Regulatory T cells enhance Th17 migration in psoriatic arthritis which is reversed by anti-TNF

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Summary

Regulatory T cells (Treg) prevent the migration of effector T cells toward sites of inflammation thereby limiting disease progression. We investigated this aspect of Treg function using psoriatic arthritis (PsA) as an exemplar of chronic inflammation. Patients with PsA had an increased Th17: Treg ratio which was reversed by anti-TNF therapy. Utilising an *in vitro* migration assay, Treg from patients with PsA treated with conventional therapy paradoxically boosted CCR6$^+$ effector T cell (a surrogate for Th17) migration towards CCL20. In contrast, Treg from PsA patients treated with anti-TNF suppressed CCL20 driven effector T cell migration. The boosting effect of TNF blockade upon Treg suppression of migration was accompanied by increased effector T cell CCL20 production and enhanced interaction between Treg and effector T cells. This study provides mechanistic insight into Treg modulation of effector T cell migration in patients with chronic inflammation and how this can be targeted by therapy.

Introduction

CD4$^+$FoxP3$^+$ regulatory T cells (Treg) play a critical role in maintaining autoimmunity through limiting effector T cell proliferation and cytokine production (Miyara et al., 2014). Analysis of Treg function in humans has been dominated by *in vitro* assays measuring suppression of effector T cell proliferation, and more recently inhibition of pro-inflammatory cytokine production. A less well documented, but potentially important function is the ability of Treg to inhibit effector T cell migration, which has been explored in murine models of inflammation (Paust et al., 2016; Ring et al., 2006; Tischner et al., 2006). Several studies have demonstrated the important role of Treg chemokine receptor expression in guiding Treg to sites of inflammation (Afshar et al., 2013; Ding et al., 2012). However, how Treg control effector T cell migration in patients with chronic inflammatory disease and the impact of therapy remain unknown.

Psoriatic arthritis (PsA) is a chronic inflammatory condition, which develops in 10-30% of patients with psoriasis (Durham et al., 2015). CCR6 mediated Th17 migration is thought to play an important role in the pathogenesis of the disease, and Th17 cells have been detected at increased numbers in synovial fluid and peripheral blood of these patients (Benham et al., 2013). The anti-IL-17 monoclonal antibody secukinumab has demonstrated efficacy in treating PsA validating IL-17 as an important driver of disease (McInnes et al., 2015). Therefore, we utilised an *in vitro* assay to study inhibition of CCR6$^+$ effector T cell migration by Treg as a surrogate of Treg suppression of T cell migration in human inflammation and applied this assay to samples from patients with PsA. We hypothesised that Treg would be impaired in their ability to control CCR6$^+$ effector T cell, as a surrogate indicator of Th17 (Annunziato et al., 2007), migration in PsA thereby failing to prevent the accumulation of IL-17 within inflamed joints.

Results

The Th17:Treg ratio was increased in patients with psoriatic arthritis (PsA) but reversed by anti-TNF therapy

The percentage of CD4$^+$IL-17$^+$ T cells was greater in the peripheral blood of PsA patients (Supplementary Table S1) receiving conventional therapy (disease modifying anti-rheumatic drugs, DMARD) compared to healthy controls (Fig. 1A). This increase in Th17 in PsA patients was reversed by anti-TNF therapy (adalimumab) (Fig. 1A). The percentage (Fig. 1A) and absolute number (Fig. 1B) of CD4$^+$IL-17$^+$ cells in synovial fluid were significantly elevated compared to peripheral blood from PsA DMARD treated patients. In contrast, CD4$^+$FoxP3$^+$ Treg frequency and number were reduced in the peripheral blood of DMARD treated patients compared to healthy controls (Fig. 1C-D) which has been noted before (Wang et al., 2020). Peripheral blood Treg numbers were restored to normal in patients treated with anti-TNF (Fig. 1C-D). Higher numbers of CD4$^+$FoxP3$^+$ Treg were present within synovial fluid compared to peripheral blood from patients with PsA treated with conventional therapy (Fig. 1C-D). These changes in Treg and Th17 numbers in PsA led to a heightened Th17:Treg ratio in the peripheral blood of DMARD PsA patients which was substantially reduced by anti-TNF therapy (Fig. 1E). Indeed, there was a negative correlation between the frequency of Th17 and Treg in the peripheral blood of PsA patients (Fig. 1F). There was also a marked increase in the Th17:Treg ratio in PsA synovial fluid compared to the peripheral blood (Fig. 1E).

