Studies on role of dendritic cells in HIV infection

AIDS was identified as a distinct clinical entity in year 1981 in United States. The virus that causes AIDS was isolated in 1983 and was given nomenclature as HIV in 1986. Subsequently two types of HIV (type 1 and type 2) were identified. It has been estimated that HIV-1 was probably transmitted from chimpanzee (*Pan troglodytes*) to humans in central Africa in early thirties whereas HIV-2 was transmitted to humans about 60 years ago from sooty mangabey (*Cercocebus atys*) monkeys of West Africa. HIV/AIDS is now major public health concern in many countries. UNAIDS global estimates indicate that the number of people living with HIV in 2008 globally was 33.4 million (31.1-35.8 million) and 2 million (1.7-2.4 million) people died of AIDS. Of the 2.7 million (2.4-3 million) people who were newly infected with HIV in 2008, (i.e. over 7400 new infections in a day) more that 96% are from low and middle income countries. Approximately 2.4 million (2 million–3.1 million) people in India were living with HIV in 2008 (UNAIDS 2009 report).

The major hallmarks of HIV infection include destruction of helper CD4+ T cells and subsequent loss of immune competence. Considerable efforts have gone into understanding the mechanism by which HIV causes the disease and two major hypotheses have been forwarded, first, that HIV causes loss of CD4+ T lymphocytes by directly infecting and killing those cells. The second is that HIV infection indirectly impairs cell function and is based on the observation that uninfected and bystander cells are affected. A deeper understanding of viral replication and the reservoirs that contribute to it, and of how the virus-host interact with each other are important for understanding the basis of viral pathogenesis. The DC reservoir is the most enigmatic of all as DCs are among the first cellular targets of HIV-1 during sexual transmission.

Dendritic cells (DCs) are most versatile antigen presenting cells that are central to induction and regulation of adaptive immune responses. DCs reside in different tissues and mucosal surfaces as immature cells and possess capacity to acquire, process and present antigens to T cells; induce tolerance in self reactive T cells, interact with B and NK cells and play a very significant role in antiviral innate
immune response. Such a large degree of functional plasticity in DCs can be attributed to a combination of specialized DC subtypes, their distribution in tissues, different maturation stages and various functional/effector molecules.

Sexual transmission across the genital mucosa is the main route of HIV infection and accounts for 80% of the infections worldwide. Immature DCs residing within the epithelial surfaces (Langerhans cells, LCs) and sub epithelial surfaces (dermal DCs) are believed to be the initial targets for HIV-1 after mucosal exposure and to be involved in early events in primary HIV infection in the mucosa. The DC-HIV interaction may result in processing and presentation of the virus particles, or rapid internalization into non-lysosomal compartments or productive infection of DCs. Transmission of HIV from DCs which are not infected but have engulfed the virus is called as in trans transmission while the transmission of de novo virus particles is known as in cis. While DCs can help in transport of virus they are also important for DC-T cell interaction for generating adaptive immune response specific to HIV. In this way, the central role of DCs in stimulating T-cell activation not only provides a route for viral transmission but also represents a vulnerable point at which HIV-1 can interfere with the initiation of primary T-cell immunity.

HIV interferes with DC function by affecting their T cell stimulatory capacity, cytokine secretion and expression of functionally important molecules. HIV infection also induces numerical and functional defects in the two major circulating DC subsets, myeloid DCs (mDC) and plasmacytoid DCs (pDC). mDCs are proficient at antigen uptake and presentation while pDCs are thought to play an important role in innate immune responses to different viruses by producing interferon alpha (IFN-α) and support antigen presentation function of mDCs. Groot et al found opposing roles for mDCs and pDCs during HIV infection of T cells i.e mDCs facilitate transmission while pDCs inhibit HIV replication.

Though a plethora of studies have focused on role of DCs in HIV immunopathogenesis, our understanding of DC-HIV interaction is far from complete. In this study we have concentrated on two such areas, with the following specific objectives,
1. To study the mechanisms responsible for depletion of circulating mDC and pDC populations at different stages of HIV infection.

2. To study the effect of HIV infection on expression of co-stimulatory molecules CD80 and CD86 and other genes important for DC function.

*In vivo* study to assess the quantitative and qualitative defects in circulating DC subsets, mDCs and pDCs at different stages of HIV infection.

