

TNF-alpha in Combination with TWEAK Induces Cell Death in Keratinocytes

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INTRODUCTION/BACKGROUND

Cell death plays an important role in several skin diseases, e.g. in atopic dermatitis (AD) and psoriasis. Both skin diseases are characterized by an infiltration of T cells and other inflammatory cells into the skin. However, the psoriatic epidermis shows a different skin keratinocyte response compared to AD. In atopic dermatitis, keratinocyte (KC)-death and spongiosis are the main characteristics, whereas psoriasis is characterized by thickening, hyperproliferation and hyperkeratinization (Bieber 2008; Lowes et al. 2007). In both skin diseases a variety of cytokines (e.g. IFN-gamma and TNF-alpha) secreted by T cells and other inflammatory cells are upregulated. In the present study, primary keratinocyte lines from non-lesional skin of five patients with atopic dermatitis, five patients with psoriasis and five healthy individuals were generated and treated with several death inducing ligands like TNF-alpha, Fas-ligand, TNF-related apoptosis-inducing ligand (TRAIL) and TNF-like weak inducer of apoptosis (TWEAK).

MATERIAL & METHODS

Keratinocytes viability was measured four days after incubation with cytokines by flow cytometry after staining with 7 amino-actinomycin D (7AAD) and annexin-V. Both early (annexin- V positive) and late apoptotic cells (annexin- V and 7AAD positive cells) and necrotic cells (7AAD positive) were excluded for the determination of viable keratinocytes. Receptor-expression was investigated by flow cytometry two days after incubation with cytokines and by quantitative RT-PCR 4, 8, 12, 18 and 24 hours after incubation with cytokines.

RESULTS

Our investigation shows that TNF-alpha in combination with TWEAK induces cell death in primary keratinocytes of healthy individuals, patient with atopic dermatitis and psoriasis, whereas the combination of TNF-alpha with anti-Fas mAb (molecular antibody) or TRAIL does not have a similar effect. FN-14 (TWEAK-receptor) and TNF-RI are expressed on the surface of all keratinocytes, whereas TNF-RII is not or only weakly expressed. The death inducing effect of TNF-alpha in combination with TWEAK does not result from the regulation of these receptors, which was investigated on the protein-expression level by flow cytometry and on the mRNA level by quantitative RT-PCR. However, FN-14 and TNF-RI are the main involved receptors in the death inducing effect of TNF-alpha in combination with TWEAK.

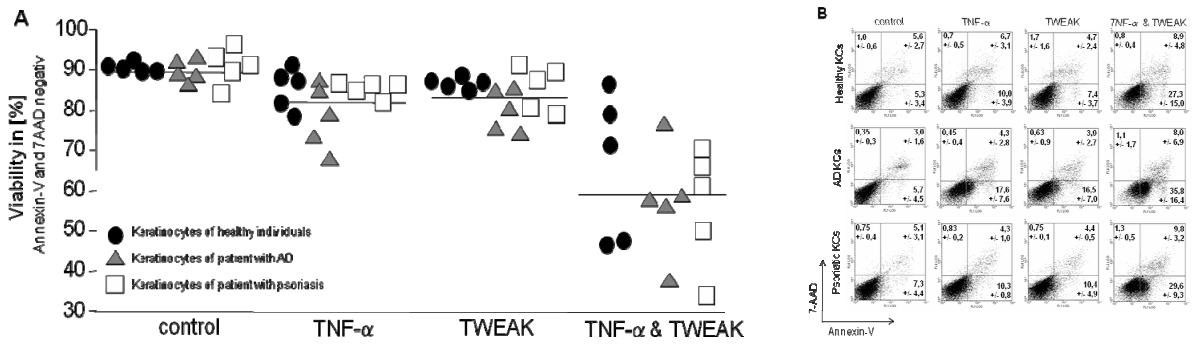


Fig. 1: TNF-alpha in combination with TWEAK can induce cell death in keratinocytes. Viability of keratinocytes four days after treatment with cytokines. A. Cell death of KCs from healthy individuals, patients with AD and psoriasis. B. Raw data, one example per group.

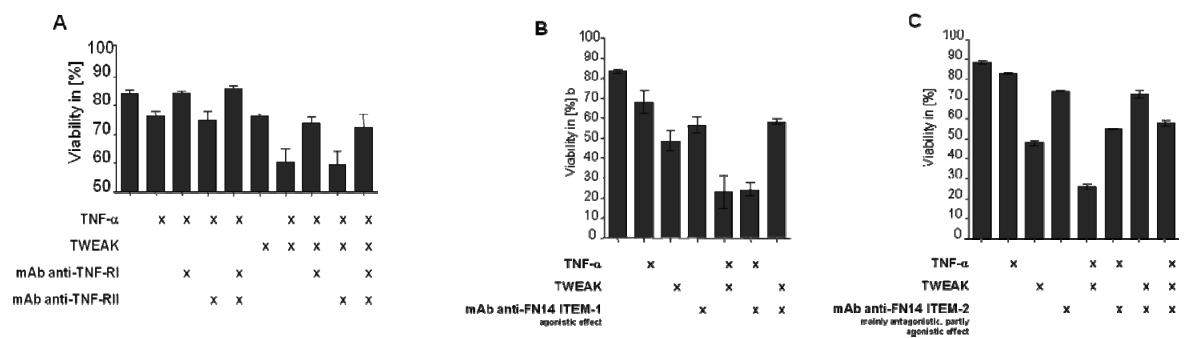


Fig.2: FN-14 and TNF-RI are the main involved receptors. A. mAb anti-TNF-RI but not mAb anti-TNF-RII neutralizes the cell death inducing effect of TNF-alpha in combination with TWEAK. B. An agonistic mAb anti-FN14 can induce cell death in combination with TNF-alpha similar to TNF-alpha in combination with TWEAK. C. A mainly antagonistic, partly agonistic mAb anti-FN14 neutralizes the cell death inducing effect of TWEAK in combination with TNF-alpha.

CONCLUSIONS & OUTLOOK

Cell death plays an important role in several skin diseases like atopic dermatitis (AD) and psoriasis. We could show that TNF-alpha in combination with TWEAK induced cell death in keratinocytes. TNF-RI and FN14 were the main involved receptors but the death inducing effect of TNF-alpha in combination with TWEAK did not result from their regulation. However, these results show an interesting new way to induce cell death in keratinocytes.

REFERENCES

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