The activation status of nuclear factor κ B (NF- κ B) in type 2 conventional dendritic cells (cDC2) before therapy, correlates with clinical response to adalimumab at week 12

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INTRODUCTION

Adalimumab is a human monoclonal antibody approved for the treatment of psoriasis which exerts its action through the binding and neutralization of tumour necrosis factor (TNF). TNF is a cytokine involved in local and systemic inflammation facilitating entry of inflammatory cells into lesional skin, stimulating keratinocyte production of other pro-inflammatory mediators and activating dermal macrophages and dendritic cells. These effects are mediated by the activation of nuclear factor κ B (NF- κ B)¹, although the specific cellular target has not been elucidated in psoriasis. Moreover, despite the broad success of anti-TNF therapies for the treatment of moderate-to-severe psoriasis, they are still ineffective in up to 30% of patients, which represents a burden to patient wellbeing and the health care system². The Psoriasis Stratification to Optimise Relevant Therapy (PSORT) Consortium aims at better understanding the determinants of response to biologic therapies and ultimately deliver a stratifier algorithm to guide psoriasis management. Using longitudinal single-cell immune profiling of psoriasis patients receiving adalimumab as part of their standard care, we investigate the effect of TNF neutralization on NF- κ B activation in key immune cell types to identify the immune determinant of clinical response to adalimumab.

HYPOTHESIS

Monitoring the activation of NF- κ B in key immune cells will provide insights into adalimumab's cellular and molecular targets in psoriasis and may lead to the identification of biomarkers of response to treatment

METHODS

Methods. The discovery cohort consisted in 16 patients undergoing adalimumab treatment. Blood was collected at baseline (before start of therapy), week 1, week 4 and week 12 after the initiation of the treatment. Blood was used to measure NF- κ B translocation and for PBMC isolation and cryopreservation. For NF- κ B translocation assessment, blood was stimulated for 30 min, stained and acquired using ImageStream. Nuclear translocation was measured using IDEAS® image analysis software and differences among translocated and untranslocated populations were measured using the Fischer discriminant ratio (rd score). For NF- κ B phosphorylation assessment, cryopreserved PBMCs were rested overnight, stimulated for 15 min and stained for phosphoflow analysis. The same phosphoflow protocol was applied to an independent replication cohort (n = 25). Finally, monocyte-derived dendritic cells (moDCs) were generated from cryopreserved PBMCs of selected adalimumab responders (PASI75) and non-responders (mechanistic cohort, n=15) by culturing CD14+ monocytes with IL4 and GM-CSF for one week to generate immature dendritic cells (iDCs). Maturation was induced with LPS for 48 h in the presence or absence of adalimumab. Cells were then collected and stained for the assessment of the expression of co-stimulatory molecules. Data analysis was done in Flowjo®. Generalized linear models were fitted using the R stats package.

Results 1: Adalimumab inhibits TNF induced NF- κ B translocation in lymphoid cells and dendritic cells but not in monocytes

Results 2: LPS-induced NF- κ B translocation in dendritic cells (DCs) at baseline correlates with response to adalimumab at week 12

Results 3: NF- κ B phosphorylation in type 2 conventional dendritic cells (cDC2) correlates with response to adalimumab at week 12

Results 4: moDCs generated from adalimumab non-responders have a significant increased upregulation of co-stimulatory molecules in response to LPS maturation, even in presence of adalimumab

Figure 4. Co-stimulatory molecule expression in moDCs generated from adalimumab responders (R) and non-responders (NR) at baseline visit and matured with LPS in presence or absence of adalimumab. R: PASI75 responder. NR: PASI75 non-responder $n = 15$, *** $p < 0.01$, ** $p < 0.01$, * $p < 0.05$. Wilcoxon test.

RESULTS

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Figure 4. Co-stimulatory molecule expression in moDCs generated from adalimumab responders (R) and non-responders (NR) at baseline visit and matured with LPS in presence or absence of adalimumab. R: PASI75 responder. NR: PASI75 non-responder $n = 15$, *** $p < 0.01$, ** $p < 0.01$, * $p < 0.05$. Wilcoxon test.

CONCLUSIONS

- Adalimumab inhibits TNF-induced NF- κ B translocation in lymphoid cells and, to a lesser extent, in dendritic cells, but has no effect on monocytes or neutrophils, in the blood of psoriasis patients
- Increased NF- κ B phosphorylation and nuclear translocation in type 2 conventional dendritic cells before the start of the therapy significantly correlates with diminished clinical response to adalimumab at week 12
- moDCs generated from adalimumab PASI75 non-responders before the start of the therapy induce an increased expression of the co-stimulatory molecules CD54, CD80, CD83 and CD86 after maturation with LPS even in the presence of adalimumab.

PERSPECTIVE

Our study sheds light into adalimumab's cellular targets as well as identifies a potential mechanism of therapeutic failure. In addition, NF- κ B activation in dendritic cells may constitute an early biomarker of treatment efficacy

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