Serum proteomics identifies interferon gamma signaling in idiopathic multicentric Castleman disease

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Abstract

Idiopathic multicentric Castleman disease (iMCD) is a rare and life-threatening hematologic illness involving uncontrolled systemic inflammation due to unknown causes. Hallmark features of iMCD include multiple enlarged lymph nodes with characteristic histopathology, polyclonal lymphoproliferation, and the release of pro-inflammatory cytokines including interleukin-6 (IL-6) that can lead to multi-organ dysfunction and death. The only FDA-approved drug for iMCD, siltuximab, blocks IL-6 and is effective in treating a portion of patients suggesting other key cytokines may contribute to iMCD pathogenesis.

To uncover other potential pathogenic mediators, we analyzed 1,178 serum analytes in the blood of iMCD patients (n=88) compared to healthy donors (n=42) and other inflammatory disease controls, including rheumatoid arthritis (n=20), Hodgkin's lymphoma (n=20), and HHV-8-associated multicentric Castleman disease (n=20). We discovered evidence of elevated activity of interferon gamma (IFN-ɣ), a pro-inflammatory cytokine critical for innate and adaptive immunity. Included among the top most up-regulated cytokines and chemokines in iMCD compared to healthy donors were several IFN-ɣ-inducible proteins such as interleukin-18 binding protein (IL-18BP) and chemokines CXCL9 and CXCL11 that are well defined markers of IFN-ɣ activity. These markers are considered to be more robust indicators of IFN-ɣ activity than circulating levels of IFNɣ due to the often locally acting and complicated nature of IFN-ɣ signaling. Expression of IL-18BP was also significantly up-regulated in iMCD compared to rheumatoid arthritis and Hodgkin's lymphoma. These data suggest that increased IFN-ɣ signaling may contribute to the cytokine storm in iMCD, potentially unlocking a new mechanistic regulator of disease.

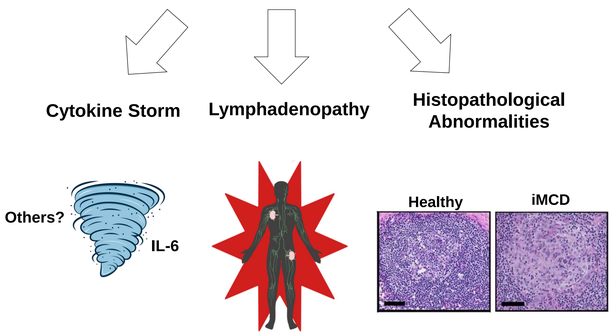
To determine if IL-6, the only cytokine known to be involved in iMCD pathogenesis, influenced the expression of IFN-ɣ-induced proteins, we quantified IL-18BP in iMCD patients before and after IL-6 blockade with siltuximab (n=52). We discovered that expression of IL-18BP was associated with response to treatment: expression of IL-18BP decreased in iMCD patients who improved with IL-6 inhibition whereas levels of IL-18BP remained elevated among non-responders. These data indicated that IL-18BP levels and thus IFN-ɣ activity could serve as potential indicators of treatment response. Furthermore, these data show that IL-18BP levels in iMCD can be either IL-6 dependent or independent suggesting that IFN-ɣ signaling is a major pathway involved in iMCD and a novel therapeutic target.

The clinical presentation of iMCD is similar to that of hemophagocytic lymphohistiocytosis (HLH), a cytokine-storm disorder resulting from the over production of IFN-ɣ that can be treated with IFN-ɣ inhibition. In addition, both inflammatory disorders are associated with an increase in cell populations that produce IFN-ɣ, including activated CD8 T cells and NK cells.[1,2] Given the similarities between iMCD and HLH, we compared the expression of the IFN-ɣ-induced protein, IL-18BP, in iMCD to a previously published dataset that included serum from patients with macrophage activated syndrome (MAS),[3] a subtype of HLH associated with autoimmune disorders. We discovered comparable levels of IL-18BP between iMCD and MAS further indicating that similar to HLH, high levels of IFN-ɣ activity are characteristic of iMCD and may play a mechanistic role in disease pathogenesis.

