Molecular Dissection of Human Antibody Responses Following Prefusion-Stabilized RSV F Vaccination

Maryam Mukhamedova[1], Daniel Wrapp[2], Chen-Hsiang Shen[1], Morgan S.A. Gilman[2], Tracy J Ruckwardt[1], Chaim A. Schramm[1], Larissa Ault[1], Lauren Chang[1], Alexandrine Derrien-Colemyn[1], Emily Phung[1], Pamela J. Costner[1], LaSonji A. Holman[1], Somia P. Hickman[1], Nina M. Berkowitz[1], Kaitlyn M. Morabito[1], Michelle C. Crank[1], Barney S. Graham[1], Jason S. McLellan[2], John R. Mascola[1]

National Institute of Allergy and Infectious Diseases
Dale and Betty Bumpers Vaccine Research Center

[1] Vaccine Research Center, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA
[2] Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX 78712, USA

Any questions can be addressed to: maryam.mukhamedova@nih.gov

Abstract

Background: An effective vaccine for RSV is an unrealized public health goal. Recently, a single dose of the prefusion-stabilized fusion (F) glycoprotein subunit vaccine (DS-Cav1) was shown to substantially increase serum neutralizing activity in healthy adults. We sought to determine if vaccination with a stabilized prefusion conformation of F (pre-F) induces a repertoire mirroring the pre-existing diversity from natural infection or whether certain antibody lineages targeting specific epitopes predominate. We performed repertoire analysis of RSV F-specific antibodies in six DS-Cav1-vaccinated individuals before and after vaccination.

Methods: To understand the induced B-cell repertoire at the single-cell level, we evaluated RSV F-specific B-cell responses before and after vaccination in six participants. Using a combination of antigen-specific memory B-cell sorting, paired heavy and light chain sequencing of plasmablasts, and unpaired heavy and light chain sequencing of naïve and memory B-cell transcripts, we identified and characterized RSV F-specific antibody lineages. Using cryo-electron microscopy, we structurally defined two neutralizing public clonotypes targeting site Ø and V on pre-F.

Results: DS-Cav1-induced lineages recognized pre-F and were genetically diverse (555 RSV F clonal lineages were identified in total). Expressed antibodies recognized all six previously defined antigenic sites on the pre-F trimer. Among the six vaccinees, we identified 34 public clonotypes. Structural analysis of two antibodies from a predominant clonotype revealed a common mode of recognition.

Conclusion: Collectively, these findings demonstrate that vaccination with DS-Cav1 generates a diverse polyclonal response targeting all known antigenic sites present on pre-F. In addition, multiple neutralizing public clonotypes were identified, and a predominant prefusion-specific, vaccine-boosted public clonotypes was structurally defined.

B Cell Datasets For Six VRC317 Clinical Trial Participants

A

Group	Subjects	Dose	Day 0	Week 12
1	15	50 mcg	DS-Cav1	DS-Cav1
2	15		DS-Cav1 + alum	DS-Cav1 + alum
3	15	150 mcg	DS-Cav1	DS-Cav1
4	15		DS-Cav1 + alum	DS-Cav1 + alum
5	15	500 mcg	DS-Cav1	DS-Cav1
6	15		DS-Cav1 + alum	DS-Cav1 + alum
Total	90*	All DS-Cav1 vaccinations are administered with needle and syringe into the deltoid muscle. *Up to 100 subjects may be enrolled if needed to evaluate safety or immunogenicity.		

B

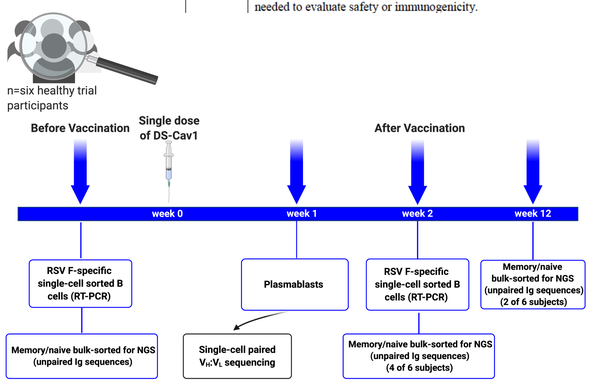


Figure 1: VRC317 Phase I Clinical Trial in Healthy Adults and Approaches for Antibody Repertoire in Six Vaccinated Adults
A) The full VRC 317 clinical trial includes 15 subjects in each of 6 groups. The goal of this study is to dissect the RSV F-specific B cell antibody repertoire elicited by the native prefusion (DS-Cav1) vaccine in six trial participants from VRC317 group 4 trial. **(B)** The approaches and datasets generated for each of the six participants from VRC317 group 4 trial.

