BACKGROUND. Herpes simplex virus lymphadenitis (HSVL) is an unusual presentation of HSV reactivation in chronic lymphocytic leukemia (CLL) patients characterized by systemic symptoms and no herpetic lesions. The immune responses during HSVL have not been studied.

METHODS. Peripheral blood and lymph node samples of a patient with HSVL were obtained. HSV-2 viral load, antibody levels, B and T cell responses, cytokine levels, and tumor burden were measured.

RESULTS. This patient showed HSV-2 viremia for at least 6 weeks. During this period, she had a robust HSV-specific antibody response with neutralizing and antibody-dependent cellular phagocytosis activity. Activated (HLA-DR+, CD38+) CD4+ and CD8+ T cells increased 18-fold and HSV-specific CD8+ T cells were detected in the blood at higher numbers. HSV-specific B and T cell responses in the lymph node were also detected. Markedly elevated levels of pro-inflammatory cytokines in the blood were also observed. Surprisingly, a sustained decrease in CLL tumor burden without CLL-directed therapy was observed with this and also a prior episode of HSVL.

CONCLUSION. HSVL should be considered as part of the differential diagnosis in CLL patients who present with signs and symptoms of aggressive lymphoma transformation. An […]
Herpes Simplex Virus Lymphadenitis is Associated with Tumor Reduction in a Chronic Lymphocytic Leukemia Patient

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AC, AMS, AW, DLJ, MC, MLH, HX, KRJ, PV, JLK, and LC have declared that no conflict of interest exists.

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RA receives research funding from Merck and holds patents on PD-1-directed immunotherapy. He is also listed as a coinventor on an Emory University-held patent for SARS-CoV-2 serology assays.
Abstract:

Background: Herpes simplex virus lymphadenitis (HSVL) is an unusual presentation of HSV reactivation in chronic lymphocytic leukemia (CLL) patients characterized by systemic symptoms and no herpetic lesions. The immune responses during HSVL have not been studied.

Methods: Peripheral blood and lymph node samples of a patient with HSVL were obtained. HSV-2 viral load, antibody levels, B and T cell responses, cytokine levels, and tumor burden were measured.

Results: This patient showed HSV-2 viremia for at least 6 weeks. During this period, she had a robust HSV-specific antibody response with neutralizing and antibody-dependent cellular phagocytosis activity. Activated (HLA-DR+, CD38+) CD4+ and CD8+ T cells increased 18-fold and HSV-specific CD8+ T cells were detected in the blood at higher numbers. HSV-specific B and T cell responses in the lymph node were also detected. Markedly elevated levels of pro-inflammatory cytokines in the blood were also observed. Surprisingly, a sustained decrease in CLL tumor burden without CLL-directed therapy was observed with this and also a prior episode of HSVL.

Conclusion: HSVL should be considered as part of the differential diagnosis in CLL patients who present with signs and symptoms of aggressive lymphoma transformation. An interesting finding was the sustained tumor control after 2 episodes of HSVL in this patient. This tumor burden reduction may be due to the HSV-specific response serving as an adjuvant for activating tumor-specific or bystander T cells. Studies in additional CLL patients are needed to confirm and extend these findings.

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Introduction

Herpes simplex virus 2 (HSV-2) is a sexually transmitted infection that establishes a chronic latent infection in the associated dorsal root ganglion, with episodes of reactivation and viral shedding that are asymptomatic or result in typical, localized herpetic lesions(1, 2). However, in patients with chronic lymphocytic leukemia (CLL), HSV reactivation may not lead to the development of herpetic lesions but instead can result in HSV lymphadenitis (HSVL). Patients with HSVL usually present with fevers, night sweats, and worsening lymphadenopathy(3). Because of these signs and symptoms, and the absent herpetic lesions, HSVL is often mistaken as aggressive lymphoma transformation. The pathophysiology of this unusual and challenging diagnosis is unknown but is not associated with CLL subtype or therapy(4). It is hypothesized that immunological defects caused by CLL contributes to this manifestation, but the immune responses in HSVL have not been examined. Here we present a detailed analysis of the HSV-specific adaptive immune responses in a CLL patient with recurrent HSVL, report the concomitant presence of viremia by a typical HSV-2 strain, and describe a sustained decrease in tumor burden after each occurrence.

