Identification of Autoantibodies Associated with Connective Tissue Disorders in Idiopathic Multicentric Castleman Disease

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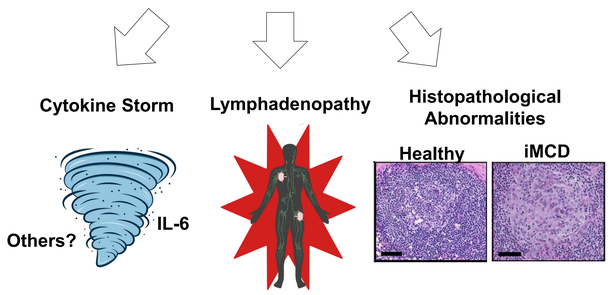
Background

- Idiopathic multicentric Castleman Disease (iMCD) is a rare, atypical lymphoproliferative disorder with significant morbidity and mortality and unknown etiology. (**Fig. 1**)
- A fundamental question is whether iMCD should be considered an infectious disease caused by an as-yet-unknown pathogen, an autoimmune disease caused by autoantibodies, an autoinflammatory disease caused by germline mutations, or a neoplastic disease caused by somatic mutations in a clonal cell population.

Idiopathic Multicentric Castleman Disease (iMCD)



Do autoantibodies contribute to iMCD pathogenesis?

Figure 1: Characteristics of idiopathic multicentric Castleman disease (iMCD). iMCD 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), but the pathology is not fully understood. The goal of this project is to identify autoantibodies that may be contributing to disease pathogenesis.

- We constructed two custom bead-based protein arrays to screen serum samples for autoantibodies from iMCD patients and healthy controls. (**Fig. 2, Table 1, and Fig. 3**)

1) The Connective Tissue Disease (CTD) array consisted of 52 antigens associated with traditional CTDs. Antigens are separated by six subcategories of CTDs (Scleroderma, Myositis/Overlap Syndromes, Systemic lupus erythematosus SLE)/Sjögren's Syndrome, GI/Endocrine, DNA-Associated, and Inflammation/Stress).

2) The Anti-Cytokine Autoantibody (ACA) array included 38 cytokines, chemokines, and cell surface proteins.

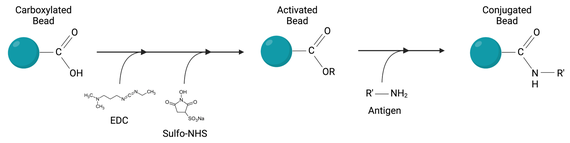


Figure 2: Two custom, bead-based antigen arrays were constructed using Sulfo-NHS/EDC (carbodiimide) crosslinking between uniquely color-coded carboxylated magnetic and recombinant protein antigens.

Clinical Cohort and Experimental Workflow

	UP (n = 38)	UAMS (n = 45)	OU (n = 18)
Sex [Percent; (N)]			
Female	51.3% (19)	40.0% (18)	33.3% (6)
Male	48.7% (18)	60.0% (27)	66.7% (12)
Age at diagnosis [Median (IQR)]	37.9 (25.6-47.8)	45.7 (35.5-57.3)	56.5 (36.3-63.0)
Subtype [Percent; (N)]			
TAFRO	56.8% (21)	28.9% (13)	0.0% (0)
IPL	10.8% (4)	17.8% (8)	5.6% (1)
POEMS	2.7% (1)	0.0% (0)	0.0% (0)
NOS	29.7% (11)	53.3% (24)	94.4% (17)
Autoimmune comorbidity [Percent; (N)]	10.8% (4)	13.3% (6)	NA
Flare/Remission [Percent, (N)]			
Flare	41.9% (18)	37.3% (19)	50.0% (13)
Remission	58.1% (25)	62.7% (32)	50.0% (13)

Table 1, Three cohorts of iMCD patients from the University of Pennsylvania (UP, n = 44 total samples from 38 subjects), the University of Arkansas for Medical Sciences (UAMS, n = 51 samples from 45 subjects), and Osaka University (OU, n = 26 samples from 18 subjects) as well as healthy controls (n = 30). Note: A number of patients were on a variety of medications including siltuximab, tocilizumab, rituximab, and chemotherapy/corticosteroids at time of blood draw.

