

### 3 DUAL EFFECTS OF SOLUBLE FASL AND MEMBRANE BOUND FASL ON FIBROBLAST-LIKE SYNOVIOCYTES CELLS FROM RHEUMATOID ARTHRITIS PATIENTS

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**Introduction** Membrane bound FasL (mFasL) is able to induce fibroblast-like synoviocytes (FLS) cell death. In experimental arthritis mouse models, injection of agonistic antibody (Ab) anti-Fas decreased the symptoms. However, soluble FasL (sFasL) is increased in rheumatoid arthritis (RA) patients serum and correlated with disease activity. These results indicated that mFasL could be protective whereas sFasL could be deleterious (O'Reilly *et al.* 2009) suggesting that they could have different functions. The authors therefore analysed the effect of different FasL preparation mimicking sFasL or mFasL on RAFLS proliferation or apoptosis.

**Materials and methods** RAFLS were treated with different FasL preparations (FasL-Flag±Ab anti-Flag, FasL-Fc or sFasL) or with agonistic Ab anti-Fas. Apoptosis was then analysed by FACS on basis of the annexin V-FITC binding and TOPRO-3 up-take. Proliferation was measured using tritiated (<sup>3</sup>H) thymidine. Activation of signaling pathways was analysed by western blot and their influences was assessed using chemical inhibitors.

**Results** FasL-Flag alone (mimicking sFasL) was not able to induce FLS apoptosis (8%±8 n=5) while proliferation was significantly activated (3.3±1 fold; n=5; p<0.05). Similarly, sFasL was only able to strongly induce RAFLS proliferation (8.1±3.3 fold; n=3). In an other hand, membrane bound FasL (FasL-Flag+Ab α-Flag) significantly induced RAFLS apoptosis (52%±18; n=5) but also a slighter but significant proliferation (2.2±0.3 fold; n=4). Duality of mFasL was confirmed using agonistic Ab anti-Fas. (mimicking mFasL) with pro-apoptotic (38%±18; n=2) and proliferative effect (2.5±0.15 fold). Finally, growing concentration of FasL-Fc leads to aggregation of the protein, mimicking mFas or sFasL at high and low concentration respectively. Dose responses confirmed mFasL and sFasL effects. FasL activated Akt and ERK (n=5) but also activated caspase-8. A pan-caspases inhibitor (z-VAD-FMK) prevented mFasL-induced apoptosis, but also blocked mFasL and sFasL-induced proliferation (n=4). Using specific inhibitors for caspases 8, 3 and 9, the authors found that caspase 8 is involved in FasL proliferation. Because ERK and Akt pathways are involved in TRAIL induced proliferation, the authors tested whether caspases participate on MAPK and Akt activation after FasL stimulation. This was not confirmed, since caspases inhibition did not inhibit FasL-induced kinases activation (n=3).

**Conclusion** mFasL induced preferentially RAFLS apoptosis, whereas sFasL only induce RAFLS proliferation. According to what the authors have already described for TRAIL, caspases are involved in FasL-induced apoptosis and proliferation. This is the first demonstration of sFasL and mFasL have different effects on RAFLS proliferation. sFasL by enhancing RAFLS proliferation could have a deleterious role in RA. Therefore, its blockage could be a therapeutic tool to prevent RA.