

**DIVISION OF
IMMUNOLOGY AND SEROLOGY**

Division of Immunology and Serology

The year under report was significant for the Division of Immunology and Serology as the Immunogenicity Laboratory received international accreditation for Good Clinical Laboratory Practices and the division led the successful conduct of the symposium on Immune Response to HIV Infection and 34th Annual conference of Indian Immunology Society. The conference was attended by leading lights in the HIV field such as Dr. John Fahey, Dr. Richard Koup, Dr. Adrian Mcdermot, Dr. Ronald Mitsuyasu etc as well as about 400 senior and young scientists from India.

The two main research focuses in the division are the immunological responses in early HIV infection and the role of dendritic cells (DCs) in HIV infection. The former is aimed at identifying the immunological and virological determinants of slow disease progression. The studies in DCs are aimed at assessing the DC functions in HIV infected, the role of DCs in disease progression and impact of the anti-retroviral treatment on DC function.

Besides research activity the department provides CD4 estimations for all referred patients and participants in the research protocols in the Institute. The Division has also been running External Quality Assessment Service for laboratories for last 3 years. The division has also supported the National AIDS Control Programme through EQAS for serological diagnosis of HIV infection for Western Region of the country and has supported the diagnosis in the sentinel surveillance programme in the Western Region.

While continuing the present research, service and programme related activities the Division plans to initiate investigations in the field of mucosal immunity and neutralizing antibody response in HIV infection.

A. Immunological and virological characterization of recent HIV infection

[Lead Investigators: Dr. Madhuri Thakar, and Dr. Manisha Ghate,]

The study on characterization of the immunological and virological parameters in the recent HIV infection was started in 2006 with the objective of exploring and identifying the factors related to low viral set point i.e., a surrogate marker for slow disease progression. The patients with recent HIV infection were identified by using the following criteria;

- a. p24 antigenemic and HIV antibody negative
- b. Documented negative HIV test result within previous six months of the first positive result and
- c. HIV antibody positive and detuned ELISA showing recent HIV infection (within 285 days after infection in case of HIV -1 subtype C)

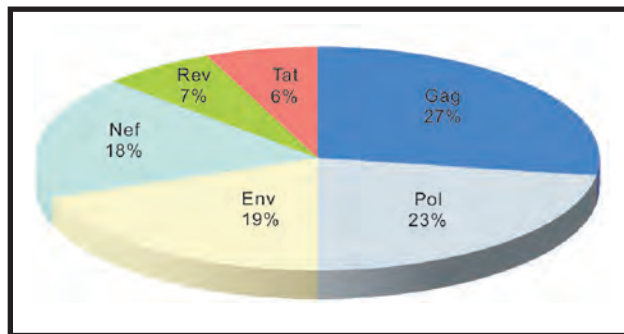
The participants were followed once in every three months for a period of one year and the peripheral blood mononuclear cells (PBMCs) and part of the freshly isolated PBMCs were used for estimation of various activation markers on the lymphocytes. For further analysis, the remaining cells and plasma samples were stored at 156°C and 70°C respectively.

During the period of April 2007 to March 2008, 7 patients were enrolled in the study within a range of 105 to 308 days after infection. The patients had a median CD4 count of 586 cells/cumm (range: 350-1196) and plasma median viral load 9275 RNA copies /ml (range: 400-139000) at the time of enrollment.

Characterization of HIV-specific immune response:

The HIV-specific T cell interferon- response was estimated against the structural and regulatory genes and the positive responses were mostly against Nef, Gag, Pol and Env antigens (Figure 3.1). The commonly identified peptides were from P24, gp120 and Pol antigens. The breadth and magnitude of the response were found to be less in recently infected individuals as compared to the response seen in HIV infected individuals with chronic HIV infection; however the observations have to be substantiated with more number of samples.