Patient case

A 79-year-old woman with treatment-naïve CLL (del13q and trisomy 12) presented approximately 7 years after diagnosis with a 5-day history of fatigue, malaise, and tender, rapidly enlarging left inguinal adenopathy but no mucocutaneous lesions. Laboratory testing revealed new onset anemia and thrombocytopenia but normal lactate dehydrogenase (LDH). Bone marrow biopsy showed involvement by CLL without evidence of aggressive lymphoma. Fluorodeoxyglucose Positron Emission Tomography-Computer Tomography (FDG-PET/CT) showed retroperitoneal and left iliac adenopathy, and a left groin mass measuring 7.4 x 4.3 cm with a maximum standardized uptake value (SUV) of 14.3 (Figure 1A). Excisional biopsy of the mass 17 days after symptom onset revealed a lymph node (LN) involved by CLL with prominent necrotic areas but no aggressive lymphoma (Figure 1B). Prior reports indicated that HSV reactivation in CLL patients
can in rare instances cause these symptoms (4). Thus, to confirm this diagnosis, we performed
immunohistochemical staining for HSV-1/2 which showed HSV-infected cells (Figure 1C). PCR
of the LN was also positive for HSV-2. The patient was treated with corticosteroids and
valacyclovir followed by acyclovir prophylaxis with complete symptom resolution and no
recurrence for over 3 years.
Results

HSV-2 Viremia and Viral Sequence

The availability of baseline and longitudinal samples collected within 270 days of presentation (Figure S1) allowed us to study the virology and the immunological responses in HSV Lymphadenitis. Plasma real-time quantitative PCR revealed HSV-2 viremia, with detectable viral DNA for at least 6 weeks (Figure 1D). High-resolution sequencing recovered >99% of known protein-coding regions of the HSV-2 genome from the blood at Day +5 and Day +12, which showed no significant differences between samples, displayed high similarities with common U.S. strains, and showed no genotypic evidence of acyclovir resistance (Figure 1E)(5, 6). These findings indicate that reactivation of a conventional HSV-2 strain and not a distinct HSV-2 variant, produced a systemic infection in this patient.

HSV-specific antibody responses in HSV Lymphadenitis

CLL patients are known to have a dysregulated immune system(7) but the immunological factors that allowed for this patient’s clinical presentation were unknown. Thus, we set out to further evaluate the HSV-specific adaptive immune responses in this patient. Flow cytometric analysis (FACS) of peripheral blood mononuclear cells (PBMCs) detected higher numbers of antibody-secreting cells (plasmablasts), defined as CD19+CD3-CD20-CD38hiIgD- lymphocytes, at presentation compared to other timepoints (Figure 2A)(8). Plasmablasts lacked CD5 expression indicating that these were not CLL cells. Assessment of the LN sample obtained at day +17 also showed high numbers of plasmablasts by FACS (Figure 2B, top). Using an ELISPOT assay, we confirmed that plasmablasts in the LN secreted IgG antibodies that recognized HSV-2 (Figure 2B, bottom)(8).

Despite the patient’s persistent hypogammaglobulinemia (Figure 2C), as often seen in CLL, HSV-2-specific IgG titers were detectable at baseline, started to increase by day 12, and peaked after
6 weeks, resulting in a 14-fold increase (p=0.0015, Figure 2D). These antibodies reacted against the 4 major HSV-2 glycoproteins (gB2, gC2, gD2 and gH2/gL2 heterodimer) and remained elevated for at least 270 days (Figure 2E). Increases in HSV-2-specific IgA titers were similar to IgG while IgM titers only increased 1-2 fold (Figure 2E).

