Fas ligand induces cell-autonomous NF-κB activation and IL-8 production.

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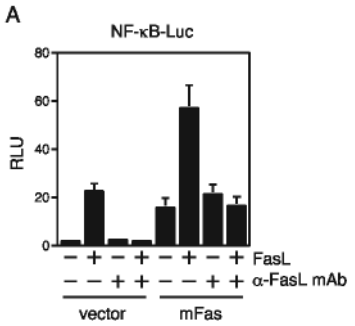
Fas ligand (FasL) has been well characterized as a death factor. However, recent studies revealed that FasL possesses inflammatory activity. We here found that FasL induces production of inflammatory chemokine IL-8 without inducing apoptosis in HEK293 cells. Reporter gene assays involving wild-type and mutated IL-8 promoters and NF-κB- and AP-1 reporter constructs indicated that induction of NF-κB and AP-1 activity by FasL is required for the maximum promoter activity. FasL induced NF-κB activation with slower kinetics compared to TNF-α. Yet, this response was cell autonomous and not mediated by secondary paracrine factors. In addition, we demonstrated that between the two physiological forms of FasL, the membrane-bound and soluble forms, the former is mainly responsible for these activities. A proteasome inhibitor or a proteasome resistant mutant of IκBα completely abrogated both FasL- and TNF-α-induced NF-κB activation. However, interestingly, FasL did not induce detectable phosphorylation at Ser32 and degradation of IκBα, while TNF-α rapidly induced these responses. Pancaspase and caspase-8 inhibitors partially inhibited NF-κB activation but enhanced IL-8 production by FasL stimulation, suggesting that there are both caspase-8 dependent and independent mechanisms, and that caspase-8 delivers both positive and negative signals for IL-8 production. In contrast, caspase inhibitors did not affect these responses to TNF-α. These results revealed that FasL induces NF-κB activation and IL-8 production by a mechanism distinct from that of TNF-α.



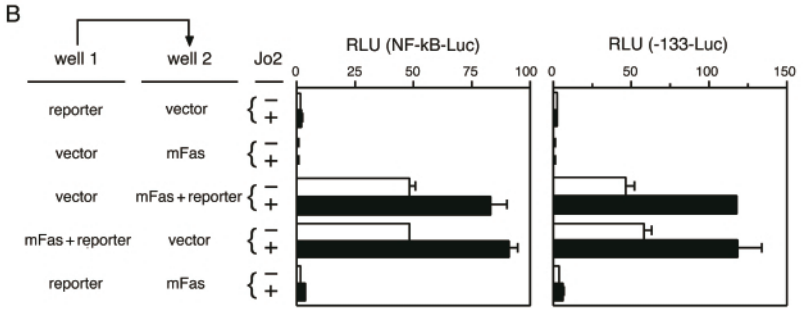


Figure. FasL directly induces the transcriptional activities of NF-κB. A, HEK293 cells were transiently transfected with pNF-κB-Luc and an expression plasmid for mouse Fas as indicated, or a control vector plasmid. Transfectants were treated with or without FasL in the presence or absence of an anti-FasL mAb, and cultured for 7 hrs. B, Two wells of HEK293 cell culture (well 1 and 2) were separately transfected with the indicated plasmid(s). Seventeen hours later, the cells in well 1 were transferred to well 2, and the mixed cells were further cultured in the presence or absence of anti-mouse Fas mAb (Jo2) for 7 hrs. The normalized firefly luciferase activity in lane 1 (A, left side bar; B, top bar) in each panel is set as 1 for RLU. Vertical lines indicate standard deviations.