19-21 GIUGNO ROMA
UNIVERSITA' CATTOLICA DEL SACRO CUORE
CENTRO CONGRESSI EUROPA
RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY
Promosso da SIMIT
16° CONGRESSO NAZIONALE ICAR
Italian Conference on AIDS and Antiviral Research
P 193
POSTER 4 YOU ICAR 2024

Evaluation of functional antibodies against HIV Env and Tat proteins: analysis of antibody-dependent cell-mediated cytotoxicity (ADCC)

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Introduction

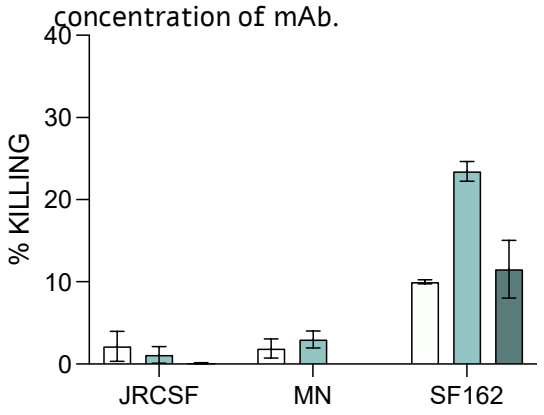
- Antibody-dependent cell-mediated cytotoxicity (ADCC), which exploits the effector activity of macrophages, neutrophils, and natural killer lymphocytes, is a relevant immune defense mechanism for the contol of viral infection.
- In detail, ADCC against the HIV envelope was found to correlate with protection from infection in the RV144 Thay trial.
- Nevertheless, several questions remain unresolved, such as: i) the effectiveness of ADCC induced by different Env forms and ii) the role of ADCC against regulatory HIV antigens (such as Tat).

Methods

- The capacity of antibodies against Env or Tat to mediate ADCC was investigated by the Rapid and Fluorometric assessment of ADCC (RFADCC) assay, using peripheral blood mononuclear cells (PBMCs) from healthy donors as effectors and CEM NKr CCR5+, pulsed with Env or Tat and opsonized by specific antibodies, as target cells.

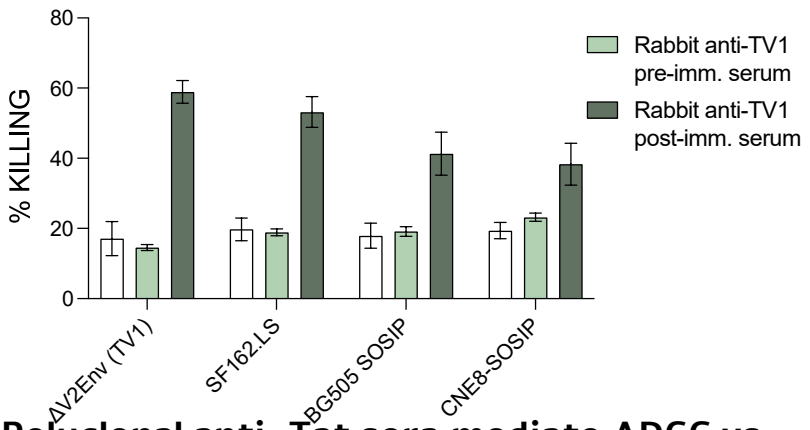
The mAb 17b mediates ADCC vs monomeric Env

- Target cells were pulsed with three monomeric B clade gp120 proteins (SF162, JRCSF, MN) and then incubated with two different concentrations (0.1 and 1 µg/ml) of mAb 17b prior to addition of effector cells. A significant ADCC activity was detected only against SF162 protein, in target cells incubated with the lower concentration of mAb.



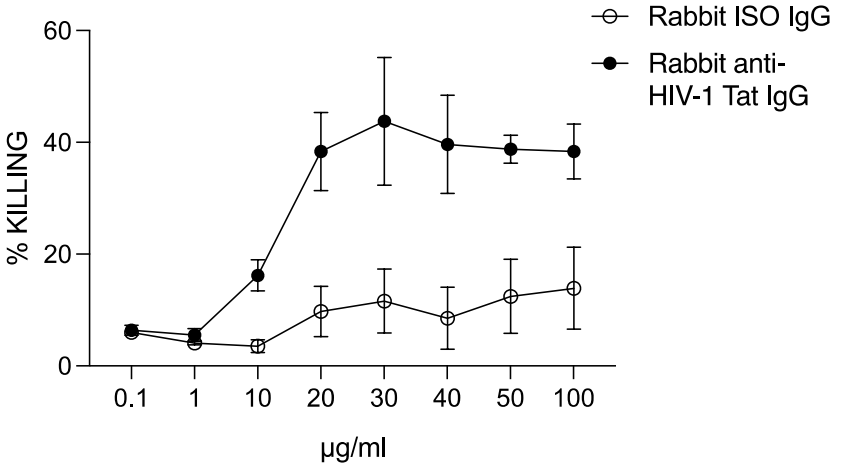
Polyclonal anti-Env sera mediate ADCC vs trimer Envs

