HIV-1 infection in macrophages and genes involved throughout: Big eaters versus small invaders

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HIV-1 infection in macrophages and genes involved throughout: Big eaters versus Small invaders

Macrophages are effector cells of the innate immune system and are the first line of the defence against invading microorganisms. These cells are responsible for sensing and clearing pathogens and damaged tissue, thus contributing to regulation of tissue homeostasis and inflammation. Due to their localization in mucosal tissues, macrophages constitute first target cells for a broad range of pathogens, including the Human Immunodeficiency virus type 1 (HIV-1). Macrophages are important target cells for HIV-1 since these cells are resistant to the cytopathic effects of viral replication, and therefore contribute greatly to the spread of the viral infection to CD4+ T cells.

HIV-1 exploits several components of the cellular machinery of macrophages, in order to replicate and produce new virus particles. Therefore it is important to identify cellular factors that interact with HIV-1 during infection, to further understand how the virus is able to replicate and disseminate throughout the body. Additionally, certain populations of phenotypically different macrophages are able to restrict viral replication. Cytokines that are produced by either innate or adaptive immune cells are responsible for skewing macrophages into two main phenotypic subpopulations: M1 or M2. M1 polarized macrophages are generated by stimulation with IFN-\(\gamma\) and TNF-\(\alpha\) or LPS, and have pro-inflammatory profile and are responsible for mediating inflammatory responses together with Th1 helper cells. M2 or alternatively activated macrophages comprise a broad variety of cells and are mediators of Th2 or anti-inflammatory responses.

To gain insight into the functional landscape of polarized macrophages, we have characterized in vitro stimulated cells and analyzed their microRNA expression profiles. microRNAs are small non-coding RNAs that regulate gene expression at a post-transcriptional level, and play a crucial role modulation of macrophage function, which is critical for maintaining tissue homeostasis. In chapter 2, we described expression signatures of miRNAs displayed during maturation of monocytes and polarization of macrophages into M1 or M2 phenotypic populations, using next-generation sequencing. Furthermore, we showed that miRNA expression signatures are closely related to several functional aspects of macrophages and may be involved in shaping the diverse phenotypes of these cells. The miRNAs identified in this chapter contribute to our understanding of the role of miRNAs in determining the macrophage function in healthy and diseased tissues.

To understand the infection process of HIV-1 in macrophages, we investigated how replication is modulated in phenotypically different subpopulations of macrophages. In chapter 3, we described how HIV-1 replication is restricted upon macrophage activation by type I interferons and polarizing cytokines. We observed
increased expression of the cellular factors Trim5α, CypA, APOBEC3G, SAMHD-1, Trim22, tetherin and TREX-1, and the anti-HIV miRNAs miR-28, miR-150, miR-223 and miR-382 by IFN-α and IFN-β, which may account for the inhibiting effect on viral replication and the antiviral state of these cells. Expression of these factors was increased to a lesser extent in IFN-γ+TNF-α stimulated macrophages, where replication was blocked at a step after reverse transcription. IL-4, IL-10 or IL-32 polarization did not affect the expression of these cellular factors and miRNAs. These results indicated that the expression of known cellular factors that interact with HIV-1 during infection of macrophages, does not entirely account for the restriction of viral replication observed in polarized macrophages. Therefore, there must be other cellular proteins that are able to inhibit HIV-1 replication.

To identify other cellular factors involved in HIV-1 infection, we further analyzed gene expression profiles of macrophages upon stimulation with IFN-γ+TNF-α, in which HIV-1 replication is restricted after proviral integration. In chapter 4, we described how we identified the GTPase activating protein (SH3 domain) binding protein 1 (G3BP1) as a novel HIV-1 restriction factor. G3BP1 was expressed in macrophages and T cells and was exclusively increased upon macrophage stimulation with IFNγ in combination with TNFα. We described that down-regulation of G3BP1 increased HIV-1 replication and HIV-1 LTR-mediated gene expression, and demonstrated that G3BP1 was able to bind HIV-1 RNA transcripts. G3BP1 may sequester viral transcripts and decrease viral protein synthesis and the production of viral particles. G3BP1 was also highly expressed in resting T cells but expression levels decreased significantly upon T cell activation by IL-2. Since G3BP1 was able to restrict post-integration steps in the HIV-1 replication cycle, this may suggest that G3BP1 contributes to the establishment of the viral reservoir in macrophages and T cells.

Other cellular factors are instead involved in facilitating HIV-1 replication in macrophages. Genetic polymorphisms in phosphodiesterase 8a (PDE8A) are strongly associated with HIV-1 replication in primary macrophages. In chapter 5, we analyzed the regulation of PDE8A in macrophages and how PDE8A affects HIV-1 replication. PDE8A mRNA expression was strongly upregulated during differentiation of monocytes into macrophages, which correlated with macrophage susceptibility to HIV-1. We also demonstrated that miR-145-5p regulates PDE8A mRNA expression levels throughout macrophage maturation. Down-regulation of PDE8A decreased HIV-1 replication and proviral DNA levels upon infection in macrophages. These observations confirm that PDE8A supports HIV-1 replication in macrophages and that it’s effect is mediated by enhancing early steps in the viral replication cycle. Identification of cellular factors that interact with HIV-1 during the infectious process that either restrict or enhance viral replication, contributes to our understanding of HIV-1 replication in macrophages, latency and establishment of the viral reservoir. This knowledge provides novel therapeutic alternatives that could help eliminate the HIV-1 reservoir.
Additional to the effects of cytokine stimulation of macrophage on expression of cellular factors important for HIV-1 replication, the sole interaction of the virus with the macrophage is able to modulate gene expression. Therefore, we investigated the ability of HIV-1 to induce changes in gene expression in unpolarized macrophages, in comparison to cytokine-polarized cells. In chapter 6, we described how CCR5-using HIV-1 is able to regulate the expression of genes that are also regulated by IL-4 in macrophages. These genes are involved in dampening pro-inflammatory responses in macrophages, which favors replication and may facilitate HIV-1 to evade antiviral responses from other immune cells. Changes in macrophage gene expression triggered by CCR5-using HIV-1 were differed from those regulated by a CXCR4-using virus, which may explain why CCR5-using viruses are preferentially transmitted to a new host.

The findings described in this thesis provide insight into the complex interplay between macrophages and HIV-1, i.e. “the big eaters” and “the small invaders”. In chapter 7, we have addressed some of the implications that cellular factors have on HIV-1 replication, dissemination throughout the body and the establishment of the viral reservoir. We have reviewed the role of activated macrophages in concomitant HIV-1 and bacterial or parasitic infections and how this can contribute to a faster progression to AIDS and other related diseases. This knowledge will not only contribute to our understanding of HIV-1 infection of cells of the immune system, but will also contribute to the design of new therapeutic alternatives towards achieving a cure for HIV-1 infection.