Syed Hani Abidi

Syed Hani Abidi's initial training, doctoral and post-doctoral work was in viral immunology and immuno-evolution. He is currently an Assistant Professor at the Aga Khan University, Karachi-Pakistan. The research interests of his group have expanded into five different thematic areas, namely: 1) viral and vaccine immunology, and immunopathogenesis; 2) viral evolution, divergence, and molecular epidemiology; 3) viral oncology; 4) drug design, and structure-function analysis of drug-protein complexes; and 5) Immunoinformatics and Bioinformatics.

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ISID/ESCMID Fellowship Grant Report

Epitope Evolution of HIV in Genetically Distant Populations

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Introduction

The T cell-mediated immune response is involved in the control of HIV-1 infection, and is considered crucial in the acute phases of infection, before the appearance of neutralizing antibodies [1]. The cytotoxic T lymphocytes (CTL) recognizes infected cells that display CTL-specific epitopes bound with its specific major histocompatibility complex class I (MHC-I), also referred as human leucocytes antigen (HLA), proteins [2, 3]. The production of epitope from intracellular antigen and their subsequent display is carried out by the MHC class Iprocessing and presentation pathway [4], which comprise of 1) proteasomal degradation of HIV immunogenic protein, followed by 2) binding of peptides with the transporter associated with antigen processing (TAP) molecules on the surface of endoplasmic reticulum (ER), followed by 3) binding of specific peptides with MHC-I heavy chains and β 2 -microglobulin in the lumen of ER and finally 4) glycosylation of the peptide-MHC complex, and its delivery to the surface of the infected cell. These CTLs are highly specific to the peptide-MHC-I complex and upon recognizing the correct peptide-MHC complex induce death of the infected cells [5]. In response to the protective host selection pressure, HIV evolves rapidly, which allows the virus to escape the host immune system by affecting HLA binding of epitopes or epitope interaction with T cell receptors (TCR), resulting in CTL activation defects [6]. HIV also evolves in a population-specific manner, which is the consequence of selective pressure exerted by population-specific HLA alleles that results in selection and amplification of immune escape mutations [7]. Accumulation of these mutations ultimately affects the HIV epitope repertoire in a given population, resulting in the emergence of population-specific antigenic patterns [7]. These observation warrants that population-specific evolution of HIV should be considered while designing treatment or vaccine strategies against HIV. Gag is an immunodominant protein of HIV-1 [8, 9]. It has been also reported that HIV constantly accumulates immune escape mutations in gag, which helps the virus to escape HLA restricted CTL responses [8, 9].

The aim of the research project was to understand 1) how HIV evolves in a population-specific manner, due to differences in selection pressures exerted by immunogenetically divergent genes from genetically diverse population, and 2) how the population-specific mutations affect the processing and presentation of HIV epitopes. My project was focused on the HIV-1 Gag protein sequences from two genetically diverse cohorts, namely Pakistani and Kenyan.

Materials and Methods

For this project, I used an integrated *in silico – in vitro* approach to investigate the research questions established earlier. I amplified HIV gag sequences from HIV-infected Pakistani and Kenyan patients. The amplified sequences were subjected to *in silico* analyses to identify common and population-specific (unique to each population) T cell epitopes in HIV gag protein sequences from Pakistani and Kenyan cohorts. I next performed immunogenetic profiling of the HIV patients to find out whether certain HLA molecules are enriched in each population,

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followed by HLA restriction analysis to identify whether these HLA molecules restrict population-specific epitopes. In the next step, we collaborated with Prof Benedikt Kessler at the NDM research building to analyze differential processing of the population-specific Gag epitopes at the first step of the antigen processing pathway, i.e. proteasomal degradation. For this purpose, synthetic Gag peptides (harboring population-specific mutations) were used in proteasomal degradation assay, performed using both constitutive- and immuno- proteasomes. The products (samples) from this assay were collected and analysed using MS/MS mass spectrometry. Our next goal was to study the effect of these mutations on subsequent steps of the antigen processing pathway. For that, peripheral Blood Mononuclear cells (PBMCs) were isolated from the blood of HIV infected patients, and used to generate short-term T cell lines. The short-term T cell lines were exposed to population-specific epitopes and the T cell response, gauged by interferon-γ production, was determined using EliSpot assay.

Results and Discussion

The bioinformatics analysis identified several regions in HIV-1 Gag protein that had mutations unique to each cohort. Out of these, HIV-1 Gag region from 290-310 was of particular interest, because this region contained three population-specific epitopes. The three Pakistani epitopes, containing Valine as single point mutations, from this region were restricted by HLA molecules B*51 and B*14. The epitopes exhibited strong interaction with these HLA molecules and with the T cell receptor. Conversely, the three Kenyan epitopes, containing Threonine in place of Valine, exhibited weak interaction with HLA B*51 and B*14 and with the T cell receptor, indicating Threonine to be the escape mutation in Kenyan sequences. In the next step, we synthesized long peptides, representing Gag region 290-310, from two different cohorts (Pakistani and Kenyan), and used the peptides to study the proteasomal degradation step of the antigen presentation pathway. This assay corroborated our earlier findings, where mutations observed in Gag epitopes from Kenyan cohorts were found to escape the proteasomal processing, while the mutations observed in Gag epitopes from Pakistani cohort were found to increase the affinity of the peptides (epitopes) towards host proteasome. In the next step we tested these epitopes in Elispot assay to determine the efficacy of T cell response against the population-specific epitopes. The preliminary screening from these experiments revealed some interesting observations, where differences were observed in the efficacy of T cell response against population-specific epitopes. This observation, however, warrants further investigation.

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