In Rheumatoid Arthritis (RA) Decreases in Conventional Dendritic Cell Lineages are Associated with Adverse Measures of Myocardial Function and Expansions of Anomalous HLA-DR$^+$ Myeloid subsets

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Abstract

Background/Purpose:

Dendritic cells (DC) are specialized antigen-presenting cells (APC), considered to have a central role in regulating immune responses. Prior studies, which we confirm, showed numeric decreases of 'canonical' myeloid (mDC) and plasmacytoid (pDC) DC in RA, and led us to hypothesize that depletion of canonical DC is only one manifestation of a more fundamental alteration in these important immunoregulatory cells. We further hypothesized that alterations in DC subsets are associated with aspects of RA including those driving RA-associated cardiovascular disease and that the totality of non-lymphoid APC candidate cells (CD3$^-$CD19$^-$HLA-DR$^+$) would not decrease in RA and include anomalous candidate APC.

Methods:

RA patients enrolled in a cohort study and healthy donors underwent immunophenotyping to define known DC subsets, including CD1c$^+$ mDC, CD141$^+$ mDC and CD303$^+$ pDC in peripheral blood mononuclear cells (PMBCs). We correlated DC subset features with disease characteristics and cardiac function. To screen for non-lymphoid cells with putative APC potential not conforming to conventional DC paradigms we defined 'candidate nonlymphoid APC' (DR$^+$CD3$^-$CD19$^-$CD56$^-$) as a DC superset. We used t-Distributed Stochastic Neighbor Embedding (t-SNE) to categorize the entirety of non-lymphoid APC candidate cells and identify possible novel RA APC subsets.

Results:

Circulating CD1c$^+$ frequencies were remarkably decreased in RA (mean 1.0% of PBMCs vs controls (2.6%);; p=0.009). Mean CD141$^+$ frequencies were 0.2% vs 0.1%; p=0.019. CD303$^+$ pDC frequencies did not differ (p=0.285). HLA-DR intensity in both myeloid subsets was significantly lower in RA whereas CCR2 intensity was higher in CD1c$^+$ DC. In RA, a lower CD1c$^+$ frequency was associated with lower cardiac index (correlation=-0.54, p=0.0078; n=24) and CCR2 expression of CD1c$^+$ DC was associated with lower Ejection Fraction (EF) (corr.=-0.695, p=0.0003; n=22). Interestingly, despite the decrease in canonical DC, the overall frequencies of 'candidate nonlymphoid APC' and 'candidate mDC APC' did not differ (Table 1) suggesting that the declines of canonical DC were compensated for by increases in other populations of this DC superset. t-SNE analysis of healthy controls allowed clear categorization with distinct clusters of APC subsets as well as a homogenous cluster of CD14$^-$CD16$^-$CD11c$^+$(Figure 2; orange circle). RA t-SNE showed a decrease of canonical DC subsets (yellow ellipses) and the appearance of five clusters with some DC features that did not fit accepted definitions (Figure 2a-e)

Conclusion:

We found extensive alterations in the DC compartment in RA, including a significant decrease in the major CD1c$^+$ subset and the emergence of anomalous HLA-DR$^+$ non-lymphoid cells with antigen-presenting potential that are candidate DC subsets. The novel subsets included one resembling CD14+ monocyte-derived DC, a subset of which also expressed CD1c in addition to plasmacytoid markers; CD303, CD123. The remaining populations were distinguished by combinations of CCR2, CD11c, CD123 and CD172a (SIRPα). The association of adverse measures of cardiac function with changes of the DC compartment, including decreases of CD1c$^+$ mDC, emphasize their potential clinical significance.

