Supplemental Information

Title: Post-vaccination graft dysfunction/aplastic anemia relapse with massive clonal expansion of autologous CD8+ lymphocytes

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Materials and Methods

Patient Recruitment

The study was conducted as a part of the prospective/retrospective Penn-CHOP Bone Marrow Failure Syndrome (BMFS) cohort to elucidate molecular mechanisms of BMFS, approved by the Institutional Review Boards of Children's Hospital of Philadelphia and the University of Pennsylvania (IRB # 10-007569). Informed consent from the patient was obtained in accordance with the Declaration of Helsinki. The diagnosis of AA was determined by standard criteria[12,13]. Peripheral blood mononuclear cells from a de-identified healthy donor were purchased from the University of Pennsylvania Human Immunology Core Facility.

Flow Cytometry

Mononuclear cells from peripheral blood (PBMCs) were isolated by density gradient separation using Ficoll-Paque (Millipore-Sigma, St. Louis, MO) according to the manufacturer's protocol. Surface staining was carried out in FACS buffer with the following monoclonal antibodies: CD3 (UCHT1) (Biolegend, Dedham, MA), CD4 (OKT4) (Biolegend, Dedham, MA), CD8 (RPA-T8) (BD Biosciences, San Jose, CA), CD45RA (HI100) (Biolegend, Dedham, MA), CD27 (L128) (BD Biosciences, San Jose, CA), CCR7 (3D12) (BD Biosciences, San Jose, CA), CD95 (DX) Biolegend, Dedham, MA), TCR V beta 19 (older nomenclature TCR V beta 17; E17.5F3.15.13) (Beckman Coulter Life Sciences, Park Ridge, IL), FoxP3 (259D/C7) (BD Biosciences, San Jose, CA), and CD127 (A7R34) (Biolegend, Dedham, MA). Flow cytometry was performed on a BD LSR II instrument and data were analyzed using FlowJo10.2 software (BD Biosciences, Franklin Lakes, NJ).

Chimerism Analysis

The chimerism analysis for this patient was performed in the CLIA-approved clinical laboratory at the Hospital of the University of Pennsylvania in the course of this patient's routine clinical care. CD3+ subset enrichment was achieved by positive selection from whole blood using immunomagnetic separation (STEMCELL Technologies, Cambridge MA). As part of the laboratory operations, the clinical laboratory performs quality control for purity analysis with each lot of immunomagnetic separation reagent and every 6 months. The quality control for the lot of immune separation reagent used in this patient's analysis showed successful enrichment to 99.45% in the CD3+ fractionated subset. Chimerism analysis was performed on DNA extracted from the fractionated and unfractionated post-transplant peripheral blood samples and evaluated by polymerase chain reaction (PCR) amplification of the informative tandem repeat loci using fluorescently-labeled primers (HS16 PowerPlex or CS7, Promega). PCR products were electrophoresed using a Life Technologies 3500xL Genetic Analyzer, followed by chimerism analysis with ChimerMarker software (SoftGenetics). The alleles at the informative loci were evaluated for the presence of donor and recipient alleles, with the relative peak areas used to quantify the percent contribution of donor and recipient components.

Bulk TCR Vβ Gene Rearrangement Analysis

Cells were sorted directly into lysis buffer and DNA was extracted using the Gentra Puregene cell kit following the manufacturer's directions (Qiagen, Valencia, CA, Cat. No. 158388). TCR rearrangements were amplified from 100ng of gDNA with two replicates per sample using a cocktail of 23 Vβ families from framework region 2 (FR2) forward primers, and 13 Jβ region reverse primers, modified for NGS from the BIOMED2 primer series[1] (primer sequences are provided in Supplemental Table S3). The PCR was performed with two mixes, both of which used the same 23 Vβ forward primers, but different Jβ mixes. The Vβ and Jβ primers mixes were used at 0.6 µM in a reaction volume of 25 µL using a Multiplex PCR kit (Qiagen, Valencia, CA, Cat. No. 158388). Amplification conditions for the PCR were: 95^{o}C for 10 minutes, cycling

