Trafficking, not lymphoproliferation, promotes lymphadenopathy in idiopathic multicentric Castleman disease

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Abstract

Idiopathic multicentric Castleman disease (iMCD) is a rare and life-threatening hematologic illness characterized by multifocal lymphadenopathy for an unknown cause. Patients with iMCD experience periods of systemic inflammation due to cytokine release that includes interleukin-6 (IL-6). Treatment with IL-6 inhibition is effective in only one-half of patients and thus, a more complete understanding of the disease process of iMCD is urgently needed to advance the development of treatment options for refractory patients. Though not well understood, iMCD is often described as a lymphoproliferative disorder and assumed to be due to expansion of lymphocytes in lymph nodes. To determine what contributes to the characteristic lymphadenopathy in iMCD, we investigated factors that influence lymph node size including cell proliferation and chemotaxis. We analyzed fluorodeoxyglucose (FDG) uptake from radiology reports and performed immunohistochemistry with Ki67 in lymph nodes from iMCD patients. Compared to controls that included lymphoma, we discovered lower FDG avidity and Ki-67 staining in iMCD indicating that mechanisms other than local cell expansion, such as increased cellular mobilization, may contribute to lymphadenopathy in iMCD. We previously showed that 3 of the top 20 cytokines or chemokines found in iMCD directly promote chemotaxis (CXCL13, CCL19 and CCL21) suggesting that increased trafficking to the tissue may influence lymph node size in iMCD. As CXCL13, CCL19, and CCL21 function to attract B and T cells to specific regions of secondary lymphoid organs, we profiled gene expression in lymph node tissue and discovered increased gene expression of CXCL13, CCL19, and CCL21 in iMCD and that the expression pattern was dysregulated. Furthermore, flow cytometry analysis of circulating immune cell types showed that expression of CXCR5, the cognate receptor to CXCL13, was significantly reduced among circulating B and T cells in iMCD during disease flare and restored in remission. Other chemokine receptors were also decreased, including CXCR3 and CCR6 in lymphocyte subsets. Together, our data suggest that lymphocyte mobilization, rather than lymphoproliferation, contributes to lymphadenopathy in iMCD. Dysregulated lymphocyte trafficking and disorganization in secondary lymphoid organs may contribute to flare episodes and iMCD pathogenesis. As such, targeting the CXCR5/CXCL13 axis is a potentially interesting therapeutic strategy for the treatment of iMCD.

Background

Idiopathic Multicentric Castleman Disease (iMCD)

TAFRO

Thrombocytopenia (<100 k/uL)
Anasarca
Fever/ elevated CRP (>20 mg/L)
Renal dysfunction
(Creatinine >1.1 (F) >1.3 (M) mg/dL)
/ **R**eticulin fibrosis
Organomegaly (includes lymphadenopathy)

Definition: (T+A+F+O) + (R|R)

IPL

Idiopathic **P**lasmacytic **L**ymphadenopathy

Hypergammaglobulinemia
(γ globulin > 1.7 g/dL; IgG > 1700 mg/dL)
Thrombocytosis (>400 k/uL)

Definition:
Hypergammaglobulinemia + thrombocytosis

NOS

Not **O**therwise **S**pecified

Defintion: >1 enlarged LN but does not achieve criteria for TAFRO or IPL

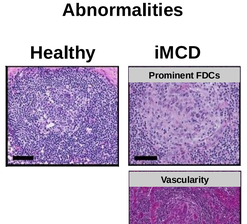
Cytokine Storm

IL-6

Others?

Lymphadenopathy

Histopathological Abnormalities



What mechanisms promote iMCD?

Figure 1: Characteristics of idiopathic multicentric Castleman disease (iMCD) and research question. Idiopathic multicentric Castleman disease is an inflammatory disorder that presents with clinical heterogeneity and varying severity. iMCD can cause intense flares that could life-threatening. Three clinical subtypes have been defined: TAFRO, NOS and IPL. Unifying features of iMCD include enlarged lymph nodes with associated histopathological abnormalities including prominent follicular dendritic cells and increased vascularity. Patients can also exhibit a life-threatening cytokine release syndrome often involving interleukin-6 (IL-6). The goal of this project is to identify other factors and pathways that may be contributing to iMCD pathogenesis.