Introduction

- **<u>Dendritic cells</u>** (DC) **<u>are highly plastic antigen-presenting cells</u>** (APC), considered to have a central role in initiating and regulating immune responses[1] including T cell activation, an established pathophysiologic pathway in RA (Fig. 0)

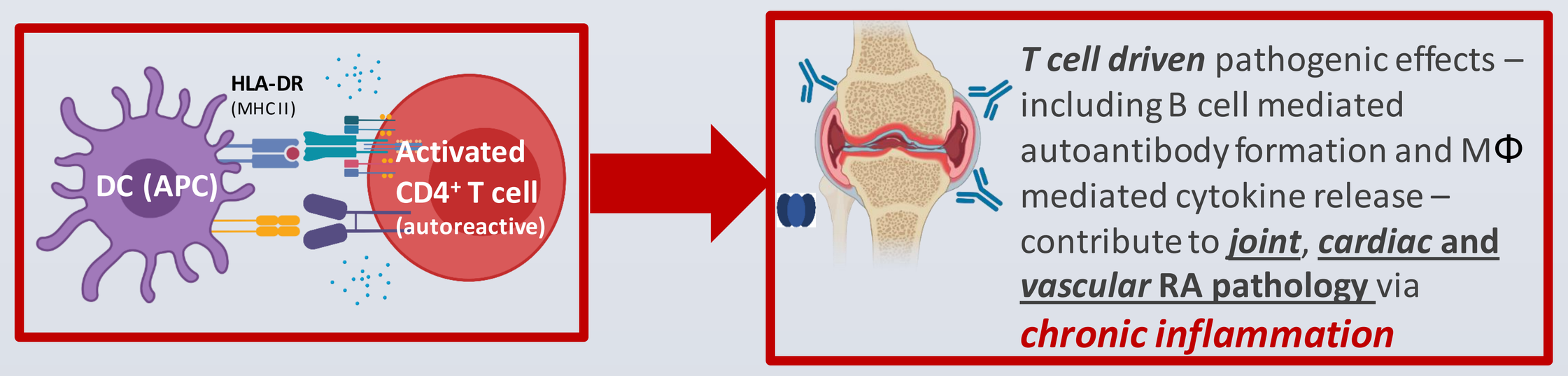

Fig. 0: DC via the DC/T cell Interface have the potential to activate pathogenic T cells in RA

- Prior studies showed, unexpectedly, numeric ***decreases* of '*canonical' DC*** subsets [myeloid (mDC) and plasmacytoid (pDC) DC] in RA[2]. We suspected that the decrease of ***canonical DC*** may not be the only change within this highly plastic immuno-regulatory compartment
- ***By defining a 'non-lymphoid candidate APC'*** DC superset inclusive of *all* non-lymphoid cells with APC potential [HLA-DR$^+$CD3$^-$CD19$^-$CD56$^-$CD14^{lo}] ***we set out to test the hypotheses that in RA***:

1. The <u>totality</u> of ***non-lymphoid candidate APC*** would ***NOT*** decrease
2. *Non-lymphoid candidate APC* would be characterized by **the appearance of '*anomalous*' phenotypes** [not conforming to established definitions]
3. That shifts within the APC compartment **would be associated with clinical RA characteristics**, including adverse measures of cardiovascular health

Methods

31 RA patients enrolled in a cohort study and healthy donors underwent immunophenotyping to define known DC subsets, including **CD1c$^+$ mDC**, **CD141$^+$ mDC** and **CD303$^+$ pDC** in peripheral blood mononuclear cells (PMBCs). We correlated DC subset features with disease characteristics and cardiac function. To screen for non-lymphoid cells with putative APC potential not conforming to conventional DC paradigms we defined 'candidate nonlymphoid APC' (DR$^+$CD3$^-$CD19$^-$CD56$^-$) as a DC superset. We used t-Distributed Stochastic Neighbor Embedding (t-SNE) to categorize the entirety of non-lymphoid APC candidate cells and identify possible novel RA APC subsets.

