Supplemental Information:

Hydroa vacciniforme-like lymphoproliferative disorder: an EBV disease with a low risk of systemic illness in Caucasians

Methods

EBV antibodies

Antibodies to EBV EBNA-1, BHFR1, BMRF1, BMLF1, BLRF2, BFRF3, gp350, and gH/gL were measured using a luciferase immunoprecipitation system (LIPS) assay (Cohen et al. 2011). Serum cytokines (IL-6, IL-8, IL-10, IL-18, IFN-α2, IFN-γ, IP-10, MIG, MCP-1, MCP3, MDC, TNF-α, MIP-1α, MIP-1β, I-TAC) were measured (Cohen et al. 2011); IL-18 was measured by ELISA (R&D). Only p values $\leq .01$ were considered significant.

Pathology and Sequencing

All cases of HVLPD from the NIH Clinical Center were reviewed in the Hematopathology Section of the National Cancer Institute at NIH. EBV RNA was detected in the skin using an EBV encoded RNA (EBER1) probe by in situ hybridization (Quintanilla-Martinez et al. 2000). Clonality of T cell receptor gamma chain genes was performed by PCR (Quintanilla-Martinez et al. 2000).

All patients had targeted sequencing for genes associated with immune deficiencies and all but one had whole exome sequencing. Next-generation (Agilent HaloPlex Target Enrichment and Life Technologies Ion Proton) genomic DNA sequencing of genes associated with immunodeficiencies or DNA repair were performed using peripheral blood mononuclear cell (PBMC) DNA from the patients (Stoddard et al. 2014). Whole exome sequencing was performed for all patients except patient 15 and both parents of patients 1-6, 9, 10, and 14 also had whole

exome sequencing patients 1 and 2 using one of two targeted capture platforms, Roche NimbleGen SeqCap EZ Exome Library v2.0 or Illumina TruSeq 1.0, followed by sequencing on the Illumina HiSeq 2000/2500.

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Lymphocyte subsets

PBMCs were stained with antibodies to CD3, CD4, CD8, CD20, CD27, CD56, CD57, CD45RA, CD62L, CD127, Ki67, PD1, and CCR7. Double negative T cells ($CD3^{+}$, $CD4^{-}$, $CD8^{+}$), naïve CD4 or CD8 T cells ($CD62L^{+}$, $CD45RA^{+}$), central memory CD4 or CD8 cells ($CD62L^{+}$, $CD45RA^{-}$), effector memory CD4 or CD8 cells ($CD62L^{-}$, $CD45RA^{-}$), and terminally differentiated effector memory CD4 and CD8 cells ($CD62L^{-}$, $CD45RA^{+}$) were quantified.

Lymphocyte surface antigens and tetramer staining

Cryopreserved PBMCs from HLA A02 patients were thawed and cultured in RPMI-c media with 10% fetal bovine sera (FBS) for 1 hour before counting. 2-5 million cells were stained with tetramers for 30 minutes at 37°C in RPMI-c+ with 5% FBS, washed in PBS, centrifuged and stained with live/dead near IR stain (BD Biosciences) for 10 minutes at room temperature. Cells were washed in PBS, spun down and stained with antibodies to lymphocyte surface antigens for 20 minutes at room temperature. Cells were washed in PBS, centrifuged, and fixed in 0.5% paraformaldehyde and analyzed on a BD Symphony flow cytometer the same day. Tetramers used for staining were EBV LMP2 (FLYALALLL-APC), BRLF1 (TVLDHLIVV-PE), and influenza M1 (GILGFVFTL-BV421) prepared by the NIH tetramer facility. Antibodies to the following lymphocyte surface antigens were used: CD3-BV650 (BioLegend), CD4-PerCP-eFluor710 (eBioscience), CD8-BV785 (BioLegend), CD27-BV510 (BioLegend), CD57-FITC

(BioLegend), CD127- APC-R700 (HIL-7R-M21, BD Biosciences), CCR7-PE /Dazzle 594 (BioLegend), and PD1-BV605 (BioLegend).

