Longitudinal analysis of plasma antibodies in antiretroviral naive subtype-C HIV-1 infected children in India

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Abstract: Delineating the factors leading to development of broadly neutralizing antibodies (bnAbs) during natural HIV-1 infection and dissecting their epitope specificities generates useful information for vaccine design. There is little information available on the humoral response in HIV-1 infected children. Children with controlled infection, who do not progress to AIDS in the absence of antiretroviral therapy for more than 7 to 10 years post-infection are termed long term non-progressors (LTNPs) and are potential candidates for identification of correlates of protection. This is the first longitudinal study to assess the plasma neutralizing antibody response in HIV-1 infected children from India. We enrolled twenty six and followed-up twenty antiretroviral (ART) naïve, asymptomatic, chronic HIV-1 infected children. Five (19.2%) baseline and ten (50%) follow-up plasma samples neutralized ≥50% of subtypes-A, B and C tier 2 viruses at ID50titre≥150. A modest improvement in neutralization breadth and potency was observed with time. At baseline, subtype-C specific neutralization predominated (p=0.026); interestingly, follow-up samples exhibited cross neutralizing activity (p=0.360). Overall, we observed an improvement in plasma neutralizing activity with time in HIV-1 infected children that suggests the evolution of bnAbs.

Key words: bnAbs, HIV-1, Vaccine, LTNP, Antiretroviral, plasma

Introduction

Neutralizing antibodies (NAbs) are considered to substantially contribute to an effective immune response developed against HIV-1(Doria-Rose, 2010). Several passive immunization studies in non-human primates have shown NAbs to confer protection against viral challenge (Barouch et al., 2012, Ferrantelli et al., 2003, Hessell et al., 2009). The HIV-1 infections in India are caused predominantly by subtype-C viruses. Disease progression is faster in HIV-1 infected children compared to adults (Richardson et al., 2003). Few studies have been conducted in HIV-1 infected children to assess their immune status(Pananghat et al., 2016, Ssewanyana et al., 2007) and plasma NAb activity (Prakash et al., 2012, Prakash et al., 2011, Goo et al., 2014). So far, most of the studies in children have evaluated NAb response either early in the acute phase (at infancy) (Goo et al., 2014), or in the context of mother-child humoral immune response, in non-subtype-C HIV-1infected individuals (Barin et al., 2006, Lynch et al., 2011, Omenda et al., 2013, Chaillon et al., 2012). In the present study, we have for the first time evaluated the NAb response over time in a cohort of 26 chronic HIV-1 infected ART naïve children (LTNPs) from India against a panel of viruses of multiple subtypes.

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Methodology

Study Subjects and ethics statement

A total of twenty six (26) chronic HIV-1 infected antiretroviral (ART) naïve children, were recruited and followed-up for up to three time points at the Pediatric Chest Clinic, Department of Pediatrics, All India Institute of Medical Sciences (AIIMS), New Delhi. A written informed consent was obtained from the legally authorized representative (LAR) of each of the infected donors, prior to blood sampling. The study was started after obtaining approval from the AIIMS human ethics committee (File No: IEC/NP-295/2011).

Viral neutralization assays

The plasma samples from 26 antiretroviral naïve HIV-1 infected children at baseline and follow up were assessed for cross neutralization activity in a single-round HIV-1 envelope pseudovirus (200 $TCID_{50}$) infection of TZM-bl cells as described elsewhere(Maitra et al., 1999). HIV-1 envelope pseudoviruses were produced by co-transfecting HEK293T cells with HIV-1 envelope containing expression vector and an HIV-1 genomic vector (pSG3 delta *env* backbone) as detailed earlier (Seaman et al., 2010). Murine leukemiapseudotyped virus (MuLV) was used a negative control.

Results and discussion

Characteristics of HIV-1 infected children

There were 18 males and 8 females within the age range of 5-17 years. The median CD4 count of baseline samples of the HIV-1 infected children was 662 (range=308-1680) cells/cubic millimeter and the median viral load was 31250 (range=3410-899000) RNA copies/ml plasma.

Evolution of plasma cross-neutralizing antibodies

Of the 26 infected children recruited at baseline, follow-up sampling at an interval of 3 or 6 months was completed for 20 children up to the third time point. ART was initiated after first follow-up sampling in the AIIMS_520 and AIIMS_530 infected children and after 2nd follow-up in AIIMS_515 and AIIMS_516, while AIIMS_515 and AIIMS_525 did not consent for blood sampling at follow-up time points.

