ABSTRACT

Human Immunodeficiency virus (HIV-1), with a diversity of numerous subtypes and recombinant forms, is the etiological agent of the globally spread Acquired immunodeficiency syndrome (AIDS) and is one of the fastest growing epidemic in sub-Saharan Africa, parts of Asia including India and China. HIV-1 subtype C, belonging to group M- the major HIV group responsible for current AIDS pandemic, is particularly predominant in India as compared to subtype B which is more common in North America and Europe and is estimated to be responsible for about 50% or more of the current infections worldwide.

Massive and progressive T cell depletion is the hallmark and the key to the immunodeficiency caused by HIV-1 infection, which ultimately leads to crippling of host immune system and the consequences associated thereof. The mechanisms of T cell death in HIV infection have been studied over a decade but have still remained an intriguing area of research. Despite evidences for several pathways by which HIV can induce cell death, apoptosis has been in the forefront of research implicated in HIV-1 induced T cell depletion. Various viral proteins have been implicated in initiation and/or intensification of the death process; however, recent evidences suggest that the connections between HIV-1 and apoptosis induction are highly complex, involving multiple viral genes and diverse signaling pathways. HIV-1 is known to manipulate cellular gene expression and biochemical processes in diverse ways not only to achieve optimal replication efficiency but also to induce T cell death.

The present work attempts to understand the biochemical process of T cell death in HIV-1 infection using differential gene expression approach. Using CEM-GFP cell line, and based on gag specific PCR and flow cytometry analysis of the HIV infected reporter T cell line, we conclusively show that both infected and uninfected cells undergo apoptotic cell death. We have also shown induction of apoptosis by Indian subtype C isolate in a newly established cell line named CGC5.1, which differs from CEM-GFP by only CCR5 chemokine co-receptor expression. This cell line would be valuable not only to understand

the changes in the gene expression and mechanism of cell death induced by HIV-1 subtype C viruses in future but also could be a useful tool in high throughput anti-HIV drug discovery assays. We have employed annexin V magnetic beads based separation of apoptotic cells for the first time in HIV infection to purify the apoptotic and non-apoptotic population of cells from the pool of HIV-1 infected cells exploiting the phenomena of phosphotidyl serine flipping during apoptosis to study differential gene expression. We have identified a gene that is a part of mitochondrial complex I (NADH Dehydrogenase) and is specifically down regulated in HIV-1 induced apoptosis. Down regulation of NDUFA6, a subunit from 46 subunit complex I, leads to impairment of the mitochondrial complex I activity, imbalance in the oxidative phosphorylation and reduced but not absolute cessation of ATP levels. This is in accordance with the previously hypothesized role of ATP levels acting as sensors to induce apoptotic cell death. Downstream effects of this mitochondrial dysfunction are evident as studied by loss of transmembrane potential and increase in the cellular reactive oxygen species (ROS) production. Our observation provides a novel molecular basis as to how HIV-1 targets a small part of a huge complex to its own benefit and identifies a viral strategy to down modulate important cellular activity by impairing the host cell energy generating system and exemplifies yet another host pathogen relationship.

Furthermore to identify genes specifically regulated in infection and apoptosis, we have uniquely separated the infected cells into four populations based on GFP fluorescence for infection and annexin V staining for apoptosis by fluorescence activated cell sorting, e.g., infected and apoptotic, infected and non-apoptotic, uninfected and non-apoptotic and uninfected and apoptotic populations. Microarray analysis with 28K chips using RNA from these four populations and control non-infected cells indicate that HIV-1 induces distinct gene expression changes in individual subsets of the sorted cells. Further analysis of the generated data results in identification of genes specifically modulated due to infection and apoptosis. These results provide a novel database of host cell factors for further studies in HIV induced T cell apoptosis.