To determine the functional capacity of these antibodies, we measured their neutralizing potential and their capacity to elicit antibody-dependent cellular phagocytosis (ADCP). Neutralizing antibody titers increased 13.5-fold by 6 weeks (Figure 2D). ADCP capacity against gD2-covered microspheres also increased, peaking at 90 days (Figure 2F). We also measured IgG subclasses of the HSV-2-specific antibodies and showed a predominant IgG1 response (Figure 2G), consistent with ADCP and neutralizing activity. Significant IgG2 and IgG3 responses were also observed. IgG1 and IgG3 antibodies have potent Fc effector functions and usually increase in response to viral infections(9). Changes in IgA and IgM levels were also detected. Overall, our findings indicate that this CLL patient mounted robust and functional humoral immune responses after HSV reactivation.

*Activation of CD4+ and CD8+ T cells in response to HSV Lymphadenitis*

T cell responses were also characterized given their important role at controlling HSV(10). We observed an 18-fold increase in the frequency of activated CD4+ and CD8+ T cells at presentation compared to baseline (18% and 43.8% of total CD4+ and CD8+ T cells respectively), as defined by co-expression of HLA-DR and CD38 (Figures 3A-B). Activated CD4+ and CD8+ T cells were significantly less numerous in the blood by 6 weeks (Figure 3B). High numbers of activated CD4+ and CD8+ T cells were also observed in the LN (Figure 3C). To further characterize these cells, we analyzed several markers associated with effector T cell differentiation(11). Consistent with an effector phenotype, activated T cells showed high levels of Ki-67 (indicating active proliferation). CD8+ T cells also expressed high levels of perforin, and granzyme B (GzmB), indicating cytotoxic ability (Figure 3D). These cells also showed lower expression of the anti-
apoptotic protein Bcl-2 and lacked CD45RA and CCR7, consistent with an effector CD8+ T cell phenotype.

To further characterize the HSV-2-specific CD8+ T cell response, we generated a major histocompatibility complex I (MHC-I) tetramer using an epitope known to be presented by an MHC-I allele similar to the patient’s allele (Figure 3E)(12). CD8+Tetramer+ cells were found in the blood at all timepoints and expanded 4-fold by day +12 (Figure 3F). CD8+Tetramer+ cells at day +5 also showed an effector phenotype (Figure 3G). CD8+Tetramer+ cells were also detected in the LN (not shown). These data show that this patient had HSV-specific CD8+ T cells at baseline and mounted a robust CD4+ and CD8+ T cell response in the blood and LN upon HSV reactivation.

The levels of pro-inflammatory cytokines were also measured. We observed a 21-fold increase in IFN-γ at presentation compared to baseline (Figure 3H). Levels of IL-18 also increased (Figure 3I). IL-18 is a cytokine that can enhance antigen-independent IFN-γ production by effector and memory CD8+ T cells, improving their activity(13). Plasma levels of other pro-inflammatory cytokines such as IL-6 (Figure 3J), TNF-α, IP-10, IL-27, IL-1RA, IL-22, and MIP-1α (Figure S2) also increased significantly at presentation. Proinflammatory cytokine levels returned to baseline around the time the patient’s symptoms resolved. These results show a profound pro-inflammatory state in this patient during the viremic stage.

Association between HSV lymphadenitis and decreased CLL tumor burden

Interestingly and surprisingly, we observed a significant reduction in circulating CLL cell counts at presentation which remained stable without the use of CLL-directed therapies even after resolution of viremia (Figure 4A). Moreover, further review of the patient’s records showed that she experienced a similar episode 2 years prior that also resulted in a significant and sustained reduction in her white blood cell count (WBC, Figure 4B, Episode #1). This decreased in WBC
was mostly attributed to decreases in lymphocyte counts as her neutrophil counts remained largely stable (Figure 4C). At that time, she presented with similar symptoms including rapidly enlarging right inguinal adenopathy. FDG-PET/CT revealed FDG-avid retroperitoneal, right iliac and inguinal lymphadenopathy, and a 5.1 x 2.1 cm right obturator LN with a SUV of 11.3 (Figure S3A). Excisional biopsy of this LN at that time showed CLL, prominent necrosis and no aggressive lymphoma (Figure S3B) so she received corticosteroids leading to symptom resolution. We retrieved her archived LN and staining showed HSV-1/2 positive cells (Figure S3C), establishing her initial diagnosis of HSVL and documenting subsequent disease recurrence. Our findings therefore show sustained tumor control after each episode of HSVL.
Discussion

