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The role of monocytes in the development of Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome

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ABSTRACT

Background: Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome (TB-IRIS) is a common complication of combined antiretroviral therapy (cART) in HIV-TB co-infected patients. However, the disease mechanism is poorly understood, prognosis of TB-IRIS is currently impossible, and diagnosis is highly challenging. We analyzed whether the gene expression of monocytes could be correlated with TB-IRIS pathogenesis and could be used to classify patients predisposed to TB-IRIS.
Methods: Monocyte gene expression was compared between patients who developed TB-IRIS and matched controls. We carried out whole-genome expression profiling using Affymetrix GeneChip® ST 1.1 arrays at two time-points: before cART initiation (baseline) and at week two post-cART initiation. For each time-point, we used different statistical approaches to identify molecular signatures which could be used as classifiers. We also functionally mapped the modulated cellular pathways using the software package Ingenuity Pathway Analysis.
Results: At baseline, before introduction of cART and before onset of symptoms, monocyte gene expression was already perturbed in patients who subsequently developed TB-IRIS, indicating a possible involvement of monocytes in TB-IRIS predisposition. The differences in monocyte gene expression in TB-IRIS patients became even more clear after two weeks of cART (when TB-IRIS commonly occurs), with more than 100 genes for which expression showed a fold change greater than 1.5. Both at baseline and at week two post-cART initiation, a classifier of 8 and 9 genes, respectively could be built, which allowed discrimination of TB-IRIS cases and controls. Pathway analyses revealed that the majority of the dysregulated genes in TB-IRIS – at the time of the IRIS episode, but also already at baseline – are associated with infection and inflammation. Relevant biological functions which were perturbed before/during TB-IRIS included "Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses" and "Complement System".
Conclusion: Our results indicate an involvement of monocytes in predisposition to/development of TB-IRIS, and suggest a number of functional pathways which may play a role in TB-IRIS development. This comprehensive study of gene regulation in monocytes provides baseline data for further studies into biomarkers for prognosis and diagnosis of TB-IRIS.

Abbreviations: cART, combined antiretroviral therapy; DABG, detection above background; HIV, human immunodeficiency virus; IPA, Ingenuity Pathway Analysis; LOOCV, leave-one-out cross-validation; Mtb, *Mycobacterium tuberculosis*; PBMC, peripheral blood mononuclear cells; PCA, principal component analysis; PPD, purified protein derivative; RMA, robust multichip average; ROC, receiver operating characteristic; R-SVM, Recursive Support Vector Machines; TB, tuberculosis; TB-IRIS, Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome.

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Introduction

Tuberculosis (TB) and the human immunodeficiency virus (HIV) are, individually, two of the world's greatest public health threats. Combined, the two diseases can be even more devastating (Pawlowski et al. 2012), and create unique treatment challenges for health care providers. One of the most common of these challenges is the TB-associated Immune Reconstitution Inflammatory Syndrome (TB-IRIS), which typically occurs during the initial weeks or months of combined antiretroviral therapy (cART). It presents as an exacerbated inflammatory response toward *Mycobacterium tuberculosis* (Mtb) antigens, resulting in paradoxical clinical deterioration in patients undergoing immune recovery during early cART (Lawn et al. 2005; Meintjes et al. 2008a,b). TB-IRIS can occur as paradoxical or as unmasking condition. Paradoxical TB-IRIS occurs in patients on TB treatment before cART. Unmasking TB-IRIS occurs in patients who are not on TB treatment 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. Due to the population in our study (Worodria et al. 2012), we limited our study to cases of paradoxical TB-IRIS.

Since the first report of TB-IRIS in the early 1990s (French et al. 1992), no prognostic/diagnostic test for TB-IRIS has been developed, and precise mechanisms underlying the development of TB-IRIS remain incompletely understood (Meintjes et al. 2008a,b). 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 (Bourgarit et al. 2006, 2009; Elliott et al. 2009; Meintjes et al. 2008a,b; Tan et al. 2008; Tieu et al. 2009), Th1/Th2 cytokine profiling (Bourgarit et al. 2006; Ruhwald and Ravn 2007; Tadokera et al. 2011; Meintjes et al. 2008a,b), and T-regulatory cells (Meintjes et al. 2008a,b; Seddiki et al. 2009). However, findings have frequently been confusing and contradictory between studies and/or study populations, for example in the case of purified protein derivative (PPD) specific-T cells (Meintjes et al. 2008a,b; Bourgarit et al. 2006). 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 immunosupressed patients. This notion has been increasingly supported by recent evidence, including the overwhelming presence of macrophages in TB-IRIS lung biopsy (Lawn et al. 2009), natural killer cell activation during TB-IRIS (Conradie et al. 2011; Pean et al. 2012), and dysregulation of the proinflammatory cytokines IL-18 and CXCL10 (Oliver et al. 2010).