We defined the plasma cross neutralizing activity as the ability to neutralize at ID_{50}titre≥150 for at least 50% of the tier 2 viruses of subtypes-A, B and C. The baseline plasma samples of 5 out of 26 (19.2%) infected children had HIV-1 specific cross NAbs. Among the 20 infected children sampled at all three follow-up time points, plasma of 10 children (50%) demonstrated cross NAbs as per the criteria defined above. Interestingly, at the third time point follow-up, plasma antibodies of six children were able to neutralize >80% of the tier 2 viruses tested at an ID_{50}≥150; AIIMS_519 (95%), AIIMS_346 (95%), AIIMS_518 (86%), AIIMS_523 (95%), AIIMS_517 (86%), AIIMS_353 (81%). Furthermore, a significant improvement in the neutralization breadth (Figure 1a) and potency (Figure 1b) was observed with time, in the above 20 children. Overall, the neutralization frequency of the plasma antibodies in these children increased from 47.2% to 74.8% in 500 virus/plasma combinations.

Development of cross-neutralizing antibodies against multiple HIV-1 subtypes

We compared GMTs(Geometric mean titres) of the plasma neutralizing activity of HIV-1 infected children *(n=26)* at baseline and in follow-up samples. The baseline plasma samples exhibited significantly higher GMTs for neutralization of subtype-C viruses than non-subtype-C viruses (Figure 2, *p=0.02).* In the3rd follow-up, there was nosignificant difference in the plasma GMTs against subtype-C and non-subtype-C viruses in comparison with the baseline, suggesting the

development of cross-NAbs in the HIV-1 infected children over time.

Discussion

The strategy of reverse vaccinology employs information gained from extensive mapping of epitope specificities of potent and bnAbs, present in the plasma of select HIV-1 infected donors, for the designof effective immunogens that can elicit similar antibodies in the vaccines (Burton, 2002).

Most of the studies on plasma NAb mapping have been conducted in HIV-1 subtype-B infected adults, with little information being available from non-subtype-B infected adults and children. This is the first longitudinal study conducted in this direction to evaluate the neutralizing activity and map epitope specificities of plasma antibodies in a cohort of antiretroviral naïve, chronic HIV-1 infected children from North India; the predominant viruses circulating in the Indian population belonging to subtype-C (Neogi et al., 2012). Fifty percent of the infected children demonstrated high titres of plasma NAbs at their baseline sampling that showed improvement in terms of breadth as well as potency with time,against different HIV-1 subtypes. Interestingly, we observed that the plasma antibodies of the baseline samples had relatively strong neutralizing potential against the subtype-C as compared to non-subtype-C pseudoviruses, with a significant correlation between viral load and neutralization potency against multiple subtype-C but not against non-subtype-C viruses. This subtype matched neutralization by the baseline plasma antibodies of the HIV-1 infected children, as has also been observed in earlier studies conducted in infected children and adults (Prakash et al., 2012, Andrabi et al., 2012, Lakhashe et al., 2007), suggests that the epitopes eliciting such immune responses are both present as well as exposed on the circulating natural viruses.

Remarkably, in the follow-up (3^{rd}follow-up) six plasma samples exhibited enhanced neutralizing activity (neutralized >80% of tier 2 viruses at an $ID_{50}\geq150$) and three of them (AIIMS_346, AIIMS_353 and AIIMS_517) demonstrated a significant positive correlation between viremia and GMT, irrespective of the viral subtype tested. Furthermore, an incremental increase in the neutralization breadth and potency was observedover time, which points at a possible evolution of virus in these infected individuals. Our findings corroborate with earlier observations implicating that certain common epitopes elicit subtype specific NAbs and that a higher antigenic stimulation dictates evolution of bnAbs(Doria-Rose et al., 2010, Piantadosi et al., 2009), suggesting the need for repetitive immunizations with the vaccine to ensure continued antigen specific B cell stimulation and affinity maturation, an important feature of bnAbs(Pancera et al., 2010, Zhou et al., 2010).

