A142 REGULATION OF PLEITROPIC EFFECT OF TRAIL ON RAFLS BY TRAIL-R1 AND -R2

R Audo, A Bruyer, B Combe, M Hahne, J Morel *Department of Immuno-rheumatology, CHU Lapeyronie, Montpellier, France*

10.1136/ard.2010.129643s

Rationale Induction of apoptosis in fibroblast-like synovio-cytes (FLS) has been proposed as a therapeutic approach. Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has been described as a pro-apoptotic factor on rheumatoid arthritis FLS (RAFLS) and suggested as a potential drug. We have previously shown that exposure to TRAIL induces apoptosis only in a portion of RAFLS. In the surviving cells, TRAIL induced RAFLS proliferation. To evaluate possibilities to overcome TRAIL resistance in RAFLS, we compared FLS resistant (RAFLS-R) and FLS sensitive (RAFLS-S) to TRAIL-induced apoptosis including expression levels of the TRAIL receptors (TRAIL-R).

Material and Methods Cell surface expression of the four membrane-bound TRAIL-R was measured by FACS. To examine the implication of TRAIL-R in TRAIL apoptosis and proliferation, RAFLS were transfected with siRNA against TRAIL-R1 and TRAIL-R2. After 3 days, efficiency of silencing was assessed by western blot and FACS analysis and cells were cultured with TRAIL for another 24h. Apoptosis was analysed by flow cytometry using annexin V-FITC binding and TOPRO-3 uptake. Proliferation was measured using incorporation of [3H] thymidine.

Results Depending on the patient, we observed inter-individual variability in sensitivity of RAFLS to TRAIL-induced apoptosis. We therefore classified the cultures depending on their sensitivity (<10%; RAFLS-R and >30%, RAFLS-S). We observed significantly higher expression of TRAIL-R4 and TRAIL-R1 in RAFLS-R ($p=0.08$ and $p=0.14$ respectively). Silencing of TRAIL-R2 significantly reduced TRAIL-induced apoptosis or proliferation (25.2 ± 2.6 to 8.9 ± 5.9 ; $p<0.01$). In contrast, silencing of TRAIL-R1 sensitised RAFLS for TRAIL-induced apoptosis (25.2 ± 2.6 to 35.8 ± 6.7 ; $p<0.05$) but had no influence on TRAIL-induced proliferation.

Conclusion Using siRNA silencing, we confirmed the implication of TRAIL-R2 in apoptosis and proliferation induced by TRAIL. We also found that TRAIL-R1 is not involved in RAFLS apoptosis or proliferation induced by TRAIL but, surprisingly, appears to be a survival factor protecting RAFLS against TRAIL-induced apoptosis. This is consistent with our observation that TRAIL-R1 is significantly more expressed in the cell surface of RAFLS-R.