Summary and conclusions
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- HIV-1 envelope contains virally encoded Env proteins (gp160) and large number of host cell proteins including HLA molecules. According to few earlier reported studies, antibodies that bind to HLA proteins can reduce viral infectivity due to steric hindrance to virus-cell interaction. However in some studies protective role of anti-HLA antibodies against HIV-1 was not observed. This potential mechanism of HIV-1 neutralization is not likely to be affected by genetic diversity observed among HIV-1 isolates. Considering this major advantage, the potential of anti-HLA antibodies to neutralize HIV-1 in-vitro was investigated.

- Binding of antibodies to specific determinants on the Env protein of HIV-1 may render the virus non infective. Cross-reactivity of neutralizing antibodies is critical in HIV infection due to extreme genetic diversity of env gene. Several studies have shown that HIV-1 neutralizing antibodies show limited cross-reactivity, due to extensive diversity in the Env proteins. HIV-1 neutralizing antibodies may show higher cross-reactivity in a setting where limited genetic diversity is observed. Several studies suggest that subtype C viruses from India show limited genetic diversity. Therefore cross-reactivity of neutralizing antibodies generated among HIV-1 infected individuals from India was investigated.
Anti-HLA antibodies (obtained from HIV seronegative individuals) and HIV-1 neutralizing antibodies (obtained from HIV-1 infected individuals) were studied with following objectives.

- To determine whether antibodies specific to HLA proteins incorporated in the HIV-1 envelope reduce viral infectivity and mediate virus neutralization.

- To determine whether neutralizing antibody response generated in HIV-1 subtype C infected individuals from India show extensive intraclade cross-neutralization.

**Study of anti-HLA antibodies for their potential to neutralize HIV-1:**

- Plasma from multiparous, HIV seronegative women was screened for anti-HLA antibodies by ELISA and 14 women carrying anti-HLA antibodies were identified. To confirm that the antibodies are specific to HLA proteins expressed by their husband, plasma was incubated with husband’s PBMCs. Binding of specific antibodies to HLA proteins on the surface of PBMCs (detected in flow-cytometry analysis) resulted in greater than 75% reduction in level of anti-HLA antibodies in the plasma.

- Since anti-HLA antibodies detected in wife’s plasma were specific to husband’s HLA proteins, HIV-1 was cultured in PBMCs from husband so that the virus carried husband’s HLA proteins.
HIV-1 neutralizing activity of anti-HLA antibodies was assessed by incubating HIV-1 cultured in husband’s PBMCs with the anti-HLA antibody positive plasma from wife and looking for reduction of infectivity of the virus in a GHOST cell assay.

No reduction in infectivity of virus was seen in any of the 14 pairs of virus and plasma.

The anti-HLA antibody titer that was seen in multi-parous women may not sufficient to neutralize the virus. IgG was purified from two plasma samples and was tested against respective virus at highest possible concentration. Still the virus neutralization was not observed. The complement-mediated neutralization was also not observed.

HLA proteins incorporated in the HIV-1 virions were quantitated. It was found that HIV-1 incorporated more number of HLA proteins than the Envelope trimers.

The complexes of virus and anti-HLA antibodies were trapped with anti-human IgG antibodies on a microtitre plate. Only virus cultured in husband’s PBMCs bound to the well but the virus grown in other PBMCs was not trapped. This confirmed that anti-HLA antibodies in wife’s plasma specifically bound to the virus that carried husband’s HLA proteins.

The study showed that HIV-1 incorporates significant amount of HLA proteins. However binding of antibodies to HLA proteins does not reduce virus infectivity.
Study of cross-reactivity of neutralizing antibody response

- Plasma samples from 235 HIV seropositive individuals were tested for neutralizing activity against a primary, HIV-1 subtype C isolate.

- Nineteen plasma samples that showed ≥90% neutralization and 2 randomly selected plasma samples that showed 50-60% neutralization during primary screening were tested against a panel of 12 primary HIV-1 subtype C isolates.

- All plasma samples showed extensive cross-neutralization of HIV-1 isolates from the panel. The profile of neutralization sensitivity of the viruses present in the panel and the capacity of the sera/plasma to neutralize different viruses in the panel showed a very complex pattern.

- There is need for optimally constituted panel of HIV viruses for use in the neutralization assay. The basis of extensive cross-reactivity seen in Indian subtype C neutralization assay needs to be explored.

- Diversity among HIV-1 subtype C envelope (gp120) nucleotide sequences from different subtype C prevalent countries (Burundi, Botswana, South Africa, Tanzania and Zambia) was investigated. The analysis showed significantly low genetic diversity among sequences from India.
In brief, the findings suggest that

- HIV-1 primary isolate cultured in PBMCs incorporates significant number of HLA proteins in the envelope. Binding of antibodies to HLA proteins incorporated by HIV-1 does not reduce their infectivity.

- HIV-1 neutralizing antibody response in patients from India extensively cross-neutralize subtype C isolates obtained from unlinked individuals. This is in agreement with significantly low genetic diversity among HIV-1 subtype C virus from India.