Treg from PsA patients treated with anti-TNF were more potent suppressors of effector T cell IFN-γ production compared to Treg from PsA patients receiving conventional therapy.

We next tested whether PsA Treg could limit effector T cell cytokine production. *In vitro* suppression assays were performed using effector T cells and Treg purified from peripheral blood (Fig. S1). The percentage suppression of IFN-γ effector T cell production by Treg from adalimumab treated PsA patients was greater than their counterparts from DMARD treated patients (Fig. 2A). Healthy Treg were unable to suppress effector T cell IL-17 production and both DMARD and adalimumab PsA Treg were similarly impaired (Fig. 2B). Indeed, IL-17 production tended to increase when Treg were added to effector T cells in the presence of monocytes.

Treg from anti-TNF treated PsA patients suppressed effector T cell migration which was associated with enhanced Treg-effector T cell interaction.

We proceeded to address whether Treg suppressor function extended to limiting effector T cell migration towards CCL20, which is an essential chemoattractant for CCR6+ Th17 cells (Hirota et al., 2007). We found that healthy Treg had no significant effect on effector T cell migration towards CCL20 *in vitro* (Fig. 3A). Unexpectedly, Treg from untreated and DMARD treated PsA patients enhanced effector T cell migration. In contrast, Treg from adalimumab treated patients suppressed effector T cell migration (Fig. 3A). The chemotactic index of effector T cells, in the absence of Treg, in both DMARD and adalimumab treated PsA were significantly higher than in healthy controls (Fig 3B). Treg migration from adalimumab treated patients, in the presence of their corresponding effector T cells, was significantly reduced compared to Treg from DMARD treated PsA, and healthy controls (Fig. 3C). Thus, the ratio of effector T cell to Treg migration was significantly increased in patients treated with adalimumab compared to DMARD treated patients and healthy individuals (Fig S2B). We used forward and side scatter analysis to identify cell doublets as a surrogate for Treg and effector T cell interaction in the upper chamber of the transwell (Fig S2C). The upper compartment of the transwell contained significantly more cell doublets when Treg and effector T cells from adalimumab treated patients were co-cultured, compared to T cells from PsA patients receiving DMARDs and healthy controls (Fig. 3D and Fig. S2C). Effector T cells and Treg were added at a 3:1 ratio to the top compartment of the transwell, but these proportions shifted towards parity after co-culture when effector T cells and Treg were isolated from adalimumab treated patients (Fig. 3E).

We postulated that the production of CCL20 by Th17 cells (Hirota et al., 2007; Wilson et al., 2007) in the upper chamber was competing with the CCL20 in the lower compartment to attract the co-cultured Treg. Effector T cells from patients treated with adalimumab showed increased CCL20 production compared to DMARD treated patients, reaching similar levels found in effector T cells from synovial fluid (Fig 3F). Effector T cells in the doublets analysed from the upper chamber of the transwell had higher expression of CCL20 compared to effector T cells in peripheral blood, with the greatest increase in those from adalimumab treated patients (Fig 3G). The frequency of CCR6+ Treg (and CCR6 Treg expression) was significantly reduced in the peripheral blood of adalimumab treated patients compared to untreated and DMARD PsA (Fig S3B and D). Similar levels of frequency and expression of CCR6 were found in effector T cells in PsA irrespective of treatment (Fig S3A and C).

