Human Immunology 77 (2016) 490–497

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

HLA-A68 and *HLA-B15* alleles correlate with poor immune response among AIDS patients on combined antiretroviral therapy

Marah El-Beeli [a], Samira Hamad Al-Mahrooqi [a], Randa Mahmoud Youssef [b], Fahad Zadjali [c], Abdullah Balkhair [d], Mohammed Said Al-Balushi [a], Elias Anthony Said [a], Sidgi Syed Hasson [a], Ali Abdullah Al-Jabri [a,*]

[a] Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, P.O. Box 35, Al Khod, Muscat, Oman
[b] Department of Family Medicine and Public Health, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman
[c] Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman
[d] Infection Control Unit, Department of Medicine, SQUH, Sultan Qaboos University, P.O. Box 35, Al Khod, Muscat, Oman

ARTICLE INFO

Article history:
Received 5 February 2014
Revised 17 December 2015
Accepted 8 April 2016
Available online 8 April 2016

Keywords:
HIV
AIDS
HLA
cART
Oman
Immune response

ABSTRACT

Around 15–30% of AIDS patients fail to recover their $CD4^+$ T cell levels following combined antiretroviral therapy despite successful inhibition of HIV-1 replication. The exact reasons for this immune recovery failure are not completely understood. HLA alleles are among the candidate that may explain this failure. A total of 65 adult AIDS patients, with viral load of <50 copies per ml were investigated. Viral load and CD4 T cells counts were performed following standard techniques. HLA genotyping was performed using PCR-SSP technique. The Statistical Package for Social Sciences (SPSS version 19) was used for data processing and analysis. A significantly higher proportion of poor immune responders were carrying *HLA-A68* (4.8% compared to 25.0%, $P = 0.025$) and *HLA-B15* (2.4% compared to 20.8%, $P = 0.023$). The etiological fraction (Efe%) among carriers of *HLA-A68* was 57.89% (95% CI = 26.79, 75.79) and was 61.35% (95% CI = 35.33, 76.91) among carriers of *HLA-B15*.

1. Introduction

The introduction of antiretroviral drugs has minimized the suffering and saved the lives of millions of acquired immunodeficiency (AIDS) patients. It has also contributed to the reduction of the transmission of the human immunodeficiency virus (HIV) among community members. Combined Antiretroviral Therapy (cART) control plasma viremia by interfering with viral replication resulting in a considerable reduction of viral load (VL) to an undetectable level namely less than 50 copies per ml [1–3]. The effectiveness of AIDS treatment is reflected on the immunological profile of the patient indicated by the increase in CD4 T cells by 50–150 cells/mm^3 per annum. CD4 T helper cells are the coordinators of any immunological cascade during the immunological response and their count in healthy individuals are in the range of 500–1200 cells/mm^3 [4].

It has been reported that 20–30% of AIDS patients show a "discordant immune response" to cART; CD4 T cell counts among these patients remains low despite the decrease of VL to undetectable levels [5]. Several studies have been conducted in the last decade to better understand this discordant immune response which seems to be the result of the interaction of multitude of viral, host and treatment related factors [6,7].

The variation in the immunological response was attributed to several host genes that control retroviral replication and pathogenesis [1,7–9]. Some genes coding for proteins which are involved in HIV entry including CCR5 and CXCR4 while others such as TRIM 5α, APOBEC3G and TSG101, MIP-1, RANTES, SDF and MCP1 are genes coding for co-receptor ligands [8,9]. Genes involved in the innate and acquired immune response against HIV include cytokine genes and cytokine receptors genes as well as genes coding for the Human Leukocyte Antigens (HLA class I & II) [6,9]. HLA molecules are essential for the proper immune response by presenting antigen derived peptides to the effective immune cells. HLA class I molecules presents intracellular peptides, whereas HLA class II molecules present exogenous peptides. Failure in antigen presentation may account for the association between HLA and discordant individuals. The classical HLA class I molecules includes

* Corresponding author.
E-mail addresses: marah.eltahir@yahoo.com (M. El-Beeli), samira.almahruqi@gmail.com (S.H. Al-Mahrooqi), rahman.randa@gmail.com (R.M. Youssef), fahad.alzadjali@gmail.com (F. Zadjali), balkhair2020@gmail.com (A. Balkhair), albuloshi@hotmail.com (M.S. Al-Balushi), esaid@squ.edu.om (E.A. Said), shyahasson@squ.edu.om (S.S. Hasson), aaljabri@squ.edu.om (A.A. Al-Jabri).

http://dx.doi.org/10.1016/j.humimm.2016.04.009
0198-8859/

HLA-A, *-B*, and *-C*, widely expressed in most tissues. The non-classical HLA molecules include *HLA-E*, *-F*, and *-G*: they are less polymorphic and often exhibit a relatively restricted tissue distribution.

The reasons of poor immunological response to cART among a proportion of Omani AIDS patients with undetectable viral load have not been investigated. This study focuses on the identification of HLA class I and II group of alleles among Omani AIDS patients on cART followed in Sultan Qaboos University Hospital (SQUH) and their possible correlation with poor immunological response. Understanding the contribution of HLA molecules among AIDS patients will help care providers in predicting treatment outcome among individual patients.

