A translational approach to identifying and targeting TNF signaling in idiopathic multicentric Castleman disease

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Abstract

Idiopathic multicentric Castleman disease (iMCD) is a rare, immunological illness with an unknown etiology. It is characterized by multiple enlarged lymph nodes with unique histopathological features and systemic inflammation resulting from cytokine release syndrome, which can rapidly develop into multiple organ failure and death. Inhibition of the pro-inflammatory cytokine interleukin-6 (IL-6) with siltuximab, the only FDA-approved therapy for iMCD, is an effective treatment for approximately one-half of patients. A better understanding of the pathogenic mechanisms underlying iMCD is critically needed to uncover new potential treatments for patients. To characterize the immune dysregulation in iMCD, previous analysis of patient blood during active disease identified tumor necrosis factor (TNF) signaling as a highly enriched pathway in iMCD and indicated that T cells, which can produce TNF, were activated in iMCD. However, whether TNF production by activated T cells was a key mechanism promoting iMCD was unclear. To determine if T cells contribute to the characteristic cytokine storm in iMCD, we investigated the ability of T cells from iMCD patients to produce different inflammatory cytokines including TNF. Upon stimulation with phorbol myristate acetate and ionomycin (PMA/I), naïve CD4⁺ T cells from iMCD patients produced significantly more TNF compared to healthy controls suggesting that CD4⁺ T cells in iMCD patients hyper-respond to stimulation specifically by producing excess TNF.

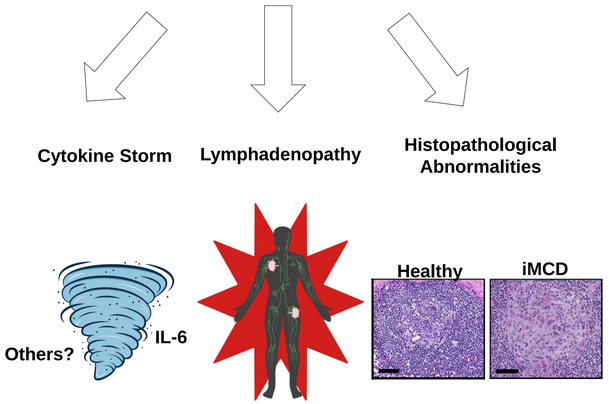
In parallel to these analyses, we leveraged KGML-xDTD, a knowledge graph- based machine learning framework to predict potential novel drug treatments for iMCD. The top three novel predicted treatments for iMCD included two TNF inhibitors, adalimumab and certolizumab pegol. We then used the reinforcement learning module of KGML-xDTD to predict the mechanism linking adalimumab to iMCD, which included IL-4, IL-6, IL-8, IL-10, STAT3, and CD4 further implicating TNF by CD4-expressing T cells in iMCD pathogenesis.

As proof-of concept that TNF promotes iMCD pathogenesis and a relevant therapeutic target, we report the successful treatment of a highly refractory iMCD patient with off-label use of a TNF blocker. When a 50-year old iMCD patient was experiencing multi-organ system dysfunction and preparing for hospice care after not responding to IL-6 inhibition, IL-1 inhibition, Bruton's tyrosine kinase (BTK) inhibition, chemotherapy, and autologous stem cell transplantation, we initiated treatment with adalimumab alongside BTK inhibition. Within 3 days of the first infusion, the patient's symptoms and organ dysfunction began to improve and the patient has been in remission for over 6 months.

We utilized a translational research approach including experimental and unbiased machine learning approaches to identify TNF as a novel therapeutic target that we inhibited to treat a highly refractory iMCD patient. Together, our data suggest that over-production of TNF, in part by activated T cells, promotes iMCD pathogenesis and highlight that further research is needed into TNF inhibition as a potential treatment strategy for iMCD.

Background

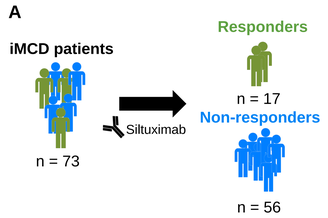
Idiopathic Multicentric Castleman Disease (iMCD)



What other pathways 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. Serum proteomic analysis reveals up-regulation of TNF signaling



B

Pathway	Nominal *P* value	FDR q value
Responders vs. Healthy Donors		
TNFα signaling via NF-κB	.004	0.090
Estrogen response early	.013	0.137
IFN-γ response	.033	0.149
Allograft rejection signature	.033	0.167
IL-6-JAK STAT3 signaling	.020	0.184
Non-Responders vs. Healthy Donors		
KRAS signaling up	.029	0.118
IL-6-JAK STAT3 signaling	.031	0.144
TNFα signaling via NF-κB	.006	0.173
Allograft rejection signature	.043	0.177
IL2 STAT5 signaling	.018	0.179

Figure 2: Analysis of serum proteome using Somalogic's Somoscan in iMCD patients compared to controls. (A) Groups and number of individuals analyzed in this study. Serum was collected from patients as part of a siltuximab clinical trial.[1] iMCD = idiopathic multicentric Castleman disease. (B) Using serum collected prior to siltuximab treatment, pathway analysis of proteome was performed on siltuximab responders or non-responders compared to healthy controls.[2] Top up-regulated pathways are shown.

