Distinct cell-bound complement activation signatures are observed in patients with systemic lupus erythematosus

Rebecca E. Schriefer[1], Gabriel R. Arguelles[1], Lynne M. Mitchell[1], Stephen T. Oh[2], Gregory F. Wu[3], Elisha D.O. Roberson[1], John P. Atkinson[1], Dennis E. Hourcade[1], and Alfred H.J. Kim[1]

Division of [1]Rheumatology, [2]Hematology, Department of Medicine, [3]Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

ACR 2019: #1038

Introduction

- Complement activation and consumption is a hallmark of SLE pathophysiology.
- Cell-bound complement activation products (CB-CAPs) have previously been shown to associate with SLE disease activity, but only a small fraction of total CB-CAPs has been examined.
- Leveraging mass cytometry, we have developed and validated a panel capable of comprehensively characterizing the types and quantities of CB-CAPs and complement receptors on human PBMCs.
- This will uniquely position us to identify SLE patient stratifications based on CAPs signatures.

Methods

- Paired PBMC samples (flare and remission) were obtained from three consented subjects with classified SLE (using 1997 ACR or 2012 SLICC criteria) from the Lupus Clinic at Washington University.
- Mass-tag barcoded PBMCs were stained using a validated set of antibodies (Table 1) and run on a Helios-upgraded CyTOF2 mass cytometer.
- Data were analyzed in Cytobank (tSNE [t-Distributed Stochastic Neighbor Embedding], FlowSOM [Flow Self-Organizing Maps]) and R (UMAP [Uniform Manifold Approximation and Projection], MEM [Marker Enrichment Modeling]).

Results

- Using a 30-marker panel designed to identify immune cell subsets and CAPs (Table 1), we found that SLE B cells handled the greatest load of CB-CAPs compared to T cells and monocytes.
- Evidence of classical, alternative, and common pathway activation was observed on SLE B cells, but not every cell activated completely through the complement cascade (Figure 1). This suggests that certain cell types may be able to regulate subsequent complement activation.
- Transitional, mature/naïve, and certain memory B cell pools possessed CB-CAPs during flare, which substantially decreased after remission (Figure 2).
- After dimensionality reduction (UMAP), clustering analysis (FlowSOM, with up to ten clusters generated), and identifying variables contributing to each cluster's formation (MEM), unique complement activation signatures were identified in B cells from flaring SLE subjects (Figure 3).

Discussion

- A nontrivial proportion (~25%) of B cells activated either classical or alternative pathway but did not have evidence of common pathway activation. Alternatively, certain signature subsets were able to activate C5 without clear evidence of classical or alternative pathway activation. These data strongly suggest that each step in complement activation may be regulated. The physiologic meaning of this remains unknown.
- Further improvements to this panel are underway, including:
  - Additional complement receptors (i.e. C3aR, C5aR1, C5aR2, CR1, CR3, CR4)
  - Ability to detect *intracellular* CAPs
  - Functional/activation markers of B cells

Results (cont.)

Table 1. Complement antibody panel for mass cytometry

Antibody	Label	Antibody	Label	Antibody	Label
CD45	089Y	CD21	152Sm	CD40	165Ho
CD46 (MCP)	141Pr	MAC (C5b-9)	153Eu	CD24	166Er
CD19	142Nd	CD3	154Sm	CD27	167Er
CD55 (DAF)	143Nd	Properdin (Factor P)	155Gd	iC3b (neo)	169Tm
CD38	144Nd	C4c	156Gd	C3d (C3b-iC3b-C3d)	170Er
CD138	145Nd	CD10	158Gd	Bb (Bb-FB)	171Yb
IgD	146Nd	CD22	159Tb	IgM	172Yb
CD20	147Sm	CD11c	161Dy	C5	174Yb
C4d (C4c-C4d)	148Nd	CD79b	162Dy	C1q	175Lu
CD14	151Eu	CD95	164Dy	CD59	176Yb

Figure 1. B cell-bound complement activation products correlate with SLE disease activity

