Characterization of cell-bound complement activation products on SLE PBMCs using mass cytometry

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Introduction

o Complement activation and consumption is a hallmark of SLE pathophysiology.

o 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.

o 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.

o This will uniquely position us to identify SLE patient stratifications based on CAPs signatures.

Methods

o 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.

o Mass-tag barcoded PBMCs were stained using a validated set of antibodies (Table 1) and run on a Helios-upgraded CyTOF2 mass cytometer.

o Data were analyzed in Cytobank (viSNE, FlowSOM).

Results

o Using a 30-marker panel designed to identify immune cell subsets and CAPs, we found that SLE B cells handled the greatest load of CB-CAPs compared to T cells and monocytes.

o Evidence of classical, alternative, and common pathway activation was observed on SLE B cells, but not every cell activated completely through the complement cascade. This suggests that certain cell types may be able to regulate subsequent complement activation.

o Transitional, mature/naïve, and certain memory B cell pools possessed CB-CAPs during flare, which substantially decreased after remission.

o Using FlowSOM, 9 metaclusters each representing an unique complement activation signature were identified in B cells from flaring SLE subjects.

Discussion

o We found that 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 the regulation of each step in complement activation can be regulated. The physiologic meaning of this remains unknown.

o Further improvements to this panel are underway, including:

  o Additional complement receptors (i.e. C3aR, C5aR1, C5aR2, CR1, CR3, CR4)
  o Ability to detect *intracellular* CAPs
  o Functional/activation markers of B cells

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

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Results (cont.)

Table 1. Complement antibody panel

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

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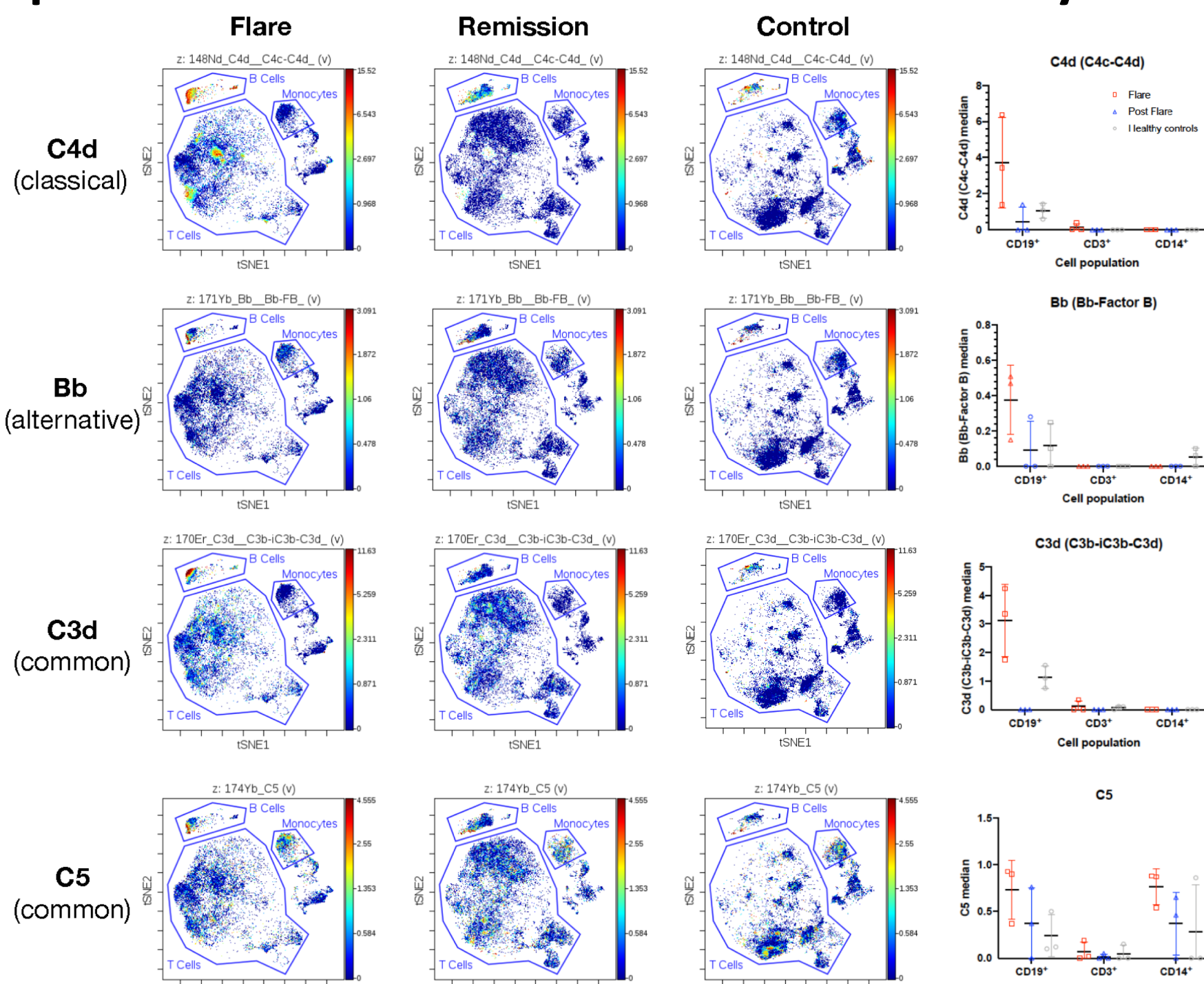