I. Lymphadenopathy is not associated with proliferation in iMCD lymph nodes

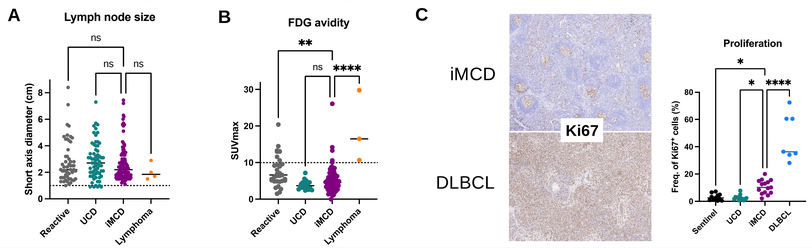


Figure 2: Lymph node size, metabolic activity, and proliferation in iMCD. Clinical radiological data was analyzed for (A) lymph node size (reactive, n=53; unicentric Castleman disease (UCD), n=65; iMCD, n=108; lymphoma, n=4) and (B) fluorodeoxyglucose (FDG) uptake and compared between different groups (reactive, n=33; UCD, n=18; iMCD, n=75; lymphoma, n=3). (C) Representative immunohistochemstry stains for Ki67 and quantification. Ki-67 positive and negative nuclei were determined by developing an algorithm to automatedly segment nuclei and measure Ki67 levels. Sentinel, n=11; UCD, n=15; iMCD, n=12; DLBCL, n=7. *p<0.05. **p<0.01. ****p<0.0001.

II. Elevated chemokine expression in the blood of iMCD patients

A

Top up-regulated cytokines/chemokines in iMCD vs. healthy

Protein	Log_2FC	Adj.p-value
CXCL13	2.85	9.01E-07
VEGFA	2.03	2.68E-06
CCL23	1.82	3.27E-05
CCL21	1.62	0.002694
TNFRSF1A	1.55	7.28E-06
IL18	1.45	0.00456984
INHBB	1.44	5.25E-05
TNFRSF1B	1.28	0.00017583
CCL15	1.27	3.30E-05
IL18R1	1.15	3.41E-07
CSF1	1.12	0.00222095
CCL15	1.11	3.44E-05
IL18R1	1.10	2.75E-06
CXCL10	1.07	0.04109918
CCL19	1.05	0.03966604
HGF	1.00	0.0008596
IL21R	0.99	0.00820716
TNFRSF1B	0.95	0.0021243
IL15RA	0.95	0.00288326

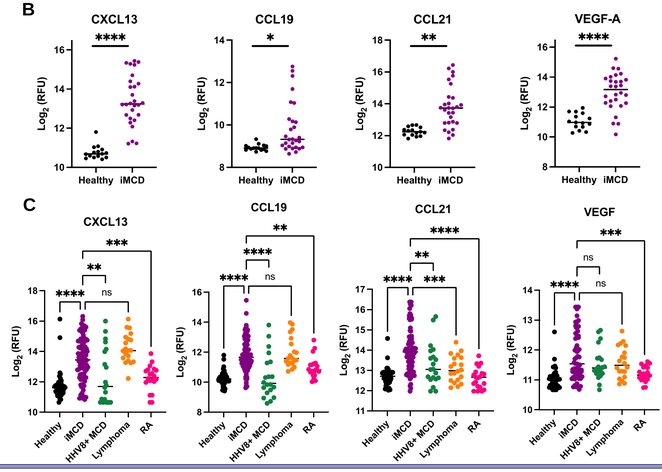


Figure 3: Serum cytokines and chemokines analyzed using Somalogic's Somascan in iMCD patients compared to controls. (A) Top up-regulated cytokines and chemokines in iMCD compared to healthy donor controls. Four of the top twenty included proteins directly or indirectly involved with lymphocyte trafficking. (B) Comparison of individual analytes in iMCD patient samples versus healthy donor controls. Healthy, n=15. iMCD, n=28. (C) In a second study,[1] CXCL13, CCL19, CCL21, and VEGF-A levels were compared between iMCD and other inflammatory diseases (Healthy, n=42; iMCD, n=88; HHV8+ MCD = human herpesvirus-8 associated MCD, n=20; Lymphoma = Hodgkin's lymphoma, n=20; RA = rheumatoid arthritis, n=20. *p<0.05. **p<0.01 ***p<0.001 ****p<0.0001.