at 95°C 45s, annealing at (57°C for Jβ mix 1, 61°C for Jβ mix 2) for 90s, extension at 72°C for 90s for 35 cycles, and a final step at 72°C for 10 minutes. Amplicons were purified using the Agencourt AMPure XP beads system (Beckman Coulter, Inc., Indianapolis, IN) in a 1:1 ratio of beads to sample and eluted in 40 µL of TE (0.1mM EDTA) buffer. Second-round PCRs to generate the sequencing libraries were carried out using 4 µL of the first-round PCR product and 2.5 µL each of NexteraXT Index Primers S5XX and N7XX, using the Qiagen Multiplex PCR kit in a reaction volume of 25 µL. Amplification conditions were 95°C for 10 minutes, followed by cycling at 95°C 30s, 60°C 30s, extension at 72°C 45s for 8 cycles, and a final step at 72°C for 10 minutes. To confirm the adequacy of amplification, aliquots of both the 1st and the 2nd round PCR products were run on agarose gels. Library quality was evaluated using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) and quantified by Qubit Fluorometric Quantitation (Thermo Fisher Scientific, Grand Island, NY) using the dsDNA HS (high sensitivity) assay kit (Cat. No. Q32851). Libraries were loaded onto an Illumina MiSeq in the Human Immunology Core Facility at the University of Pennsylvania using an Illumina MiSeq Reagent Kit v3, 600 cycles, Illumina Inc., San Diego, Cat. No. MS-102-3003. Raw sequences were quality filtered as previously described[2] and clone assemblies were processed with MiXCR[3] (v.2.1.10) and VDJtools[4] (v1.2.1).

Single Cell V(D)J Immune Profiling

10X Genomics Chromium Single Cell GEM and T cell V(D)J library preparation were carried out in accordance with the manufacturer's protocol (10X Genomics, Pleasanton, CA), followed by next-generation sequencing on MiSeq (Illumina, San Diego, CA) at a depth of 30 million reads at the Children's Hospital of Philadelphia Center of Applied Genomics. The sequencing data were analyzed using the Loupe V(D)J Browser (10X Genomics).

Identifying TCR Antigen Specificity Groups (GLIPH Analysis)

To evaluate for antigen convergence between our patient's expanded T cell clones, paired TCR α and β sequences from our patient were analyzed for antigen convergence using the "Grouping Lymphocyte Interactions by Paratope Hotspots" (GLIPH) algorithm. GLIPH uses multiple metrics of convergence including conserved motifs, CDR3 length, gene usage, and global similarity of complementarity-determining region 3 (CDR3) sequences to cluster TCR sequences into groups with a high probability of sharing antigen specificity[5]. The 78 paired TCR α and β CDR3 sequences identified by 10X Genomics single-cell V(D)J immune profiling were analyzed using GLIPH 1.0 with default parameters. Twelve of the 78 paired TCR α and β sequences mapped to one of the 5 GLIPH convergence groups (Supplemental Dataset S1). The two expanded clones--Clone #1 (TRA: CAASVTGDGNKLVF, (TRAV29DV5-J47) and TRB: CASSIGGIGELFF (TCRVB19-D2-J2-2)) and Clone #2 (CAGSGGLIDSYGNKLVF(TRAV35-J47) and CASSIGGIAGAYF (TRB19-D2-J2-5))—clustered together into a single convergence group with a GLIPH CRG score of $p=2.2*10^{-5}$, indicating a high probability of antigen convergence.

