Reconstruction of the ADCC Antibody Repertoire of an HIV-1 Non-Transmitting Mother

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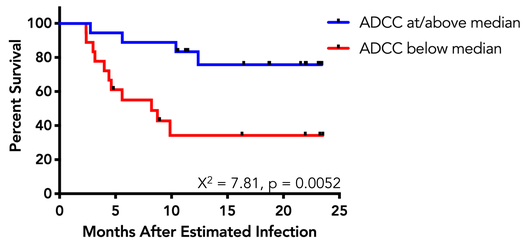
Introduction

- Plasma ADCC mediated by passively-transferred Abs is associated with reduced HIV acquisition risk and improved survival in HIV+ infants, but the antibodies comprising the plasma ADCC repertoire are not well understood

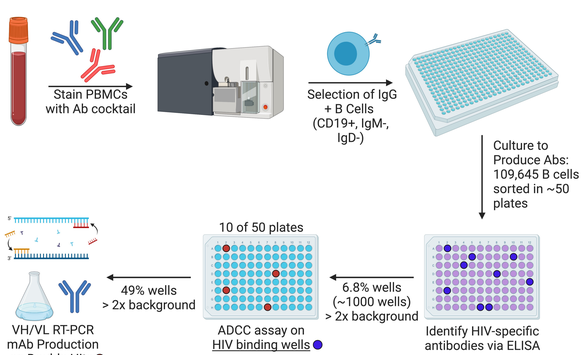


Hypothesis: Reconstructing ADCC antibodies from memory B cells circulating during late pregnancy can provide insight into ADCC-capable antibodies in infants exposed to HIV. This is a first step in better understanding the ADCC repertoire in the setting of vertical transmission.

Methods: Sample Selection and Sorting



A mother living with HIV, MG540, who was enrolled in the Nairobi Breastfeeding Trial (NBT; 1992-1998) and did not transmit HIV to her infant, BG540, was selected for reconstruction of HIV-specific ADCC antibodies based on the following criteria: 1) PBMCs available from late pregnancy (when placental antibody transfer occurs), 2) high plasma viral load (associated with vertical transmission risk), and 3) high late pregnancy and infant passively-acquired plasma ADCC activity.



IgG-expressing memory B cells were sorted from a late pregnancy PBMC sample and cultured to produce antibodies. Culture supernatants were subsequently screened for HIV-specific activity via ELISA. Wells with HIV-specific antibodies were then tested for ADCC activity. Any wells containing HIV-specific and ADCC-capable antibodies were considered for mAb reconstruction via RT-PCR.

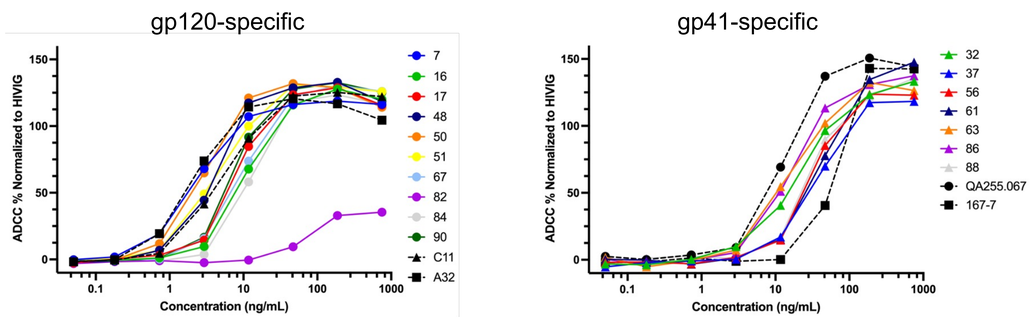
Antibodies targeting multiple epitopes are required to recapitulate the plasma ADCC activity of mother MG540 and her infant

Results: Characteristics of 17 mAbs isolated from MG540

			Rearrangement Characteristics		Epitope Mapping		RFADCC Activity
Family	ELISA Target	Antibody ID	Heavy Chain	Light Chain	Phage Display	ELISA	EC_{50}
1	gp120	MG540.7*	V1-69 D3-9 J3	KV1-9 J5	negative	C11	2.4
2		MG540.16	V5-51 D2-15 J4	LV3-1 J2	V3	NT	9.9
		MG540.67			V3	V3	8.0
		MG540.90*			V3	V3	6.2
3		MG540.17*	V3-21 D3-9 J4	KV1-5 J2	negative	negative	6.7
4		MG540.48	V1-2 D3-10 J5	LV8-61 J3	negative	A32	3.7
5		MG540.50*	V1-69 D3-10 J5	KV3-20 J4	negative	A32	2.8
6		MG540.51	V1-69 D3-16 J4	KV3-20 J3	negative	C11	3.8
7		MG540.82*	V1-69 D6-13 J4	KV3-11 J5	negative	17b	59
8		MG540.84	V5-51 D3-16 J4	LV1-51 J2	V3	V3	11
9	gp41	MG540.32	V1-3 D2-15 J3	KV3-15 J2	IDE	NT	33
		MG540.86*			IDE	IDE	23
10		MG540.37*	V1-2 D2-21 J6	KV4-1 K2	negative	Cluster II	47
11		MG540.56*	V4-39 D1-26 J4	LV2-14 J3	negative	Cluster II	37
12		MG540.61	V1-69 D4-17 J6	KV2-28 J3	negative	Cluster II	56
13		MG540.63*	V3-64 D5-12 J6	KV2-29 J4	IDE	IDE	19
14		MG540.88	V3-30 D3-3 J6	LV1-40 J3	negative	Cluster II	42