II. Increase in T cell activation

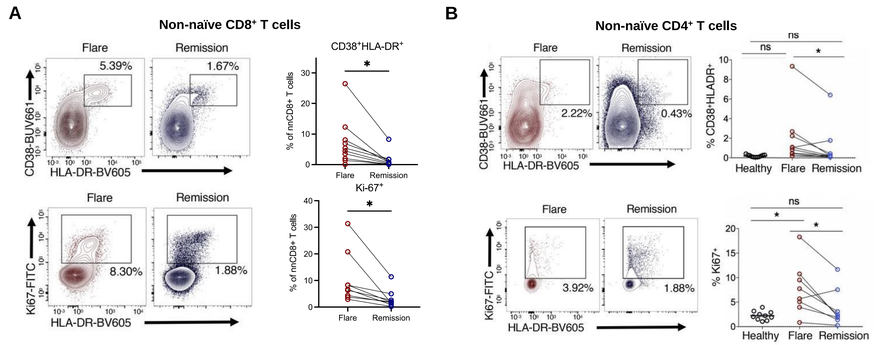


Figure 3: Flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) from iMCD patients in flare or remission.[1] (A) Non-naïve CD8⁺ T cells (CD45⁺CD3⁺CD45RA⁻CCR7⁻) and (B) non-naïve CD4⁺ T cells show markers of activation (CD38⁺HLA-DR⁺) and proliferation (Ki-67⁺) during iMCD flare. n=10. *p<0.05.

III. Naïve CD4⁺ T cells produce excess TNF

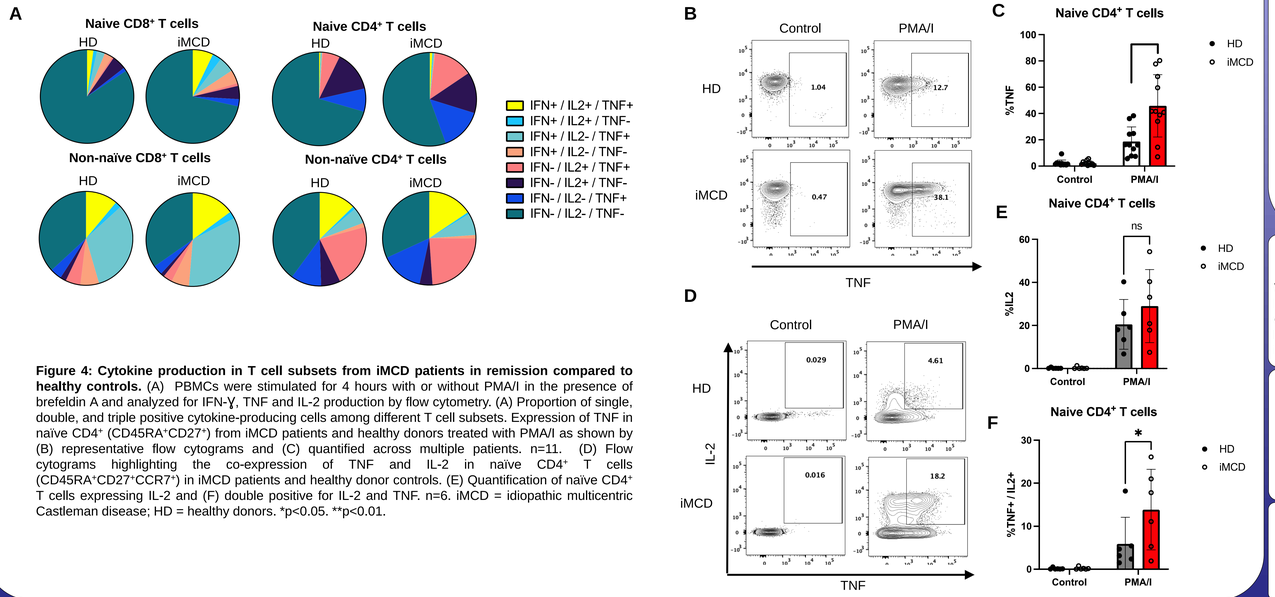


Figure 4: Cytokine production in T cell subsets from iMCD patients in remission compared to healthy controls. (A) PBMCs were stimulated for 4 hours with or without PMA/I in the presence of brefeldin A and analyzed for IFN-γ, TNF and IL-2 production by flow cytometry. (A) Proportion of single, double, and triple positive cytokine-producing cells among different T cell subsets. Expression of TNF in naïve CD4⁺ (CD45RA⁺CD27⁺) from iMCD patients and healthy donors treated with PMA/I as shown by (B) representative flow cytograms and (C) quantified across multiple patients. n=11. (D) Flow cytograms highlighting the co-expression of TNF and IL-2 in naïve CD4⁺ T cells (CD45RA⁺CD27⁺CCR7⁺) in iMCD patients and healthy donor controls. (E) Quantification of naïve CD4⁺ T cells expressing IL-2 and (F) double positive for IL-2 and TNF. n=6. iMCD = idiopathic multicentric Castleman disease; HD = healthy donors. *p<0.05. **p<0.01.