Results

1A) We confirm decreases of 'canonical' mDC subsets in RA (Fig. 1)

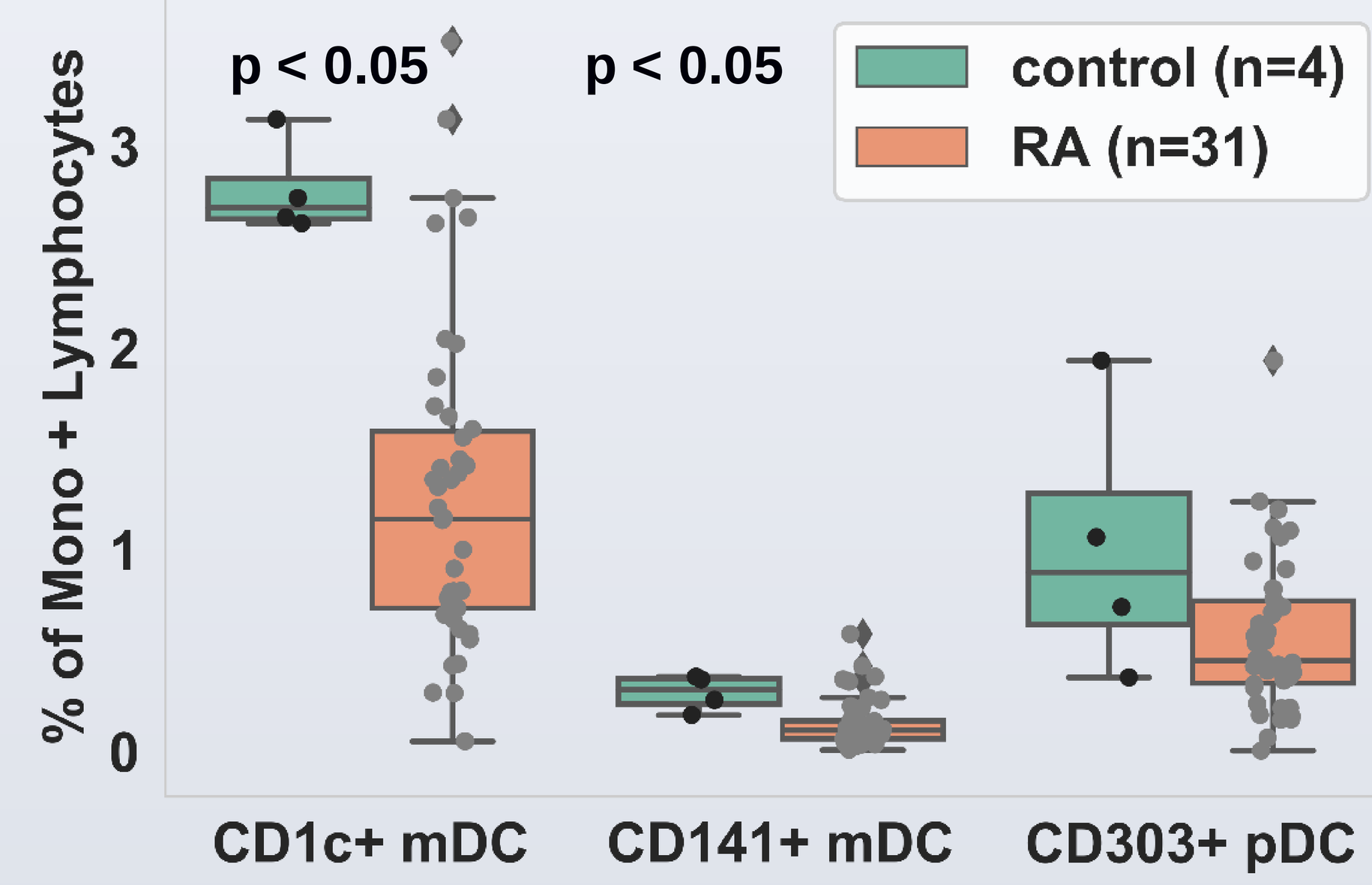

Fig. 1: Enumeration of 'canonical' DC subsets.

