Exploring Liver X Receptor role in human macrophage polarization

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Abstract

Macrophages are a heterogeneous population of myeloid cells that display a variety of phenotypes (usually referred to as polarization states) whose balance is critical for tissue homeostasis. In particular, pro- and anti-inflammatory macrophages are crucial for initiation and resolution of inflammatory processes, and their absent or exacerbated functions contribute to chronic inflammatory diseases. Several nuclear receptors regulate macrophage behaviour, but the role of Liver X Receptors (LXR), LXRα and LXRβ, in the plasticity and biology of human macrophages is still unknown. Using a well-stablished *in vitro* system in which blood monocytes differentiate into pro- or anti-inflammatory macrophages, we aimed to clarify the function of LXR in human macrophage polarization. We found that activation or inhibition of LXR during macrophage differentiation has a great impact on the transcriptomic and functional (cytokine production) profile of pro- or anti-inflammatory macrophages. Besides, siRNA-mediated depletion of LXR in differentiated macrophages evidenced that the activity of both isoforms is important for macrophage functions, and that LXRβ is upregulated upon LXRα knockdown. Our next steps will address the specific role of each LXR isoform during macrophage differentiation at the transcriptional and functional levels, and their respective interactions with other transcription factors involved in human macrophage polarization.

Introduction

Macrophages constitute a very diverse population of cells that are crucial for the proper function of the organism[1]. Macrophages exhibit a large diversity of polarization states and their misbalance promotes or contributes to several inflammatory pathologies[2]. Therefore, it is essential to know the distinct factors and molecules that regulate the functional macrophage plasticity.

Many nuclear receptors contribute to macrophage biology but the role of Liver X Receptors (LXR) in human macrophage behaviour is currently unknown. LXR family, LXRα (NR1H3 gene) and LXRβ (NR1H2 gene), regulate many important processes in macrophages like cholesterol transport or phagocytosis of apoptotic cells and contribute to inflammatory responses and autoimmunity[3,4]. Besides, LXRα is the main isoform expressed in macrophages[5] and LXRα is autoregulated by LXRs itself in human macrophages but not in murine macrophages[6]. Altogether, this makes LXRs relevant targets for the study of human macrophage polarization plasticity.

Objectives

We aim to determine LXR function in human macrophage plasticity, emphasizing on their role in macrophage differentiation and to discern the particular contribution of LXRα and LXRβ to these processes.

Material & Methods

We use an *in vitro* system in which we isolate CD14⁺ monocytes from buffy coats (leukocyte concentrated suspensions from healthy blood donors) and differentiate them into anti-inflammatory or pro-inflammatory macrophages using M-CSF (M macrophages; M-MΦ) and using GM-CSF (GM macrophages; GM-MΦ), respectively. After 7 days in culture we analyze gene or protein expression[7].

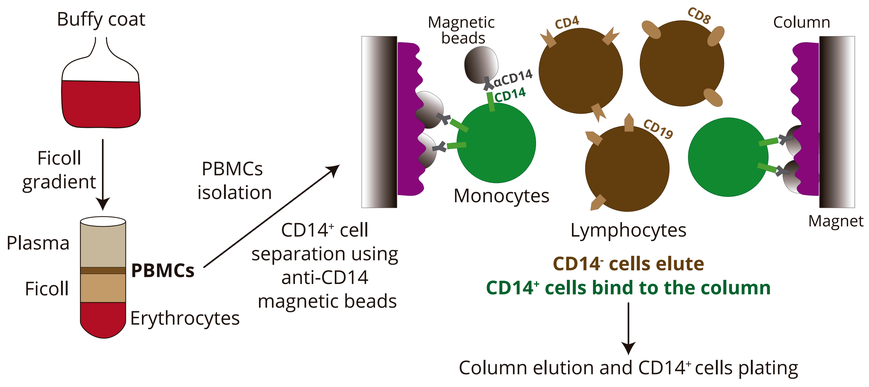

Figure 1. Protocol for isolation of CD14⁺ cells (>95% monocytes) from buffy coats.