This study provides several insights in the clinical presentation and the breadth of the adaptive immune responses in HSVL, with the key finding that each episode of HSVL in this patient was associated with reductions in CLL counts lasting long after resolution of the acute process. Clinically, the finding of viremia in the acute setting is intriguing, since HSV viremia is rare and the patient had no herpetic lesions. The presence of HSV viremia in this patient suggests that HSVL could be a manifestation of a systemic HSV infection. Given the similar clinical presentation between HSVL and aggressive lymphoma transformation, testing for HSV viremia during workup could facilitate its prompt diagnosis and treatment, and help elucidate its true incidence as less than 50 cases have been reported(14). FACS showing prominent T cell activation could also help support the diagnosis of HSVL. Nonetheless, further studies are needed to determine if these tests combined with localized photopenia on FDG-PET/CT(15, 16) in the appropriate clinical context could be diagnostic for HSVL and safely prevent invasive procedures.

The pathophysiology of HSVL remains unclear, but the identification of a conventional HSV-2 strain in this patient suggests that host factors rather than variations in HSV viral sequences are primarily responsible for this unusual clinical presentation of HSV reactivation. A robust humoral and T cell response in the blood and LN was observed in this episode of HSVL. Notably, even though CLL patients are known to have IgG deficiencies(17) and our patient had low total IgG levels throughout the time of analysis, baseline IgG levels against some HSV-2 surface glycoproteins were equivalent to healthy HSV-2-seropositive controls and titers against most of these proteins increased significantly soon after the acute phase. These observations contrast with antibody responses after local reactivation in otherwise healthy individuals, where no measurable changes in circulating antibody titers were detected(18).

The interesting observation that the patient’s circulating tumor burden decreased, establishing a new, lower baseline after each HSVL episode without any CLL-directed therapies suggest that
the immune response triggered by this systemic infection helped control the CLL. Indeed, the phenomenon of tumor control after a severe infection served as early evidence of the immune system’s ability to control cancer(19-21). One potential explanation is that the systemic HSV-2 reactivation experienced by this patient is serving as an “adjuvant” for tumor-specific T cells. Consistent with this, recent studies showed that cell death induced by virus-like-particles can generate potent pro-immunogenic conditions and result in prolonged tumor control in a T cell dependent manner(22). Given that HSV-2 can infect certain B cells in vitro(23), it is conceivable that CLL cell death after infection could also generate such pro-immunogenic conditions, promoting sustained tumor reduction. In addition, several reports have shown that the immune responses triggered by a systemic viral infection can result in the activation of “bystander” CD8+ T cells present in the tumor microenvironment resulting in delayed tumor progression(24-27). Thus, some bystander effect by HSV-2-specific T cells could have also contributed to the decrease in tumor burden. Activation of tumor-specific or bystander CD8+ T cells could be further enhanced by the high levels of cytokines like IL-18, augmenting antigen-independent IFN-γ production(13) and creating a more proinflammatory environment. Aside from its immunostimulatory effects, IFN-γ can also reprogram nurse-like cells to produce a less supportive tumor microenvironment(28). Elevated IL-6 levels, on the other hand, can slow leukemia progression by antagonizing toll-like receptor signaling(29). Other mechanisms including reinvigoration of CLL-specific or other viral-specific T cells by proinflammatory cytokines, or that a common event simultaneously triggered both tumor regression and HSV-2 reactivation leading to HSVL, are also possibilities. Although single patient studies preclude the identification of a specific mechanism that explains this patient clinical presentation and disease course, our study provides some insight into the pathogenesis and immunobiology of HSVL and evidence that virus-specific immune responses can promote sustained control of cancer cells. Further research with larger number of patients is needed to better understand the impact of infections and the ensuing immune response on cancer control.
Materials and Methods

