidt-Arras and coworkers that inhibition of FLT3-ITD catalytic activity promotes the maturation of the receptor with acquisition of complex glycosylation, leading to an increase of FLT3-ITD molecular mass from 130 to 150 kDa.[6] We further showed that both AC-220 and SGI-1776 increased annexin V binding (Figure 1B) and induced the cleavage of caspase-3 (Figure 1C) in the MOLM-14 (FLT3-ITD) but not in the OCI-AML3 (FLT3-WT) AML cell lines. Although Chen and colleagues observed apoptosis in SGI-1776–treated OCI-AML3