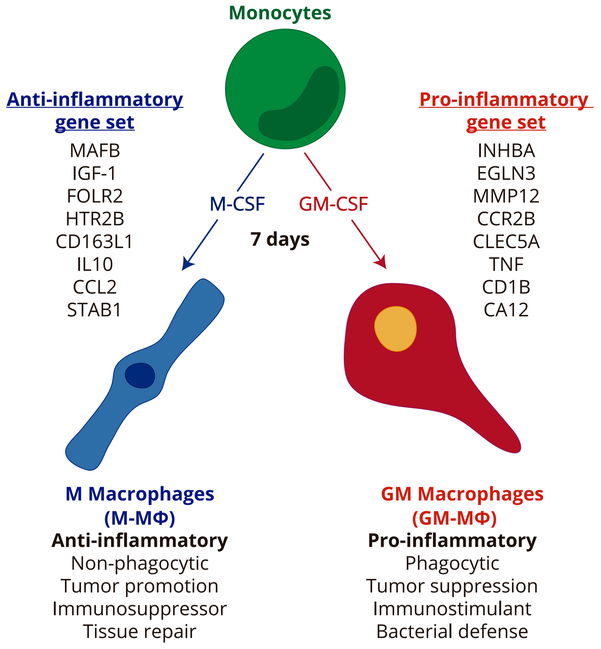

Figure 2. Protocol for *in vitro* generation of human anti- or pro-inflammatory macrophages (M-MΦ and GM-MΦ respectively)

Results

Modulation of LXR during differentiation alters the transcriptomic and cytokine profile of human macrophages

To study the contribution of LXR in human macrophage differentiation, we treated monocytes at day 0, 2 or 5 of the differentiation with only one dose of LXR synthetic **agonist GW3965** (1μM) or **antagonist GSK2033** (1μM) and analyzed gene expression at day 7. The expression of LXR target gene ABCA1 was analyzed as a control.