2. Methods

2.1. AIDS cohort

The study included 65 AIDS patients on cART for at least one year and attending SQUH every 3 months for follow up. All patients were Omani nationals and above the age of 18 years. All patients have achieved full viral suppression (VL $\leqslant$ 50 copies per ml) and 36.9% of them were immunologically poor responders (increase of CD4 T cell count by less than 50 cell/mm^3 per year). Women constituted more than half of the good (53.7%) and poor immune responders (58.3%). Good and poor immune responders were comparable in respect to sex ($P = 0.458$), age (40.02 ± 11.33 years compared to 45.83 ± 11.98 years, $P = 0.055$), the duration between diagnosis of HIV and onset of treatment (1.61 ± 2.16 years compared to 1.65 ± 2.78 years, $P = 0.625$) and the duration between treatment and full suppression of viral replication (3.71 ± 3.22 years compared to 3.04 ± 3.14 years, $P = 0.420$).

2.2. Ethical consideration

The study was approved by the Ethics Review Committee of Sultan Qaboos University (SQU) and The Research Council (TRC) in Oman. Patients gave a written informed consent certified by the reference senior consultant. Information sheet provided a detailed explanation of the purpose of the study as well as the risks and benefits involved. Patients had the right to refuse participation without affecting their treatment programme and continue receiving the best care. Confidentiality and anonymity were maintained; specimens were labelled numerically to match the databases following sample analysis without reference to names or hospital identification number.

2.3. HIV-1 viral load measurement

The Cobas Ampliprep (AmpliPrep/COBAS TaqMan HIV-1 Test v2.0) is an automated nucleic acid sample preparation equipment which extracts RNA using protease enzyme, Lysis buffer and positively charged magnetic beads particle. The extracted RNA is added to a master mix comprising of forward and reverse primers from the conserved HIV-1gag gene, probes specific for HIV-1 and Quantitation Standard for viral load calculation, nucleotides, d-ATP, d-CTP, d-GTP, d-UTP and Manganese solution. Being RNA, the special enzyme, thermus species ZO5 DNA polymerase with both reverse transcriptase and DNA polymerase activity is used for reverse transcription to convert RNA to complimentary-DNA prior to the Real Time PCR (RT-PCR). After 60 cycles of PCR the viral load of positive samples are calculated based on comparison between the cycles thresholds of the samples against the cycle threshold of the Quantitation Standard incorporated in the PCR assay. The assay range for the kit is 15 copies per ml to10 million copies per ml.

2.4. Measurement of CD4 T cells

CD4 T cells count was conducted in the Hematology Laboratory of SQUH using (FC 500 Bekman coulter) flow cytometry. Blood was added and incubated with monoclonal antibodies conjugated to fluorescent indicators specific for CD4 T lymphocytes and the measurement was performed according to standard methods.

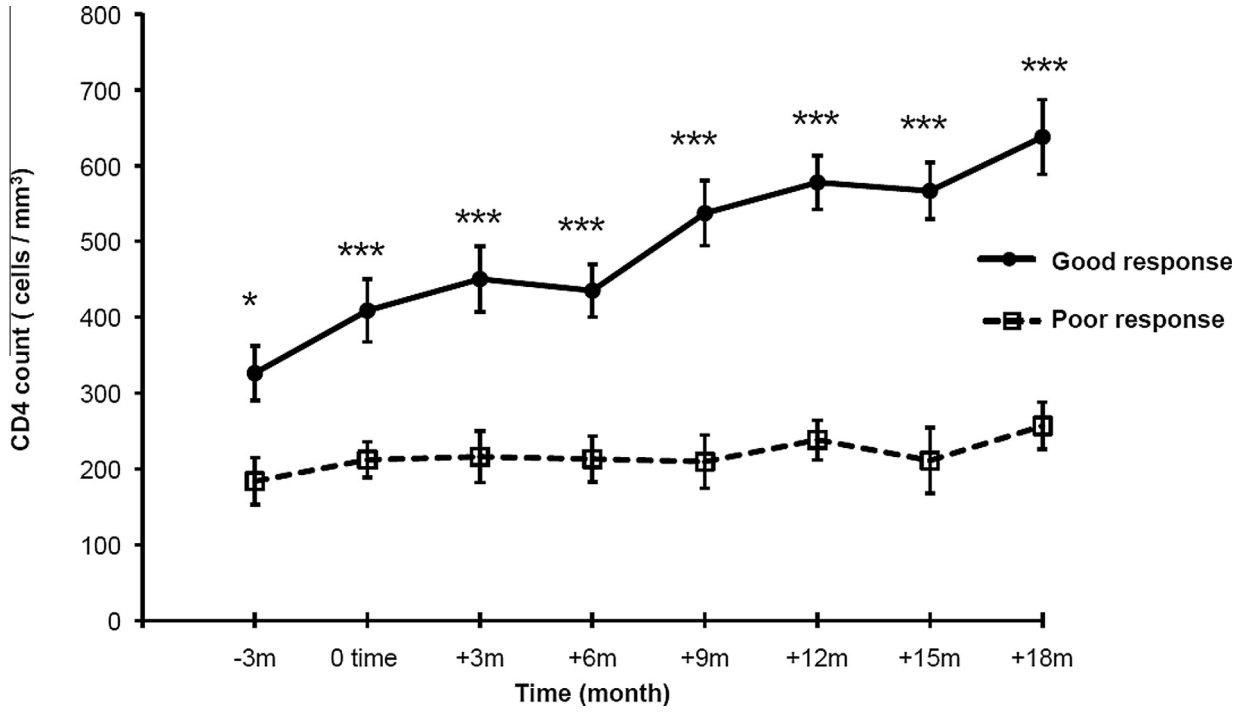

Fig. 1. Variation in the trend of CD4 T cells count between immunologically poor and good responders among Omani AIDS patients on cART with undetectable viral load (<50 copies/ml).