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Research Article

Antiviral Potency of HIV Long term Survivors Sera

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ABSTRACT

The survival of a small fraction (5-15%) of individuals without immunologic deterioration after being infected with HIV for more than 8-10 years without any therapy is still elusive. Long-term non-progressors of HIV -1 infected children serve as ideal models to study virological, genetic and immunological characteristics to determine factors associated with slow progression of disease and death due to their natural control of HIV-1 infection. LTNP and LTPs whole blood were collected by venipuncture in CPT tubes. Blood was transported to the lab within 3- 6 hours in a leek-proof container. IgG was purified from the plasma by absorption with protein A- agarose and virus quantified by P^{24} Assay. Plasma from all the groups LTNP, SP, and FP neutralized or reduced HIV replication above 50% inhibition at low dilutions (10^{-1}). The neutralization was statistically significant ($P < 0.01$) between LTNP and SP, FP cohort at higher dilutions (10^{-2} to 10^{-4}) where LTNP (ART Naïve) plasma showed higher neutralization activity than SP, FP plasma. 6 non-progressors and 6 fast progressor's samples were used for evaluation. It showed that plasma from fast progressors could neutralize only one strain NL4-3. No neutralization of the other two isolates HIV-$1_{92BR025}$ and HIV-$1_{93IN101}$ was observed with the plasmas from fast progressors, while minimum 3 samples (50%) of LTNP could neutralize all the 3 isolates and 4 (66.6%) of LTNP neutralized minimum 2 isolates. The neutralization breadth of plasmas from LTNP was greater than FP. Plasmas were again re-evaluated for virus neutralization with both Ig G and non-IgG fractions. Flow-through fractions showed only 10 to 20% neutralizing activity, whereas antibody fraction displayed 60 to 80% neutralizing activity. Neutralizing activity of plasma revealed that LTNP had higher levels of neutralizing antibodies and broad neutralization activity against primary isolates and the neutralizing activity of plasma is attributed to antibody IgG fraction. Our results provide some insight into antibody titer and the breadth of neutralization of diseaseprogression.

1. Introduction

During the developmental period from fetal life to adolescence, humans are exposed to a plethora of pathogenic organisms making them more susceptible and vulnerable to chronic viral infections (Prendergast et al. 2012). HIV-1 pathogenesis in children is characterized by rapid disease progression and shorter time to progress to AIDS and ultimately to death, compared to adults. The immune system in early life is more tolerogenic and fails to control viral replication, leading to persistent viraemia, co infections, microbial translocation, immune dysregulation and immune exhaustion subsequently to disease progression. It is reported that, of the estimated 35.3 million people living with HIV globally, approximately 3.2 million are children infected with HIV below 15 years of age as of 2013. 190,000 AIDS deaths of children below 15 years of age were claimed in 2012, approximately 530 child deaths every day despite about 770,000 children were on antiretroviral therapy (ART). An estimated 210,000 children were newly infected within 2012 and nearly 700 children are newly infected with HIV every day (UNAIDS, Global Report, 2014). HIV transmission to the children can occur in two ways, one is the mother to child transmission (MTCT) or vertical transmission, 95% of the pediatric infections occur by vertical transmission. HIV transmits from an HIV- positive mother to her child during pregnancy, labor, delivery or breast feeding (Burger et al. 1990) (Walker et al. 2004). 5% of the pediatric infections occur by horizontal transmission.The rate of disease progression in

an HIV- infected pediatric population progresses more rapidly than adults and their survival pattern follows a bimodal distribution (Scott et al. 1989) (Blanche et al. 1990). Some children, who develop early onset of disease are often associated with AIDS defining illnesses and die within the first few years of life; these are called as rapid progressors or fast progressors. However, children who have survived beyond 8 years of age after diagnosis of HIV infection are known as long term survivors (LTS). In this group two sub populations have emerged, the long term non progressors (LTNP) who have remained asymptomatic or mildly symptomatic over a period of years in the absence of any therapy, while those who have survived despite clinical and laboratory evidence of disease progression are long term progressors (LTP) or slow progressors (SP) (Radhakrishna et al. 2013). Long-term non progressors of HIV -1 infected children serve as ideal models to study virological, genetic and immunological characteristics to determine factors associated with slow progression to disease and death due to their natural control of HIV-1 infection.

