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Naturally processed soluble CD95L activates a Src kinase/PI3K-driven cell migration pathway

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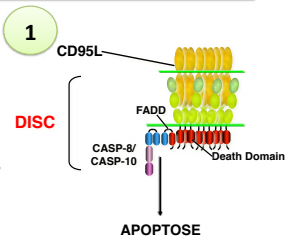
State of The Art

CD95 belongs to the TNF (Tumor necrosis factor) receptor family. Its cognate ligand, CD95 ligand (CD95L) is expressed at the surface of activated T lymphocytes and NK cells where it plays a crucial role in the elimination of infected and transformed cells. The membrane bound CD95L (m-CD95L) can be cleaved by metalloproteases (cl-CD95L) and released in bloodstream. CD95 engagement drives the formation of the Death Inducing Signaling Complex (DISC) that ultimately leads to the induction of apoptosis (fig. 1). In contrast, the patho-physiological role of cl-CD95L remains unknown.



cl-CD95L activates cell migration through a caspase-independent and Src kinase-dependent signaling pathway.

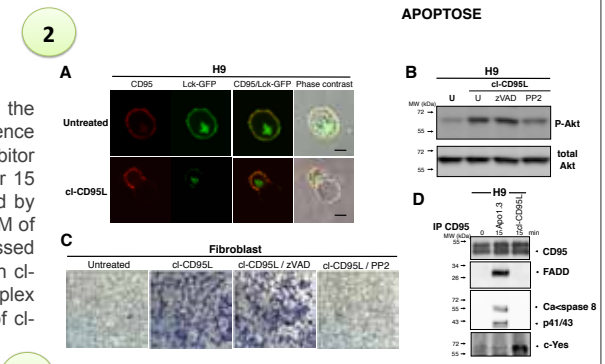


Figure 2. A. T-leukemic cell line H9 was transfected with a lipid raft marker (Lck-GFP) and the distribution of CD95 and lipid rafts was analyzed by confocal microscopy in presence or absence of cl-CD95L. **B.** H9 T-cells were pre-incubated with DMSO (U), 10 μM of the src kinase inhibitor PP2 or 40 μM of the caspases inhibitor zVAD-fmk (zVAD) and then treated or untreated for 15 minutes with cl-CD95L. The amounts of Akt phosphorylation and whole Akt were assessed by immunoblots. **C.** PS120 cells were pre-incubated for 30 min with 40 μM of zVAD-fmk or 10 μM of PP2 and then treated or untreated with 100 ng/ml of cl-CD95L. Cell migration was assessed using Boyden Chamber assay. **D.** The T-cell line H9 was incubated for indicated times with cl-CD95L or APO1-3. Cells were lyzed, CD95 was immunoprecipitated and the associated complex was analyzed by immunoblots. Although the DISC formation is not achieved in presence of cl-CD95L, the src kinase c-yes is found associated to CD95.

cl-CD95L is increased in SLE patients and promotes endothelial transmigration of activated peripheral blood T-lymphocytes.

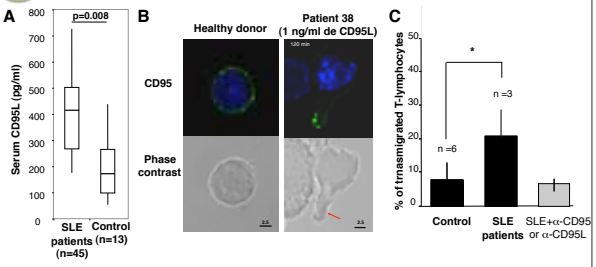


Figure 3. A. The amount of soluble CD95L in sera of healthy donors (control) and systemic lupus erythematic (SLE) patients was quantified by ELISA. **B.** Activated peripheral blood lymphocytes (PBLs) were incubated for the indicated times with sera of healthy donors or SLE patients and the distribution of CD95 was analyzed by confocal microscopy. **C.** Activated PBLs were incubated in presence of sera from SLE patients or healthy donors and the endothelial transmigration of activated PBLs was assessed through a confluent monolayer of endothelial cells deposited in a Boyden Chamber assay.

CONCLUSION

Here, we demonstrate that CD95 engagement promotes T-cell migration in the presence of the metalloprotease-cleaved CD95L. In addition, we show that cl-CD95L elicits the compartmentalization of CD95 into lipid rafts and the recruitment of the src kinase c-yes, whose activity participates in activating the PI3K/Akt signaling pathway. Finally, we found that cl-CD95L is increased in SLE patients compared to healthy individuals and this cytokine enhances the transmigration of T-lymphocyte in the inflamed area, fuelling local and/or systemic tissue damage.

Publications in which ANR support has been acknowledged

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