Patient Samples

All peripheral blood samples were collected in sodium citrate CPT tubes (BD) and PBMCs were isolated according to the manufacturer’s protocol. Patient plasma and peripheral blood mononuclear cells (PBMCs) were stored at -80° C and in liquid nitrogen, respectively, until the time of analysis. LN sample was collected and processed as previously published(30).

Antibodies, cell lines, viruses, and proteins.

Antibodies used in this study are listed in Table S1. The following cell lines were used: HaCaT keratinocytes (Addex Bio) and THP-1 (ATCC TIB-202). Recombinant HSV-2 strain 333 that encodes the β-galactosidase gene (β-Gal) (HSV-2/Gal) under cytomegalovirus (CMV) promoter control inserted between virus UL3 and UL4 genes was kindly provided by Drs. Patricia Spear and Richard Longenecker(31). Stem of influenza hemagglutinin (Flu-HA) strain H1 1999 NC was expressed using vector VRC-3925(32) in 293F cells and was kindly provided by Drs. M. Gray and L. Stamatatos. HSV-2 proteins gB2, gC2, and gH2/gL2 were kindly provided by Drs. G. Cohen and R. Eisenberg. HSV-2 proteins UL19ud, UL25 and gD2(33) were provided by Immune Design Corp.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded lymph node biopsy samples using anti-HSV1/HSV2 ICP5 major capsid protein antibody (clone 3B6; Abcam). Bound antibody was detected using EnVision™ FLEX+ secondary reagents on a Dako Autostainer Link48 per manufacturer's instructions.

Real Time Quantitative PCR
DNA was extracted using QIAamp 96 DNA HT kit (Qiagen) from 100 ul of serum and eluted into 100 ul of AE buffer. Ten ul of extracted DNA was used to perform HSV quantitative real-time PCR (34).

**HSV-2 Sequencing**

HSV-2 was sequenced from two PCR-positive samples using hybridization-based target enrichment and next-generation sequencing (5). Genomes were assembled using a previously described pipeline (35) and deposited in GenBank as MT461026 (Day +5, 2020-3449AC) and MT461027 (Day +12, 2020-3450AC). Whole genome sequences for the two samples were aligned against reference sequences from Genbank (36) using MAFFT v7.450 (37). UL-US regions were extracted and concatenated in Geneious Prime v 2020.1.2 (Biomatters, Auckland, NZ).

**Flow cytometry**

FACS analysis was performed using either fresh or cryo-preserved PBMCs. Cells were incubated with appropriate surface antibody mix for 15 mins then 1x FACS/Lyse (BD) was added to each sample. After a 10-min incubation, the sample was washed once, permeabilized with FOXP3 buffer kit, and stained for intracellular markers per manufacturer’s protocol (eBiosciences). Cells were then washed once with 1x FOXP3 wash buffer followed by 1x PBS supplemented with 2% FBS and 1 mM EDTA prior to sample analysis on a BD LSR II instrument.

**Melon gel IgG isolation and total IgG concentration measurement**

Melon gel (ThermoScientific) was used to isolate IgG from plasma according to manufacturer’s instructions. Briefly, plasma was buffer exchanged into Melon gel Purification buffer using Zeba Desalting spin columns (ThermoScientific) and loaded onto Melon gel. Flowthrough fraction containing IgG was buffer exchanged into PBS using Zeba spin columns. Final IgG preparation was sterilized and IgG concentration was measured using Nanodrop 200 (Thermo).
Enzyme-linked Immunosorbent Assay (ELISA)

HSV-2 antigens (diluted 1:100, Meridian Lifesciences) were coated on ELISA plates overnight. Plates were then washed, incubated with patient serum, and virus-specific IgG detected as previously published(9).