Detailed Case Description

A 31-year-old Chinese male was diagnosed with severe AA, after presenting with fatigue and bruising. His peripheral blood count revealed severe pancytopenia, with a white blood cell count of 1.5 $*10^3$ cells/μL, a neutrophil count of $0.57*10^3$ cells/μL, a hemoglobin of 3.7 g/dL with an absolute reticulocyte count of $9*10^3$ cells/μL, and a platelet count of $4*10^3$ cells/μL. Bone marrow biopsy showed a markedly hypocellular aplastic marrow with ~5% cellularity consisting predominantly of scattered plasma cells and small lymphocytes. Median lymphocyte telomere lengths measured by fluorescent in situ hybridization with flow cytometry (Flow FISH) were normal. Chromosome breakage studies were within normal limits. Metaphase cytogenetics and targeted next-generation sequencing (NGS) of hematologic malignancies-associated genes revealed no abnormalities. Two months after initial presentation, the patient underwent

treatment with fludarabine 30 mg/m^2 intravenously (IV) daily for 4 days, cyclophosphamide 300 mg/m^2 IV daily for 4 days, and rabbit anti-thymocyte globulin (Thymoglobulin, Sanofi Genzyme, USA) 3.75 mg/kg IV for 4 days, followed by an infusion of bone marrow from his HLA-identical, ABO-matched sister for an administered cell dose of $2.1*10^8$ total nucleated cells ($1.1*10^6$ CD34+ cells) per kilogram. Donor and recipient were both CMV IgG positive. A short course of post-transplant methotrexate and ongoing immunosuppression with tacrolimus were used as graft versus host disease (GVHD) prophylaxis. Neutrophil engraftment of $>1*10^3$ cells/μL and platelet engraftment of $>50*10^3$ cells/μL occurred on days +26 and +33, respectively. The patient was discharged on day +28. Day +34 chimerism analysis revealed a total 46% peripheral blood donor DNA, with 0% CD3+ donor chimerism. By day +100, the patient had nearly normal blood counts with a total white blood count of $4.0*10^3$ cells/μL consisting of 73% neutrophils, 21% lymphocytes, 5.0% monocytes, 1% eosinophils, 0% basophils, hemoglobin of 11.8 g/dL, and platelets of $123*10^3$ cells/μL. A bone marrow biopsy showed a variably cellular (5-30%) marrow with trilineage hematopoiesis, with a total bone marrow donor chimerism of 93%. The cytogenetic analysis confirmed normal female donor's cells in all twenty metaphases analyzed. The only early post-transplant complication was a drug-associated, Coombs-negative, non-microangiopathic hemolytic anemia to tacrolimus noted on day +130, which resolved after switching GVHD prophylaxis to cyclosporine, targeting a trough of 200-300 μg/L.

By day +200, the patient achieved nearly normal blood counts, with a white cell count of $3.8*10^3$ cells/μL, hemoglobin of 11.8 g/dL with 64% neutrophils, an absolute reticulocyte count of $71*10^3$ cells/μL and a platelet count of $217 *10^3$ cells/μL. Peripheral blood chimerism was 82% donor in total cells, with >99% chimerism in CD33/CD66b+ cells; CD3+ donor cell fraction was persistently low at 7%. At this time, his cyclosporine dose was reduced by 15%, and, as per the standard post-transplant vaccination schedule, the patient received pneumococcal conjugate and inactivated influenza vaccines.

