ANTI-TUMOR NECROSIS FACTOR MECHANISM OF ACTION: WHAT IS THE ROLE OF CD69IN INFLIXIMAB RESPONSE?

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Background and aim: Although anti-tumor necrosis factor (TNF) agents, such as infliximab (IFX) are approved for the therapy of inflammatory bowel disease (IBD), their mechanism of action is still not well known. Recent studies have identified in IBD patients some genes that were regulated by anti-TNF treatment such as interleukin-6(IL-6) and CD69. CD69is an early membrane receptor transiently expressed on activated lymphocytes at inflammatory sites but its role during inflammation must be clarified yet. T cell activation induces several signalling cascades such as extracellular signal-regulated kinase (ERK), c-JUN N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB) pathways that enhance the release of proinflammatory cytokines, which maintain and upregulate CD69expression on cell surface. The aim of this study is to evaluate IFX mechanism of action on Jurkat cells, an activated immortalized T cell line.

Methods: Jurkat cells were activated with PMA (0.05μg/mL) plus ionomycin (0.96μg/mL) (eBioscience™ Cell Stimulation Cocktail) for 4hours and then treated with different concentrations of IFX (0, 1, 10 and 100 μg/mL) overnight. The effect of IFX was evaluated both in term of decrease of CD69by flow cytometry and NF-κB protein expression by western blot analysis. Statistical analyses were performed by one-way ANOVA and Bonferroni's multiple comparisons test using GraphPad Prism 6software. The threshold level of statistical significance was set to ≤ 0.05. The data are reported as means ± standard error of the mean (SEM) of three independent experiments.

Results: CD69cell surface expression decreased from 100 to 91.7± 3.61, 87.4± 6and 73.1± 0.98% in activated Jurkat cells treated with 0 (control), 1, 10 and 100 μg/mL of IFX overnight, respectively (one-way ANOVA: p-value = 0.0042; Bonferroni's multiple comparisons test: Jurkat activated vs Jurkat activated + IFX100 μg/mL p-value ≤ 0.01). Western blot analysis revealed that after activation, Jurkat cells have a higher NF-κB protein expression than non-activated T-cells (+ 51.5%). The treatment of activated Jurkat cells with IFX 100 μg/mL led to a decrease of NF-κB protein expression in comparison to control.

Discussion and conclusion: The NF-κB signalling pathway is highly induced in activated T cell lines (CD69+) and plays an important role in the initiation of inflammatory response. Its activation occurs in response to a variety of stimuli including TNF. In vitro, the synergy of ionomycin with phorbol esters in triggering T cell activation enhances activation of protein kinase C (PKC), that promotes proliferative (ERK/JNK) and NF-κB signalling pathways, leading to release of pro-inflammatory cytokines (among which TNF), that up-regulate and maintain the expression of the T-cell activation marker, CD69. The results obtained in vitro have shown that when T-cells are treated with IFX, probably the inhibition of TNF and, consequently, the reduced of NF-κB protein expression, leads to the diminished cell surface expression of CD69making it a possible marker of IFX response.