EBioMedicine 5 (2016) 161–166

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Role of Natural Autoantibodies in Ugandans With Rheumatic Heart Disease and HIV☆

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ARTICLE INFO

Article history:
Received 25 December 2015
Received in revised form 28 January 2016
Accepted 3 February 2016
Available online 12 February 2016

ABSTRACT

Background: Rheumatic heart disease (RHD) and HIV are prevalent diseases in sub-Saharan Africa, but little is known about their potential interrelationships. The objective of this study was to assess the prevalence of protective natural autoantibodies among patients with RHD in Uganda, and to determine whether the levels of these autoantibodies are affected by HIV status.

Methods: Participants were grouped according to RHD and HIV status. The three control groups (RHD − HIV −, RHD − HIV +, RHD + HIV −) were age-matched to the RHD + HIV + participants. All participants underwent HIV testing and echocardiography to evaluate for RHD. Natural autoantibody levels reactive with phosphorylcholine (PC) and malondialdehyde (MDA) were measured.

Findings: We enrolled 220 participants; 21 with both RHD and HIV. Ages ranged from 10 to 60 years, with female predominance (144/220, 65%). After adjusting for age and gender, HIV infection and RHD were each associated with low IgM anti-PC (HIV: $p < 0.0001$ and RHD: $p = 0.01$). A distinct HIV∗RHD interaction was identified ($p = 0.045$) with increased IgG anti-MDA levels in HIV infected subjects without RHD, whereas IgG anti-MDA levels were decreased in HIV infected subjects with RHD.

Interpretation: We found that HIV and RHD are associated with alterations in natural autoantibody responses previously linked to an increased risk for atherosclerosis and autoimmune inflammatory disease.

1. Introduction

Rheumatic heart disease (RHD) and HIV are prevalent in Uganda and other sub-Saharan African countries. Rheumatic heart disease (RHD) affects 15 million people worldwide with an estimated 1.4 million deaths annually. In Uganda RHD is the most common cause of heart disease within the 15 to 49 age group (Remenyi et al., 2012; Okello et al., 2012; Marijan et al., 2012; Sliwa and Zilla, 2012). The immunopathogenesis of chronic valvular inflammation in RHD including the role of cellular vs. humoral immune responses, and the identity of the responsible immunodominant epitopes that drive the progression of disease are debated (Tandon et al., 2013). HIV currently affects 5–10% of Ugandan adults, which is improved from nearly 30% in the early 1990s (The Republic of Uganda, 2014). The immune deficiency and dysfunction caused by HIV and AIDS has been shown to impact susceptibility to and progression of other autoimmune diseases (Zandman-Goddard and Shoenfeld, 2002); however, the impact of HIV infection on RHD pathogenesis has not been investigated.

Natural arising autoantibodies to oxidation-associated epitopes have been shown to modulate the initiation and progression of a range of immune-mediated inflammatory diseases including atherosclerosis. These autoantibodies are postulated to play roles in homeostasis and immune regulation (Gronwall and Silverman, 2014), and altered serum levels correlate with certain disease states. IgM natural autoantibodies reactive with the phosphorylcholine (PC) head group are commonly cross-reactive with PC determinants on oxidatively modified

☆ Funding sources: DMH was supported by the IDSA Medical Scholars Program, the Rottman Fund of Case Western Reserve University, the ASTMH Benjamin H. Kean Travel Fellowship, and the Cleveland Clinic Richard J. Fasenmyer Medical Student Fellowship in HIV Research. CTL is supported by the National Institutes of Health (K23 HL123341), a Wolf Family Foundation Scholars Grant, and Medtronic Philanthropy. GJS is supported by the NIH-NIAID R01 AI090118, R01 A1068063, and the American College of Rheumatology Research Education Foundation Within Our Reach campaign.

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http://dx.doi.org/10.1016/j.ebiom.2016.02.006

low density lipoprotein (LDL), and apoptotic cells but not with healthy cells. In mice and humans, the antibody genes for dominant B-cell clones to PC determinants show evidence of clonal restriction and convergent somatic evolution (Silverman, 2015). Malondialdehyde (MDA) is a small chemically reactive compound that readily forms adducts on proteins, and IgM anti-MDA antibodies are common at birth and show great structural and clonal diversity (Chou et al., 2009; Unpublished data). Induction of high levels of IgM anti-PC antibodies has been shown to protect hyperlipidemic mice from atherosclerosis (Binder et al., 2003), and low IgM anti-PC correlates with increased risk for stroke and myocardial infarction in population-based cohort studies (Fiskesund et al., 2010; Gronlund et al., 2009). These natural autoantibodies may also modulate the pathogenesis of experimental and clinical autoimmune disease. IgM antibodies to PC enhance the clearance of damaged and apoptotic cells by binding to oxidation-associated epitopes, enhancing recruitment of C1q and mannose binding lectin and suppressing Toll-like receptor mediated inflammatory responses (Chen et al., 2009a; Chen et al., 2009b; Vas et al., 2012). In systemic lupus erythematous (SLE) patients, higher levels of anti-PC IgM have been associated with lower clinical disease activity, lower rates of MI and stroke, as well as reduced preclinical atherosclerosis burden based on carotid ultrasound (Gronwall et al., 2012; Gronwall et al., 2014).