Within one week of vaccination, the patient's absolute reticulocyte count dropped to $27*10^9$ cells/L and plummeted into an undetectable range within four weeks of vaccination. There was no evidence of hemolysis, a negative Coombs test, and a normal serum lactate dehydrogenase. In agreement with a presumptive diagnosis of recurrent marrow failure/graft failure, an erythropoietin level was elevated at 750 mIU/mL. Other cell lines showed new moderate thrombocytopenia, with a platelet nadir of $81*10^9$ cells within 10 weeks of vaccination. Apart from anemia-associated fatigue, the patient was asymptomatic. There was no evidence of GVHD. Infectious and nutritional studies were unrevealing, including negative polymerase chain reaction (PCR) testing for Parvovirus B19, Cytomegalovirus, Ebstein-Barr Virus, and Hepatitis B. Bone marrow biopsy showed a hypocellular marrow with a reduction in erythroid elements without dysplasia, normal female cytogenetics and persistently poor donor T cell chimerism (9% CD3+ donor chimerism) in the face of nearly complete myeloid donor chimerism (>99% CD33/CD66b+ donor chimerism). Notably, the patient also exhibited a progressive expansion of CD3+ lymphocytes with CD8+ cell predominance (CD4/CD8 ratio of 0.2). Clinical T cell receptor (TCR)-γ rearrangement testing using PCR amplification of the consensus TCR-γ V and J regions revealed 184 and 195 bp peaks, with the same size of TCR-γ clonal rearrangement as was detected pre-transplant. Evaluation for markers of immune escape revealed a new minute glycosylphosphatidylinositol (GPI)-negative granulocyte population of 0.03%. A presumed diagnosis of immune-mediated graft dysfunction was made. The patient was treated by increasing the dose of cyclosporine, leading to a gradual resolution of reticulocytopenia and stabilization of platelet count at ~$100*10^9$ cells/L three months following vaccination. Given persistent poor donor T cell chimerism and inability to taper immunosuppression due to recurrent graft dysfunction upon cyclosporine taper, the patient was eventually treated with a donor lymphocyte infusion (DLI) of $1x10^7$ mononuclear cells per kilogram, which resulted in gradual improvement to nearly complete CD3+ donor chimerism, with no evidence of GVHD.

Supplemental Tables

Supplemental Table S1: Laboratory Values

Variable	Reference Range, Adults	At vaccination	3 weeks post-vaccination	6 weeks post-vaccination	8 weeks post-vaccination
Hemoglobin (g/dL)	13.5-17.5	11.8	11.8	9.1	9.3
Hematocrit (%)	40-52	34	33	25	26
Absolute Reticulocytes (THO/uL)	22.0-116.0	71	1.0*	1.0	2.0
Reticulocytes (%)		2.4	<0.1*	<0.1	0.1
Mean Corpuscular Volume (fL)	80-100	110	108	104	96
White Blood Count (THO/uL)	4.0-11.0	3.8	3.8	3.8	3.7
Differential count (%)					
Neutrophils		64.1	58.8	61	59.3
Lymphocytes		28.3	32.5	32.4	34.2
Monocytes		7.3	8.6	6.4	6.3
Eosinophils		0.1	0	0	0
Basophils		0.2	0.1	0.2	0.2
Platelet count (THO/uL)	150-400	217	187	102	84
Sodium (mmol/L)	136-144	138	139	140	140
Potassium (mmol/L)	3.6-5.1	4.5	4.1	4.1	5.1
Chloride (mmol/L)	101-111	105	105	106	105
Carbon dioxide (mmol/L)	22-32	27	25	25	29
Creatinine (mg/dL)	0.64-1.27	1.26	1.19	1.15	1.40
Glucose (mg/dL)	70-99	136	109	137	119
Calcium (mg/dL)	8.9-10.3	9.8	10	9.7	9.7
Protein Total (g/dL)	6.1-7.9	8	8.1	7.7	8.0
Albumin Level (g/dL)	3.5-5.1	4.8	4.9	4.5	4.6
Alanine aminotransferase (U/L)	17-63	50	41	80	56
Aspartate aminotransferase (U/L)	15-41	35	30	51	41
Alkaline phosphatase (U/L)	38-126	102	98	101	121
Lactate dehydrogenase (U/L)	98-192	182	179	203	
Vitamin B12 Level (pg/mL)	232-1245	481			