Discussion

The main finding of these data is that Treg differentially modulate CCR6 mediated cell migration in PsA providing insight into the properties of Treg in an inflammatory disease and the impact of therapy. Treg from DMARD treated PsA patients promoted CCR6 mediated effector T cell migration towards the chemokine CCL20 which could promote inflammation and worsen disease. In contrast, Treg from anti-TNF treated patients suppressed CCL20 driven effector T cell migration. Our data supports the hypothesis that the increased interaction between effector T cells and Treg, from anti-TNF treated patients, in the upper chamber of the transwell reduced the numbers of effector T cells migrating towards the CCL20 in the lower compartment. We postulate that the increased CCL20 production by effector T cells competes with the CCL20 added to the lower compartment, reducing the migration of Treg and promoting the formation of Treg-effector T cell conjugates, thereby also reducing their own transmigration to the lower chamber. The reduced CCR6 expression on circulating Treg from anti-TNF treated patients could contribute to their reduced migration towards the distant CCL20 in the bottom compartment of the transwell, and may favour an interaction with neighbouring CCL20 producing effector T cells. Moreover, the lower CCR6 expression on Treg from patients receiving anti-TNF therapy could account for their heightened suppressive properties, both in respect of inhibiting effector T cell migration and cytokine production, in light of previous data showing expression of this chemokine receptor can disable Treg function (Kulkarni et al., 2018). It remains unclear where Treg are exerting their effects on migration of effector T cells. Data from murine models of arthritis suggest Treg may act in the draining lymph nodes rather than the inflamed joints (or peripheral blood) (Oh et al., 2010; Ohata et al., 2007), which would explain our observations that Th17 cells were reduced in the peripheral blood after anti-TNF therapy.

The enhanced migration of effector T cells driven by Treg in PsA is consistent with the enrichment of Th17 cells in synovial fluid, and associated with increased production of IL-17 at the site of inflammation (Benham et al., 2013). This pro-inflammatory effect of Treg in PsA may represent another manifestation of their instability during inflammation, in addition to their well described propensity to release IL-17 (Beriou et al., 2009; Koenen et al., 2008). Treg accelerated CCR6 mediated Th17 migration, compounded by their inability to control Th17 production which has also been reported in rheumatoid arthritis (RA) (McGovern et al., 2012), would likely worsen disease. Indeed, Treg can themselves produce IL-17, which could exacerbate inflammation (Koenen et al., 2008). Of note, anti-TNF did not endow Treg with the ability to suppress IL-17 production by

effector T cells, in contrast to patients with RA (McGovern et al., 2012). However, anti-TNF did increase peripheral blood Treg numbers in patients with PsA compared to those treated with conventional therapy reminiscent of findings in RA (McGovern et al., 2012).

Our findings suggesting that changes in effector T cell chemokine production in anti-TNF treated patients could modulate the ability of Treg to function at sites of inflammation are reminiscent of the boosting properties of effector T cells upon Treg in a murine model of diabetes (Grinberg-Bleyer et al., 2010). Th17 cells produce CCL20 (Wilson et al., 2007) which could represent a negative feedback mechanism to attract Treg and dampen inflammation. Increased interaction between Treg and effector T cells would likely limit migration into the joint and pro-inflammatory cytokine release. Anti-TNF therapy confers upon Treg the ability to suppress effector T cell migration as well as more efficient suppression of IFN-γ, revealing a potential mechanism of action for this treatment in PsA. Resistance to Treg suppression by effector T cells may contribute to the differences observed in effector T cell migration in the presence of Treg from the different patient groups. Additional studies are needed to demonstrate the clinical utility of the *in vitro* migration assay utilised here as a measure of Treg function in other chronic inflammatory diseases, and whether Treg suppressing effector T cell migration is an important mechanism of action of anti-TNF therapy. Further examination of this axis of regulation could provide new therapeutic avenues to treat psoriatic arthritis as well as extend these findings to other diseases characterised by chronic inflammation.

Limitations of the study

The principle limitation of this research is the use of an *in vitro* assay to study control of effector T cell migration by Treg. This was necessary as we wanted to study human Treg rather than utilise an animal model of disease, where relevance to disease us unclear and response to targeted therapy does not always match mechanisms in patients. The findings presented here do not prove that anti-TNF works via Treg suppressing effector T cell migration. In addition, if Treg are acting in draining lymph nodes to prevent egress of effector T cells into the inflamed joint, it would have been valuable to have data from this tissue. Finally, it would have been useful to study the synovial tissue of patients treated with anti-TNF to ascertain the changes in Treg and Th17 cells, although these patients rarely have synovial fluid due to their response to treatment

Figure Legends

Figure 1. Anti-TNF therapy reversed the increased Th17:Treg ratio in patients with psoriatic arthritis.

