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POSTER ABSTRACTS

623.MANTLE CELL, FOLLICULAR, AND OTHER INDOLENT B CELL LYMPHOMAS: CLINICAL AND EPIDEMIOLOGICAL

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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Background : 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 (HHV-8-associated MCD) infection, POEMS syndrome (POEMS-associated MCD), and those with an idiopathic cause (iMCD). The underlying pathological mechanisms and etiologies of UCD and iMCD are not well understood. Given that a viral pathogen, HHV-8, causes HHV-8-associated MCD, we pursued multiple approaches to identify potential pathogens that may trigger iMCD and UCD.
Methods: We used 2 distinct detection methods to identify potential pathogens in CD. PathoChIP is a microarray-based assay that uses ~60,000 DNA/RNA probes to detect 4,000+ pathogens associated with human diseases. Viral Track is a computational method that can be applied to unmapped next-generation sequencing (NGS) reads to search for the presence of viral RNA. Formalin-fixed paraffin-embedded (FFPE) lymph node tissue was analyzed by PathoChIP from iMCD (n=6), UCD (n=8), HHV8-associated MCD (n=1, HHV8-MCD), EBV-driven post-transplant lymphoproliferative disorder (n=1, EBV-PTLD), reactive samples without a clear etiology (n=1), and POEMS-associated MCD (n=2) patients. We also analyzed serum from iMCD patients (n=2) and healthy donors (n=8). A 'positive' probe for a given microorganism was defined as exceeding the 95 th percentile on a per-probe basis of the optical signal captured across all probes in the dataset.
Data from single-cell RNA sequencing of cryopreserved peripheral blood mononuclear cells (iMCD, n=4; EBV, n=2; HHV8, n=1; healthy donor, n=2) and bulk RNA sequencing of FFPE lymph node tissue (iMCD, n=9; sentinel, n=7; systemic lupus erythematosus (SLE), n=3; Diffuse large B-cell lymphoma (DLBCL), n=3) were used as input into the Viral Track program. To detect a positive viral signal, standard thresholds were required: >50 uniquely mapped reads to a given virus, a read complexity threshold >1.2, and >10% coverage of the length of the respective viral genome.
Results: After quality control, 49,657 probes from the PathoCHIP assay were mapped to 4,000+ known pathogens. As expected, the HHV-8 MCD and EBV-PTLD samples identified significantly more positive probes compared to the rest of the cohort for HHV-8 (65/259 vs 1/259) and EBV (38/235 vs 1/235), respectively. Importantly, no other microorganisms were detected in UCD or MCD lymph nodes or serum samples at a significantly increased rate than controls.
For Viral Track, human endogenous retrovirus K113 and Escherichia phages were detected at low levels throughout all results. Excluding these viruses, positive controls including HHV-8-MCD, and EBV solely identified their respective predicted virally mapped NGS reads and no viral signature was detected in healthy donors. Importantly, there was no significant viral signature specific to CD in PBMCs or LN tissue. Interestingly, 1 iMCD sample did contain a significant number of NGS sequencing reads mapping to HHV5. These results were confirmed with clinical data reporting a positive IgG, negative PCR, and negative IgM test within 2 weeks of the biopsy date, suggesting that the HHV5 positive result was likely detecting a previous, now latent infection rather than a potential pathological driver of CD.
Conclusions: Although there are limitations to this study, including small cohorts and viral mRNA single-cell capture limitations, we were able to use 2 orthogonal pathogen detection methods on multiple tissue types from iMCD patients. Taken together, our results suggest that an active infectious agent is unlikely to be a major contributor or pathological mechanism driving

UCD or iMCD. The continued search for potential causative microorganisms in iMCD patients should continue, but our results suggest that alternative hypotheses related to the pathology and etiology of iMCD and UCD should be prioritized.

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