Thyroid Stimulating Hormone (uIU/mL)	0.27-4.20	2.83			
Thyroxine (ug/dL)	4.60-12.00	6.08			
CD3 Lymphocytes (%)	62-84	64	66		
CD3 Absolute Count (cells/uL)	900-3245	658	794		
CD4 Lymphocytes (%)	32-56	12	12		
CD4 Absolute Count (cells/uL)	560-1840	121	141		
CD8 Lymphocytes (%)	15-40	51	52		
CD8 Absolute Count (cells/uL)	260-1230	524	634		
CD4/CD8 Ratio	0.9-3.4	0.2	0.2		
Immunoglobulin A	50-500		126		
Immunoglobulin G	650-2000		1618		
Immunoglobulin M	40-270		67		
Cyclosporine Trough Level	101-198*	116	103	198	320
Chimerism					
CD3+ % Donor		7	8	8	
Unfractionated % Donor		82	76	79	
Cytomegalovirus (dsDNA)		Not detected	Not detected	Not detected	Not detected
Epstein-Barr Virus (dsDNA)				Not detected	
PCR Parvovirus B19				Not detected	
HLA Type	HLA-A 11:01; 24:02; HLA-B 40:01; 48:01; HLA-C 03:04; 01:02; HLA-DR 08:03, 09:01				

* Cyclosporine trough level ranged from 101-198 throughout 4-week post-vaccination treatment period and then was increased due to presumed graft failure

Supplemental Table S2: Dominant paired TCRαβ clones identified through single-cell sequencing

Paired Ranking	Barcode Frequency	Proportion	TRA CDR3	TRB CDR3
	144	0.1195		CASSIGGIGELFF
	91	0.0755		CASSIGGIAGAYF
1	83	0.0689	CAASVTGDGNKLVF	CASSIGGIGELFF
	54	0.0448		CASSLERGGKSYEQFF
2	48	0.0398	CVVNPGSTLGRLYF	CASSLGLAGAYAYEQYF
	33	0.0274	CAGSGGLIDSYGNKLVF	
	30	0.0249		CASSLGLAGAYAYEQYF
	28	0.0232		CASSLGGPSGNTIYF
3	25	0.0207	CAVFFSGGYNKLIF	CASSLERGGKSYEQFF
	20	0.0166		CASSMGYNEQFF
	20	0.0166		CASSLGTGRDNEQFF
4	15	0.0124	CVVNRGSTLGRLYF	CASSWTSGSSYEQYF
5	15	0.0124	CAENWETDKLIF	CASSLGTGRDNEQFF
6	15	0.0124	CAGSGGLIDSYGNKLVF	CASSIGGIAGAYF
	14	0.0116		CASSPPLAGGPGTVETQYF
7	13	0.0108	CAASYGNQFYF	CASSPPLAGGPGTVETQYF
8	10	0.0083	CAVNRGNNDMRF	CARTQENTGELFF
9	10	0.0083	CAVNDVNTDKLIF	CASSYGTGNSYEQYF
10	9	0.0075	CALSQGGKLIF	CASSLGTGRDNEQFF
	9	0.0075		CASSYGTGNSYEQYF

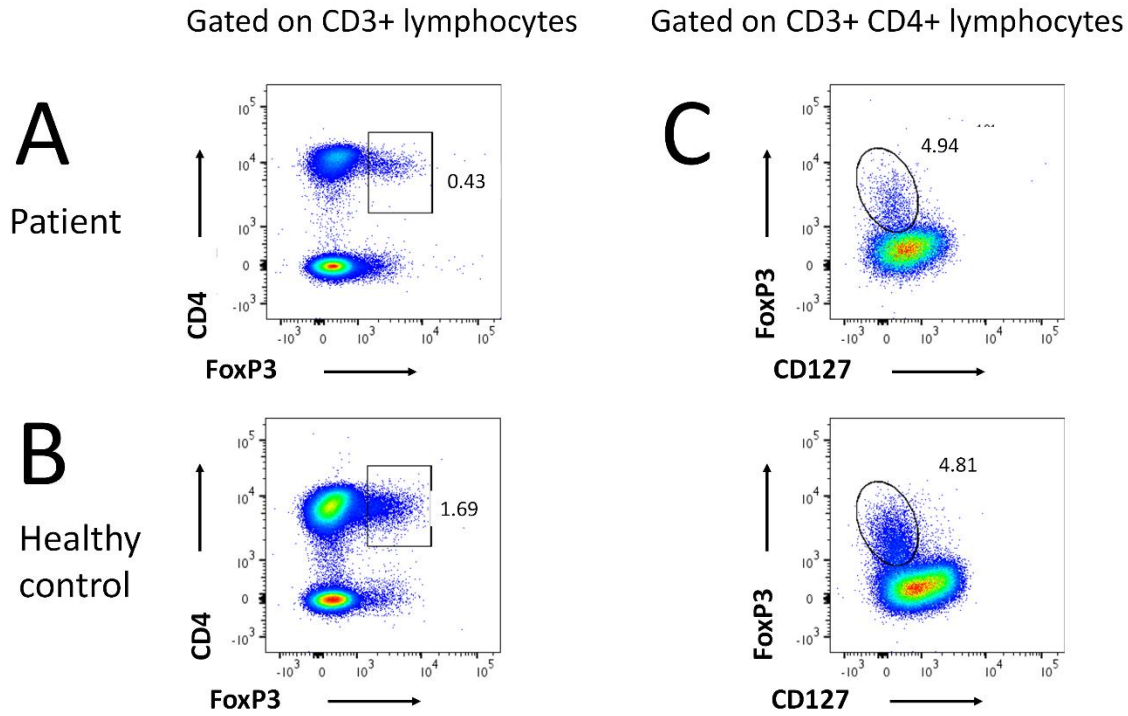
A clone containing TCRα/β from #1 Clone Clone containing TCRα/β from #2 Clone