2. Materials and Methods

2.1 Population characteristics

A total of 26 children were enrolled in the study. Among 26 HIV infected children, 6 were LTNP, 20 were LTP or SP and RP or FP, classified based on clinical and laboratory outcome. Children who have survived more than 8 years of age, without any clinical symptoms and had a CD4 count >350 cells/µl without any treatment were classified as LTNP (n=6), while those who have survived for 8 years, showed opportunistic infections and the CD4 count < 300 were categorized as SP or LTP and RP or FP (n=20), (Radhakrishna et al. 2013).

2.2 Sample Collection & IgG purification from plasma

LTNP and LTPs whole blood was collected by venipuncture in CPT tubes. Blood was transported to lab within in 3- 6 hours in a leek proof container. Plasma and other cell components were obtained after gradient centrifugation at 1000 rpm for 10 min and all cell components stored at -80 until use. IgG was purified from the plasma by absorption with protein A- agarose. Protein A agarose was supplied as a suspension in PBS. The resin was washed 4 times, the supernatant was removed and the resin was packed in a column. Protein A agarose was equilibrated with 5ml of equilibration buffer and the buffer was allowed to drain out completely. 1 ml of a diluted (1:2) plasma sample with PBS was loaded onto the column and allowed to drain off completely and the column was again reloaded with the collected flow through to ensure complete removal of IgG from plasma. The column was washed with 5ml of 1x equilibration buffer. Bound IgG was eluted by using 1 ml elution buffer and the collected IgG was neutralized by neutralization buffer.

2.3 Plasma and IgG and non - IgG fractions of plasma neutralization assay with different viral isolates

Plasma was heat inactivated (56°C/30 min), serially diluted 10-fold and incubated with virus NL4-3 for 30 min at 37°C. The virus-serum mixtures were added to the sup T1 cells incubated for 4 hours at 37°C in a 5% CO2 incubator. The plasma and virus mix was removed and the cells washed, replenished with fresh media and incubated for 4 days at 37°C. On the 5th day, supernatants were collected to quantify p24 antigen using ELISA. To determine the neutralization efficiency and broad neutralization of plasma of both LTNP and FP antiviral assay was performed with three different isolates NL4-3, HIV-1 93IN101 and HIV-1 92BR025.

2.4 HIV-1 p24 antigen assay

HIV-1 p24 present in the supernatant was determined by using an ELISA kit and the protocol was followed according to manufacturer's instructions. Supernatants collected were diluted and the 100µl diluted supernatant was added to each well of ELISA plate containing 25µl of disruption buffer and the plate was incubated at 37°c for 1 hour. After 1 hour of incubation, the plate was aspirated, washed with wash buffer and 100µl of the conjugate solution was added and incubated the plate at 37°c for 1 hour. After incubation, plate was washed and 100µl of substrate solution was added and the plate was incubated at room temperature for 20 min. After 20 minutes, the reaction was stopped by adding 100µl of stop solution or 1N HCl and reading was taken at 450 nm.

3. Results and Discussion

3.1 Neutralization activity of whole plasma

Neutralizing activity of plasma was examined by different HIV cohorts, plasma from all the groups neutralized or reduced HIV replication above 50% inhibition at low dilutions (10^{-1}). The neutralization was statistically significant ($P < 0.01$) between LTNP and SP, FP cohort at higher dilutions (10^{-2} to 10^{-4}) where LTNP (ART Naïve) plasma showed higher neutralization activity than SP, FP plasma (Figure-1).