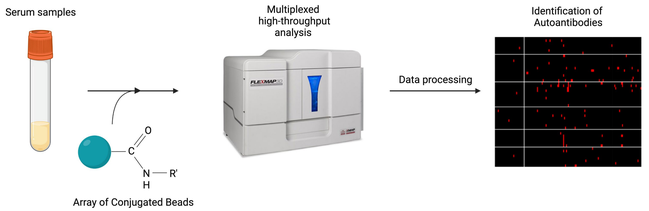
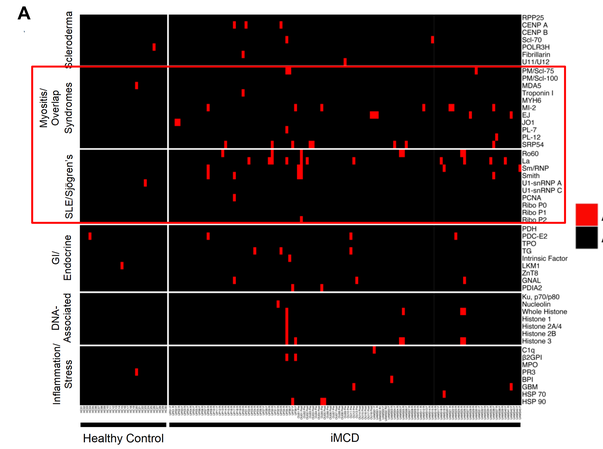


Figure 3: Serum samples were probed with each of the two arrays and analyzed using a FlexMap3DTM instrument (Luminex Corp.). Binding events were measured as Median Fluorescence Intensity (MFI). Serum samples were considered "positive" for autoantibodies targeting a specific antigen if the normalized MFI was greater than 5 standard deviations (SDs) above the average MFI for HC for that antigen and greater than 3,000 units

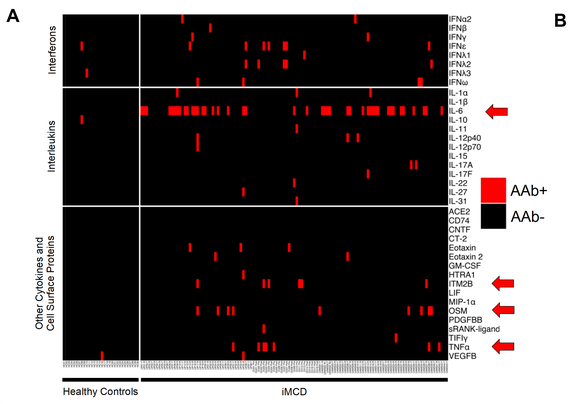
Autoantibodies associated with Connective Tissue Diseases (CTD) are prevalent in iMCD patients



- Overall, 46% of iMCD subjects were positive for a CTD autoantibody compared to 17% of HCs (OR 4.1, *P* = 0.005). (**Fig 4A**)
- Autoantibodies associated with Myositis and SLE (red box) trended higher in iMCD patients (but did not pass multiple testing correction).
  - IgG autoantibodies associated with CTDs, including anti-Mi2, anti-SRP54, anti-La, anti-Ro, and anti-Histone H3 were common in iMCD compared to HC (**Fig 4 B, C**)

Figure 4. (**A**) Heatmap displaying serum IgG AAbs identified by a 52-plex array of autoantigens associated with traditional CTDs. Red indicates an AAb positive hit for a specific antigen and sample. The cutoff for AAb positivity was an MFI value > 5 SD above the average MFI for HC and an MFI value > 3,000 units. (**B**) Dot plots comparing MFI values for five of the most targeted autoantigens associated with myositis in iMCD patients (n = 101) and HC (n = 30). MFI values above the red line indicate an autoantibody positive hit. (**C**) Dot plots displaying five of the most targeted autoantigens associated with SLE.