Finally, previously published single-cell RNA sequencing data from our group showed that IFN-ɣ signaling was up-regulated in every circulating cell population profiled in iMCD during active disease suggesting that IFN-ɣ signaling is activated in immune cells in the blood, likely from high levels of IFN-ɣ.[1] Herein, we present evidence that increased IFNɣ activity is characteristic of iMCD and propose IFN-ɣ signaling as an underappreciated pathway and key mechanism underlying iMCD and potential therapeutic target.

Background

Idiopathic Multicentric Castleman Disease (iMCD)



What factors contribute to cytokine storms in iMCD?

Figure 1: Characteristics of idiopathic multicentric Castleman disease (iMCD) and research question. Idiopathic multicentric Castleman disease is an inflammatory disorder characterized by enlarged lymph nodes with associated histopathological abnormalities including prominent follicular dendritic cells and increased vascularity. Patients 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. Elevated interferon-ɣ activity in iMCD

A

Group	# of patients
Healthy	42
iMCD	88
HHV8+ MCD	20
Lymphoma	20
RA	20

C

IFN-ɣ ⇨ CXCL9, CXCL10, CXCL11, IL-18BP

B

Top up-regulated cytokines in iMCD vs. healthy

Protein	Type	Log_2FC	Adj.p-value
CXCL13	Chemokine	1.70	1.95E-09
NAMPT	Cytokine	1.36	7.35E-12
IL36A	Interleukin	1.35	3.04E-09
CCL21	Chemokine	1.30	5.99E-10
IL5RA	Interleukin	1.00	2.20E-06
IL1RL1	Interleukin	0.91	0.000820216
CXCL11	Chemokine	0.91	1.81E-05
CCL28	Chemokine	0.87	4.01E-06
CCL23	Chemokine	0.87	6.43E-08
CCL18	Chemokine	0.85	3.29E-06
IL18BP	Interleukin	0.75	1.54E-09
GDF15	Cytokine	0.74	1.22E-08
IL1R1	Interleukin	0.74	6.26E-06
CCL14	Chemokine	0.70	7.59E-07
CCL15	Chemokine	0.68	4.62E-07
CCL23	Chemokine	0.65	1.52E-06
IL2	Interleukin	0.61	7.10E-08
THPO	Cytokine	0.60	2.96E-06
SPP1	Cytokine	0.55	2.00E-08
CXCL9	Chemokine	0.53	0.010478099

D

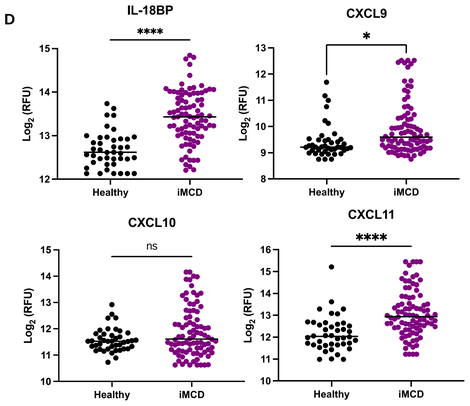


Figure 2: Serum cytokines and chemokines analyzed using Somalogic's Somoscan in iMCD patients compared to controls. (A) Groups and number of individuals analyzed in this study. iMCD = idiopathic multicentric Castleman disease; HHV8+ MCD = human herpesvirus-8 associated MCD. Lymphoma = Hodgkin's lymphoma; RA = rheumatoid arthritis. (B) Top up-regulated cytokines and chemokines in iMCD compared to healthy donor controls. (C) Three of the top twenty included proteins induced by IFN-ɣ: CXCL9, CXCL11, and IL-18 binding protein (IL-18BP). (D) Comparison of individual IFN-ɣ induced proteins in iMCD patient samples versus healthy donor controls. *p<0.05. ****p<0.0001.