In this study, we have considered patients from different stages of HIV disease progression, like the recently infected patients (within one year of acquiring HIV infection), slow progressors (with CD4+ T cell counts more than 500 cells/mm³ for more than 5 years) and AIDS patients (with CD4+ T cell counts less than 200 cells/mm³). The recently infected and the AIDS patients (i.e. before and after ART) were followed longitudinally. We determined percentages of pDCs and mDCs in circulation and studied markers of DC function, such as co-stimulatory molecules (CD80/CD86) and IFN-α secretion in study participants and compared with HIV uninfected individuals. We also studied the expression of apoptotic markers and the migratory capacity of mDCs and pDCs. The findings from this study are,

- HIV infection results not only in persistent decrease in pDC numbers by induction of apoptosis but also impede their function. Since pDCs are important mediators of innate antiviral immune response, early irreversible loss of pDCs, largely through apoptosis may be responsible for impaired innate anti-HIV immune responses.

- Maintaining normal levels of functional pDCs by slow progressors in our study to retain functional pDCs, imply that pDCs may play an important role in HIV virus control and subsequent progression to AIDS.

- The loss of circulating mDCs during HIV infection might be due to migration to lymphoid areas as well as apoptosis.
In vitro studies to assess the effect of HIV infection on gene expression by dendritic cells

In this study we have used monocyte-derived dendritic cells from HIV uninfected individuals and were infected in vitro using pNL4.3 (HIV-1 subtype B) and pIndieC (HIV-1 subtype C) as wild type viruses and as viruses pseudotyped with VSV envelope protein. DCs matured with LPS and with VSV-g were used as controls. Expression of various important co-stimulatory molecules, i.e. CD80 and CD86 was assessed at protein and RNA level in these cells. At the same time, effect of HIV infection on other genes important for DC function, like transcription factors (TFs), antigen uptake and processing, induction and regulation of T cell response, innate immune activation and interaction with B and NK cells, apoptosis and migration was also studied using microarray. The findings from this study are,

- Reduced surface expression of CD80 and CD86 on DCs during HIV infection may be regulated at transcription level by multiple TFs, including NFκB subunits and MYB.
- HIV infection also hampers the expression of other members of the co-stimulatory molecules (TNFRSF8, TNFRSF9, TNFRSF11B, and CD70) and this may be a major mechanism by which the virus abrogates generation of HIV-specific immune response.
- HIV is capable of impairing DC function even without productively infecting the DCs.

The findings from this study indicate that by virtue of quantitative and qualitative depletion of pDC the innate immune responses are down regulated during the early HIV infection. pDCs may play a crucial role in viral control early on and may have implication on the disease progression infection. Apoptosis is the major cause of depletion of pDCs. Identifying the triggers for pDC apoptosis may be crucial to reverse the decline. mDCs were depleted only during AIDS stage and may be associated with progression to AIDS. The in vitro experiments indicated that HIV infection or mere exposure to HIV may lead to down regulation of genes critical for
antigen processing and antigen presentation especially co-stimulatory molecules. The role of DCs in disease progression needs to be studied further and possible interventions for reversing the loss of pDCs and mDCs as well as restoring the co-stimulatory function of mDCs need to be explored.

**Publications**

1. Irreversible loss of pDCs by apoptosis during early HIV infection may be critical determinant of immune dysfunction; **Meera Singh**, Madhuri Thakar, Manisha Ghate and Ramesh Paranjape; Viral Immunolog, *In Press*.

2. Poster presentation at XVII International AIDS Conference held at Mexico City, Mexico from 3-8 August 2008. **Meera Singh**, Madhuri Thakar, Snehal Suregaonkar, Manisha Ghate, Ramesh Paranjape. Plasmacytoid Dendritic Cells (pDCs) Play Role in the Disease Progression among Persons Infected with HIV-1

3. Poster presentation at 15th Conference on Retroviruses and Opportunistic Infections (CROI 2008) held at Boston, MA, USA from 3-6 Feb 2008 **Meera Singh**, Madhuri Thakar, Snehal Suregaonkar, Manisha Ghate, Ramesh Paranjape. Reduction in The Absolute Number Myeloid Dendritic Cells (mDCs) And Plasmacytoid Dendritic Cells (pDCs) In HIV Infection Correlates With The Disease Progression.

4. HIV impairs antigen presentation by dendritic cells through transcriptional suppression of co-stimulatory molecules, CD80 and CD86; **Meera Singh**, Madhuri Thakar, Ramesh Paranjape; manuscript under preparation.