Enzyme-linked Immune Absorbent Spot (ELISPOT)

ELISPOT plates were coated with HSV-2 or VZV antigens (control) overnight. To assess for the presence of HSV-2-specific IgG-secreting plasmablasts in the LN, mononuclear cells were incubated in the ELISPOT plate for 18 h and secreted antibodies were analyzed as reported(8).

HSV-2 binding antibody assay.

HSV-2 proteins and control antigens were coupled to MagPlex beads (Luminex Corp.) using Antibody Coupling kit (Luminex Corp.) according to manufacturer recommendations. Beads were blocked with phosphate buffered saline (PBS; Gibco) containing 5% Blotto (Bio-Rad) and 0.05% Tween-20 (Sigma), and then incubated with serially diluted plasma samples. Pooled sera from HSV-2 positive and HSV-2 negative donors were included as positive and negative controls, respectively. Beads were washed and incubated with subclass-specific secondary antibodies conjugated to phycoerythrin (all from Southern Biotech). Beads were then washed and resuspended in PBS with 1% BSA and 0.05% Tween-20 and binding data were collected on Luminex 200 instrument (Luminex Corp.) operated by MagPlex software (Hitachi). Bead regions were detected, and fluorescence was measured for at least 70 beads per region to calculate Median Fluorescence Intensity (MFI) for each region. MFI measured with unconjugated beads was considered background and was subtracted from all experimental sample measurements.

HSV-2 neutralizing antibody assay.
This assay was adapted from Baccari et al. (38). Briefly, HaCaT keratinocytes (Addex Bio) were seeded in 96-well plates 24 h prior to infection with HSV-2/Gal, which expresses β-galactosidase in an amount that is proportional to the infection. The next day, HSV-2/Gal at multiplicity of infection (MOI)=0.5 was mixed with plasma or IgG samples serially diluted in growth media with 2% FBS and the mixture was incubated for 1 h at 37 °C. HaCaT growth medium was replaced with virus-plasma or virus-IgG mixtures afterwards and incubated at 37° C for 20 h. Cells were subsequently lysed and incubated with a solution containing 0.5 mM chlorophenol red-β-D-galactopyranoside (Sigma), 70 mM Na$_2$HPO$_4$, 31.6 mM NaH$_2$PO$_4$, 10 mM KCl, 2 mM MgSO$_4$ and 20 mM 2-mercaptoethanol for 60 min at 37° C. A colorimetric readout of β-galactosidase activity was then measured on SpectraMax M2 microplate reader (Molecular Devices). The 50% neutralizing titer (NT) against HSV-2 was defined as the reciprocal of the dilution for which the virus infectivity was reduced by 50% relative to control infection without plasma or antibodies and was calculated using the formula: [(Average OD of “cells+virus” control - Average OD of cell only control)/2 + Average OD of “cells only” control]. The 50% NT for each test sample was interpolated by calculating the slope and intercept using the last dilution with an OD below the 50% neutralization point and the first dilution with an OD above the 50% neutralization point. The following calculation was then used to determine the 50% NT: [50% NT = (50% neutralization point - intercept)/slope].

HSV-2 antibody-dependent cellular phagocytosis (ADCP) assay.

The ADCP assay was performed as previously described (39). Briefly, gD2 protein was biotinylated using a sulfo-NHS-LC biotin (ThermoScientific) according to the manufacturer’s instructions. Unreacted biotin was removed by buffer exchange using Amicon centrifugal concentration devices (Millipore) and biotinylated antigen (2.5 µg) was incubated with 9.6 x 10$^7$ fluorescent neutravidin beads (Invitrogen) overnight at 4° C. Beads were washed and resuspended in PBS with 1% BSA (Sigma; PBS-BSA). Serially diluted plasma and control
samples were prepared in round bottom 96-well plate, $1 \times 10^6$ beads were added to each well, and the plate was incubated for 1 h at 37° C. Following equilibration, $2 \times 10^4$ THP-1 cells were added to each well and the plate was incubated overnight under standard culture conditions. The next day, the plate was centrifuged and 100 µl of supernatant was replaced with 100 µl of 4% paraformaldehyde. Cells were washed and analyzed on a BD FACS Canto II. A phagocytic score was determined by gating the samples on events representing cells and calculated as follows:

$$\text{Phagocytic Score} = (\text{MFI of bead positive cells}) \times \text{(Frequency of bead positive cells, %)}.$$  

Corrected Phagocytic Score was calculated based on the difference between the results of Phagocytic Score of each sample and the Phagocytic Score of the control sample containing cells treated with neutravidin beads not conjugated to an antigen.

**HSV-2 UL25 tetramer generation**

HLA class I loci were genotyped using a sequence-based typing method. The Immune Epitope Database (IEDB) was queried for known HSV-2 epitopes as well as conserved HSV-1/2 epitopes with confirmed binding to the determined HLA class I alleles including HLA-B*14:02, which exhibits comparable peptide binding characteristics as the patient’s HLA-B*14:01. Twelve peptides were identified in IEDB, synthesized (Genscript) with >90% purity, and resuspended in DMSO. To identify recognized CD8 T cell epitopes, interferon-γ ELISPOT assays using PBMCs were performed as described previously(40). Of the peptides tested, one (DRLDNRLQL) elicited a positive interferon-γ response and was used for monomer generation. HLA-B*14:01/UL25$_{405-413}$ (DRLDNRLQL) monomers were obtained from ImmunAware and tetramerized in-house per manufacturer’s instructions.

**Cytokine measurement**

Plasma cytokine levels were measured using a custom electrochemiluminescence multi-array assay according to manufacturer’s protocol (Meso Scale Diagnostics, Rockville, MD).
Statistical Analysis

Statistical analysis was conducted using Graphpad Prism V9 (GraphPad Software, San Diego, CA). Frequency and percentage or mean and standard deviation were reported based on the data.

Study approval

The patient provided written informed consent prior to enrolling in the study, which received Institutional Regulatory Board (IRB) approval at Emory University (IRB00057236).
Author contributions

AC conceptualized the study, designed and performed the experiments, performed the clinical data abstraction, provided patient samples, analyzed the results, generated the figures, and wrote the manuscript. AMS designed and performed experiments, analyzed the results, generated the figures, and wrote the manuscript. AW, DLJ, MC, MH, and HX performed the experiments. KRJ, PR, and ALG performed experiments and analyzed the results. JLK, and JBC provided patient samples and clinical support, analyzed the data, and contributed to writing the manuscript. DMK, LC, CRF, and RA conceptualized the study, analyzed the data, provided funding, and wrote the manuscript.
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References