III. Elevated chemokine expression in lymph node tissue of iMCD patients

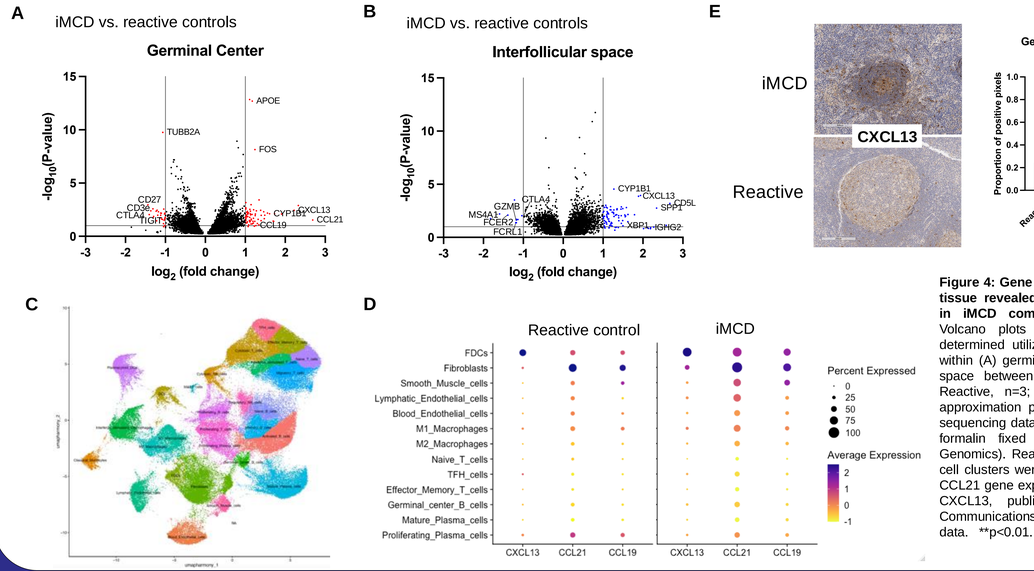
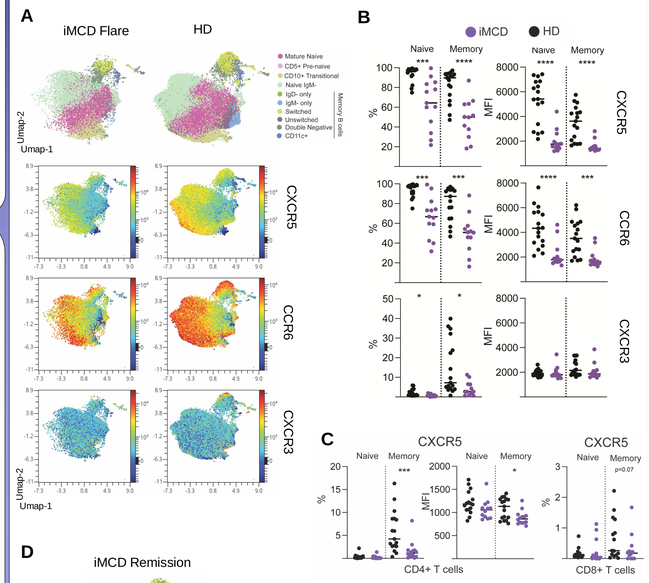


Figure 4: Gene expression analysis of lymph node tissue revealed increased chemokine expression in iMCD compared to inflammatory controls. Volcano plots comparing gene expression as determined utilizing Nanostring's GeoMx technology within (A) germinal centers and the (B) interfollicular space between iMCD and reactive lymph nodes. Reactive, n=3; iMCD n=6. (C) Uniform manifold approximation projection (UMAP) of single-cell RNA sequencing data of lymph node cells dissociated from formalin fixed paraffin embedded sections (10X Genomics). Reactive, n=4; iMCD, n=4. (D) Individual cell clusters were analyzed for CXCL13, CCL19, and CCL21 gene expression. (E) Immunohistochemistry of CXCL13, published in Pierson et al. Nature Communications 2022,[2] validated gene expression data. **p<0.01. ***p<0.001.

IV. Loss of chemokine receptor expression in circulating cells of iMCD patients



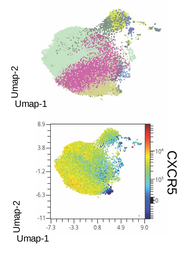


Figure 5: Flow cytometric analyses of PBMCs from iMCD patients during disease flare compared to healthy donor controls. (A) UMAP projections of B cell subsets in iMCD flare and healthy PBMC samples showing different B cell subsets and cell surface expression of CXCR5, CCR6, and CXCR3. (B) Quantification and comparison of chemokine receptor expression in naïve and memory B cell subsets. (C) Summary plots showing the expression of CXCR5 in T cell subsets, confirming previously published data.[3] (D) UMAP projections of B cell subsets and cell surface expression of CXCR5 in iMCD during remission. Healthy donor (HD), n=17; iMCD, n=13 flare and n=7 remission. *p<0.05. ***p<0.001 ****p<0.0001.

Conclusions

1) Lymph nodes from iMCD and lymphoma patients were similar in size, yet iMCD lymph nodes were not as proliferative.
2) The most up-regulated cytokines and chemokines during iMCD disease flare were proteins involved in chemotaxis to secondary lymphoid organs (ie CXCL13, CCL19 and CCL21).
3) Chemokine expression was also elevated in the lymph node tissue of iMCD patients.
4) The expression levels of cognate chemokine receptor expression, notably CXCR5, was decreased in circulating B cells and T cells in iMCD.

References

1. Pierson, S.K., et al., *Discovery and validation of a novel subgroup and therapeutic target in idiopathic multicentric Castleman disease*. Blood Adv, 2021. **5**(17):3445-56.
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3. Pai, R.L., et al., *Type I IFN response associated with mTOR activation in the TAFRO subtype of idiopathic multicentric Castleman disease.* JCI Insight, 2020. **5**(9).

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