1B) RA DC subsets showed lower HLA-DR expression and higher CCR2 expression compared with controls. (Table 1)

	RA (n=31)	controls (n=4)	p
Live PBMC analyzed	188255 [146909,290563]	163257 [147186,194461]	0.593
Dendritic cells analyzed	3889 [1465.8,5601.5]	5974 [3916.0,8670.7]	0.165
CD1c$^+$ cDC			
% of PBMCs	1.2 [0.7,1.6]	2.6 [2.2,2.9]	0.006
HLA-DR MFI	35067 [24107,40366]	50513 [47347,52586]	0.009
CCR2 MFI	17702 [14611,19381]	13188 [12840,13975]	0.033
CD141$^+$ cDC			
% of PBMCs	0.1 [0.1,0.2]	0.2 [0.2,0.3]	0.019
HLA-DR MFI	22279 [14672,25703]	46890 [43845,50418]	0.002
CCR2 MFI	5506 [4190,6937]	3669 [3617,4017]	0.069
CD303$^+$ pDC			
% of PBMCs	0.5 [0.3,0.7]	0.8 [0.6,1.1]	0.285
HLA-DR MFI	19115 [14493,23608]	18504 [17768,19496]	0.915
CCR2 MFI	14794 [12798,15978]	11782 [11205,12054]	0.028
Reference APCs			
'Candidate nonlymphoid APC' Frequency in % PBMC	13.7 (5.6)	12.6 (4.3)	0.667
'Candidate mDC APC' Frequency in % PBMC	8.2 (3.7)	8.5 (2.9)	0.857
B cell (CD19$^+$) HLA-DR MFI	18943 [16549,23917]	22991 [21605,24727]	0.149
Monocytes (CD14$^+$) HLA-DR MFI	12780 [10321,14755]	16264 [15287,18343]	0.022

Table 1: Phenotypic characteristics of DC subsets in RA vs controls. Numbers in average +-(SD) resp. mean [IQR] **MFI: median fluorescence intensity**

2A) The *<u>total</u> non-lymphoid candidate APC* population **<u>was *not* decreased</u>** (Table 2)

	RA (n=31)	controls (n=4)	p
Live PBMC analyzed	188255 [146909,290563]	163257 [147186,194461]	0.593
Dendritic cells analyzed	3889 [1465.8,5601.5]	5974 [3916.0,8670.7]	0.165
'Candidate nonlymphoid APC' Frequency in % PBMC	13.7 (5.6)	12.6 (4.3)	0.667
'Candidate mDC APC' Frequency in % PBMC	8.2 (3.7)	8.5 (2.9)	0.857

Table 2: Enumeration of DR$^+$ DC supersets
'Candidate nonlymphoid APC' defined as HLA-DR$^+$CD3$^-$CD19$^-$CD56$^-$CD14lo
'Candidate mDC APC' defined as HLA-DR$^+$CD3$^-$CD19$^-$CD56$^-$CD14^{lo}CD11c$^+$]

2B) In RA, the *non-lymphoid candidate APC* superset included APC that clustered near canonical DC but did not meet currently accepted definitions (Fig. 2; a-e)