Conclusion and Future directions

In summary, this longitudinal study provides the first estimate of the prevalence of plasma NAbs and their epitope specificities in Indian HIV-1 infected children, exhibiting plasma cross-neutralizing activity. In majority of the antiretroviral naïve chronic HIV-1 infected children, an improvement in the plasma neutralization breadth and potency was observed over time.

Acknowledgments

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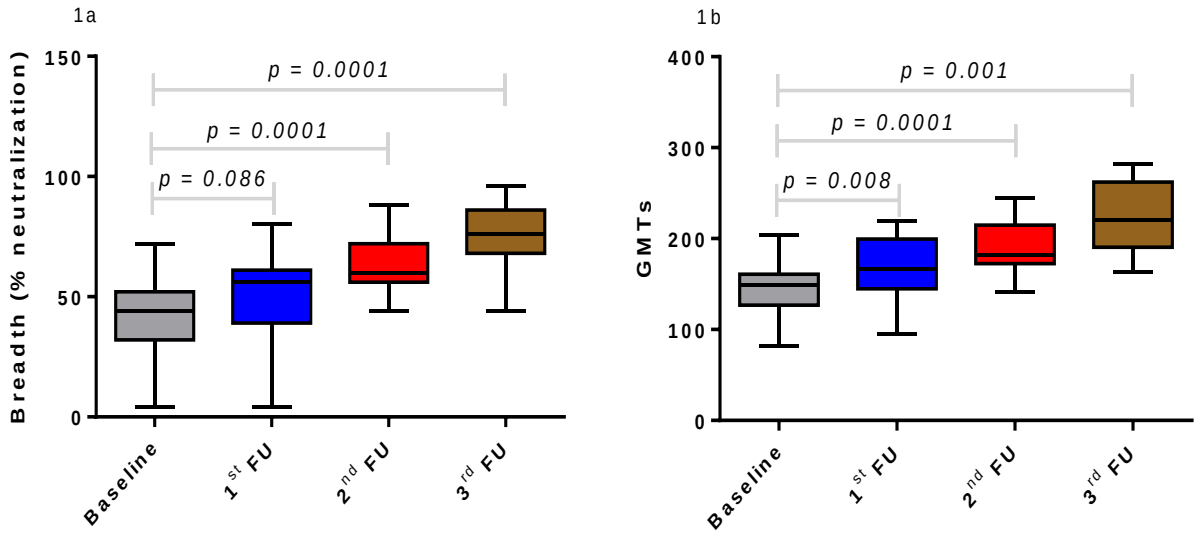

Figure 1: Evaluation of neutralization breadth and potency over time: 1a and 1b depict the % neutralization and potency achieved against a panel of 25 pseudoviruses at baseline and three follow-up time points. *P*-values (two sided) are based on Mann–Whitney U test. The error bars show the median with the interquartile range. Significant differences between the four time points are indicated. FU: Follow-up

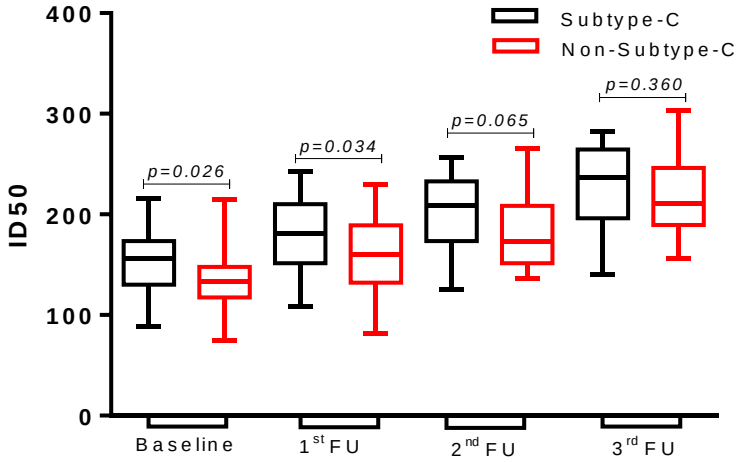

Figure 2: Box-and-Whisker plot comparing GMTs of plasma NAbs for subtype-C and non-subtype-C virusesat baseline and follow ups.*P*-values (two-sided) are based on the Mann–Whitney U test. X-axis indicates the subtype-specific virus groups. Y-axis values represent GMTs. FU: Follow-up

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