II. IFN-ɣ activity is up-regulated in iMCD compared to other inflammatory diseases

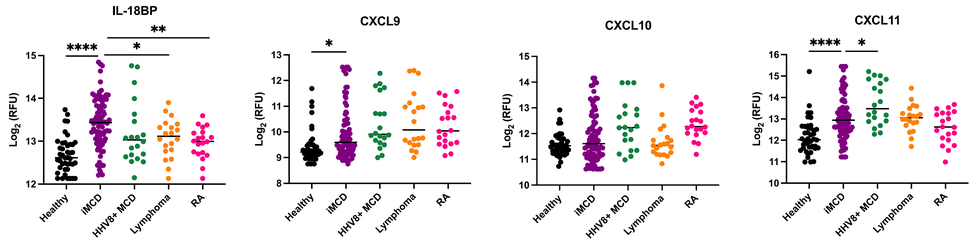
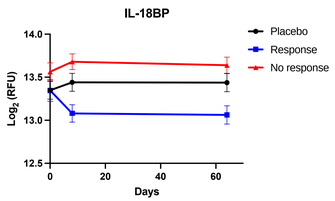


Figure 3: IFN-ɣ responsive proteins in iMCD compared to other inflammatory disorders. IL-18BP, CXCL9, CXCL10, and CXCL11 expression in iMCD versus other inflammatory disorders. iMCD = idiopathic multicentric Castleman disease; HHV8+ MCD = human herpesvirus-8 associated MCD. Lymphoma = Hodgkin's lymphoma; RA = rheumatoid arthritis. *p<0.05. **p<0.01. ****p<0.0001.

III. IFN-ɣ activity indicates treatment response to anti-IL-6 therapy

A



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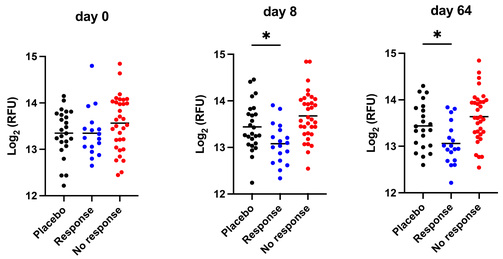

Figure 4: Expression of IL-18BP in the sera of iMCD patients treated with the anti-IL6 therapy siltuximab or placebo. Blood was collected from iMCD patients at 0, 8 and 64 days after treatment and analyzed for IL-18BP by Somascan. (A) IL-18 BP expression in patient groups separated by their clinical response to siltuximab. (B) Baseline levels of IL-18BP at day 0 were comparable between groups where as IL-18BP expression tracked with response to siltuximab at day 8 and day 64 post-treatment. *p<0.05.

IV. Comparable IFN-ɣ activity in cytokine storm disorders

A

Group	# of patients
Healthy	70
iMCD	88
HHV8+ MCD	20
Lymphoma	20
RA	20
Active MAS	17
Active sJIA, no MAS	25
sJIA-LD	30
Inactive sJIA	35
Active NOMID	5
Active SAVI	4
PAP	14
PJP colonized	11

B

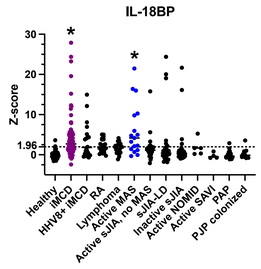

Figure 5: Expression of IL-18BP in the sera of various patient groups measured via Somascan across two different studies. (A) Patient groups analyzed for serum analytes including IL-18BP. (B) To compare datasets from the 2 studies, Z-scores for each sample were generated for IL-18BP. iMCD = idiopathic multicentric Castleman disease; HHV8+ MCD = human herpesvirus-8-associated MCD. Lymphoma = Hodgkin's lymphoma; RA = rheumatoid arthritis. MAS = macrophage activation syndrome; sJIA = systemic juvenile idiopathic arthritis; sJIA-LD = lung disease associated with systemic juvenile idiopathic arthritis; NOMID = neonatal-onset multisystem inflammatory disease; SAVI = STING-associated vasculopathy with onset in infancy; PAP = pulmonary alveolar proteinosis; PJP = pneumocystis jiroveci pneumonia. * indicates groups where the median Z-score reached significance.