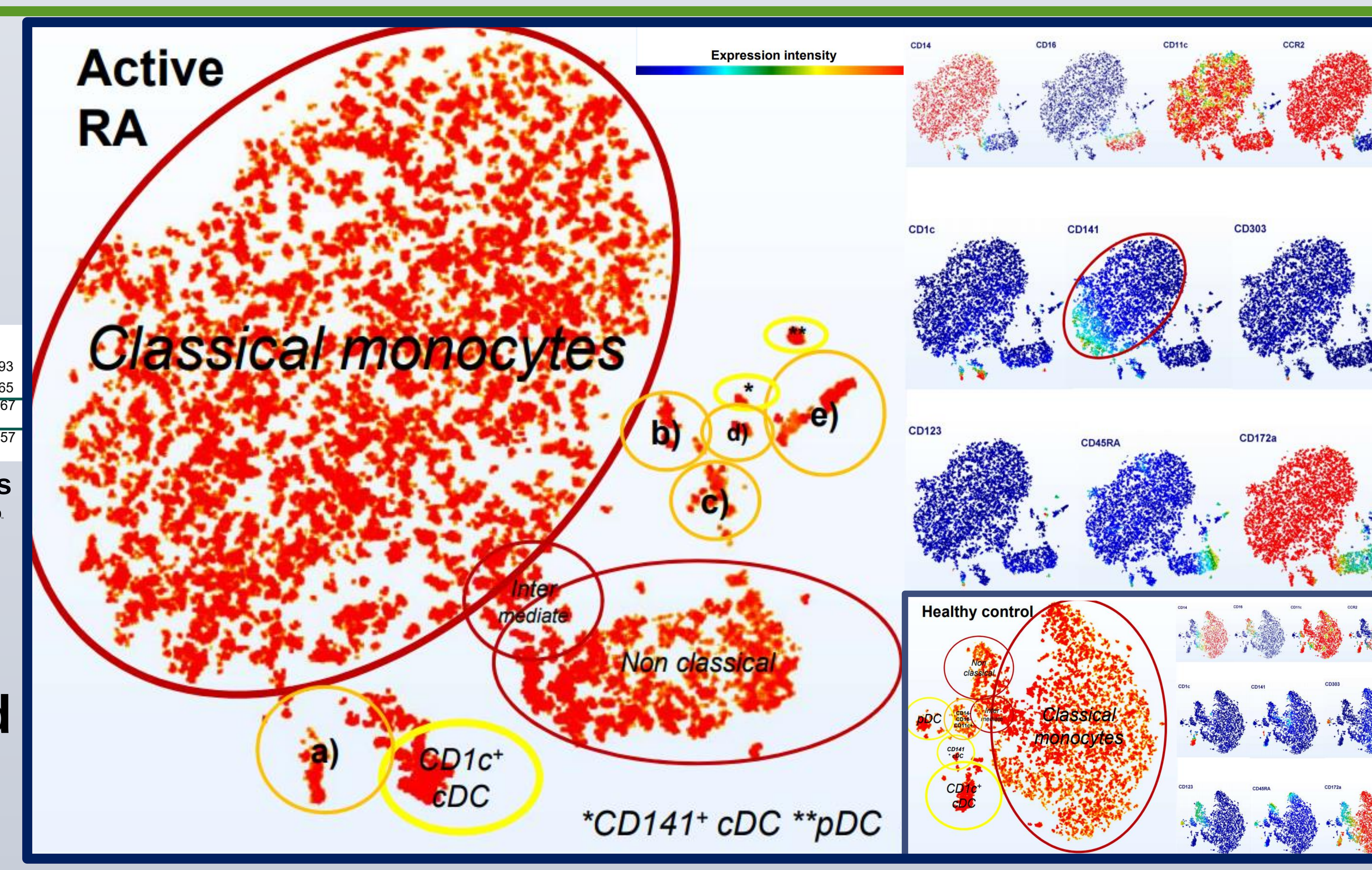

Fig. 2: Totality of *non-lymphoid candidate APC (DR$^+$CD3$^-$CD19$^-$) of a patient with active RA (CDAI 29) and a healthy donor (gray insert).* Representative *t-SNE* plots shown.

3) A higher fraction of non-canonical APC was associated with lower EF (Fig. 3a). Lower canonical CD1c$^+$ cDC percentages showed reciprocal associations with **lower systolic function; increased aortic FDG uptake** (Fig. 3b-c)

