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Optimization of cytokine milieu to reproduce atopic dermatitis-related gene expression in HaCaT keratinocytes

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Introduction

Atopic dermatitis (AD) and Th1 / Th2 pathway

- AD has been considered a Th2 disease characterized by predominant Th2 cytokine production, including interleukin (IL)-4, IL-5, and IL-13, elevated serum Ig E, and eosinophilia.
- However, Th1 cells are also involved in the pathogenesis of AD. Th1 cytokines, like interferon (IFN)-γ and IL-12, are expressed in chronic AD lesions. High numbers of IFN-γ-expressing CD4+ and CD8+ T cells infiltrate acute AD lesions, and IFN-γ-producing CD8+ T cells are required for the development of an AD-like phenotype in mice. Skin expression of IFN-γ, but not IL-4, correlates with the clinical course of AD and is downregulated with AD improvement. AD patients have elevated levels of CC chemokine receptor 4 ligands, including thymus and activation regulated chemokine (TARC) or macrophage derived chemokine (MDC), which mediate preferential Th2 recruitment. Additionally, CXC chemokine receptor 3 ligands, which induce Th1 polarization, such as monokine induced by IFN-γ (Mig), are also elevated in AD patients compared to normal controls.
- In addition to inflammatory microenvironment imbalance, important barrier-related proteins including filaggrin (FLG) and loricrin (LOR) are decreased in AD. In primary keratinocytes, tumor necrosis factor (TNF)-α downregulates FLG and LOR through c-Jun N-terminal kinase. These results suggest that complex inflammatory networks, involving both Th1 and Th2, orchestrate AD pathogenesis.

Aim of Study

- This study was to identify in vitro cytokine milieu that can accurately reproduce the expression profile of genes important in AD pathogenesis.
- We used qRT-PCR to identify the in vitro cytokine milieu involved in the pathogenesis of AD and to accurately reproduce the expression profile of genes important in AD.

Materials and Methods

- Cytokine-stimulated HaCaT cells were examined for morphological changes using a phase-contrast microscopy, and the effects of cytokine stimulation on HaCaT cell growth was measured by a water-soluble tetrazolium salt (WST-1) assay with cell viability determined by measuring the absorbance at 450 nm.
- mRNA levels of CCL22 (encoding MDC), CCL17 (encoding TARC), IL5, IL13, FLG, and LOR were evaluated by qRT-PCR in skin samples from six AD patients, 12 healthy controls, and in HaCaT cells cultured in the presence of various combinations of Th1 (TNF-α, 10 ng/ml; and/or IFN-γ, 10 ng/ml) and Th2 (IL-4, 50 ng/ml) cytokines (supplementary materials and methods).

Results

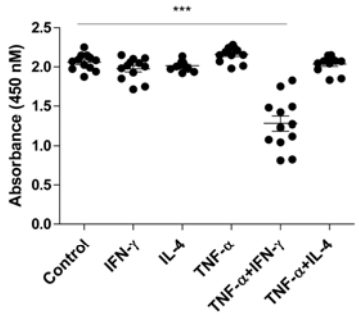

Figure 1. HaCaT cell viability following stimulation with various combinations of Th1 (IFN-γ, 10 ng/ml; or TNF-α, 10 ng/ml) and Th2 (IL-4, 50 ng/ml) cytokines for 24 hours. The effects of cytokine stimulation on HaCaT cell growth was measured using a water-soluble tetrazolium salt (WST-1) assay, and cell viability was determined by measuring the absorbance at 450 nm. ***p < 0.001

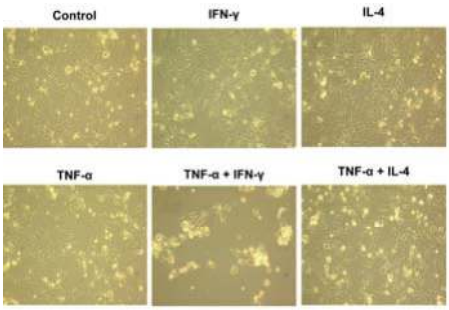

Figure 2. Evaluation of the morphological changes of cytokine-stimulated HaCaT cells using phase contrast microscopy. Original magnification × 20.

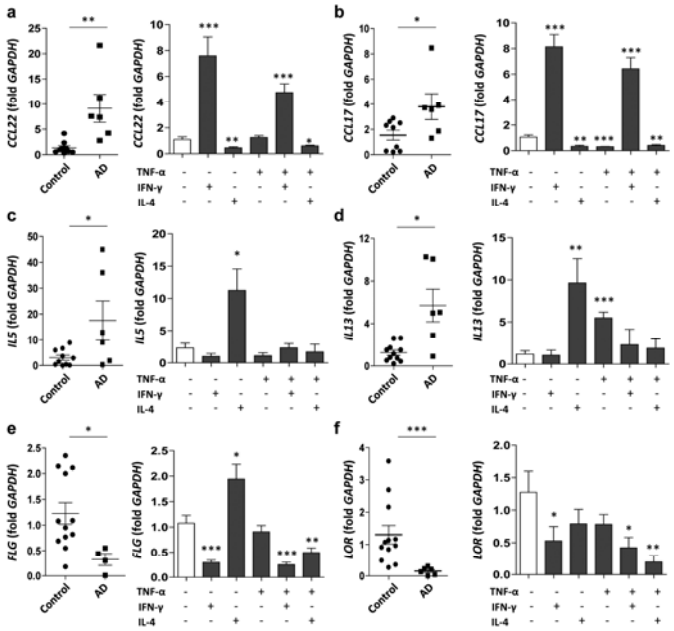

Figure 3. Correlation between atopic dermatitis (AD)-related gene expression in lesional skin and that observed in cytokine-stimulated HaCaT cells. HaCaT cells were cultured in the presence of interferon (IFN)-γ (10 ng/ml), tumor necrosis factor (TNF)-α (10 ng/ml) and/or interleukin (IL)-4 (50 ng/ml) for 24 hours. Expression of (a) CCL22 (encoding macrophage derived chemokine), (b) CCL17 (encoding thymus and activation regulated chemokine), (c) IL5, (d) IL13, (e) FLG (filaggrin), and (f) LOR (loricrin) were evaluated by qRT-PCR in skin samples from AD patients (left) and cytokine-stimulated HaCaT cells (right).

Conclusions

- Current data suggest that Th1 and Th2 cytokines do not function dichotomously, and that a complicated inflammatory network drives AD-like changes. Therefore, in further in vitro experiments using HaCaT cells to study AD-related genes, stimulation with various cytokine combinations not limited to Th2 polarization would be necessary for optimal gene expression levels.

References

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Conflicts of Interests

None to declare.

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