Supplementary material:

T-cell subset: staining, flow cytometry strategies and reported frequencies

Subset quantification was performed for naive, IRC and Treg cells. Flow cytometry was performed on 6 ml of fresh EDTA blood, using NHS-routine immunology services. Flow cytometry was performed as previously described[1]. Briefly, naïve and IRC CD4+T-cell subsets were identified based on their expression of CD45RB-FITC (antibody clone details in **Table S1**), CD45RA-PE and CD62L-APC. Treg were quantified by cell surface staining for CD4-Pacifc blue, CD25-APC and CD127-PE followed by intracellular staining for FOXP3-FITC using the anti-human Foxp3 staining kit (Insight Biotechnology, Wembley, UK).
Staining was performed according to the NHS- routine procedures which were similar to that used previously in the research laboratory, but using 250 ul of blood per panel. Flow cytometry analysis was performed on a QUANTO cytometer (BD), using BD Biosciences FACSDIVA software. The gating strategies are presented in **Figure S1a.**
Subset frequencies were recorded as percentage of gated CD3+/CD4+ T-cells. There is an age relationship between naïve and Treg frequencies as shown in **Figure S1b**. For naïve and Treg, subset frequencies were then corrected for age as previously described [1] and reported as age corrected % of CD4+T-cells using the heathy control range: [Age corrected %] = [frequency observed in patient] - [frequency expected at that age]. The latter being calculated from the age-subset frequency correlation observed in120 HC. This is not a statistical correction but a practical variable normalisation using positive vs negative parameter rather than a percentage value. We have changed this to normalised %: [Normalised %] = [frequency observed in patient] - [frequency expected at that age].

T-cell subset: no difference between cs-DMARDs and b-DMARDs

T-cell subset data were analysed with respect to drug intake (**Figure S2a**). There was no significant difference between groups for any of the subsets.
Glucocorticoid data were not recorded in this cohort. Previous data on early, DMARD naive RA at inclusion into our register showed no effect previous glucocorticoid on any of the T-cells subsets (**Figure S2b**).

Reference

1.Ponchel F, Goëb V, Parmar R, et al. An immunological biomarker to predict MTX response in early RA. Annals of the rheumatic diseases 2014;73(11):2047-53.

Table 1S: Antibody clones used for each panel.

Panel 1: naïve and IRC	Clone	company
CD4-BV421	RPA-T4	BD
CD3-V500	UCHT1	BD
CD45RB-FITC	MEM-55	Serotec
CD45RA-PE	F8-11-13	Serotec
CD62L	130-091-755	Miltenyi
Panel 2: Treg		
CD4-BV421	RPA-T4	BD
CD3-V500	UCHT1	BD
CD25-Pe-Cy7	2A3	BD
FOXP3-AF488	236/E7	BD
CD127- PERCP-Cy5.5	M21	BD