Top clones identified through single-cell sequencing (10X genomics) as ranked by barcode copy number and proportion of total sequenced cells. Clones containing TCRβ from previously identified expanded clones using bulk TCR sequencing are highlighted in blue (Clone #1) and green (Clone #2). The Clone #1 (TRA: CAASVTGDGNKLVF; TRB: CASSIGGIGELFF) and the Clone #2 (TRA: CAGSGGLIDSYGNKLVF; TRB: CASSIGGIAGAYF) have multiple similarities between CDR3 motifs, length, gene usage and global similarity and cluster into the same antigen convergence group. These clones also share similarity with several known influenza-reactive T cell clones from VDJdb[6] (see text, Supplemental Dataset S1, Supplemental Fig S2).

Supplemental Table S3. PCR Primers. Shown are the nucleotide sequences of the primers used for PCR amplifications of TCR Vβ gene rearrangements (see Supplementary Methods for PCR details).

Oligo Name	Nucleotide Sequence
Vβ mix	
NexteraR2-Hu-biomed2Vβ2	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACTATGTTTTGGTATCGTCA-3'
NexteraR2-Hu-biomed2Vβ4	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACGATGTTCTGGTACCGTCAGCA-3'
NexteraR2-Hu-biomed2Vβ5/1	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGTGTGTCCTGGTACCAACAG-3'
NexteraR2-Hu-biomed2Vβ6a/11	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACCCTTTATTGGTACCGACA-3'
NexteraR2-Hu-biomed2Vβ6b/25	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCCCTTTTTTGGTACCAACAG-3'
NexteraR2-Hu-biomed2Vβ6c	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACCCTTTATTGGTATCAACAG-3'
NexteraR2-Hu-biomed2Vβ7	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGCTATGTATTGGTACAAGCA-3'
NexteraR2-Hu-biomed2Vβ8a	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCCGTTTTCTGGTACAGACAGAC-3'
NexteraR2-Hu-biomed2Vβ9	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGCTATGTATTGGTATAAACAG-3'
NexteraR2-Hu-biomed2Vβ10	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTATGTTTACTGGTATCGTAAGAAGC-3'
NexteraR2-Hu-biomed2Vβ11	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAAATGTACTGGTATCAACAA-3'
NexteraR2-Hu-biomed2Vβ12a/3/13a/15	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATACATGTACTGGTATCGACAAGAC-3'
NexteraR2-Hu-biomed2Vβ13b	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCCATGTACTGGTATAGACAAG-3'
NexteraR2-Hu-biomed2Vβ13c/12b/14	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTATATGTCCTGGTATCGACAAGA-3'
NexteraR2-Hu-biomed2Vβ16	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAACCTTTATTGGTATCGACGTGT-3'
NexteraR2-Hu-biomed2Vβ17	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCCATGTACTGGTACCGACA-3'
NexteraR2-Hu-biomed2Vβ18	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCATGTTTACTGGTATCGGCAG-3'