Figure 3.1: HIV-specific T cell response against structural and regulatory HIV genes in recent HIV infection



The CD4 and CD8 + T cells from these patients were being analyzed for the percentages of naïve (CD45 RO+ ve), effectors (CD45 RA+ ve) and activated (HLA-DR and CD38+ ve) population using freshly isolated PBMCs using flowcytometry. The data is being analyzed. The dendritic cells of these patients were analyzed for expression of co-stimulatory molecules. The PBMC and plasma samples have been stored for further investigations that include intracellular cytokine secretion by the activated CD4+ and CD8+ T cells upon HIV-specific stimulation, assessment of functional capacity of the macrophage and NK cells etc.

Virological studies:

The preferential usage of co receptor for HIV strains isolated at enrollment was determined using GHOST cell assay. All strains were found to be CCR5 tropic. The studies on replication kinetics of these viruses are underway. Six of the viral isolates were sequenced.

B. The in situ analysis of the dendritic cells in peripheral blood of HIV infected individuals

[Ph.D. Study: Student: Mrs. Meera Singh, Guide: Dr. R. S. Paranjape]

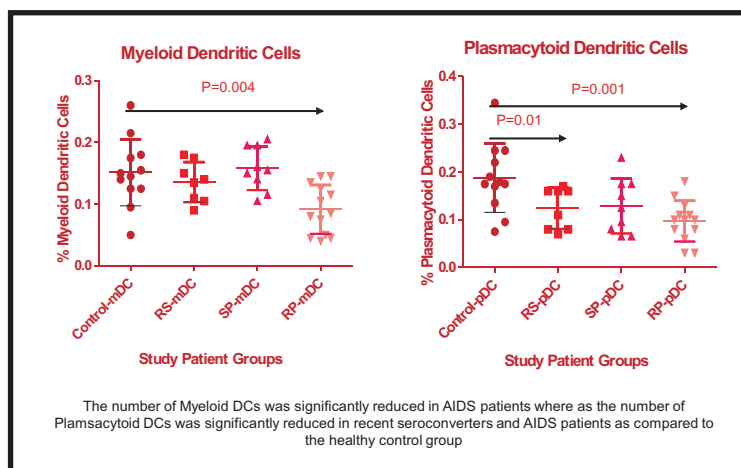
The subpopulations of Dendritic cells (DCs) play an important role in antigen presentation; mDCs in antigen uptake and T cell activation where as the pDCs secrete Interferon- α providing antiviral innate immunity. Impaired antigen presentation by dendritic cells during HIV infection may be attributed either to the depletion of DCs or the reduced expression of co-stimulatory molecules

(CD80 and CD86) that are important for antigen presentation. mDC and pDC populations and surface expression of co-stimulatory molecules (CD80 and CD86) were estimated in HIV positive individuals to study the role of DCs in HIV infection.

The study participants included ART naive HIV seropositive individuals comprising 8 recent seroconverters, 13 AIDS patients (CD4 < 200 cells/mm³) and 9 slow progressors (asymptomatic with CD4 counts > 500 cells/mm³ for more than three years). Twelve healthy HIV seronegative individuals were included as controls.

DCs were identified as the lineage negative (CD3, CD14, CD16, CD19, CD20, and CD56 negative), HLA-DR-positive population and the two subpopulations were further differentiated by CD11c expression using Flow Cytometry. The surface expression of CD80 and CD86 was quantified as mean fluorescence intensity (MFI) (Figure 3.2)

Figure 3.2: The analysis of the subpopulations of dendritic cells; myeloid (mDCs) and plasmacytoid (pDCs) in HIV infection



The number of mDCs was significantly lower in AIDS patients ($p < 0.01$) whereas the number of pDCs was significantly lower in the recently seroconverted ($p < 0.05$) and AIDS patients ($p < 0.01$) as compared to the uninfected controls. The reduction in the number of pDCs in HIV infected individuals might be responsible for the impairment of immune response against the virus.