Results

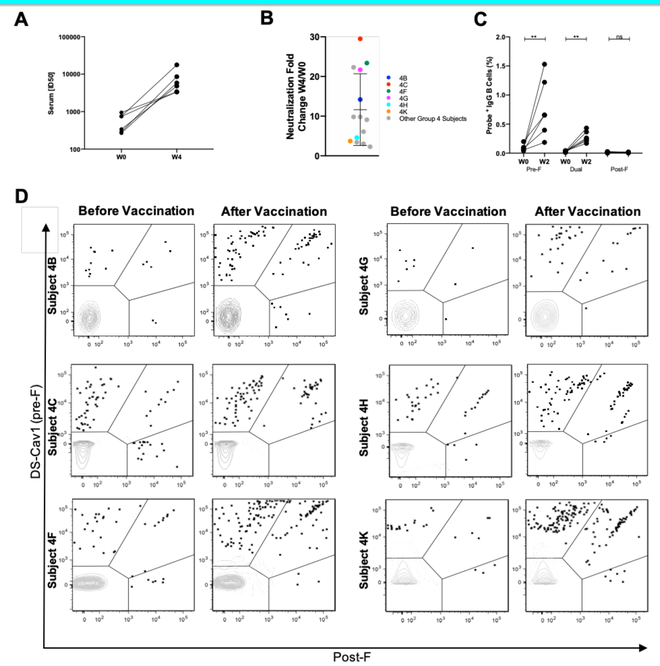
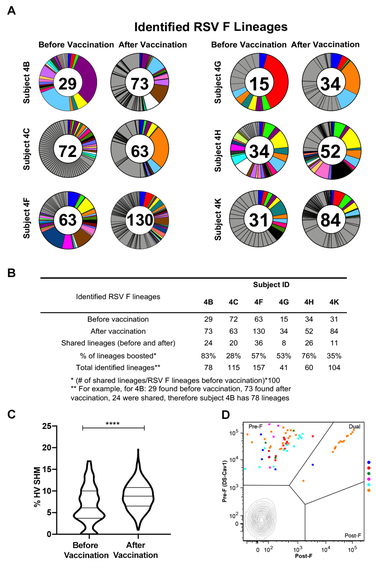


Figure 2: Identification of RSV F Memory B Cells Before and After DS-Cav1 Vaccination
(A) Serum neutralization titers for six individuals vaccinated with 150 µg DS-Cav1+ alum. Titers are shown before vaccination (W0) and 4 weeks after vaccination (W4). **(B)** Fold increase in serum neutralization titer four weeks after vaccination for all 15 subjects immunized with 150 ug DS-Cav1 + alum adjuvant. The six vaccinees in Group 4 studied (4B, 4C, 4F, 4G, 4H, 4K) are colored. **(C)** The percentage of CD19 + /CD27+ memory B cells with probe phenotype specified as pre-F-specific, dual, or post-F-specific before vaccination (W0) and 2 weeks after vaccination (W2). **(D)** FACS index sorted plots for six vaccinees (before and 2 weeks after immunization) showing RSV F memory B cells reactive with the indicated fluorescently labeled probes DS-Cav1 or Post-F. X and Y axis show MFI. Gate regions in each plot show B cells as pre-F-specific, dual, or post-F-specific binders



Identified RSV F lineages	Subject ID 4B	4C	4F	4G	4H	4K
Before vaccination	29	72	63	15	34	31
After vaccination	73	63	130	34	52	84
Shared lineages (before and after)	24	20	36	8	26	11
% of lineages boosted*	83%	28%	57%	53%	76%	35%
Total identified lineages**	78	115	157	41	60	104

* (# of shared lineages/RSV F lineages before vaccination)*100
** For example, for 4B: 29 found before vaccination, 73 found after vaccination, 24 were shared, therefore subject 4B has 78 lineages

Figure 3: DS-Cav1 boosts pre-existing B-cell lineages that target prefusion sites. (A) Circle plots show RSV F-specific B-cell lineages identified before and after vaccination with DS-Cav1. Lineages were identified from probe-sorted memory B cell heavy chain sequences and augmented with data from plasmablasts and unpaired NGS. Each slice of the plot represents a lineage, with slice size proportional to the number of sequences for the lineage. Some lineages were found both before and after vaccination as indicated by the same color for that subject. The value in the circle indicates the number of lineages found either before or after vaccination for each subject. Gray slices are remaining lineages found only before or after DS-Cav1 vaccination. **(B)** Summary of RSV F-specific lineages. For each subject, the number of lineages identified before and after vaccination is shown. Shared lineages are those with sequences identified before and after vaccination. Total identified lineages are the number of distinct RSV F lineages per subject. **(C)** Violin plots show all sequences from shared (boosted) lineages in six subjects before (n=299) or after (n=1639) vaccination. Percent nucleotide divergence from the inferred germline heavy chain gene (% HV SHM) is shown on Y axis. Statistical significance was determined by unpaired Mann-Whitney nonparametric test with p<0.0001. **(D)** Probe-sorted RSV F-binding memory B cells from the top three clonally expanded lineages per subject are overlaid on a flow plot (18 of 125 shared lineages). Probe-sorted sequences are colored by subject. X and Y axes show MFI and the indicated antigen-specific probe used. Gate regions in each plot show B cells as pre-F or post-F specific or dual binding.