NexteraR2-Hu-biomed2Vβ19	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTATGTTTATTGGTATCAACAGAATCA-3'
NexteraR2-Hu-biomed2Vβ20	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAACCTATACTGGTACCGACA-3'
NexteraR2-Hu-biomed2Vβ21	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACCCTTTACTGGTACCGGCAG-3'
NexteraR2-Hu-biomed2Vβ22	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATACTTCTATTGGTACAGACAAATCT-3'
NexteraR2-Hu-biomed2Vβ23/8b	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACGGTCTACTGGTACCAGCA-3'
NexteraR2-Hu-biomed2Vβ24	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGTCATGTACTGGTACCAGCA-3'
Jβ mix 1	
NexteraR1-Hu-biomed2Jβ1.1	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTTACCTACAACTGTGAATCTGGTG-3'
NexteraR1-Hu-biomed2Jβ1.2	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCTTACCTACAACGGTTAACCTGGTC-3'
NexteraR1-Hu-biomed2Jβ1.3	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAAACTTACCTACAACAGTGAGCCAACTT-3'
NexteraR1-Hu-biomed2Jβ1.4	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCGACATACCCAAGACAGAGAGCTGGGTTC-3'
NexteraR1-Hu-biomed2Jβ1.5	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGANCTTACCTAGGATGGAGAGTCGAGTC-3'
NexteraR1-Hu-biomed2Jβ1.6	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCATACCTGTCACAGTGAGCCTG-3'
NexteraR1-Hu-biomed2Jβ2.2	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNGACTTACCCAGTACGGTCAGCCT-3'
NexteraR1-Hu-biomed2Jβ2.6	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTCGCCCAGCACGGTCAGCCT-3'
NexteraR1-Hu-biomed2Jβ2.7	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAAAACTTACCTGTAACCGTGAGCCTG-3'
Jβ mix 2	
NexteraR1-Hu-biomed2Jβ2.1	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAACCTTCTTACCTAGCACGGTGA-3'
NexteraR1-Hu-biomed2Jβ2.3	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCCCGCTTACCGAGCACTGTCA-3'
NexteraR1-Hu-biomed2Jβ2.4	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCAGCTTACCCAGCACTGAGA-3'
NexteraR1-Hu-biomed2Jβ2.5	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNCGCGCACACCGAGCAC-3'

Supplemental Figures



Supplemental Figure S1: Decreased frequency of T regulatory cells in the patient.

(A) Immunophenotyping analysis of the patient's peripheral blood lymphocytes on day +354 post-transplant revealed low frequency of Tregs (CD4+ FoxP3+) as a percentage of CD3+ lymphocytes (0.45%). (B) A representative analysis of Tregs (CD4+ FoxP3+) in a healthy control (1.69% of CD3+ lymphocytes); published frequencies of Tregs in healthy controls range from 1-3% of total lymphocytes[7]. (C) Similar percentage of FoxP3+ CD127low Tregs as a fraction of CD4+ T cells in the patient and the control.

A

Influenza-reactive TRA CDR3 cluster	CAMSPMEYGNKLVF
	CAVSPMEYGNKLVF
	CAVSSMEYGNKLVF
	CAVRFMEYGNKLVF
	CAVSFMEYGNKLVF
Clone 1 TRA CDR3	CAASVTGDGNKLVF