RNA-seq analysis

RNA was isolated from two 4 mm biopsies on the same day from patient 1: an HVLPD vesicular skin lesion from a sun-exposed site showing infiltration of T cells, EBV RNA, and EBV LMP, and from unaffected axillary skin. RNA from approximately 10 mg of tissue was processed with an OMNI disruptor (240 sec for HVLPD skin lesion on forearm; 180 sec for axillary skin control) followed by cell lysis with TRIZOL and RNeasy Fibrous Tissue Kit (Qiagen) to yield 6.2 ug (HVLPD skin lesion) and 1.3 ug (axillary skin control) total RNA. RNA integrity measured using a Bioanalyzer with RIN values of 6.8 (HVLPD skin lesion) and 5.0 (axillary skin control).

RNA-seq libraries were prepared from 500ng of total RNA using Illumina TrueSeq Stranded mRNA for NeoPrep and sequenced on Illumina NextSeq500 paired-end sequencing with read length 75, and depth of 45 million read pairs per sample. Adapter and quality-based trimming, read alignment to Hg38 (Human) and strand-specific mapping to genes was computed using CLC-bio Genomics Workbench software version 10.0.1.

Supplemental References

Cohen JI, Jaffe ES, Dale JK, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. Blood. 2011;117:5835-49.

Quintanilla-Martinez L, Kumar S, Fend F, Reyes E, Teruya-Feldstein J, Kingma DW, Sorbara L, Raffeld M, Straus SE, Jaffe ES. Fulminant EBV(+) T-cell lymphoproliferative disorder

following acute/chronic EBV infection: a distinct clinicopathologic syndrome. Blood. 2000; 15;96:443-51.

Stoddard JL, Niemela JE, Fleisher TA, Rosenzweig SD. Targeted NGS: A Cost-Effective Approach to Molecular Diagnosis of PIDs. Front Immunol. 2014;3;5:531.

Table S1. T cell subsets in HVLPD patients

Patient	Double negative T cells	Naïve CD4	Central memory CD4	Effector memory CD4	Terminally differentiated effector memory CD4	Naïve CD8	Central memory CD8	Effector memory CD8	Terminally differentiated effector memory CD8
1	116	650	251	**40**	2	380	**20**	**15**	27
2	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	<u>291</u>	346	**146**	55	<u>45</u>	282	43	71	57
4	112	**66**	256	109	2	76	48	56	31
5	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	<u>311</u>	255	180	**28**	3	120	56	**20**	**8**
7	151	490	**136**	**36**	2	318	33	36	33
8	133	311	272	88	6	147	58	55	26
9	100	564	318	114	<u>54</u>	251	50	46	50
10	<u>432</u>	513	264	86	0	325	45	34	27
11	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	74	1021	448	63	0	239	70	123	70
13	**8**	**23**	**70**	**29**	0	**74**	<u>1767</u>	62	14
14	**45**	**16**	**146**	**41**	0	**53**	<u>921</u>	99	47
15	<u>1264</u>	254	251	91	0	158	174	75	**8**
16	<u>1678</u>	191	222	**31**	0	290	<u>447</u>	130	**27**

Normal values: double negative T cells 18-185, naïve CD4 ($CD62L^+$,$CD45RA^+$) 102-1041, central memory CD4 ($CD62L^+$,CD45RA-) 162-614, effector memory CD4 ($CD62L^-$, $CD45RA^-$) 42-225, terminally differentiated effector memory CD4 ($CD62L^-$, $CD45RA^+$) 0-29, naïve CD8 ($CD62L^+$, $CD45RA^+$) 85-568, central memory CD8 ($CD62L^+$, $CD45RA^-$) 25-180, effector memory CD8 ($CD62L^-$, $CD45RA^-$) 24-175, terminally differentiated effector memory CD8 ($CD62L^-$, $CD45RA^+$) 11-172. Bold=below limit of normal, Underline=above upper limit of normal, ND=not done.