AIDS patients (CD4 < 200 cells/mm³) showed significantly increased expression of CD80 in mDCs and CD86 in both mDCs and pDCs ($p < 0.01$ in all cases) as compared to the control group. Increased expression of co-stimulatory molecules in patients with advanced disease might be a result of activation of the DCs by the increased viral multiplication.

Both early HIV infection and slow progression were found to be associated with increased number of blood DCs. The expression of co-stimulatory molecules was found to be lower in these patients compared to AIDS patients indicating that DCs may have important role to play in disease progression.

It has been proposed to look at the same parameters in the mucosal DCs as well as DCs in lymph nodes to understand the mechanisms of immune pathogenesis in chronic and advanced HIV infection.

C. Cd4 count estimation

C1. CD4 count estimation provided to the patients.

From April 2007 to March 2008, CD4 count estimation was provided to 6317 patients (3719 Male and 2598 Female) attending various NARI clinics.

The laboratory successfully participated in various External Proficiency Assessment Programs like UKNEQAS, QASI, Canada and COE, Thailand. The panels provided by the external agency were tested in the laboratory. The EQC program for UKNEQAS and COE was run every second month while the other QASI panels were tested four times. The performance for all the panels in the EQC program for UKNEQAS, COE and QASI are satisfactory.

C2. The external proficiency programme conducted by NARI for Indian Laboratories

NARI is an apex laboratory for external quality assessment scheme (EQAS) being run for the laboratories linked to NACO ART centers providing ART to the eligible (CD4count <200 cells/mm³) HIV infected patients. The programme is run in collaboration with QASI, Canada. This year, three rounds of EQAS were conducted. The stabilized whole blood samples received from QASI were distributed to number of laboratories estimating CD4 counts for Patients under ART. Results were obtained from participating laboratories in the prescribed format. The results were sent to QASI. QASI analyzed the result in context with the results obtained from all participating laboratories and the reports were sent back to NARI. The reports were analyzed for the performance of each center and distributed to individual centers. The findings were reported to NACO. On the basis of analysis, a workshop for the centers using standard flowcytometer was organized at NARI to give hands on training for sample processing and analysis using flowcytometer.

Table 3.1: No. of EQAS rounds for the laboratories linked to NACO ART centers

No. of round	No. of laboratories	Month of issue
15	51	April 2007
16	60	September 2007
17	62	February 2008

The Institute additionally conducts EQAS for Indian laboratories other than NACO centers in collaboration with Center of Excellence in flowcytometry (COE), Thailand.

During the period of April 2007 to March 2008, six panels of stabilized whole blood samples received from COE were distributed to Indian laboratories. Results were obtained from participating laboratories in the prescribed format. The results were sent to COE and reports received from COE were distributed to individual laboratories.

Table 3.2: No. of rounds conducted for Indian laboratories not linked to NACO

No. of panel	No. of laboratories	Month of issue
COE 22	15	April 2007
COE23	15	June 2007
COE24	16	August 2007
COE25	14	October 2007
COE26	15	December 2007
COE27	8	February 2008

SEROLOGY

Serological diagnosis of HIV forms the major part of work conducted at the serology laboratory of the institute. HIV antibody testing is based on the algorithm suggested by NACO. ELISA Rapid test are used to screen the samples. The samples giving discordant results in these tests are subjected to western blot assay.

Blood samples obtained from the patients who attended any of the nine clinics of the institute were tested for HIV antibodies. HIV diagnosis was provided to all patients (screened/enrolled) in various projects undertaken at NARI. In addition, samples were received from various organizations in and around Pune and other parts of Maharashtra state for confirmation of HIV diagnosis.

- Serology laboratory also tested samples of foreign students admitted to Pune University.
- Being a National Reference Center, services were provided for testing of blood products for anti-HIV antibody.
- Evaluation of anti-HIV antibody kits was performed on receipt of request from the manufacturers.
- The lab provided quality control testing for samples obtained under HIV sentinel survey, 2007 in Maharashtra, Goa and Gujarat states.