Peripheral blood was taken from healthy individuals, DMARD and anti-TNF (adalimumab) treated PsA patients, and synovial fluid (SF) was harvested from PsA patients where available. Representative and cumulative data indicating the percentage (**A**) and absolute numbers (**B**) of CD4$^+$IL-17$^+$ and (**C-D**) CD4$^+$FoxP3$^+$ T cells detected by flow cytometry in peripheral blood or synovial fluid. **E**: Ratio of CD4$^+$IL-17$^+$: CD4$^+$FoxP3$^+$ T cells in healthy individuals and patients with PsA. **F**. Correlation between CD4+Foxp3+ Treg and CD4+IL-17+ in PsA patients (DMARD and anti-TNF treated). Data are represented as mean +/- SD from 17 healthy individuals, 10 DMARD, 14 adalimumab, 4 synovial fluid. Mann Whitney U tests and unpaired t tests were used to to determine significance. $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$.

Figure 2. Anti-TNF therapy enhanced Treg suppression of effector T cell IFN-γ production in psoriatic arthritis.

CD4$^+$Treg (Treg: CD4$^+$CD25^{high}CD127$^-$), CD4$^+$ effector T cells (Teff: CD4$^+$CD25$^-$CD127$^+$) and CD14$^+$ monocytes were isolated from peripheral blood of healthy individuals, DMARD and adalimumab treated PsA patients. Teff were stained with CFSE, monocytes stained with PKH-26® and Treg left unlabelled. Teff:Treg:monocytes were cultured at a 3:1:1 ratio with anti-CD3. Representative and cumulative data show percentage suppression of IFN-γ (**A**) and IL-17 (**B**) production by effector T cells in the presence of Treg. Data are represented as mean +/- SD from healthy individuals n=5, DMARD treated PsA patients n=4, adalimumab treated PsA patients n=6. Unpaired t tests were used to to determine significance. $p < 0.05^*$.

Figure 3. Regulatory T cells enhanced CCR6 driven effector T cells migration in psoriatic arthritis which was reversed by anti-TNF.

A-E: CD4$^+$Treg (Treg: CD4$^+$CD25^{high}CD127$^-$) and CD4$^+$ effector T cells (Teff: CD4$^+$CD25$^-$CD127$^+$) were purified from peripheral blood of healthy individuals, untreated, DMARD and adalimumab treated PsA patients. Effector T cells were stained with CFSE and Treg were left unlabelled. Effector T cells (150,000) were placed in the upper compartment of a transwell (with CCL20 in the lower chamber) either alone or with CD4$^+$ Treg (Effector T cell: Treg ratio was 3:1) and co-cultured for 3 hours. **A**: Representative flow cytometry plot showing the number of effector T cells migrating towards CCL20 (or media alone) in the presence or absence of Treg. Cumulative data represented as mean +/- SD, show the percentage suppression of migration of effector T cells by Treg in the different groups. Healthy individuals n=9, untreated PsA n=5, DMARD treated PsA patients n=6, adalimumab treated PsA patients n=9. **B**: Chemotactic index of effector T cells migrating towards CCL20 in the absence of Treg from healthy individuals, DMARD and adalimumab treated PsA patients. **C**: Chemotactic index of Treg in the presence of their matched effector T cells. **D-E**: The upper chambers of the transwells containing effector T cells and Treg from the chemotaxis assays were harvested and cell doublets identified based on forward and side scatter. Cumulative data showing (**D**) cell doublet number, (**E**) with effector T cells and Treg separately enumerated in the cell doublets contained within the upper chamber, with a representative flow cytometry plot gated on cell doublets discriminating effector T cell and Treg based on CFSE staining of effector T cells. Healthy individuals n=5, DMARD treated PsA patients n=4, adalimumab treated PsA patients n=5. **F**: Expression of CCL20 in peripheral blood and synovial fluid within effector T cells, healthy individuals n=12, DMARD treated PsA patients n=6, adalimumab treated PsA patients n=10. **G**: Percentage of CCL20 producing effector T cells present in the cell doublets from the upper compartment of the transwell. Healthy indivuals n=5, DMARD treated PsA patients n=5, adalimumab treated PsA patients n=5. Unpaired t-tests were used to determine significance. p<0.05*, p<0.01**, p<0.001***.

STAR★Methods

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Michael R. Ehrenstein (m.ehrenstein@ucl.ac.uk)

Materials availability
This study did not generate unique reagents.

