B-Cell Immunogenicity of a Fusion Peptide HIV Vaccine in Adults: Results From HVTN 303 Part B

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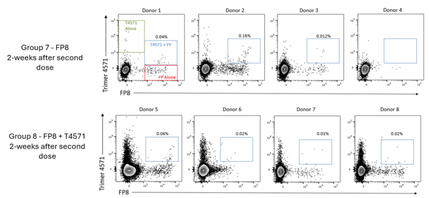
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BACKGROUND

HVTN 303, a trial of HIV-1 fusion peptide (FP)-directed vaccine, was designed to prime FP-binding antibodies with neutralizing potential. An 8-amino acid peptide (FP8) was conjugated to the heavy chain of tetanus toxoid to create a protein conjugate (FP8-rTTHC) that was given alone or in combination with Trimer 4571 (a stabilized version of Clade A BG505 SOSIP), adjuvanted with Adjuplex (20%). Due to multiple grade 3 adverse events, vaccinations were permanently discontinued in March 2023. 13 participants received one dose of Trimer 4571 (200 µg); 13 participants received one dose of FP (200 µg) and five received a 2nd dose at month 1; 9 participants received one vaccination with FP plus Trimer 4571 (each 200 µg), and 6 participants received a 2nd dose at month 1.

B cell fraction binding both FP8 & Trimer 4571



FACS isolation of B cell fractions that bind both the fusion peptide and Trimer 4571 in 7 of 8 participants (3 in group 7 & 4 in group 8) who received two vaccine doses

METHODS

8 of 11 participants who received 2 doses (4 FP only/4 FP + Trimer 4571) were selected for B cell analysis. Antigen-specific B cells 2 weeks after vaccination were sorted by flow cytometry, and their B cell receptors (BCR) were sequenced. Paired variable heavy (VH) and variable light (VL) chains were used to generate monoclonal antibodies (mAbs) and were tested for binding affinity, FP fingerprint analysis, and selected mAbs underwent cryo-EM. Epitope angle of approach and detailed epitope-paratope structural analyses were performed. The BCR sequences, genetic profile, HCDR3 length, and somatic hypermutation (SHM) were assessed.

Monoclonal Ab Binding Kinetics

Group	Monoclonal Ab	To Trimer 4571			To Fusion Peptide		
		Ka (1/Ms)	Kd (1/s)	KD (nM)	Ka (1/Ms)	Kd (1/s)	KD (nM)
Control	VRC 34.01	1.66E+05	2.45E-05	0.15	1.29E+05	6.87E-05	0.53
7	7.1-1-D10-24.1	1.66E+04	9.95E-04	27.2	1.26E+05	1.01E-02	32.90
	7.1-1-F06-11.2	1.28E+05	8.78E-04	7.54	1.26E+05	2.38E-04	1.89
	7.1-1-D03-4.2	2.19E+04	1.42E-04	6.51	2.94E+05	3.17E-05	0.11
	7.1-1-E12-19.1	4.83E+04	8.45E-04	18.2	1.41E+05	3.9E-04	2.77
	7.1-1-F07-1.4	1.77E+04	1.69E-03	95.4	2.57E+05	7.77E-05	0.30
	7.1-2wk-1.2	1.03E+04	1.08E-04	10.5	1.46E+05	9.06E-06	0.06
	7.2-1-B11-1.6 (Fab A1)	**1.54E+04**	**2.69E-05**	**1.75**	**8.59E+04**	**2.08E-05**	**0.23**
	7.2-1-E05-12.1	3.20E+04	5.11E-04	16	1.20E+05	5.62E-05	0.47
	7.3-1-A04-1.1 (Fab A3)	**1.25E+04**	**1.82E-04**	**14.6**	**1.12E+05**	**3.63E-05**	**0.33**
	7.3-2wk-1.1	1.91E+04	4.25E-03	2.12	1.47E+05	2.58E-05	0.16
	7.4-2wk-1.1	5.22E+04	2.47E-03	47.4	1.17E+05	1.26E-04	1.08
	4 participants / 11 mAbs						
8	8.1-D11-1-0.0	4.69E+03	2.58E-04	53.7	1.69E+05	1.13E-04	0.66
	8.1-1-G06-29.1	9.38E+03	2.38E-04	25.3	1.74E+05	8.09E-06	0.05
	8.1-d15-1.7	7.50E+03	2.81E-05	3.75	1.43E+05	4.08E-05	0.29
	8.2-d15-1.8	8.22E+03	1.58E-05	1.55	1.31E+05	2.63E-05	0.20
	8.3-2wk-7.1	5.68E+04	1.54E-03	27.2	1.32E+05	8.43E-05	0.64
	3 participants / 5 mAbs						

Surface plasmon resonance (SPR) determined association rate (k_a), dissociation rate (k_d) and affinity (K_D) for VRC34.01 & 16 monoclonal antibodies with Trimer 4571 or Fusion Peptide. Pink/light red/dark red coloring indicate increasingly stronger responses. Light blue/blue/dark blue coloring indicate increasingly weaker responses.