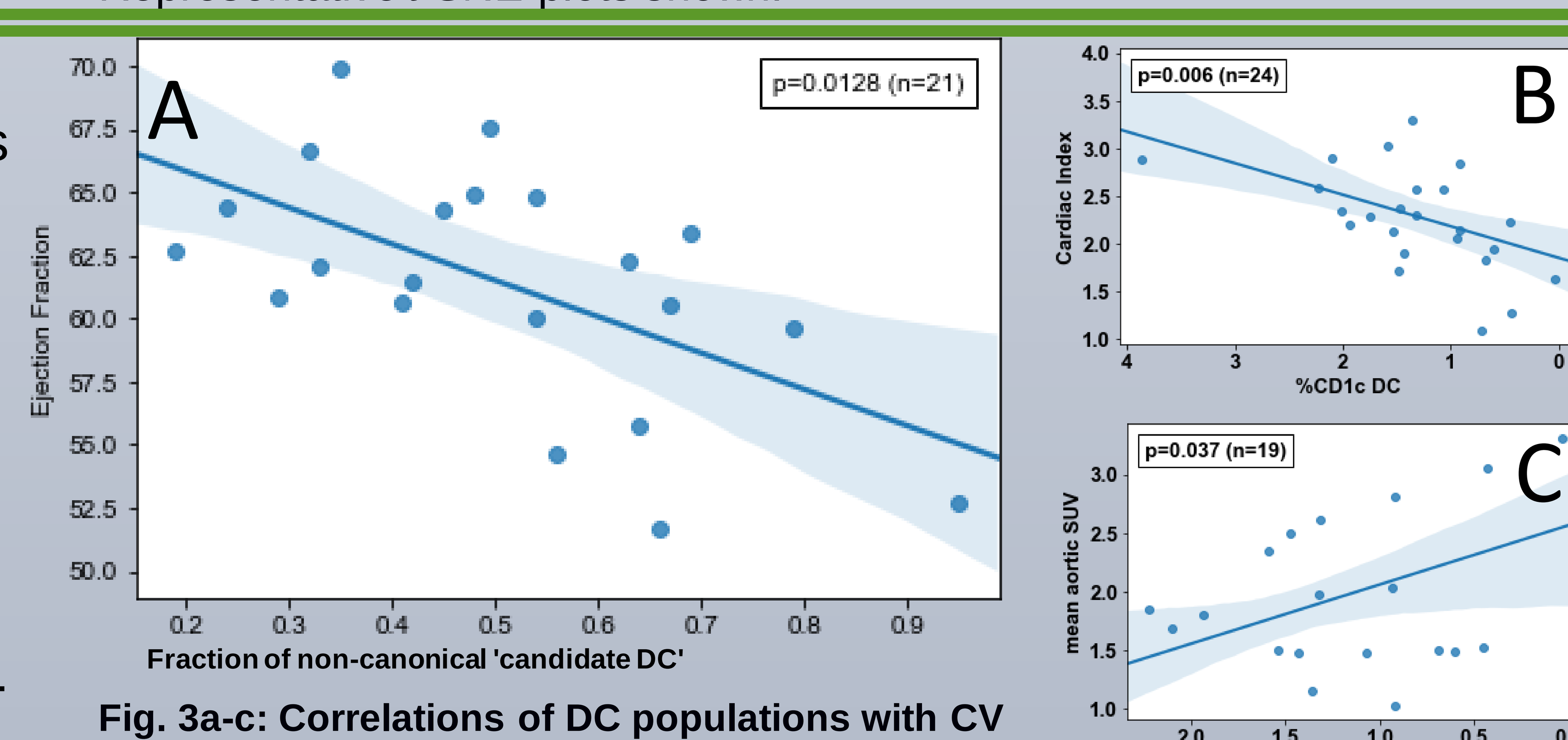

Fig. 3a-c: Correlations of DC populations with CV measures a) Ejection Fraction b) Cardiac Index c) Aortic SUV

Conclusion

We found **extensive perturbations in the non-myeloid APC compartment in RA:**

1) A **significant decrease and phenotypic alterations of 'canonical' mDC populations** – most pronounced in the major CD1c$^+$ subset – that included decreased HLA-DR and increased CCR2 expression **suggestive of increased propensity to traffic to inflamed tissues**
2) The concurrent **emergence of anomalous non-lymphoid cells** with antigen-presenting potential (HLA-DR$^+$) that are candidate DC subsets. The largest population included CD14$^+$ APC that showed a partial loss of CD1c expression resembling recently defined DC3 by *Dutertre et al*.[3] The remaining populations were distinguished by combinations of CCR2, CD11c, CD172a (SIRPα) and plasmacytoid markers; CD303, CD123
3) The **association of adverse measures of cardiovascular health with changes of the DC compartment, including decreases of CD1c$^+$ mDC**, emphasize their potential **clinical significance**.

Functional analyses of the putative DC populations are needed.

Anomalous DC may be implicated in RA rendering the DC/T cell interface a potential therapeutic target in RA

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