Due to the common expression of these natural autoantibodies, we postulated that there may be similar roles in resolution of cellular injury in damaged valvular tissues in RHD, yet this has not previously been studied. In theory, high IgM anti-PC antibody titers would be predicted to promote rapid clearance of apoptotic cells in damaged tissues, suppressing over-exuberant inflammatory responses and release of autoantigens from cells, which may also have direct or indirect influence on recruitment and activation of auto-reactive lymphocytes. Given their putative role(s) in inflammatory vascular conditions, protective natural autoantibodies may also affect the risk of atherosclerosis in patients with RHD and HIV. The chronic systemic inflammation that is a hallmark of RHD and rheumatic fever may contribute to increased risk of atherosclerosis (Habeeb and Hadidi, 2011; Zoller et al., 2012). HIV infection is a well-known risk factor for accelerated atherosclerosis independent of use of antiretroviral therapy (ART), due to chronic inflammation and immune activation (Longenecker and Triant, 2014). In a recent study, HIV infection was associated with lower protective IgM natural autoantibodies and higher levels of IgG natural autoantibodies against oxidized forms of LDL (Yilmaz et al., 2014) but the relevance to clinical cardiovascular disease was not examined (Tsimikas et al., 2007). Perturbations in the levels of these natural autoantibodies may therefore contribute to the accelerated atherosclerosis seen in HIV.

The purpose of the current study was to assess the relative prevalence of these natural autoantibodies among patients with and without RHD in Uganda and to determine whether the presence of these autoantibodies may be impacted by HIV infection. We sought to test the hypothesis that both conditions would be associated with reduced levels of oxidation-associated IgM anti-PC natural autoantibodies along with increased IgG anti-MDA antibodies.

2. Methods

2.1. Study Participants and Enrollment

Study participants were enrolled from the Joint Clinical Research Centre and the Uganda Heart Institute in Kampala, Uganda from April to June 2014. For this cross-sectional study, we recruited subjects that were then stratified by RHD and HIV status into groups (RHD − HIV −, RHD − HIV +, RHD + HIV −) that were age-matched to the RHD + HIV + group. All participants were >8 years old and had a transthoracic echocardiogram performed for valvular heart disease, due to clinical presentation or as part of a screening protocol. We included those with overt clinical RHD or definite latent RHD, as defined by World Heart Federation (WHF) criteria (Remenyi et al., 2012). Subjects were excluded from the RHD negative controls if they had elevated anti-streptolysin O, borderline RHD by WHF criteria or had congenital heart disease or heart failure other than from RHD. Written informed consent was obtained from each participant and the study was approved by the Institutional Review Boards at University Hospitals, Cleveland, Ohio and Makerere University, Kampala, Uganda.

HIV-1/2 testing was performed on all participants, unless an HIV diagnosis had previously been confirmed with rapid antibody testing and confirmed by ELISA or Western Blot. We used a primary rapid antibody test (Alere Determine HIV-1/2) with confirmation by Trinity Biotech Uni-Gold and AccuBioTech tests. Patients who were newly HIV positive were also referred for further care and confirmatory testing to the Joint Clinical Research Centre or other preferred HIV treatment center. Venous blood was obtained by venipuncture and a complete blood count, Anti-streptolysin O (ASO), and high sensitivity CRP (hsCRP) were measured at the clinical laboratory of the Uganda Heart Institute. CD4 count was measured in all HIV positive subjects. Serum samples were frozen and stored at the Joint Clinical Research Centre at −80 °C without thawing before autoantibody analysis. At the time of enrollment clinical history was obtained by chart review. All data were stored in a REDCap database hosted at University Hospitals, Cleveland (Harris et al., 2009).

2.2. Natural Autoantibodies

Levels of anti-phosphorylcholine (PC) and anti-malondialdeyhyde (MDA) antibodies were measured in duplicate from frozen serum samples by in-house assays, as previously described (Gronwall et al., 2012). Herein, ELISA wells were coated with PC6-BSA (Biosearch Technologies Inc., Novato, CA, USA) or MDA-BSA (Academic Bio-medical Co., Houston, TX, USA). Values were reported from 1:1000 dilutions in 1% BSA-PBS after detection with goat anti-IgM-HRP (Southern Biotech, Birmingham, AL, USA) or goat anti-IgG-HRP (Jackson Immunosearch, West Grove, PA, USA). An established standard curve from a SLE pool was used for calibration. Assays for IgG anti-PC or IgM anti-MDA were not included as pilot studies have shown these levels to be non-informative [data not shown] (Gronwall et al., 2012).

2.3. Statistics

Baseline demographic and clinical characteristics were described with standard descriptive statistics. Median natural autoantibody or inflammatory marker levels were compared between groups using the Kruskal–Wallis test. Linear regression was used to assess the effect of HIV and RHD on autoantibody and inflammatory marker levels while adjusting for age and sex and accounting for potential effect modification between HIV and RHD by testing their cross product term. The linearity and normality of residuals assumptions were assessed with residual by predicted plots and quantile–quantile plots respectively. Violations were rectified by log-transforming the outcome variables. P values less than 0.05 were considered significant. SAS 9.3 was used for statistical analyses (SAS Institute Inc., Cary, NC, USA).

2.4. Funding

The study was funded by the National Institutes of Health, American College of Rheumatology, and Medtronic Philanthropy. No funding source had any role in the design of the study.

3. Results

Overall, 231 participants were enrolled at the two Ugandan sites (Fig. 1); however, eleven were later excluded from the RHD negative control group because of an elevated ASO or an abnormal screening