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Classical and alternative macrophages have impaired function during acute and chronic HIV-1 infection

Leonardo J. Galvão-Lima[a], *Milena S. Espíndola*[a], *Luana S. Soares*[a], *Fabiana A. Zambuzi*[a], *Maira Cacemiro*[a], *Caroline Fontanari*[a], *Valdes R. Bollela*[b], *Fabiani G. Frantz*[a,*]

[a] *Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Laboratório de Imunologia e Epigenética, São Paulo, SP, Brazil*
[b] *Universidade de São Paulo, Hospital das Clínicas de Ribeirão Preto, Divisão de Doenças Infecciosas, São Paulo, SP, Brazil*

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ABSTRACT

Objectives: Three decades after HIV recognition and its association with AIDS development, many advances have emerged – especially related to prevention and treatment. Undoubtedly, the development of Highly Active Antiretroviral Therapy (HAART) dramatically changed the future of the syndrome that we know today. In the present study, we evaluate the impact of Highly Active Antiretroviral Therapy on macrophage function and its relevance to HIV pathogenesis.

Methods: PBMCs were isolated from blood samples and monocytes (CD14+ cells) were purified. Monocyte-Derived Macrophages (MDMs) were activated on classical ($M_{GM\text{-}CSF+IFN\text{-}\gamma}$) or alternative ($M_{IL\text{-}4+IL13}$) patterns using human recombinant cytokines for six days. After this period, Monocyte-Derived Macrophages were stimulated with TLR2/Dectin-1 or TLR4 agonists and we evaluated the influence of HIV-1 infection and Highly Active Antiretroviral Therapy on the release of cytokines/chemokines by macrophages.

Results: The data were obtained using Monocyte-Derived Macrophages derived from HIV naïve or from patients on regular Highly Active Antiretroviral Therapy. Classically Monocyte-Derived Macrophages obtained from HIV-1 infected patients on Highly Active Antiretroviral Therapy released higher levels of IL-6 and IL-12 even without PAMPs stimuli when compared to control group. On the other hand, alternative Monocyte-Derived Macrophages derived from HIV-1 infected patients on Highly Active Antiretroviral Therapy released lower levels of IL-6, IL-10, TNF-α, IP-10 and RANTES after LPS stimuli when compared to control group. Furthermore, healthy individuals have a complex network of cytokines/chemokines released by Monocyte-Derived Macrophages after PAMP stimuli, which was deeply affected in MDMs obtained from naïve HIV-1 infected patients and only partially restored in MDMs derived from HIV-1 infected patients even on regular Highly Active Antiretroviral Therapy.

* *Corresponding author.*
E-mail address: frantz@Usp.Br (F.G. Frantz).
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Conclusion: Our therapy protocols were not effective in restoring the functional alterations induced by HIV, especially those found on macrophages. These findings indicate that we still need to develop new approaches and improve the current therapy protocols, focusing on the reestablishment of cellular functions and prevention/treatment of opportunistic infections.

Introduction

Since the discovery that HIV was the cause of the AIDS and the establishment of this condition as the main cause of death associated with infectious diseases, recent data estimate that over 35 million people around the world are living with HIV.[1] During the last three decades, there have been many advances in the understanding of viral cycle replication and pathogenesis-related mechanisms. As a result several antiretroviral drugs were developed. Specifically, the combined Highly Active Antiretroviral Therapy (HAART) resulted in the reduction of deaths related to AIDS, helped controlling the viral spread and increased the quality of life and the life expectancy of HIV-1 infected patients.[2–4] However, many challenges still hamper the development of new and more effective therapeutic approaches to functional cure.

Classically, the immune dysfunction observed during HIV infection is directly associated with an intense reduction of CD4+ T cells in peripheral blood and other tissues including intestinal mucosa. In recent years several studies have demonstrated the contribution of innate immunity to viral pathogenesis and AIDS development.[5–7] This paper will focus on the role of macrophages, but other authors have explored the relevance of dendritic cells and neutrophils on HIV pathogenesis.[8–10]

Monocyte-Derived Macrophages (MDMs) play a central role in the immune response, orchestrating the development of innate and adaptive immunity to pathogens, and further, these MDMs can be infected chronically by HIV.[11] Besides remaining viable, HIV infected MDMs can be differently activated and develop several functional impairments, with reduced phagocytic and intracellular killing activity, thus allowing for the occurrence of opportunistic infections.[12–14]

In the recent years, Chihara and colleagues have demonstrated that HIV-1 proteins including gp120, Tat, and Nef induce macrophage polarization towards the classical pathway[13] and, according to Cassol and colleagues, enable macrophage support for increased viral replication.[15] Currently, few papers have evaluated the role of antiretroviral drugs on functional immune recovery after the onset of regular therapy protocols. In our study we evaluated the influence of HIV-1 infection on cytokine/chemokine release by classically ($M_{GM\text{-}CSF+IFN\text{-}\gamma}$) or alternatively ($M_{IL\text{-}4+IL13}$) activated MDMs after PAMPs stimuli on MDMs derived from treatment-naïve patients or from those on regular HAART.

Material and methods

Casuistic and selection criteria

Eight treatment-naïve HIV-1 infected patients on early stage of disease were recruited for this study. In addition, 15 HIV-1 infected patients on regular HAART (HIV-1 + HAART) for at least six months and 15 healthy blood donors were included as controls (Table 1). Informed consent was obtained from all participants included in the study. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The present study was approved by the Clinical Hospital of FMRP/USP Ethics Committee (#9817/2012).

Isolation PBMCs, purification of CD14+ cells, and MDMs activation

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque™ PLUS (GE Healthcare), following the manufacturer's instructions. The obtained PBMCs were quantified and the monocytes (CD14+ cells) were purified using positive selection with magnetic system microbeads (Miltenyi Biotec). After elution, the CD14+ cells were cultured in 48-well plates with RPMI 1640 medium containing 1 μL/mL gentamicin, supplemented with 10% of FBS (Gibco Laboratories) and incubated at 37 °C, 5% CO_2. To induce classical ($M_{GM\text{-}CSF+IFN\text{-}\gamma}$) or alternative ($M_{IL\text{-}4+IL13}$) MDMs activation, CD14+ cells were cultured for six days in medium containing GM-CSF (20 ng/mL; R&D Systems) and IFN-γ (10 ng/mL; Millipore) or IL-4 and IL-13 (50 ng/mL each; R&D Systems), respectively. Resting MDMs (M0) were cultured with standard medium without supplementation of cytokines. Every two days in culture, fresh medium containing the respective conditions (M0, $M_{GM\text{-}CSF+IFN\text{-}\gamma}$ or $M_{IL\text{-}4+IL13}$) was added to keep cell viability.

Quantification of cytokines and chemokines on supernatant after PAMPs stimuli

After the respective activation, MDMs were stimulated with 10 ng/mL of LPS (Sigma–Aldrich) or 100 μg/mL of β-glucan extracted from *Saccharomyces cerevisiae* (Calbiochem, Merck Millipore) during 24 h. After this period, the supernatant was collected and nine cytokines and chemokines released (IL-1β,