HIV-1 infection in macrophages and genes involved throughout: Big eaters versus small invaders
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chapter
SEVEN
GENERAL DISCUSSION
Macrophages constitute the first line of the defence against invading pathogens and are crucial in mediating and controlling inflammatory responses, as well as preventing tissue damage. Macrophages were first characterized as very dynamic cells with a high phagocytic ability¹, which is reflected in their name, derived from the Greek “Big eaters” (makros: Big and phagein: Eat). In vivo, heterogeneous macrophage populations are responsible for maintaining tissue homeostasis and at the same time are activated by the microenvironment that surrounds them. The multiplicity of tissue-specialized macrophages originates from their ability to trigger the appropriate response upon encountering cytokines, damaged tissue or pathogens².

microRNAs are key post-translational regulatory elements of gene expression³ and play important roles in regulating development and function of immune cells⁴. We have described how microRNAs control gene expression and therefore functional aspects in macrophages. We have identified several microRNAs that are expressed in in vitro cytokine polarized macrophages that are related to macrophage function, especially with their role in the immune system (Chapter 2). Certain microRNAs are involved in maturation of monocytic precursors into macrophages, whereas others are exclusively regulated upon cytokine stimulation and skewing of macrophages into a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. Since microRNAs can be excreted into the extracellular environment, most likely in exosomal vacuoles⁵,⁶, they serve as a reflection of the intracellular microRNA activity, and function as biomarkers to identify cellular abnormalities during disease⁷,⁸. Consequently, some circulating microRNAs have been identified as promising biomarkers for inflammation and age-related disease such as cardiovascular disease, Alzheimer’s disease, type 2 diabetes and cancers⁹.

Given the involvement of macrophages in regulation of immune responses, analysis of miRNA expression in tissues as well as in biological fluids in comparison to their expression in monocytes or stimulated macrophages described here, will help identify biomarkers associated with deregulation of inflammation, or development of macrophage-mediated pathologies during the course of infection. Therefore, the use of biomarkers will help understand the development of such diseases, and will also serve as an early diagnostic tool for timely treatment.

The interaction between macrophages and HIV-1 is complex and therefore must be analysed in detail. The heterogeneous nature of macrophages and the variety of functions they carry out in the tissue influences their interaction with the virus. Furthermore, HIV-1 relies on cellular proteins as well as the cellular transcriptional and translational machinery upon infection, in order to produce the virus progeny. Cellular mechanisms are designed to prevent replication of foreign pathogens and mediate triggering of antiviral responses in these immune cells. This is the role of recently described cellular factors like Trex-1, SAMHD1, IFI16 and MX2 among others¹⁰-¹³. HIV-1 has counteractive mechanisms of its own that allow the virus to overcome restriction by cellular components, and still make use...
of the cellular machinery for its replication. For example, accessory proteins Vif and Vpu directly target the cellular factors Tetherin and APOBEC3G and prevent them from inhibiting viral replication. Therefore, the interaction between HIV-1 and host proteins is essential for viral replication.

In addition, expression of several cellular factors is regulated during skewing of macrophages towards an effector phenotype (pro-inflammatory or anti-inflammatory macrophages), which allows macrophages to control viral replication efficiently. Among the different cytokine-polarized macrophages, infection is blocked at different stages of the replication cycle; however none of the known HIV-1 restriction factors are solely responsible for inhibition of HIV-1 in these cells (Chapter 3). Therefore, other cellular factors expressed in cells that do not support HIV-1 replication may have the intrinsic power to stop viral replication. Through the analysis of gene expression profiles in macrophages that inhibit HIV-1 replication (M1 polarized macrophages) we have identified a new HIV-restriction factor: GTPase activating protein (SH3 domain) binding protein 1 (G3BP1) which is expressed in macrophages and induced upon IFN$\gamma$+TNF$\alpha$ stimulation (Chapter 4). G3BP1 is able to bind HIV-1 RNA transcripts, and this may delay mRNA translation and viral protein production, leading to lower production of new viral particles. G3BP1 is highly expressed in resting T cells, however upon activation G3BP1 levels decrease significantly, indicating that inhibition by G3BP1 could contribute to the formation of the viral reservoir in resting T cells as well as in macrophages.

