EXPLORATORY ANALYSES OF LYMPH NODE TISSUE, SERUM, AND PERIPHERAL BLOOD MONONUCLEAR CELLS DO NOT REVEAL PATHOGEN SIGNATURES IN IDIOPATHIC MULTICENTRIC CASTLEMAN DISEASE

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INTRODUCTION

Castleman disease (CD) encompasses a group of hematologic disorders that share characteristic histopathological features. Unicentric CD (UCD) involves a solitary enlarged lymph node with typically mild symptoms. Multicentric CD (MCD) involves generalized lymphadenopathy and often severe cytokine-driven multi-organ dysfunction. MCD is further subdivided into cases caused by Human Herpesvirus-8 (HHV8-MCD) infection, POEMS (Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal, and Skin changes) syndrome (POEMS-MCD), and those with an idiopathic cause (iMCD). The underlying pathological mechanisms and etiologies of UCD and iMCD are not well understood.

AIM

Given that a viral pathogen, HHV-8, causes HHV8-MCD, we pursued multiple approaches to identify potential pathogens that may trigger iMCD and UCD.

METHODS

Two distinct methods, PathOChip[1] and Viral-Track[2], were implemented for detection of pathogens in iMCD and UCD samples in multiple tissue types.

1) PathOChip, a ~60,000 probe-based assay was used to detect 4,000+ known pathogens associated with human disease from serum and lymph node CD tissue.

2) Viral-Track, An unbiased computational method for identifying viral reads from next generation sequencing (NGS) data was used on sequencing reads from lymph node and PBMC CD tissue

RESULTS

- The PathOChIP platform successfully identified HHV8 and EBV (Epstein-Barr virus), in the HHV8-MCD and EBV post-transplant lymphoproliferative disorder (EBV-PTLD) lymph node positive controls, respectively. (**Fig 1, A-B**).
- No probes were significantly elevated for a particular pathogen from the iMCD and UCD cohorts using the PathOChIP microarray (**Fig 1, C-D**).
- Viral-Track successfully detected viral reads from HIV (human immunodeficiency virus), HHV8, EBV, and HCV (Hepatitis C) positive controls (**Table 2**).
- For the fresh lymph node tissue single-cell cohort (**Table 1, Cohort 4**) no viral reads were detected.
- Viral-Track was not able to detect a shared viral pathogen in PBMC and FFPE lymph node CD cohorts (**Fig 2).**

Table 1. Summary of PathOChIP and Viral-Track cohorts

Cohort	Method	NGS data type	Sample description	Non-CD	CD
1	PathoChIP	--	FFPE lymph node biopsy slides	1 HHV8 1 EBV-PTLD 1 Reactive	6 iMCD 8 UCD 2 POEMS-MCD
2	Viral-Track	Bulk RNA	FFPE lymph node biopsy slides	7 Sentinel 3 SLE 5 BLN	9 iMCD
3	Viral-Track	Single-cell RNA	PBMCs	2 HD	4 iMCD
4	Viral-Track	Single-cell RNA	fresh lymph node tissue	--	4 UCD

Table 2. Positive Controls for Viral-Track computational pipeline

NGS data type	Sample Description	Phenotype	In vivo	Number of samples with detected virus
Single-cell	PBMCs	2 HIV	N	2/2
Single-cell	PBMCs	2 EBV	N	2/2
Single-cell	Primary effusion lymphoma derived cell line	1 HHV8	N	1/1
Bulk	FFPE core needle liver biopsy	40 HBV	Y	35/40
Single-cell	CD4 enriched PBMCs	2 HIV	Y	2/2

Figure 1. PathOChIP results

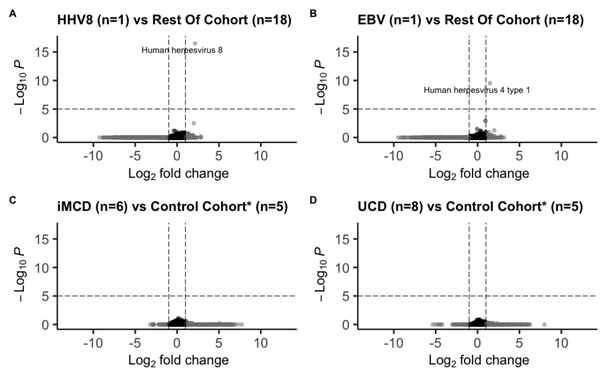

Fig 1. Volcano plot of all pathogens plotted by log2 fold change from difference in probe intensity and an adjusted p value from the comparisons of positive probe ratio (probes above 95th percentile intensity for all 50,000 probes). Black horizontal line represents require p value to survive FDR adjust with an alpha of 0.05. Control cohort (n =5) consisted of one HHV8-MCD, one EBV-PTLD, one reactive, and two POEMS-associated MCD lymph nodes.

Figure 2. Viral-Track results

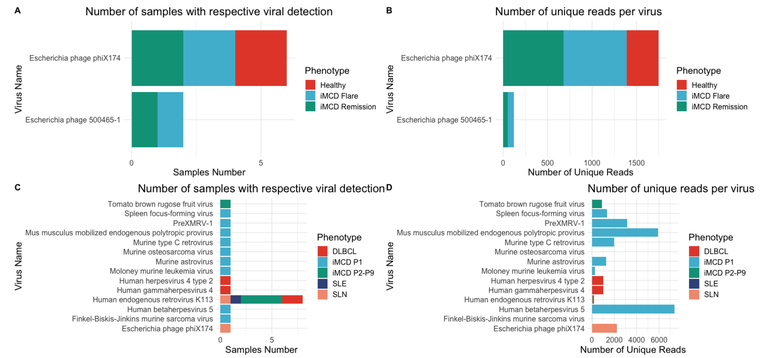

Fig 2. Summary of Viral-Track results by **A-C** number of samples with detected viruses and **B-D** total unique reads of the respective virus (strength of detection). **A-B**. Single Cell PBMC cohort consists of four iMCD samples from flare and remission disease states with two healthy donors. Results from biological replicates were combined per sample for the summary bar plot. **C-D** Bulk RNA FFPE cohort. diffuse large B-cell lymphoma (DLBCL), Idiopathic multicentric Castleman disease (iMCD), systemic lupus erythematosus (SLE), sentinel lymph node (SLN).

CONCLUSIONS

- Using two different methods for pathogen detection, we were unable to identify a pathogenic signature in PBMC and lymph node CD samples.
- This study does not completely rule out the possibility that iMCD or UCD has a viral etiology, some caveats include: 1) viral mRNA could be present at a level below our limit of detection, 2) the pathogen may be present in an untested tissue, and 3) a pathogen may have precipitated the inflammatory cascade, but has been sufficiently cleared by time of sample collection.
- The absence of a pathogenic signatures in the CD cohorts assembled here suggests that alternative etiological mechanisms potentially involving autoimmune or neoplastic processes should be further explored and prioritized

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