Gene expression profiling of monocytes in the HIV/tuberculosis-associated immune reconstitution inflammatory syndrome

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Summary

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mt), is one of the most common opportunistic infections (OIs) in HIV patients, in particular in developing countries. Restoration and preservation of immune function by combination antiretroviral therapy (cART) have led to a significant reduction in prevalence and associated mortality of OIs such as TB, and improved survival of HIV-TB coinfected patients. However, in some instances cART is associated with complications and paradoxical clinical deterioration. One of the more common but least understood complications associated with cART in HIV-TB coinfected patients is the TB-associated Immune Reconstitution Inflammatory Syndrome (TB-IRIS), which typically manifests as a paradoxical clinical deterioration in patients already stabilized on anti-TB therapy. This syndrome significantly complicates the management of both HIV and TB treatment and represents a great burden for the resource-limited health systems in developing countries.

Despite satisfactory control of viral replication and reconstitution of CD4+ T cell counts, and despite absence of active TB infection, patients with TB-IRIS show worsening symptoms of TB (such as fever, return of cough) and/or detrimentally exuberant inflammatory reactions such as abscess formation within lymph nodes, soft tissues or solid organs. TB-IRIS can occur as paradoxical or as unmasking condition. Paradoxical TB-IRIS occurs in patients on successful TB treatment before cART. Unmasking TB-IRIS occurs in patients without apparent TB when they start cART and may represent either reactivation of latent infection or worsened symptoms of TB that was not previously diagnosed as active disease or was subclinical.

The precise mechanisms underlying the development of TB-IRIS remain incompletely understood and no prognostic/diagnostic test for TB-IRIS has been developed. Most studies to date on the immunopathology of TB-IRIS have focused on components of the adaptive arm of the immune system, such as mycobacterial-specific T cells, Th1/Th2 cytokine profiling, and T-regulatory cells. However, findings have frequently been confusing and contradictory between studies and/or study populations. Other components of the immune system have only been explored cursorily, even though the predominantly early occurrence of TB-IRIS (during the initial months and even weeks of cART) suggests a possible role for the innate arm of the immune system in severely immunosuppressed patients. This notion has been increasingly supported by recent evidence, including the overwhelming presence of macrophages (Mφs) in TB-IRIS lung biopsy, Natural Killer cell activation during TB-IRIS, and dysregulation of pro-inflammatory cytokines associated with innate immune cell function. Combined with the documented wide range of monocyte/macrophage (MO/Mφ) dysfunctions during HIV infection, HIV-Mt coinfection, and cART, we considered it likely that MOs/Mφs are of importance in the development of TB-IRIS.
The current work therefore aimed to investigate the following aspects of potential involvement of myeloid cells in the development of TB-IRIS, using monocytes (MOs) as a study model: (1) to confirm that MOs are involved in TB-IRIS development; (2) to search for MO-associated factors that represent (prognostic and diagnostic) biomarkers for the development of TB-IRIS; and (3) to investigate how MOs functionally contribute to cART and combined cART/anti-TB treatment responses in patients who do and do not develop TB-IRIS. Accordingly, our work consisted of three main parts: a study on the different MO subsets present in PBMCs of TB-IRIS patients, a human genome-wide (HGW) microarray analysis, and a focused functional investigation. Our work was conducted as a nested case-control study, integrated in a larger cohort study on TB-IRIS, involving an HIV-positive patient population attending Mulago hospital in Kampala, Uganda. Due to the specifics of our study population, this work focused on the development of paradoxical TB-IRIS only.

In order to obtain an overview of the extant MO sub-populations during TB-IRIS, we performed flow cytometric analysis on PBMC samples from TB-IRIS patients and matched HIV+/TB+ non-IRIS controls. We observed proportional differences of MO subpopulations between patients and controls, specifically for the CD14\textsuperscript{hi}/CD16\textsuperscript{-} MO subset, providing the first indication of dysregulation of myeloid cell functionality during TB-IRIS. As this MO subset is also readily available in peripheral blood samples, it was selected as the focus of our study. Protocols for isolation of this MO population from cryopreserved PBMC samples and purification of high-quality RNA from minute cell samples were optimised.

To compare gene expression between patients who developed TB-IRIS and control patients, a HGW microarray profiling was conducted, in which the transcriptome of MOs of TB-IRIS patients and controls was analysed at two time-points: at cART initiation (baseline) and at week two post-cART initiation. This study was among the first to show the possibility of using gene expression to classify TB-IRIS patients and controls, both at the approximate time of the TB-IRIS-defining event and before initiation of cART (i.e. as a predictive classification). This finding is an important proof-of-concept that gene expression can be used as a classifier for predisposition to TB-IRIS, and moreover that this predisposition manifests at least partially at the level of the circulating MO population. This observation should allow a much more refined analysis of the underlying mechanisms of predisposition to TB-IRIS, such as putative genetic determinants, than the relatively general pre-cART predictors identified so far, such as a low nadir CD4 count. At the practical level, such a predisposition could be exploited for the development of a prognostic test or testing algorithm, which could allow identification of patients at high risk of developing TB-IRIS, thus greatly facilitating clinical management of co-infected patients. In addition to the relevance of MO for the purpose of classifying TB-IRIS patients, the fact that MO-associated genes are expressed differentially, combined with the functional mapping of such differentially expressed MO genes, indicates a potential functional contribution of MO/myeloid cells to the development of TB-IRIS. A number of
MO-associated biological processes or functions were found to be perturbed in TB-IRIS patients - in particular, an apparent dysregulation in both anti- and pro-inflammatory processes in MOs was identified.

Amongst these modulated pathways, the complement system was selected as focus for further investigation, due to the extent of its dysregulation and its potential relevance. An independent technological setup was used for confirmation of complement cascade dysregulation at the mRNA level, and key protein components of the complement system were examined further where the tools and samples were available. We showed that even before initiation of cART, the complement system was dysregulated in HIV-TB co-infected patients who were predisposed to TB-IRIS. Detailed analysis revealed differences between TB-IRIS patients and matched non-TB-IRIS cases, at the level of the balance between the effector C1Q and the inhibitor C1-INH, both before and two weeks after cART initiation. Our results suggest that inappropriate control of complement activation could be associated with the “flaring up” of inflammation observed during TB-IRIS.

Even though a great number of research gaps remain to be filled, the data from our microarray analysis has opened several potential directions for further functional investigation of the disease mechanisms. Integrating our findings and other reports on TB-IRIS suggests the underlying immunopathogenesis of the syndrome is likely a multifactorial process resulting from, amongst others, availability of antigen, sensitivity towards antigen, degree of reduction of microbicidal activity and aberrant activation of inflammatory effector cells leading to an imbalance between pro-and anti-inflammation.