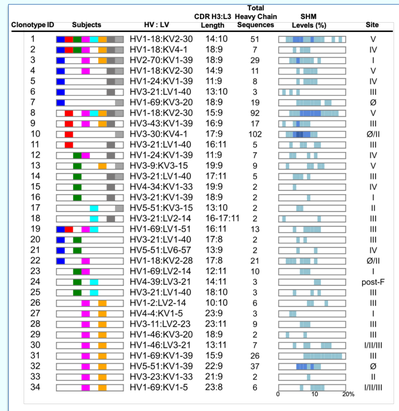


Figure 4: Identification of RSV F-specific public clonotypes. Thirty-four public clonotypes were identified by augmenting probe-sorted memory B cell heavy chain sequences with paired plasmablast and unpaired NGS data for all six subjects and by adding published RSV F specific antibody heavy chain sequences to the dataset. Public clonotypes are numbered 1 to 34. Second column shows subjects, each one with distinct color as shown in key on right, in which the clonotype was found. Third column shows the heavy and light chain germline gene pairing based on IMGT. Fourth column shows the CDR H3 and L3 amino acid length, respectively. Fifth column shows the total number of heavy chain sequences identified for each clonotype. Sixth column shows the distribution of SHM within a clonotype, based on nucleotide divergence from the germline heavy chain gene. The rectangular box indicates SHM ranging from 0% to 20% (bottom of sixth column). The intensity of the blue hue corresponds to number of heavy chain sequences (as seen on the right in bottom figure legend) at a specified SHM percent. Last column indicates the antigenic site of RSV F recognized by the clonotype.

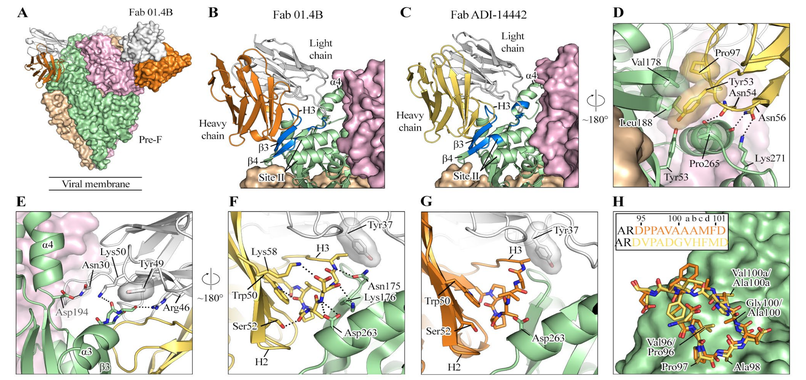


Figure 5. Structural basis of site V recognition by clonotype 1 antibodies isolated after DS-Cav1 vaccination or natural RSV infection.
(A) Cryo-EM structure of Fab 4B in complex with pre-F. The three protomers of pre-F are shown as molecular surfaces colored tan, green and pink. Two 01.4B molecules are shown as molecular surfaces and one is shown in ribbons, each with the heavy chain in orange and the light chain in white. The constant domains of the Fabs were not modeled in the structure. **(B)** Magnified view of the interface between 01.4B and pre-F. Colored as in (A), except the third protomer of pre-F is shown in ribbons with regions within 5.5 Å of 01.4B colored blue. **(C)** The interface between ADI-14442 and pre-F determined by cryo-EM is shown with the same orientation and coloring as in (B), with the exception of the ADI-14442 heavy chain, which is colored gold. **(D)** Further magnified view of the interface between the heavy chain of ADI-14442 and pre-F is shown with an approximately 180° rotation about the trimeric axis relative to the orientation shown in (C). **(E)** Magnified view of the interface between the light chain of ADI-14442 and pre-F. **(F)** Magnified view of the ADI-14442 CDR H3 interactions with CDR H2, CDR L1 and pre-F, shown with an approximately 180° rotation about the trimeric axis relative to the orientation shown in (E). **(G)** Magnified view of the 01.4B CDR H3 interactions with CDR H2, CDR L1 and pre-F, shown in the same orientation as in (F). **(H)** Pre-F is shown as green molecular surfaces. The CDR H3s of 01.4B and ADI-14442 are shown as sticks colored orange and gold, respectively. The CDR H3 sequences are shown in the inset and are colored to match the structures. For all panels, side chains of residues involved in hydrogen bonding or salt bridges are shown as sticks with oxygen and nitrogen atoms colored red and blue, respectively. Hydrogen bonds and salt bridges are depicted as black dotted lines. Transparent molecular surfaces are shown for residues involved in hydrophobic interactions.

Conclusions

- **Vaccination with DS-Cav1 boosts a diverse polyclonal repertoire of B cell lineages**
- **Vaccine stimulation of numerous public clonotypes to all known antigenic sites**
- **Common mode of recognition from most prevalent clonotype found in all six vaccinees targeting antigenic site V**

Acknowledgements: This work was supported by intramural funding from the National Institute of Allergy and Infectious Diseases