The importance of the role of cellular factors for HIV-1 infection is reflected by the impact of genetic polymorphisms on disease progression and also by evidence that some HIV-1 infected patients seem to control HIV-1 infection in the absence of therapy (long-term non-progressors and elite-controllers). Identification of genes that are required for successful HIV-1 replication in macrophages also contributes to our understanding of the interplay between the virus and cells of the immune system. Genetic polymorphisms in PDE8A were found to be associated with lower viral replication in macrophages. PDE8A expression in macrophages is regulated by miRNA miR-145-5p during differentiation, and we observed that PDE8A supports HIV-1 replication at an early post-entry step, resulting in increased proviral DNA synthesis (Chapter 5). These findings not only indicate the importance of expression levels of cellular factors for HIV-1 replication, but also highlight the significance of miRNA-mediated regulation of gene expression in this process. This may suggest that alterations in expression of cellular factors that have the potential of naturally controlling infection and disease progression, could serve as targets for antiviral therapy. Therefore, the identification of such factors will provide candidates for new gene-based therapeutic strategies that can manipulate their expression, contributing to the control HIV-1 infection and eradication of the viral reservoir.

Restriction of HIV-1 in primary cells by the effect of restriction factors could however become a double-edged sword in the efforts of the immune system...
to control HIV-1 infection. While polarized macrophages control infection, they may contribute to cell-to-cell transmission of the virus, without being detected by other cells of the immune system. Cells that are not susceptible to viral infection, can still mediate transmission of HIV-1 particles to uninfected cells through the virological synapse (discussed in chapter 1). IL-4 mediated activation of macrophages induces expression of DC-SIGN on the cell membrane, which in turn increases the ability of these cells to transmit viral particles and disseminate the infection of CCR5- but not CXCR4-using viruses\textsuperscript{25,26}. This means that while macrophages are not productively infected, they silently contribute to infection and spread of the virus in the tissues. Furthermore, HIV-1 infection induces a favourable environment for viral replication by down-modulating the cell’s ability to establish antiviral responses, and may also mediate host-to-host transmission of the virus. HIV-1 infection is able to modify gene expression and therefore alter functional aspects in macrophages and T cell that can be beneficial for viral replication\textsuperscript{27,28}. We observed that genes regulated by HIV-1 replication are similar to those regulated by IL-4 mediated polarization of these cells (\textit{Chapter 6}). This ability to modify the macrophage gene expression was dependent of the ability of the virus to bind the CCR5 coreceptor, while only minimal changes in gene expression were observed upon CXCR4 binding, which may explain why CCR5-using HIV-1 strains are preferably transmitted to a new host. Our observations also indicate that HIV-1 is able to skew cells towards an anti-inflammatory phenotype, thus avoiding triggering of innate antiviral responses and favouring continuous replication and systemic spread of the virus throughout the body.

\textit{In vivo}, the failure of infected macrophages to fulfil their primary functions is reflected by the lack of control of infections by other pathogens and their role in increasing replication of HIV-1 as well as the concomitant infectious agents, resulting in faster disease progression. Because of their function and location, macrophages are tissue-available target cells for several pathogens in addition to HIV-1, like \textit{Mycobacterium tuberculosis} and \textit{Leishmania} spp., among others. At the same time, HIV-1 infection results in severe depletion of CD4\textsuperscript{+} T cells and progression to AIDS, which increases susceptibility to secondary infections, especially in geographical areas where certain bacterial or parasitic infections are endemic and access to antiretroviral therapy is limited. Macrophages are a known source of HIV-1 replication during these co-infections\textsuperscript{29}, and are particularly associated with increased production of viral particles at the sites of infection. Protozoan parasites such as \textit{Trypanosoma} spp., \textit{Plasmodium} spp. and \textit{Leishmania} spp. also reside in macrophages, and in most cases parasitic co-infections result in higher viremia and parasitemia\textsuperscript{30}. Although some reports show that macrophage infection with \textit{Trypanosoma cruzi} and \textit{Plasmodium falciparum} inhibit HIV-1 replication\textsuperscript{31,32}, other studies describe that \textit{Leishmania} increases viral and parasitic infection in MDM\textsuperscript{33}. \textit{Schistosoma} spp. infections are also associated with increased
susceptibility to HIV-1 infection and disease progression, although the role of macrophages in this parasitic co-infection is still unclear. Bacterial tuberculosis caused by _Mycobacterium tuberculosis_ is another well described co-infection that increases HIV-1 replication in alveolar macrophages and can enable viral replication in local lymphocytes. Although macrophages mediate Th1 or Th2 responses against bacterial and parasitic pathogens and _in vitro_ activated macrophages are capable of inhibiting HIV-1 replication at the cellular level, these cells are unable to control the invading pathogens in vivo and in most cases amplify bacterial, viral or parasitic replication during co-infection scenarios, leading to faster disease progression. In the case of bacterial infections, this phenomenon has been associated with an impairment of macrophages in mounting innate responses, caused by HIV-1 infection. Additionally, many of these pathogens have other strategies to evade immune responses elicited by macrophages. Consequently, it is essential to understand the changes that macrophages undergo when they become activated by an invading infectious agent, especially during the interaction with multiple pathogens, and how these interactions affect their ability to trigger innate responses and clear the infection. Gene and microRNA expression studies described in chapter 2 and 4 will be a source of information for identifying cellular factors in anti-inflammatory macrophages (M2) that may play a role in infection of macrophages by HIV-1 and parasites.