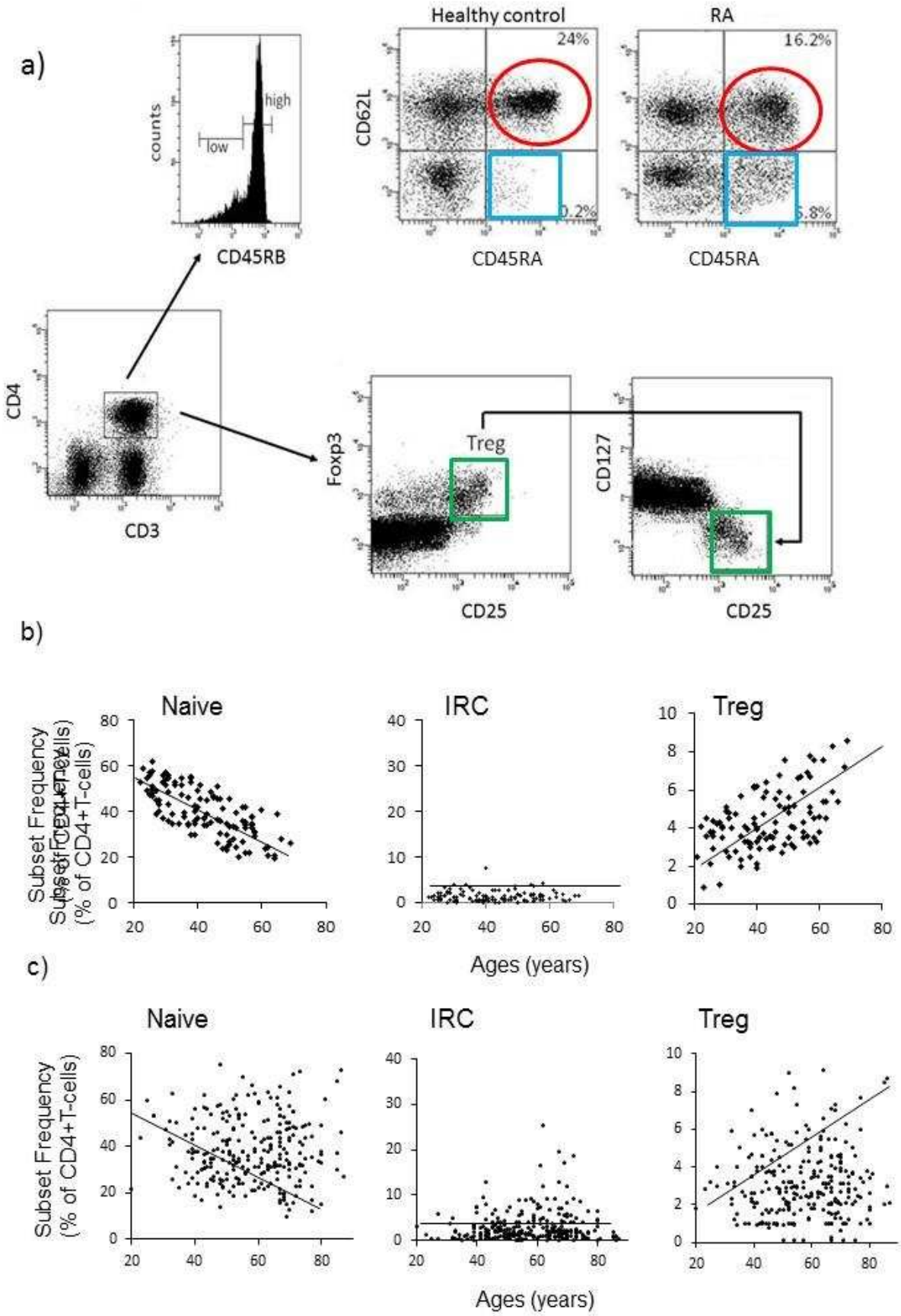
a)
Healthy control
RA
counts
low
high
CD45RB
CD62L
24%
16.2%
0.2%
5.8%
CD45RA
CD45RA
CD4
CD3
Foxp3
Treg
CD127
CD25
CD25
b)
Naive
IRC
Treg
Subset Frequency
(% of CD4+T-cells)
Ages (years)
c)
Naive
IRC
Treg
Subset Frequency
(% of CD4+T-cells)
Ages (years)

Figure S1: Flow cytometry analysis. a) Representative flow cytometry plot for naïve (red circle, CD45RBhigh/CD45RA+/CD62L+), IRC (blue box CD45RA+/CD62L-) and T-reg (green box FoxP3+/CD25+/CD127-) following gating on CD3+CD4+ T-cells. Difference between health and RA are highlighted for naïve/IRC subsets. **b)** established age relationship in 120 healthy controls for naïve and Treg CD4+T-cells. [expected naïve] = -0.63 x [age] +66.6 (rho=0.850, p<0.0001); [expected Treg] = +0.061 x [age] +1.83 (rho=0.554, p=0.001). IRC were not related to age. IRC were considered high when above the 95% CI of distribution (set at 4%). **c)** Data from RA patient in clinical remission (naïve/IRC, n=297; Treg n=260). Lines represent expected values for naïve and Treg cell with respect to age (i.e. as in HC in panel b) and the top 95% CI of the HC distribution for IRC.