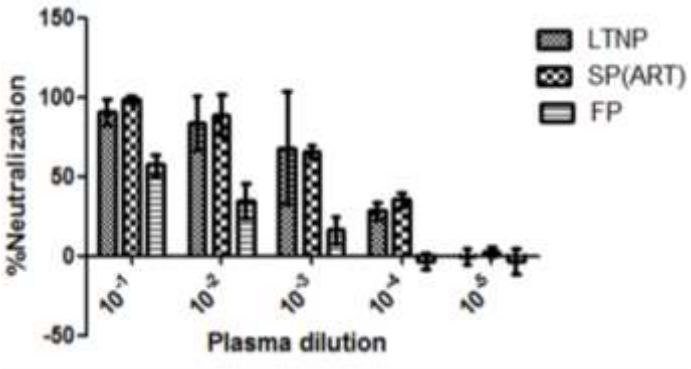

Figure-1. Neutralization of HIV-1$_{93IN101}$ replication by plasma taken in different dilutions (10 fold) from nonprogressor, slow and fast progressors. Virus replication was determined by p24 antigen (pg/ml) in culture fluids collected at 96 hours. Plasma from longterm non progressors (ART Naïve) could neutralize the virus even at higher dilution (10^{-4}).

3.2 Neutralization activity of plasma against primary isolates

Plasma samples obtained from LTNP and SP, FP were assessed for neutralization breadth with three HIV strains (NL4-3, HIV-1$_{92BR025}$ and HIV-1$_{93IN101}$). 6 non progressors and 6 fast progressor's samples were used for evaluation. From Figure-2, it can be seen that plasma from fast progressors could neutralize only one strain NL4-3. No neutralization of other two isolates HIV-1$_{92BR025}$ and HIV-1 $_{93IN101}$ was observed with the plasmas from fast progressors, while minimum 3 samples (50%) of LTNP could neutralize all the 3 isolates, and 4 (66.6%) of LTNP neutralized minimum 2 isolates. The

neutralization breadth of plasmas from LTNP was greater than FP.

