THE ORPHAN NUCLEAR RECEPTOR NR2F6AS A NOVEL REGULATOR OF IMMUNE RESPONSES

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Introduction: Nuclear receptor subfamily 2, group F, member 6(NR2F6), an orphan nuclear receptor, is a member of the COUP-TF receptor family (chicken ovalbumin upstream promoter transcription factor), whose expression is highest in immune cells, where it has a critical regulatory function in the adaptive immune system. Specifically, NR2F6acts as transcriptional repressor in Th0 and Th17cells via direct binding to the Il2and Il17a promoter loci. NR2F6also represses IL-2, IFN-g and TNF-a cytokine production in Th1CD4$^+$ as well as in CD8$^+$ effector T cells, affecting T cell activation and effector functions. In addition, it has been reported that, NF2F6may act as a novel intracellular immune checkpoint, directly suppressing transcription of cytokines relevant for cancer rejection in T cells. Recently, we found that NR2F6is also highly expressed in antigen presenting cells (APCs) types, and specifically in dendritic cells (DCs), but the function of this repressor in these immune cells is presently unknown. In this study, we found that NR2F6is highly expressed in selected DC subsets and its modulation affects DC effector functions.

Materials and methods: CD4$^+$ and CD8$^+$ T cells were purified by spleens of C57BL/6male mice, activated with anti-CD3and anti-CD28and stimulated with specific cytokines cocktail to induce differentiation of T helper or T effectors cell phenotypes. DCs cells were purified from bone marrow (BMDCs) of the same mice and cultured for 9days in vitro. Conventional type 1(cDC1) and type 2(cDC2), DCs were separated by magnetic sorting. NR2F6gene and protein expression was analyzed by RT-PCR and western blot analysis respectively. By using a series of computational and biochemical assay we identified a novel small molecule, modulating NR2F6activity. To assess the role of this novel NR2F6modulator in cDC1and cDC2, cDC1and cDC2were pre-treated with this small molecule at different concentrations in combination or not with LPS then, they were cultured with purified splenic naïve CD4$^+$ T cells. After 3days, specific cytokines released from CD4$^+$ T cells were evaluated.

Results: Using public gene databases, we checked for expression of NR2F members (NR2F1, NR2F2, NR2F6) in different immune cells. We confirmed higher expression of NR2F6compared to the two other members (NR2F1and NR2F2) in T cells. Moreover, we demonstrated that NR2F6is differentially expressed in cDC subsets. Specifically, we found that cDC1express higher NR2F6gene and protein expression compared to cDC2, both in steady state conditions and after treatment with inflammatory stimuli, such as LPS. We identified a novel small molecule able to bind NR2F6protein in both cell free assays and in specific cell lines overexpressing NR2F6. Specifically, we showed that this novel compound was able to modulate cytokine production in T cells by greatly increasing their ability to secrete Il2both in Th0 and Th17cell populations. Notably, we found that cDC1, pre-treated with this compound was also able to condition T cells to produce high levels of the cytokine Il2.

Conclusions: Overall, this study reveals that nuclear receptor NR2F6may have important implications in regulating the function not only in T cells but also in selected DC subsets. Moreover, these data suggest that innovative small molecules, targeting NR2F6are able to regulate both T and dendritic cell functions, resulting in potentiation of immune responses