Genome-wide gene transcript profiling has evolved into an overall measure of the status of the immune system in health and disease, and has been particularly useful in pinpointing genes and cellular pathways that may be involved in the pathogenesis of infectious and immune related-diseases (Chaussabel et al. 2010). Transcriptional profiling of monocytes/macrophages, which are the first line of defense in both HIV (Herbein and Varin 2010) and Mtb (Schluger 2005) infection individually, has been systematically performed in the context of HIV infection (Van den Bergh et al. 2010; Wu et al. 2013; Giri et al. 2009; Tilton et al. 2006), Mtb infection (Toossi et al. 2012; Jacobsen et al. 2007), and HIV–Mtb co-infection (Maddocks et al. 2009), and has yielded important new insights in the field. Based on our hypothesis on the possible importance of macrophages in TB-IRIS (Van den Bergh et al. 2006), we therefore attempted to address the question whether changes in monocyte gene expression correlate with disease conditions or clinical outcomes by performing genome-wide microarray analysis in a previously documented cohort of TB-IRIS patients (Worodria et al. 2012). Recently, we have reported on the use of these data in a hypothesis-generating approach, whereby a specific set of selected genes in the complement pathway was validated in a technologically independent set-up (Tran et al. 2013). In the current manuscript, we evaluate the potential to identify molecular signatures that can be used to classify patients predisposed to TB-IRIS and present a functional mapping of the modulated cellular pathways in the genome-wide data set.

Materials and methods

Study setting and population

Patient recruitment was conducted at the Mulago National Tuberculosis and Leprosy clinic and the Infectious Diseases Institute in Kampala, Uganda. Recruitment and follow-up of patients and TB-IRIS diagnosis were described previously by Worodria et al. (2012). Patients who did not develop TB-IRIS or other forms of IRIS in the first 12 months of cART were considered as potential control patients. Control patients were selected for cases by matching gender, age and pre-cART CD4 count. The study was approved by the Institutional Review Board of all involved institutions, and written informed consent was obtained from all donors in accordance with the Declaration of Helsinki.

Sample processing

Blood samples (30–40 mL) were collected in EDTA-tubes at baseline and at week two of cART. Plasma and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll–Paque Plus gradient separation and were cryopreserved. Monocytes were purified from cryopreserved PBMC samples using Miltenyi Biotec's MACS system (Bergisch Gladbach, Germany). Yields were minimally 5×10^5 monocytes with a purity of $\geq$92% and viability $\geq$88% as verified through flow cytometry (using anti-CD14 and anti-CD16 antibodies) and trypan blue dye exclusion staining, respectively. Total RNA and protein fractions were isolated from purified monocyte Trizol lysates using chloroform extraction.

Microarray analysis

Microarray analysis was performed by the VIB Nucleomics Core (www.nucleomics.be) using Affymetric Gene 1.1 ST arrays, according to the manufacturer's instructions. RNA concentration and purity were determined spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies) and RNA integrity was assessed using a Bioanalyser 2100 (Agilent, Santa Clara, CA, USA). Datasets were deposited at the EMBL-EBI repository (E-MEXP-3618; E-MEXP-3591).

Microarray data quality control and normalization

Analysis of the microarray data was performed in the R programming environment, in conjunction with the packages developed within the Bioconductor project (http://www.bioconductor.org). The analysis was based on the robust multichip average (RMA) expression levels of the probesets that had at least one present detection above background (DABG) detection call. The DABG score can be calculated for each probeset by matching perfect match probes to members of the background pool with the same GC content and measuring the relative distance between the two. Intensities below the background signal (i.e. absent from DABG detection call) were omitted. Intra-array normalization (background correction, $\log_2$-transformation, and probeset summation) and inter-array normalization (quantile normalization) were conducted following the RMA procedure (Irizarry et al. 2003; Bolstad et al. 2003).