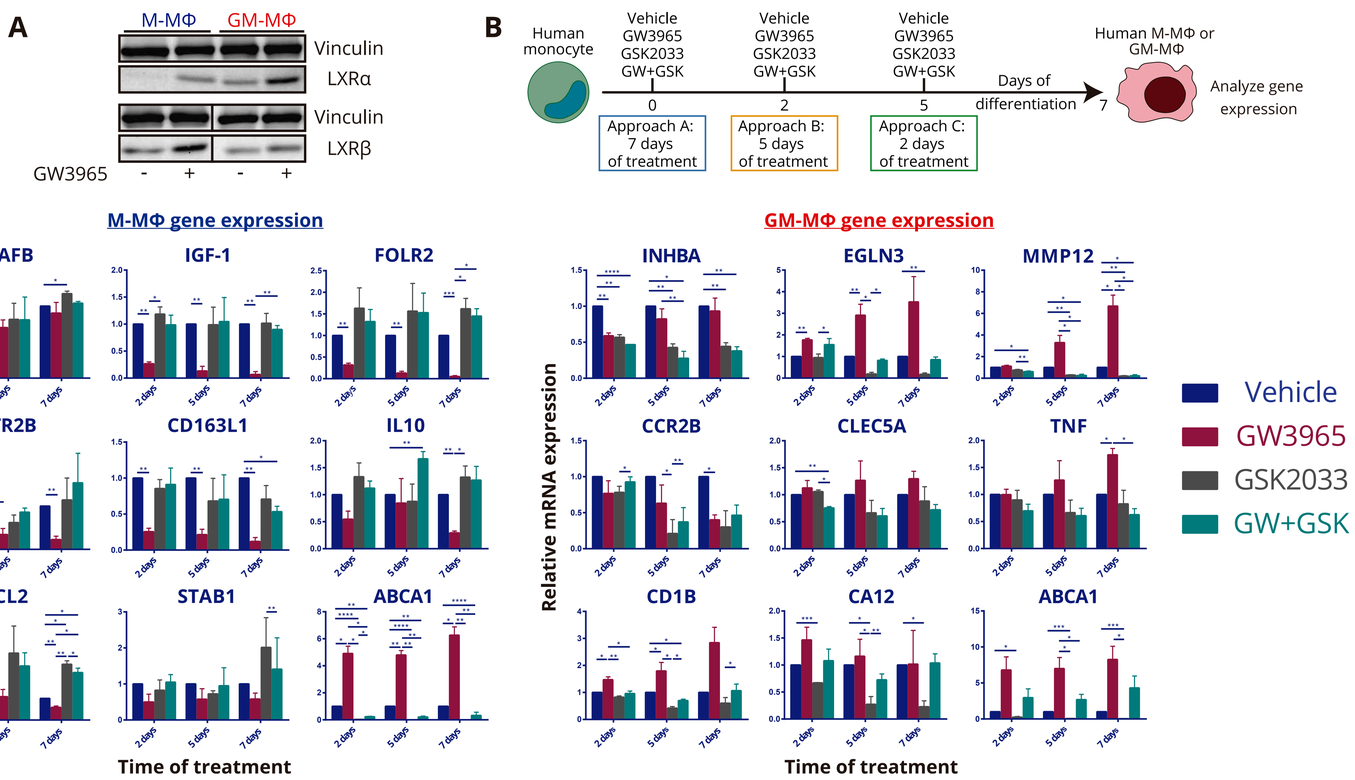

Figure 3. A) LXRα and LXRβ expression in M-MΦ and GM-MΦ in basal and LXR-activated (GW3965, 1 μM, 24 hours) conditions. One representative experiment is shown. B) Summary of experimental design. C) Gene expression in M-MΦ and GM-MΦ after modulation of LXR activity during differentiation. Results represent the mean ± SEM of three samples. Different conditions were compared using paired T student's test; *p<0.05, **p<0.01,***p<0.001, ****p<0.0001.

The greatest transcriptional changes occurred when monocytes were exposed to the agonist at day 0. To evaluate the biological relevance of these alterations, we measured the production of basal and LPS-induced (10 ng/mL; 18 hours) TNF-α and IL10 in both types of macrophages.

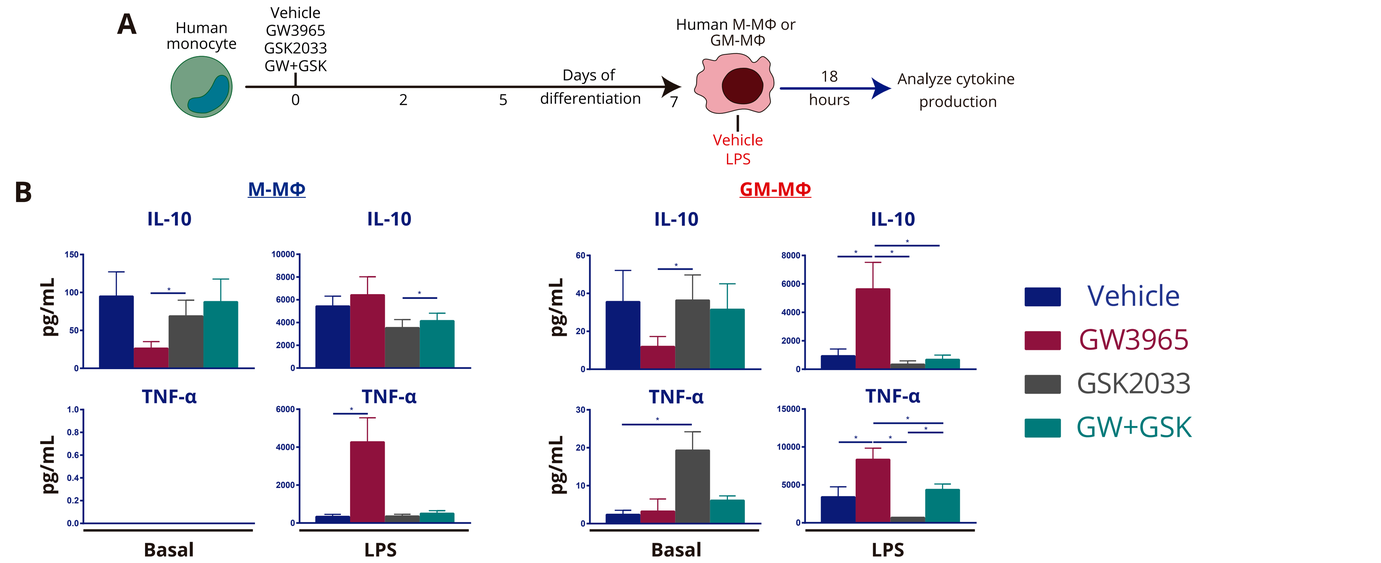

Figure 4. A) Summary of experimental design. B) TNF-α and IL10 production in M-MΦ and GM-MΦ after modulation of LXR activity during differentiation (7 days of treatment), in basal and activated (LPS) conditions. TNF-α was undetectable in basal conditions in M-MΦ. Results represent the mean ± SEM of four samples in M-MΦ and five samples in GM-MΦ. Different conditions were compared using paired T student's test; *p<0.05.