IgG Anti-Cytokine Antibodies (ACA) are common in iMCD patients



- Anti-cytokine autoantibodies (ACAs) were common in iMCD patients
  - IL-6 autoantibody was the most common in iMCD. This was expected as many were on siltuximab (IL-6 monoclonal antibody to IL-6, red arrow **Fig. 5A**)
- Anti-OSM, anti-TNF, and anti-ITM2B were also common in iMCD patients (red arrows, **Fig. 5A, Fig. 5B**)

Figure 5. (A) Heatmap displaying serum IgG ACAs identified by a 38-plex array of cytokines, chemokines, growth factors, and receptors. Red indicates an AAb positive hit for a specific antigen and sample. The cutoff for AAb positivity was an MFI value > 5 SD above the average MFI for HC and an MFI value > 3,000 units. **(B)** Dotplots comparing MFI values in iMCD subjects (n = 101) and HC (n = 30) for nine cytokines and cell surface proteins that were most commonly targeted by autoantibodies in iMCD subjects. MFI values above the red line indicate an autoantibody positive hit.

IgG autoantibodies fluctuate between flare and remission states and with anti-IL-6R treatment (Tocilizumab)

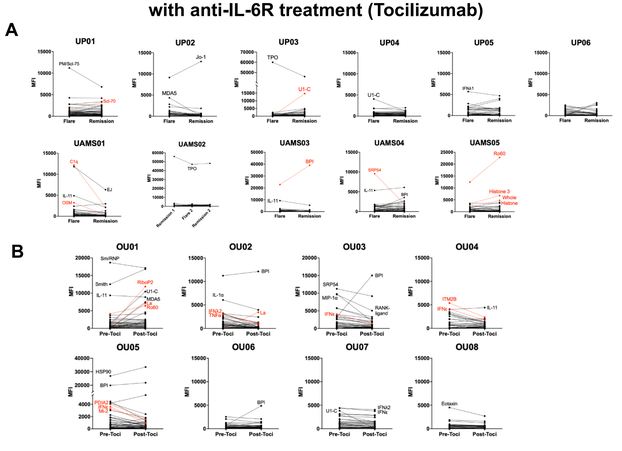


Figure 6. (A) Paired dotplots of longitudinal flare and remission serum samples. Significant increases or decreases in MFI levels between timepoints are highlighted with red text and red lines. (**B**) Paired dotplots of longitudinally paired samples before and after Tocilizumab treatment for eight OU subjects. Significant increases or decreases in MFI levels between timepoints are denoted by red text and red lines

- Longitudinal flare and remission serum samples were available for 11 iMCD subjects (n = 6 UP subjects; n = 5 UAMS subjects). Additionally, paired samples before and after Tocilizumab treatment for eight OU subjects were available from the Osaka University cohort (**Fig. 6 A,B**).
- Significant increases or decreases in MFI levels were determined by: 1) > 50% increase or decrease in MFI; 2) MFI > 3,000 for the following timepoint (increases) or for previous timepoint (decreases); and 3) MFI > 3 SDs above the mean MFI of HC for the following timepoint (increases) or for the previous timepoint (decreases). (red text, **Fig. 6A,B**)
- Autoantibody levels fluctuate over time and may fluctuate based on disease state. Investigation of specific autoantibodies in iMCD is ongoing.

Conclusions

1) Overall, 46% of iMCD subjects were positive for a Connective Tissue Disorder (CTD) autoantibody compared to 17% of HCs (OR 4.1, P = 0.005).
2) Although not surviving multiple hypothesis correction, autoantibodies associated with myositis and SLE trended higher in iMCD patients.
3) IgG autoantibodies associated with CTDs, such as anti-Mi2, anti-SRP54, anti-La, anti-Ro, and anti-Histone H3 were common in iMCD patients compared to HC.
4) Anti-cytokine autoantibodies (ACAs) such as anti-OSM, anti-TNF, and anti-ITM2B were also common in iMCD patients.
5) Autoantibody levels in iMCD subjects fluctuate over time, and autoantibodies that are associated with specific autoimmune diseases may fluctuate depending on disease state.