V. Increased activation of IFN-ɣ producers

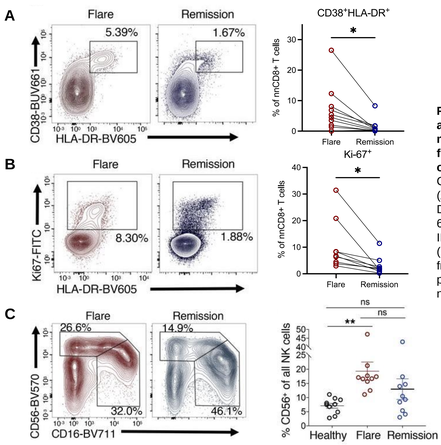

Figure 6: Flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) from iMCD patients in flare or remission.[1] Non-naïve CD8+ T cells show markers of (A) activation (CD38+HLA-DR+) and (B) proliferation (Ki-67+) during iMCD flare. (C) IFN-ɣ-producing NK cells ($CD56^{bright}$) were more frequent in the blood of iMCD patients during disease flare. n=10. *p<0.05. **p<0.01.

VI. Up-regulation of IFN-ɣ signaling in PBMCs

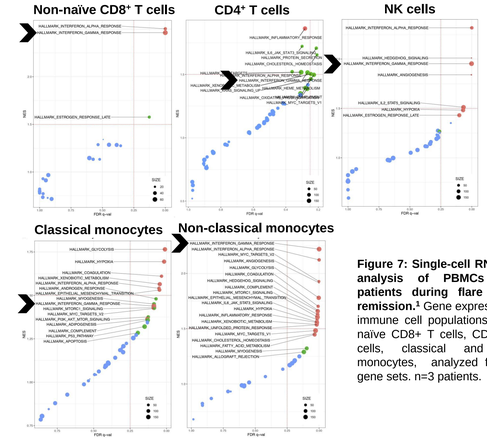

Figure 7: Single-cell RNA-sequencing analysis of PBMCs from iMCD patients during flare compared to remission.[1] Gene expression profiling of immune cell populations including non-naïve CD8+ T cells, CD4+ T cells, NK cells, classical and non-classical monocytes, analyzed for HALLMARK gene sets. n=3 patients.

Conclusions

1) Markers of IFN-ɣ activity including IL-18BP are elevated in iMCD patients as compared to healthy controls and other inflammatory disease donors.
2) Expression of IL-18BP decreased in iMCD patients that responded to anti-IL-6 therapy, but remained elevated in treatment refractory patients.
3) Serum IL-18BP was elevated in iMCD and MAS, a cytokine storm disorder known to involve IFN-ɣ.
4) Cells that produce IFN-ɣ, including non-naïve $CD8^+$ T cells and NK cells are activated in iMCD during disease flare.
5) All PBMC populations in iMCD disease flare activate IFN-ɣ gene expression programs.

References

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2. Carvelli J, Piperoglou C, Farnarier C, et al. Functional and genetic testing in adults with HLH reveals an inflammatory profile rather than a cytotoxicity defect. *Blood*. 2020;136(5):542-552.
3. Chen G, Deutsch GH, Schulert GS, et al. Identification of Distinct Inflammatory Programs and Biomarkers in Systemic Juvenile Idiopathic Arthritis and Related Lung Disease by Serum Proteome Analysis. *Arthritis Rheumatol*. 2022;74(7):1271-1283.