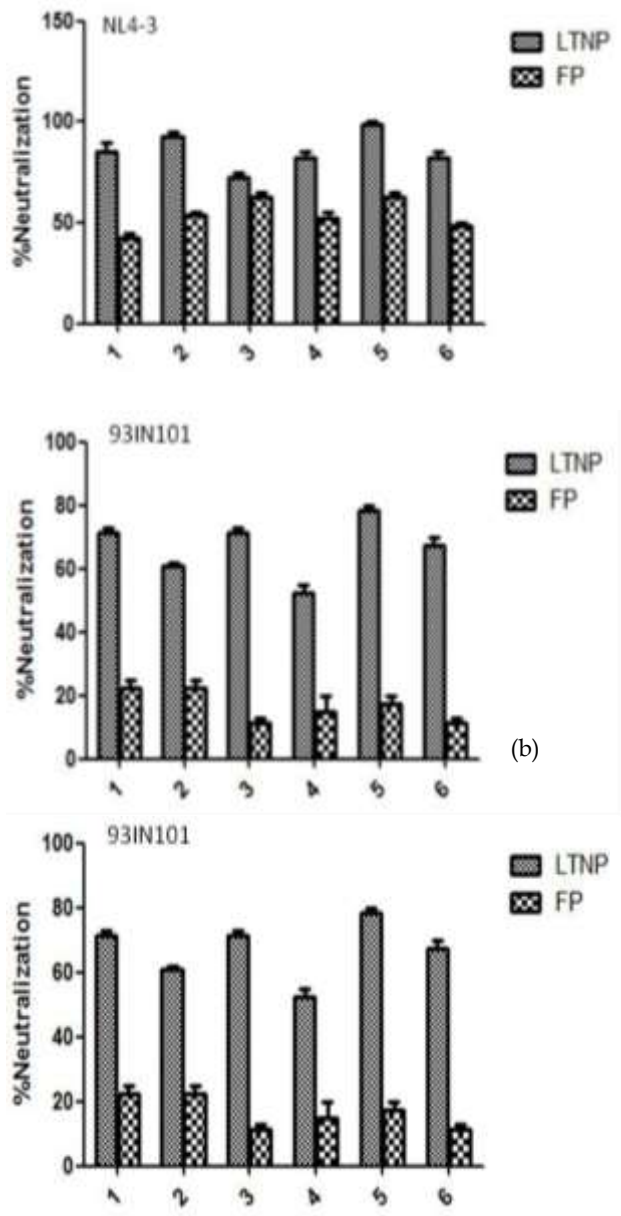

Figure-2. The neutralization activity of plasma against different isolates. (a) LTNP and FP plasma was treated with three different HIV-1 strains NL4-3 (a), HIV-1$_{93IN101}$ (b) and HIV-1 $_{92BR025}$ (c). Plasma and virus mix was added to PBMCs after 2 hours of incubation, cells were washed and replenished with fresh media and allowed to grow until day 5, and Supernatants were collected and checked for the concentration of p24 by ELISA.

3.3 Neutralization of plasma is antibody mediated

To determine whether antibodies or other factors present in the plasmas were responsible for neutralization activity, we fractioned plasma as IgG fraction and the non-IgG fraction. IgG of plasma was removed by using protein-A agarose column chromatography and flow through was used as a non-IgG fraction, separation of IgG was determined by SDS-PAGE (Figure-3). Plasmas were again re-evaluated for virus neutralization with both IgG and non-IgG fractions. Flow through fractions showed only 10 to 20% neutralizing activity, whereas antibody fraction displayed 60 to 80% neutralizing activity (Figure-4) indicating that neutralization of plasma was attributed to antibodies IgG. Our results showed that LTNP has higher levels of neutralizing antibodies, titers and increased breadth of neutralization. From our results, it was also evident that neutralization was antibody mediated. Furthermore, a neutralization assay with both IgG and non-IgG fraction revealed lack of neutralization in a non-IgG fraction of plasma. Thus, plasma neutralization is likely antibody-mediated. Also, our study about the neutralizing activity of plasma revealed that LTNP had higher levels of neutralizing antibodies and broad neutralization activity against primary isolates and the neutralizing activity of plasma is attributed to antibody IgG fraction. Our results provide some insight between levels of cytokines, antibody titer and breadth of neutralization about disease progression.

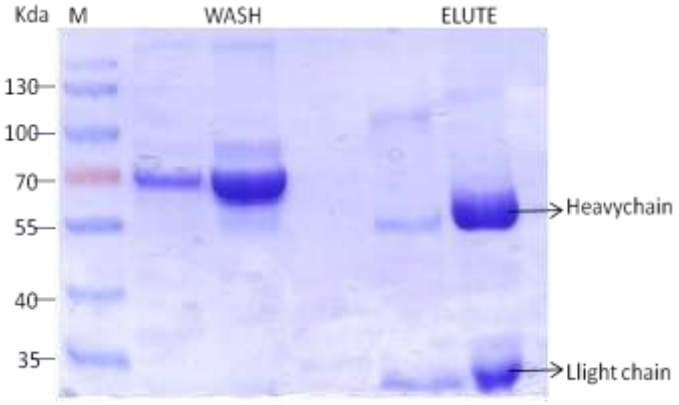

Figure-3. IgG purification from plasma. IgG purified from plasma using protein A agarose

3.4 Neutralization activity of plasma with different dilutions

Plasma neutralization with different viral isolates

LTNP and FP plasma was treated with three different HIV-1 strains NL4-3, HIV-1$_{93IN101}$ and HIV-1$_{92BR025}$. Plasma and virus mix was added to PBMCs after 2 hours of incubation, cells were washed and replenished with fresh media and allowed to grow until day 5, and supernatants were collected and checked for the concentration of p24 by ELISA.