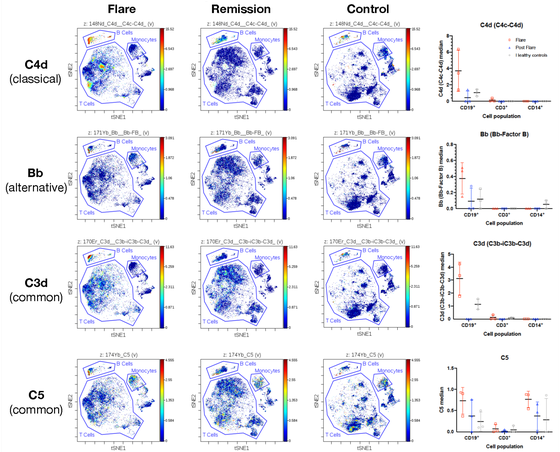


Figure 2. SLE B cell subsets differentially possess CB-CAPs

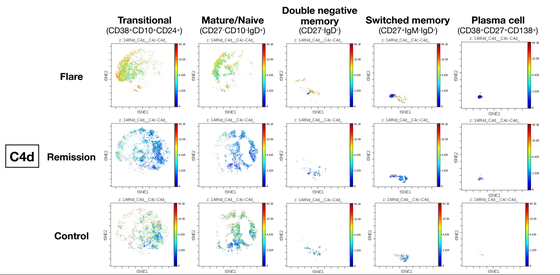
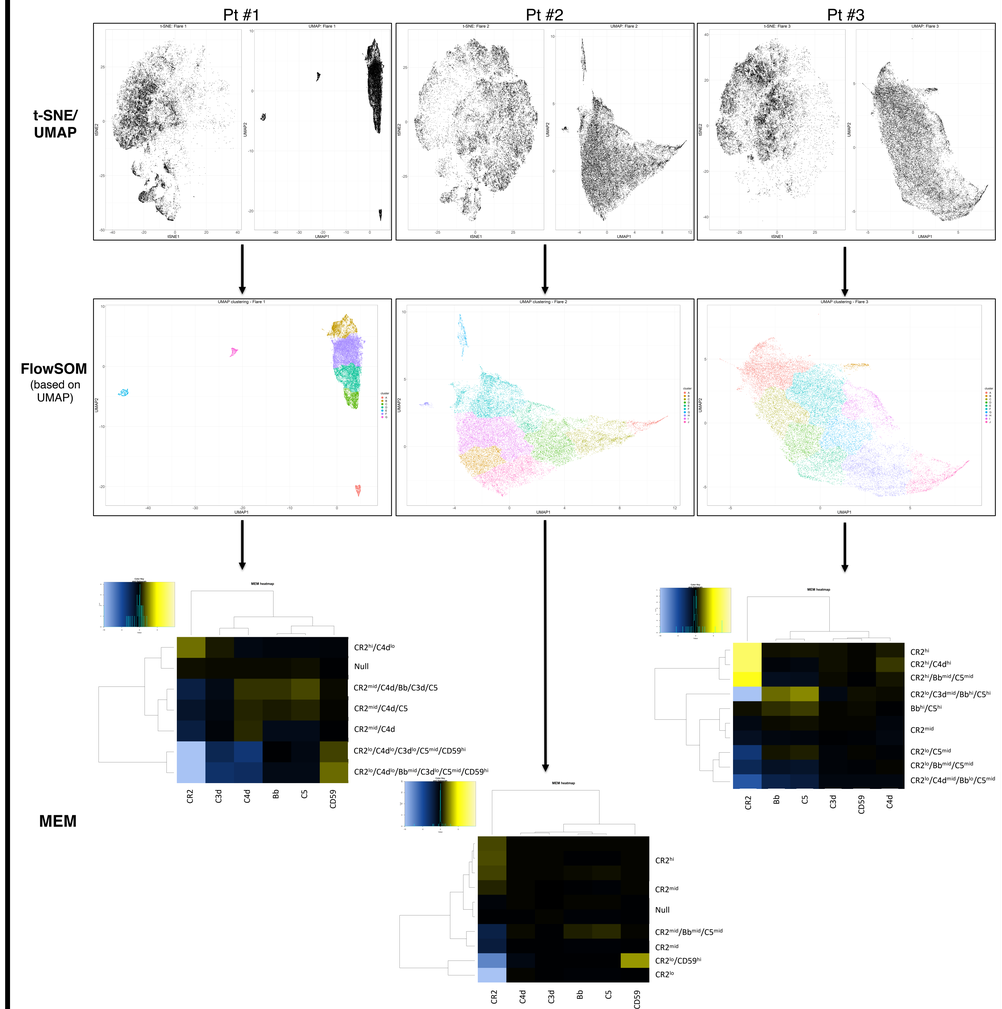


Figure 3. CB-CAPs signatures can be identified on flaring SLE B cells



Mass cytometry can define cell-bound complement activation signatures on human SLE PBMCs.

Contact:
akim@wustl.edu
@alhkim (Twitter)

Funding

This research was funded/supported by NIH/NIAMS (R21AR069833 to AHJK), NIH/NIAID (R21AI123789 to DEH) and Doris Duke Charitable Foundation (to AHJK).