Knockdown of LXRα in differentiated human macrophages leads to increased expression of LXRβ

Modulation of LXR activity at day 5 of differentiation also triggers transcriptional changes, so we analyzed LXR function in differentiated macrophages. We depleted LXRα using a siRNA-mediated strategy: human M-MΦ and GM-MΦ were treated with NR1H3 (LXRα)-siRNA or control-siRNA for 24 hours and we analyzed protein and gene expression.

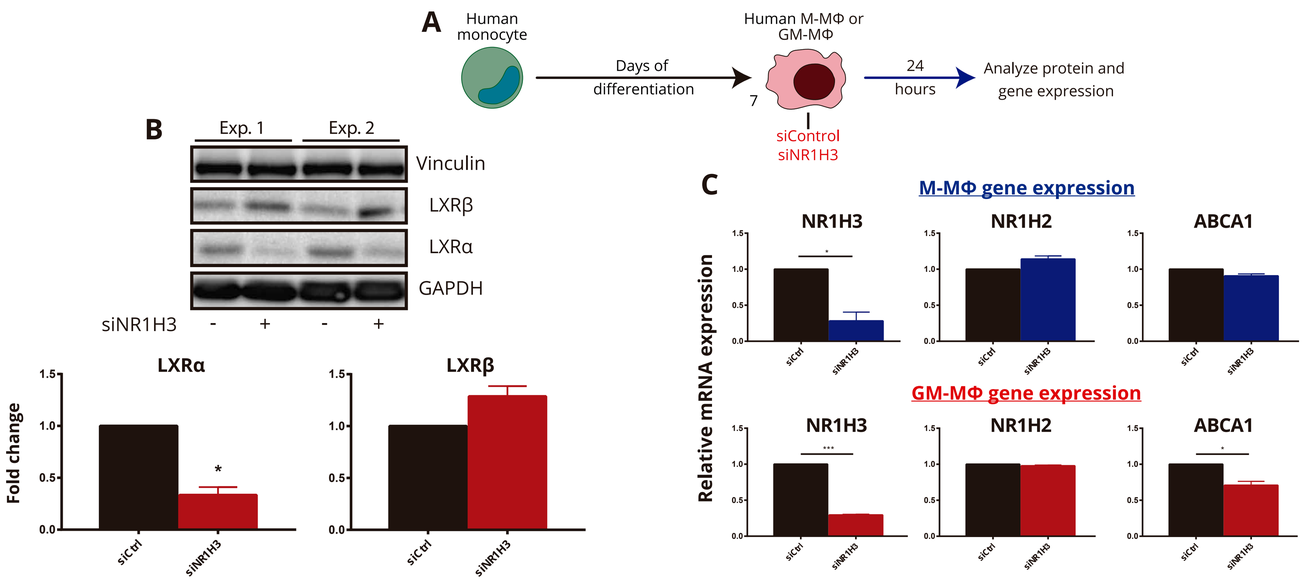

Figure 5. A) Summary of experimental design. B) LXRα and LXRβ expression in GM-MΦ (LXRα is undetectable in M-MΦ in basal conditions) after silencing NR1H3. Two representative experiments are showed. Results represent the mean ± SEM of three samples. C) Gene expression in M-MΦ and GM-MΦ after silencing NR1H3. Results represent the mean ± SEM of three samples. Control and silenced conditions were compared using paired T student's test; *p<0.05, ***p<0.001. NR1H3 is the gene symbol for LXRα and NR1H2 is the gene symbol for LXRβ.

Conclusions

Modulation of LXR activity during human M-MΦ and GM-MΦ differentiation modifies their transcriptomic signature and their biological response, at least in cytokine production in response to LPS. Altogether, results show that agonist-mediated LXR activation skews M-MΦ to a less anti-inflammatory state and skews GM-MΦ to a more pro-inflammatory state.

Knockdown of NR1H3 mRNA in M-MΦ and GM-MΦ leads to slight alterations in their respective phenotype, as it is also illustrated by the weak effect of NR1H3-silencing on ABCA1 gene expression.

In ongoing experiments, we are 1) determining the LXR-dependent transcriptome in human macrophages and 2) analyzing the contribution of LXR to various macrophage effector functions (pathogen recognition and binding, chemokine production). Besides, we are addressing the existence of NR1H2- or NR1H3-specific functions in human macrophages.

References

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