A total of 15909 samples were screened during the year

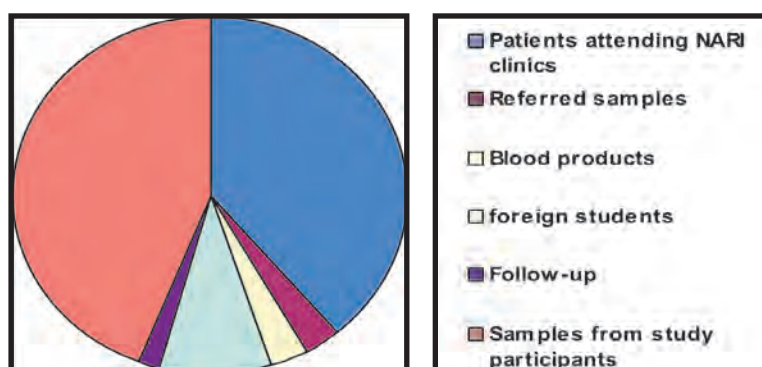
Figure 3.3: Samples screened during the year

Table 3.3: Samples collected from patients attending NARI clinics excluding patients enrolled in various studies

Risk Group	Total	Positive	Negative
Patients attending various clinics (6198)			
Promiscuous	2083	845	1238
STD patients	22	7	15
FSWs	20	14	6
Blood recipients	153	75	78
TB patients	71	20	51
Others	3849	1448	2401
Referred samples (454)			
Promiscuous	13	11	2
STD patients	4	4	-
Others	437	261	176
Blood Products	526	0	526
Foreign Students	1431	0	1431
Follow-up	284	0	284
Total	8893	2754	6139

Table 3.4: Clinic wise collection of samples from Study participants

Clinic Name	Total	Positive	Negative
ANC clinic (KNH)	2571	32	2539
TB DOTS study	258	44	214
HPTN Study (follow-up)	227	6	221
Jehangir Clinic	45	3	42
NGO (PTW)	1664	355	1309
FP Society Clinic	313	6	307
Talera Clinic	651	313	338
PMC Clinic (JSH)	229	56	173
NGO (ST)	53	4	49
NGO (VV)	1005	178	827
Total	7016	997	6019

Patients attending NARI Clinics:

A total of 6198 persons attended NARI clinics during the year 2007. HIV prevalence was estimated in different groups of patients. A majority of these patients were referred to NARI clinic for confirmation of HIV diagnosis made elsewhere which explains high seropositivity seen in them. None (1431) of the samples from foreign students was found to be positive.

Samples referred to NARI:

A total of 454 serum samples were referred to NARI during the year. A very high rate of reactivity for HIV antibody was seen in all samples from patients, belonging to different risk groups. A majority of these samples were from patients who were found to be positive in screening done previously. None (526) of the blood products, received from the Drug Controller's Office, Mumbai, were found to be positive.

Evaluation of commercial kits for detection of HIV antibody:

NARI is one of the three institutes recognized by NACO for HIV kit evaluation. A total of 6 ELISA and 10 rapid kits for detection of anti-HIV-1 & 2 antibodies, were evaluated during the year. All the kits were tested by a panel of 90 coded serum samples, prepared at this laboratory. This panel of serum samples was earlier characterized using 3 different ELISA, 2 Rapid and 1 Western Blot/LIA test.

HIV QC testing provided to National studies:**Sentinel Surveillance 2007**

NARI was identified as regional institution by NACO for overall supervision of sentinel survey in seven states of western India. Quality control testing for samples obtained and tested for HIV, from Maharashtra, Goa and Gujarat states, was performed at serology laboratory at NARI. Over 3898 samples were QC tested at serology laboratory of which results of 15 samples did not match. The matter was discussed with the concerned testing centers and guidance was provided to them to improve the quality of testing.