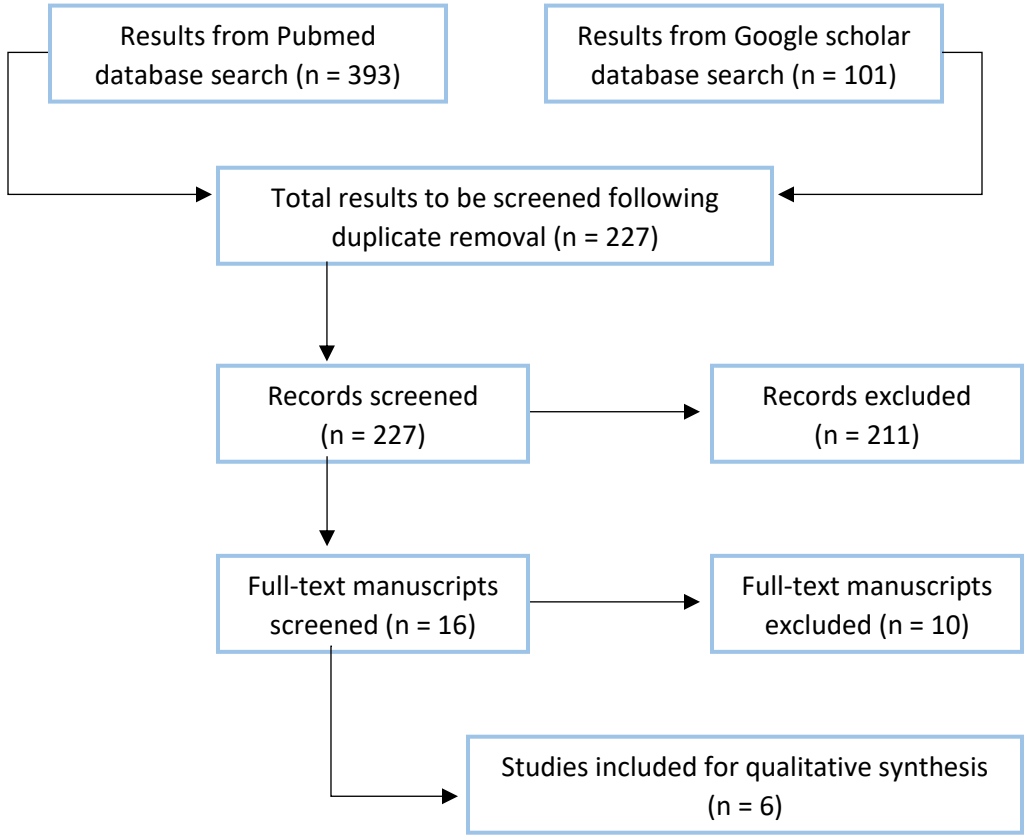
B

Influenza-reactive TRB CDR3 cluster	CASSLVETGELFF
	CASSLVGTGELFF
	CASSLVGVGEQYF
	CASSPTGVGEQFF
	CASSPVGTGELFF
	CASSPVGVGELFF
	CASSPVGVGEQFF
	CASSPVGVGEQYF
Clone 1 TRB CDR3	CASSIGGIGELFF

Supplemental Figure S2: TCR CDR3 Motif Similarity of the Patient's Expanded T Cell Clone (Clone #1 TRA: CAASVTGDGNKLVF; TRB: CASSIGGIGELFF) with Influenza-reactive CDR3 Sequences from VDJdb.

(A) A comparison of the patient's TRA CDR3 and the VDJdb[6] motif database identified a cluster of 5 closely related Influenza A-specific human TRA sequences. (B) A comparison of the patient's TRB CDR3 and the VDJdb motif database identified a cluster of 8 closely related Influenza A-specific human TRB sequences. In A and B, the respective sequence logo plots for each cluster show the CDR3 cluster consensus, with the red boxes highlighting the residues matching the patient's CDR3 sequence. Influenza-specific CDR3 sequences comprising each cluster are listed in the tables below the logo plots, and amino acids matching the patient's CDR3 sequences are indicated by the red boxes surrounding the matching amino acids.

Supplemental Figure S3: Systematic Review of Aplastic Anemia cases temporally related to vaccinations



Supplemental References

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