HIV-1 envelope fusion peptide specific B cells with features of known human bnAbs are activated with two doses of a tetanus-toxoid fusion peptide conjugate vaccine in humans. These results suggest a strategy for further development of a fusion peptide targeting sequential immunization vaccine.

RESULTS

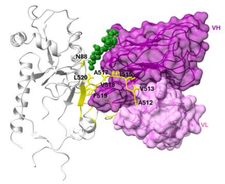
7 of 8 participants had B cells that bound both FP8 & Trimer 4571; 6 had BCR sequences from the VH3 family (VH3-23, VH3-30, VH3-30-3) – a gene group used by FP broadly neutralizing mAbs ACS202, PGT151, and PGT152. Approximately 500 mAbs were generated from the BCRs, with 17 identified as high-binders and selected for more detailed epitope characterization. Although the mAbs did not induce neutralization, cryo-EM analysis showed FP-epitope binding through the trimer glycan shield with varying angles of approach. One example is mAb Fab A1, the VH of which penetrates past the epitope canonical glycan N88 and engages multiple gp41 residues, forming 11 hydrogen bonds and 2 salt bridges. Another example is mAb Fab A3 which engages the gp41 at another angle of approach, and through 11 hydrogen bonds and 1 salt bridge stabilizes the FP in another orientation.

Monoclonal Ab Binding Fingerprint Analysis

Group	Peptide Sequence	AVGIGAVFL	AVGIGAVFL	AVGIGAVFL	AVGIGAVFL	AVGIGAVFL	AVGIGAVFL	AVGIGAVFL	AVGIGAVFL
	Amino Acid Position	512	513	514	515	516	517	518	519
	Amino Acid	A	V	G	I	G	A	V	F
Controls	VRC34.01								
	PGT151								
	PGT152								
	ASC202								
7	7.1-1-D10-24.1								
	7.1-1-F06-11.2								
	7.1-1-D03-4.2								
	7.1-1-E12-19.1								
	7.1-1-F07-1.4								
	7.1-2wk-1.2								
	7.2-1-B11-1.6 (Fab A1)								
	7.2-1-E05-12.1								
	7.3-1-A04-1.1 (Fab A3)								
	7.3-2wk-1.1								
	7.4-2wk-1.1								
8	8.1-D11-1-0.0								
	8.1-1-G06-29.1								
	8.1-d15-1.7								
	8.2-d15-1.8								
	8.3-2wk-7.1								

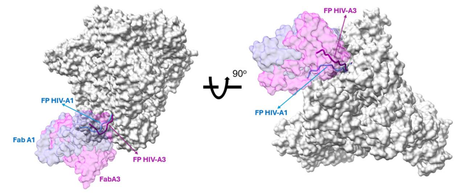
Ab binding fingerprint as determined using relative binding to truncation peptides. Variants resulting in at least a 10-fold reduction in binding relative to non-truncated fusion-peptide are shown on dark red, a 2- to 10-fold reduction are shown in light red, and with no loss of binding shown in white.

Fab A3 Interaction with Trimer 4571



CryoEM structure of Fab A3 (purple) bound in complex with Trimer 4571. The N88 glycan component of the epitope is shown in green and the rest of the epitope is shown in yellow. Compared with Fab A1, there are more FP residues interacting with Fab A3.

Overlay of Fab A1 and Fab A3 while binding to Trimer 4571



An overlay of mAb Fab A1 (blue) and Fab A3 (purple) as they interact with Trimer 4571 (grey) shows a more parallel angle of approach (with respect to the membrane) vs. basilar angle of approach of Fab A1 vs Fab A3, with the fusion peptides stabilized in two different conformations.

CONCLUSIONS

FP directed lineages, primarily using VH3 genes, were induced in most participants who received two doses of FP8-rTTHC alone or in combination with Trimer 4571. Observed binding to the intact trimer FP epitope with notable SHM and affinity suggests boosting vaccination may be able to mature FP mAb lineages with the potential for broad neutralization capacity.

IMPORTANCE OF FINDINGS

An effective HIV vaccine will likely require neutralizing antibodies targeting at least 3 different epitopes. The fusion peptide may be an effective complementary target, with antibodies stabilizing trimers in a prefusion orientation. VH3 gene using FP bnAbs are potent and broad (e.g., PGT151 IC50 0.04 µg/ml & 73% breadth). While this regimen showed frequent adverse events attributed to the adjuvant Adjuplex, new regimens using the FP8-rTTHC and an alternative adjuvant are under development. This study clearly indicates the immunological effectiveness of this priming approach.

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