IV. TNF production is immediate

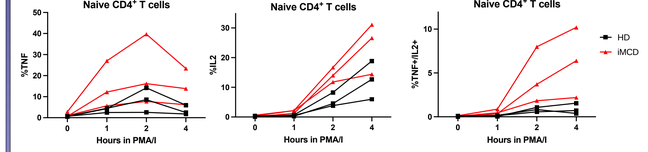
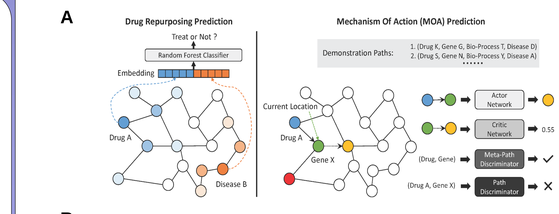


Figure 5: Cytokine production over time in naïve CD4⁺ T cells from iMCD patients in remission or healthy donor controls. PBMCs from iMCD patients or healthy donor (HD) controls were stimulated with PMA/I for indicated times and analyzed for TNF and IL-2.

V. Knowledge graph predicts TNF inhibition as novel treatment strategy



B

TOP Predicted Treatments

Rank	Drug	Mechanism	Score
1	ADALIMUMAB	anti-TNF	0.8374
2	RITUXIMAB	B cell depletion	0.8339
3	CERTOLIZUMAB PEGOL	anti-TNF	0.8261
4	CYCLOPHOSPHAMIDE	cytotoxic	0.8164
5	METHYLPREDNISOLONE	corticosteroid	0.8159
63	RUXOLITINIB	anti-JAK1/2	0.7701
177	TEMSIROLIMUS	mTOR inhibitor	0.7533
253	SARILUMAB	anti-IL6R	0.7473

C

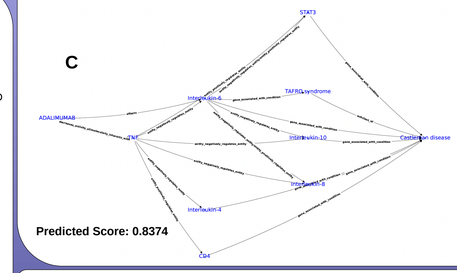


Figure 6: KGML-xDRD, a knowledge graph-based machine learning network, predicts TNF inhibition as a novel therapeutic strategy for iMCD. (A) Utilizing 3,659,165 nodes and 18,291,237 edges, from 70 public biomedical sources, we trained a random forest-based machine learning algorithm on true positive and true negative treatment relationships. (B) The top three novel predicted treatments for iMCD included two TNF inhibitors, adalimumab and certolizumab pegol, and the B cell depleting agent, rituximab, the second most prescribed drug for iMCD after siltuximab. (C). The reinforcement learning module of KGML-xDTD predicted the mechanism linking adalimumab to iMCD, which included IL-4, IL-6, IL-8, IL-10, STAT3, and CD4 .

VI. TNF inhibition treats iMCD

When a 50-year old iMCD patient was experiencing multi-organ system dysfunction and preparing for hospice care after not responding to IL-6 inhibition, IL-1 inhibition, Bruton's tyrosine kinase (BTK) inhibition, chemotherapy, and autologous stem cell transplantation, we initiated treatment with adalimumab alongside BTK inhibition. Within 3 days of the first infusion, the patient's symptoms and organ dysfunction began to improve and the patient has been in remission for over 6 months.

Conclusions

1) Experimental and unbiased machine learning approaches identify TNF as an important pathogenic mediator of iMCD and a novel therapeutic target.
2) Flow cytometric data suggest that phenotypically naïve CD4⁺ T cells contribute to iMCD pathogenesis, in part by over-producing TNF.

References

1. van Rhee F, Wong RS, Munshi N, Rossi JF, Ke XY, Fossa A, et al. Siltuximab for multicentric Castleman's disease: a randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2014;15(9):966-74.
2. Pierson SK, Shenoy S, Oromendia AB, Gorzewski AM, Langan Pai RA, Nabel CS, et al. Discovery and validation of a novel subgroup and therapeutic target in idiopathic multicentric Castleman disease. Blood Adv. 2021;5(17):3445-56.
3. Pai RL, Japp AS, Gonzalez M, Rasheed RF, Okumura M, Arenas D, et al. Type I IFN response associated with mTOR activation in the TAFRO subtype of idiopathic multicentric Castleman disease. JCI Insight. 2020;5(9).