- We next investigated the ADCC induction mediated by the an hyperimmune serum from a rabbit immunized with Env ΔV2Env TV1 against a clade C trimeric Env protein devoid of the V2 loop (DV2-TV1), the gp140 protein SF162.LS.gp140 (clade B) and two SOSIP trimers (BG505 - clade A and CNE8 - clade C). ADCC activity was detected against all proteins with the highest percentage of killing against the Env ΔV2Env TV1 protein.



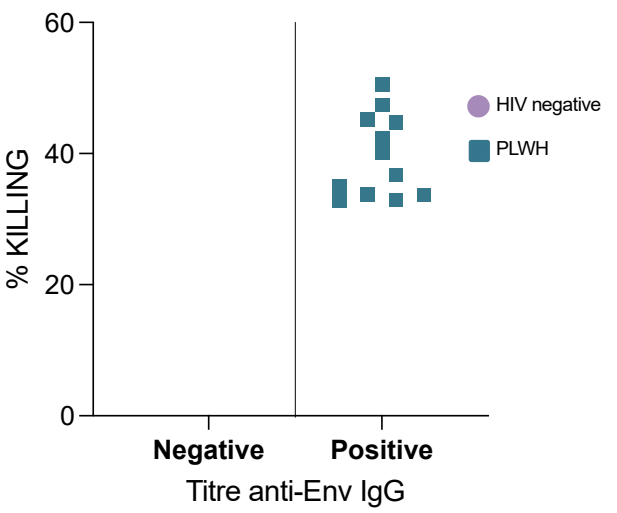
Polyclonal anti-Tat sera mediate ADCC vs Tat

- We then developed a protocol of RFADCC to assess whether Tat-specific antibodies can mediate ADCC against Tat-pulsed target cells We observed significant ADCC activity (>40% killing) in target cells that were pulsed with 30µg/ml of Tat protein and subsequently incubated with a hyperimmune serum derived from a rabbit immunized with Tat.

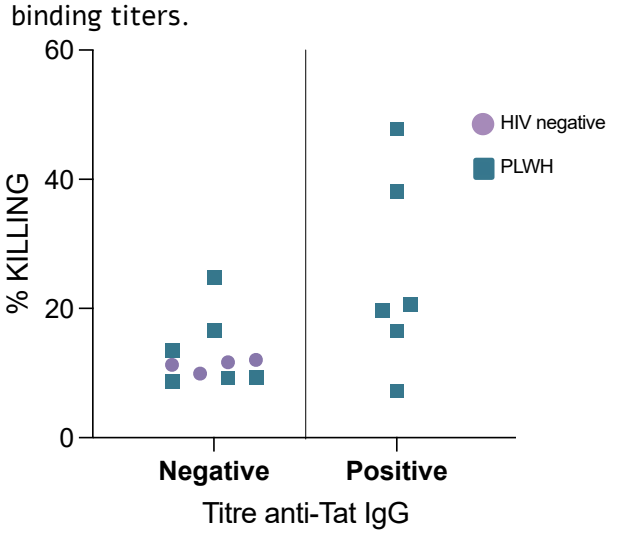


Sera from PLWH mediate ADCC vs Tat and Env

- Next, we proceed to evaluate the cell-mediated cytotoxic responses triggered by human sera from people living with HIV (PLWH). As shown, all PLWH have a strong and similar cytotoxic activity against ΔV2Env TV1protein.



- Conversely, not all PLWH have developed antibodies against Tat protein. However, patients who developed them can mediate ADCC, with different degrees of response intensity that mainly depend on the antibody binding titers.



Conclusion

- These results confirm that Env-specific mAb mediate ADCC activity;
- Our data suggest that monomeric and trimeric forms of Env from different clades exhibit different degrees of ADCC response intensity;
- We demonstrate, for the first time, that human anti-Tat antibodies from PLWH can mediate ADCC;
- Given the key role of Tat in HIV acquisition and disease progression, these results suggest that Tat should be considered a valuable antigen for developing innovative HIV vaccines and that Tat-specific ADCC can be exploited to monitor protection indicators.

Funding: Bill & Melinda Gates Foundation INV-037179: Evaluations of Tat and Tat-Env as targets for HIV