Figure 1. Clinical presentation HSVL in a patient with CLL. (A) PET/CT scan obtained at presentation showed left-sided FDG-avid adenopathy. (B) Low- and high-power micrographs of H&E stain showing areas of necrosis (arrow) in the biopsy specimen of the FDG-avid left obturator node. (C) Cells staining for HSV 1/2 major capsid protein were found in the biopsy sample (brown). (D) Plasma HSV-2 genome copies over time showing evidence of HSV-2 viremia. Day = days since symptom onset. Vertical dotted line = time of presentation. Arrow indicates time of antiviral therapy initiation. (E) Top: sequence coverage depth for plasma-derived HSV-2 DNA from Day +5 (green) and Day +12 (purple). Coordinates from HSV-2 reference strain HG52 GenBank JN561323.2. Poor coverage of UL and US inverted repeats is commonly noted with short read technologies. Bottom: Schematic of HSV-2 genomes showing inverted repeats and HSV-1 insertions in three indicated HSV open reading frames that are prevalent in North American strains(5) and were detected in both patient specimens.
Figure 2. Vigorous HSV-specific B cell responses observed in the blood and LN. (A) Quantification of plasmablasts per million live PBMCs in the blood over time. Red = viral titer as depicted in Figure 1C. (B) Flow cytometry showing plasmablasts in the LN (left). ELISPOT (right) showing that LN plasmablasts secreted HSV-2-specific IgG antibodies. (C) Persistent hypogammaglobulinemia is observed in this patient. Shaded area = expected normal range. (D) Relative IgG binding titers against HSV-2 lysates by ELISA (blue) and neutralizing antibody titers leading to 50% reduction in viral infectivity (black). Error bars = SEM. Positive control neutralizing antibody titer = 1:256. (E) IgG, IgA, and IgM antibody binding to different HSV-2 surface proteins at each timepoint vs pooled plasma from HSV-2+ and HSV-1/2- controls. White = sample not analyzed. (F) HSV-2 antibody-dependent cell phagocytosis (ADCP) score (in thousands) of gD2 covered microspheres incubated with plasma from each timepoint. ADCP score for pooled healthy
HSV-2+ control = 111320. Error bars = SD. (G) IgG subclass analysis of antibodies binding to HSV-2 surface proteins at each timepoint vs pooled plasma from HSV-2+ and HSV-1/2- controls. For D and F, antibody binding to influenza HA (FluHA) was included as control. Heatmap scale reflects endpoint titer in log2. All measurements performed in duplicate. For all applicable graphs: Day = days since symptom onset. Vertical dotted line = time of presentation.
Figure 3. Robust T cell and plasma cytokine responses were observed. (A) FACS plots of singlet, live, CD3+, CD4+ (top) and CD8+ (bottom) lymphocytes at baseline and on Day +5, identifying activated HLA-DR+CD38+ T cells. (B) Percent of activated CD4+ (purple) and CD8+ (orange) T cells over time as analyzed in A. (C) FACS analysis showing high percent of activated CD4+ and CD8+ T cells in the LN. For A-C: Numbers = percent of total CD4+ or CD8+ T cells. (D) CD38+HLA-DR+ T cells in the blood (red) have an effector phenotype. Numbers are percent of activated CD4+ or CD8+ T cells expressing or downregulating the marker of interest. Naïve (black) CD4+ or CD8+ T cells shown as control. All gates set in reference to naïve CD4+ or CD8+ T cells. (E) UL-25 tetramer stain in the blood on day +5 and day +270. Numbers = Percent of total CD8+ T cells. (F) Percent of HSV-2 UL25-specific Tetramer+CD8+ T cells in the blood over time. (G) Extended phenotype analysis of UL-25 tetramer+ CD8+ T cells in the blood at day 5. Green = UL-25 Tetramer+ CD8+ T cells. Black = naïve CD8+ T cells. Numbers = percent of tetramer+ (green) CD8+ T cells. Mean plasma levels of proinflammatory cytokines IFN-γ (H), IL-18 (I), and IL-6 (J) were elevated in the acute setting. All measurements performed in quadruplicate. Error
bars = SD. Horizontal dotted line = levels in pooled plasma from aged-matched healthy individuals. For all applicable graphs: Day = days since symptom onset. Vertical dotted line = time of presentation.
Figure 4. Decreased circulating CLL tumor burden after HSVL. (A) Absolute CLL cell count (in thousands) per μL blood as assessed by FACS. (B) White blood cell (WBC, black), hemoglobin (HgB, red), and platelet count (Plt, blue) over time show a sustained decrease in absolute leukocyte count after each HSVL episode. Vertical lines represent values at first (#1) and second (#2) presentation of HSVL. Horizontal lines = WBC count ranges between first and second HSVL episode. (C) Absolute neutrophil (black) and lymphocyte (blue) counts over time. Decrease in WBC was largely due to decreased absolute lymphocyte count (blue) after each HSVL episode as absolute neutrophil (black) count remained constant. For B and C, error bars = SD.