Macrophages have the potential to act as a viral reservoir and HIV-1 infected macrophages reside in the tissues of HIV-1 infected patients, which allows for residual viral replication in sites such as the gut-associated lymphoid tissue and the brain, even after the use of antiretroviral therapy. It is also known that HIV-1 variants, which infect macrophages very efficiently, are dominantly present in the Central Nervous System (CNS) of infected individuals, and can contribute greatly to residual viral replication and development of AIDS-related pathologies such as HIV-associated neurocognitive disorders, cardiovascular disease and lymphomas. Therefore, in addition to the possibility of enabling residual viral replication, infected macrophages contribute to the parallel deterioration of several organs during disease progression. HIV-1 infection can be controlled by antiretroviral therapy; however several diseases develop in individuals on long term therapy, of which some are associated with aging (e.g. cardiovascular disease), and seem to take place earlier in HIV-1 infected patients on therapy, compared to uninfected individuals. Ongoing research focuses on determining whether these co-morbidities are caused by the use of therapy or residual viral replication. Nevertheless, even under cART regiments, residual viral replication in compartments such as the CNS, where antiretroviral drugs are less efficiently absorbed, still contribute to the development of neurocognitive disorders.

Since reservoirs are a main source of residual viral replication, it is of paramount importance to define which cells act as reservoirs, in which form is the virus residing
(e.g. episomal DNA, integrated provirus) in such reservoirs and what mechanisms contribute to the establishment of latency in these cells. This knowledge will contribute to the design of strategies that eliminate HIV-1 infected cells and may ultimately be part of a sterilizing cure for HIV-1 infection. It is therefore necessary to study HIV-1 infection in macrophages and understand if these cells indeed contribute to residual viral replication and which cellular factors may be involved in latency and formation of the viral reservoir in macrophages. We have established an experimental strategy that allowed us to identify novel cellular factors that restrict HIV-1 by analysing gene expression in cells that support HIV-1 replication and comparing it to cells that are able to inhibit viral replication in macrophages.

Although the introduction of combined antiretroviral therapy has dramatically changed the outcome of HIV-1 infection, the continuous immune dysfunction, chronic immune activation and inflammation observed in HIV patients on effective therapy are associated with an increased risk for non-HIV related co-morbidities. Therefore there is still an urgent need to find new therapeutic alternatives. Major objectives could include the re-establishment of the appropriate immune function in macrophages that will allow them not only to control HIV-1 replication at the cellular level, but also to prevent spread of the virus, formation of viral reservoirs, and at the same time to be able to control other invading pathogens. To achieve these goals, we must first identify the cellular factors that are involved in every step of the interaction between the macrophages and HIV-1 and understand their role in detail. The identification of novel cellular factors and microRNAs described here is the result of our efforts to understand which genes are involved throughout the HIV-1 infection process of macrophages, also after cellular activation. This knowledge contributes to our understanding of how pathogens interact with macrophages and how cytokine polarization affects the outcome of this encounter.

REFERENCES