The survival of the small fraction (5-15%) of individuals without immunologic deterioration after being infected with HIV for more than 8-10 years without any therapy is still elusive. Understanding the factors that contribute to this non progression phenomenon may provide information to develop anti-HIV regimens and vaccines against HIV-1. Several reports identified the importance of neutralizing antibodies (Nabs) in long term non progression of HIV infection. Most of the studies were cross-sectional where they compared the Nab levels in AIDS patients with compromised immune function with LTNP who had an intact immune system. It has been reported that presence of higher level of neutralizing antibodies in LTNP with a comparison to fast progressors at later stages of infection is attributed to two phenomena. The first phenomenon is the low level of Nabs in the plasma of the FPs. This cannot be due to a lack of immunogen, since progressors higher viral load is associated with disease

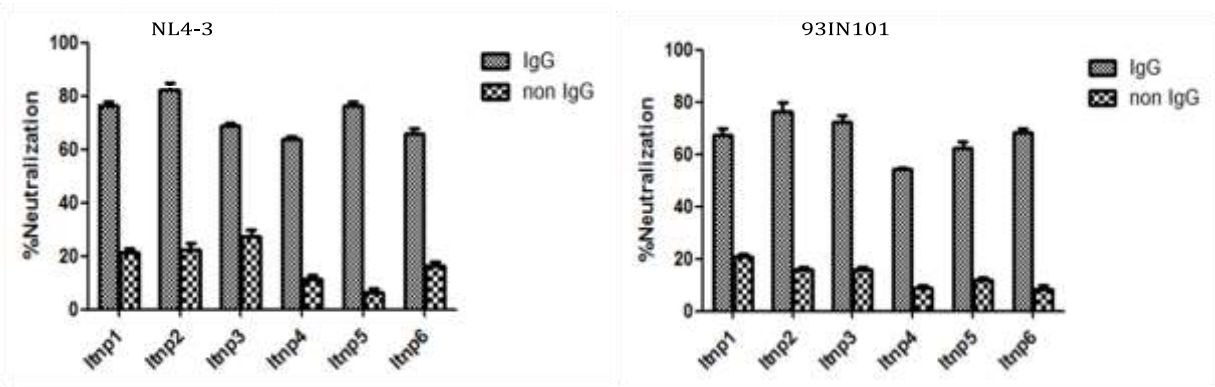

Figure-4. The neutralizing activity of IgG and non-IgG fractions of plasma. Neutralization of virus isolates NL4-3 and HIV-1$_{93IN101}$ was evaluated with both IgG and flow through (non-IgG) was done and the antiviral activity was mediated by antibody fraction of plasma

progression to AIDS (Tetali et al. 1996). The decreased antibody levels in rapid or fast progressors are certainly caused by the deterioration of the immune function, which has been reported not only in terms of decreased antibody titers, lower immunoglobulin levels, and declined CD4+T cell counts but also increased apoptosis of CD4+ T and B cells (Samuelsson et al. 1997), decreased antibody-dependent cellular cytotoxicity (ADCC) (Baum et al. 1996), and decreased proliferative responses (Dyer et al. 1997). A second phenomenon contributing to the presence of higher levels of Nabs in LTNP (Zhang et al. 1997) is attributed to low levels of virus replication, maturation of humoral immune response exhibiting an increase in the number of B cells to produce neutralizing antibodies and presence of more heterogeneous immune cell populations in LTNPs (Delwart et al. 1997), which in turn may provide stronger and more diverse antigenic stimulation.

4. Conclusion

Our study about the neutralizing activity of plasma revealed that LTNP had higher levels of neutralizing antibodies and broad neutralization activity against primary isolates and the neutralizing activity of plasma is attributed to antibody IgG fraction. Our results provide insight into antibody titer and the breadth of neutralization of disease progression.

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Conflicting Interests

The authors have declared that no conflicting interests exist.

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