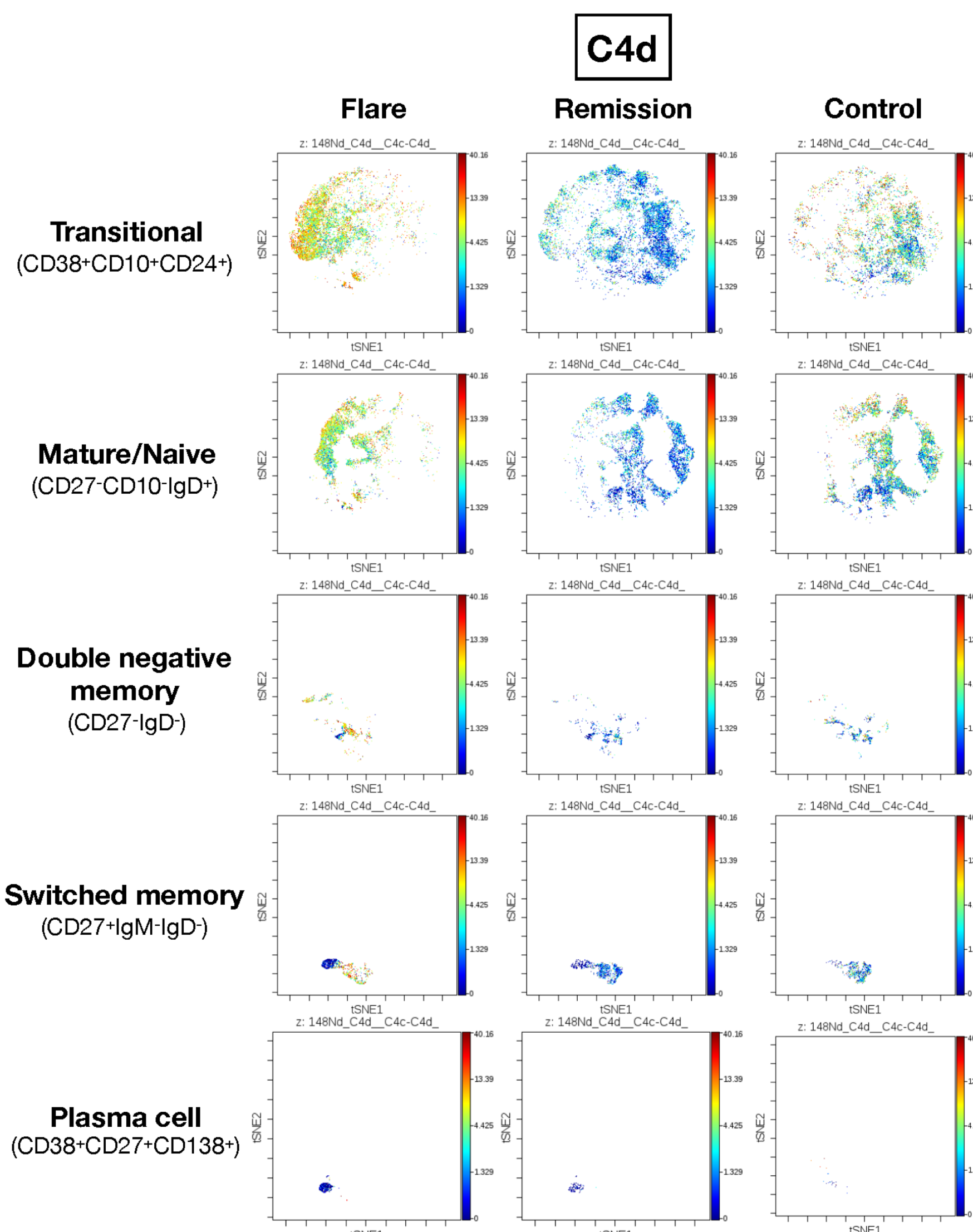
Figure 2. SLE B cell subsets differentially possess CB-CAPs

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