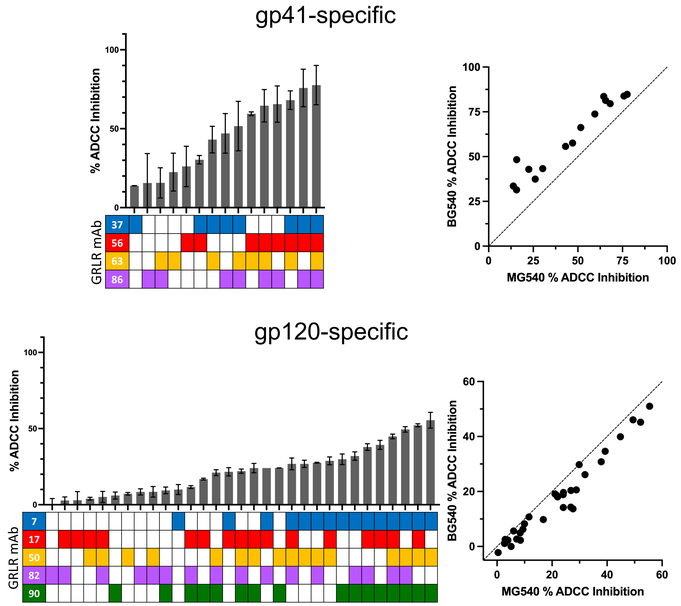
Table summarizing properties of HIV-specific antibodies isolated from mother MG540. Antibodies are grouped by HIV Env subunit target, as determined by ELISA. Antibodies grouped in a shared box in the ID column are clonally related to each other. Antibody epitope was determined by phage display immunoprecipitation of HIV Env peptides and/or competition ELISA using antibodies targeting known epitopes. Antibodies that did not map to an epitope in the indicated assay are labelled as negative. RFADCC assay EC_{50} (ng/mL) was determined via serial dilution (as shown below) and fitting of a dose-response curve. *: indicates antibodies selected for plasma competition experiments (figures on the right).

Results: ADCC activity and potency of isolated MG540 antibodies



Results from serial dilution ADCC experiments of MG540 antibodies, separated by specificity for gp120 (left) or gp41 (right). Antibodies were tested for ADCC activity via the RFADCC assay, using CEM.NKr target cells coated with BG505 (clade A) gp120 or ZA1197 (clade C) gp41, respectively. Plots show ADCC activity normalized to that of HIVIG for mAbs at the indicated concentrations. The CD4i, cluster A mAbs C11 and A32 were included as positive controls for gp120 mAbs. The gp41 cluster I and II mAbs QA255.067 and 167-D were included as positive controls for gp41 mAbs. Data are representative are results from two replicate experiments, using PBMCs from distinct donors as effector cells.

Results: MG540 antibodies recapitulate MG540 and BG540 plasma ADCC



Inhibition of MG540 late pregnancy plasma gp41- (upper left) or gp120-specific (lower left) RFADCC activity by target cell pre-incubation with the indicated combinations of GRLR mAb variants. Shaded boxes indicate mAbs present in each competition. Conditions ordered by ascending degree of plasma ADCC inhibition. Right panels show correlations between GRLR mAb plasma gp41- (upper right) or gp120-specific (lower right) ADCC competitions for MG540 and her infant, BG540. Dashed lines indicate line of identity. Data are from two replicate experiments.

Conclusions

- A targeted approach identifies ADCC-capable mAbs targeting multiple Env eptiopes from mother MG540
- mAbs targeting multiple epitopes are required to recapitulate the plasma ADCC activity of MG540 and the passively-acquired ADCC activity of her infant, suggesting that the passive ADCC repertoire was polyclonal in this mother-infant pair
- Studies of additional mother-infant pairs will be needed to determine if polyclonality is a common feature of ADCC antibodies in HIV-exposed, uninfected infants

Acknowledgments

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