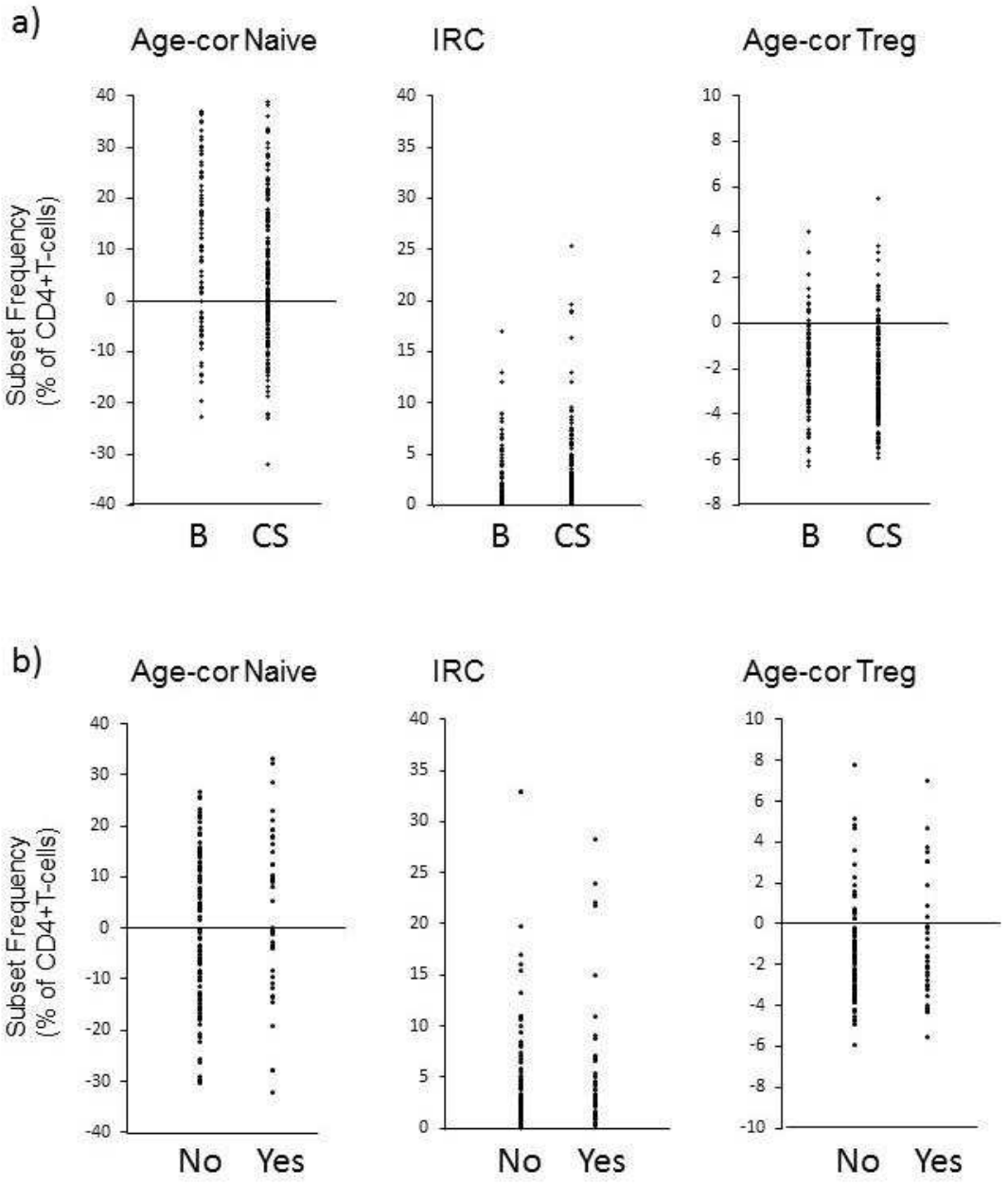

Figure S2: T-cell subset frequencies. a) Subsets frequencies in patients in DAS-remission displayed with respect to drugs : B: b-DMARDs and CS: cs-DMARDS) **b)** Subsets frequencies in early inflammatory arthritis patients, DMARDs-naive (n=154) displayed with respect to glucocorticoid (yes n=41 / no n=113) being used prior to the subset being tested. No significant difference was observed for either comparison.