Neutralization of plasma vs. antibodies: the HJ16, HGN194 and HK20 comparisons

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Introduction
Several new human antibodies (Abs) with a neutralizing potential across different subtypes have recently been described. Three Abs, HJ16, HGN194 and HK20, were obtained from patients within the HJ16 cohort of the Institute of Tropical Medicine. Patients were selected using an extended incubation PBMC based neutralization assay. Polyclonal plasma from these HIV-1 infected patients was compared to Abs obtained from their memory B-cells. PBMC based neutralization assays with varying incubation (1h vs. 24h), adsorption (1-2 h vs. 24 h) and culture phases (7 or 14 days) were performed. We compared these PBMC assays with several cell line based assays using TZMbl and GHOST cells. The role of neutralizing virus versus non-neutralizing pseudovirus was considered using a panel of tier 1 and 2 strains.

Materials & Methods

- Patients were preferentially selected from sub-Saharan regions where the subtypes A, C and/or CRF02 are prevalent. Plasma was subsequently screened for its ability to neutralize a panel of four subtype A, four subtype C and six CRF02 primary HIV-1 strains.
- HJ16, HK20 and HGN194 Abs were obtained as part of the collaboration for AIDS Vaccine Discovery program from Dr. D. Corti.
- HIV-1 neutralization assays: plasma was tested at 1/20 dilution and Abs were tested at 50 µg/ml.
  - 24/14 PBMC based:
    - incubation Ab or plasma + virus 24 hr
    - absorption to PHA/IL-2 stimulated PBMC 1 hr, wash
    - culture cells 14 days, p24 ELISA readout
  - 1/2/7 PBMC based:
    - incubation Ab or plasma + virus 1 hr
    - absorption to PHA/IL-2 stimulated PBMC 2 hr, wash
    - culture cells 7 days, p24 ELISA readout
  - 1/24/14 PBMC based:
    - incubation Ab or plasma + virus 1 hr
    - absorption to PHA/IL-2 stimulated PBMC 24 hr, wash
    - culture cells 14 days, p24 ELISA readout
  - TZMbl cell based:
    - incubation Ab or plasma + virus 1 hr
    - absorption/culture on TZMbl cells during 2 days and luciferase readout
  - GHOST cell based:
    - incubation Ab or plasma + virus 1 hr
    - absorption/culture on GHOST cells during 3 days and luciferase readout

Plasma responses:
- PBMC based assays:
  - 242315 and S29552 neutralize fewer strains (HCV even loses potential) in the classical short to PBMC assays.
  - Prolonging the incubation to 1/24/14 only ‘rescues’ some potential for 314994.
- Cell line based assays:
  - very potent neutralization profiles detected
  - pseudoviruses easily neutralized compared to the infectious strains
- Statistical correlation:
  - 242315: mostly subtype A and C (specific 3/4 A, 4/4 C, 4/4 CRF02 strains)
  - 314994: mostly subtype A specific (3/4 A, 2/4 C, 4/4 CRF02 strains)

Antibody responses:
- PBMC based assays:
  - antibody 24/1/14 responses are much more restricted compared to plasma responses
- Cell line based assays:
  - similar or higher potency detected when measured with pseudovirus based assays
- Statistical correlations: no correlations between 24/1/14 and other assays

Conclusions
The present study indicates that different neutralization assays yield different results and it is still unclear which one is most predictive of in vivo neutralizing activity. Moreover, the strong profiles in the patients’ plasma were not solely due to Abs represented by the newly isolated Abs. This better understanding of in vitro neutralization characterizations of patient plasma and Abs will hopefully lead to more effective ways of discovering new Abs that ultimately can be used for HIV-1 immunogen design and subsequent vaccine development.

References