Data and code availability
All data reported in this paper will be shared by the lead contact upon request.
This paper does not report original code.
Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects
Heparinised peripheral blood and synovial fluid were taken from 66 PsA patients (34 male and 32 female patients, mean age: 52.8 years, standard deviation: 14.2 years) (Supplementary Table 1). Synovial fluid was isolated from patients with active disease on conventional DMARD therapy. Peripheral blood from 38 healthy volunteers were also recruited. Ethical approval was granted by the North London Ethics Committee and written consent was obtained from all patients participating in the study. Patients recruited to the study were either treatment naïve (untreated) or were receiving methotrexate or sulfasalazine (DMARD), or treated with the anti-TNF therapy adalimumab. All other biologic and conventional therapies were excluded from the study.

METHOD DETAILS

Flow cytometry
Peripheral blood and synovial fluid mononuclear cells (PBMC and SFMC) were isolated by density gradient centrifugation over Ficoll (Merk) according to the manufacturer's instructions. Cells were stained with Live/Dead Blue (ThermoFisher Scientific) before staining for CD4, CCL20 and CCR6 surface expression. Samples were then fixed/permeabilised using the FoxP3 Transcription buffer staining kit (ThermoFisher Scientific) according to the manufacturer's instructions and stained for FoxP3 expression. Immune cell phenotyping was performed using an LSR2 or Fortessa flow cytometer (BD Biosciences) and Diva software, version 9.0.0, and analysed by FlowJo v10.7.1 (BD Biosciences). For intracellular cytokine staining, fresh PBMC and SFMC were stimulated for 4 hours with PMA (40 ng/ml, Merk), Ionomycin (2µg/ml, Merk) and Golgi Stop® (1µg/ml, BD Biosciences). Samples were fixed/permeabilised as above and stained for IL-17 or IFN-γ production.

Treg suppression assay

CD4 Treg (CD25^{high}CD127^{low}), CD4 effector T cells (Teff) (CD25$^-$CD127$^+$) and autologous CD14$^+$ monocytes were purified from freshly isolated PBMC using an Aria Cell Sorter (Figure S1). Monocytes were added to boost IL-17 production (McGovern et al., 2012). Teff were stained with CFSE (ThermoFisher Scientific), and CD14$^+$ monocytes with PKH-26 (Merk). Treg were left unlabelled. Cells were cultured at a

ratio of 3:1:1; Teff:Treg:monocytes in the presence of 1 µg/ml anti-CD3 (HIT-3a) for 4 days. Cells were re-stimulated with PMA, Ionomycin and Golgi Stop® and stained for intracellular cytokines as detailed above. Data represented as percentage suppression of Teff IFN-γ or IL-17 production, calculated as: [(% IFN-γ+ or IL-17+ Teff alone - % IFN-γ+ or IL-17+ Teff in presence of Treg)/ % IFN-γ+ or IL-17+ Teff alone] x100.

Treg suppression of migration assay

Treg and effector T cells (Teff) were purified from freshly isolated PBMC using an Aria Cell Sorter as above. Effector T cells were stained with CFSE and cultured at a 3:1 ratio with Treg (1.5 x 10^5 Teff:0.5 x10^5 Treg, 50 µl each; in a total volume 100 µl in the top well of a 24-well 5 µm transwell Corning). CCL20 (50 ng/ml, PeproTech) was placed in the bottom compartment in media (600 µl). After 3 hours incubation, the number of migrated cells in the bottom well was enumerated by flow cytometry using beads. Counting beads (20,000/tube, One Comp ebeads, ThermoFisher Scientific) were added to each well and analysis by flow cytometry for each sample was completed after 2000 beads were recorded. The number of migrated effector T cells and Treg were measured by flow cytometry after gating on CFSE+ (Teff) or CFSE- (Treg) cells (Figure S2A). Chemotactic Index = No. cells migrated towards CCL20/no. cells migrated to media alone. Percentage suppression = [(chemotactic index of Teff alone – chemotactic index of Teff in presence of Treg)/chemotactic index of Teff alone] x 100.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analysed using the D'Agostino & Pearson omnibus test to assess whether they were normally distributed. Parametric data were analysed using paired and unpaired t-tests, non-parametric data were analysed using the Mann-Whitney U Test. All analyses were performed using Prism software, version 8. A value of p less than 0.05 was considered significant, *p<0.05, **p<0.01, ***p<0.001.

Acknowledgements

Author Contributions

DN and HB share first authorship, with DN providing the critical experiments to complete the work that HB initiated. DN, HB, AE, RB and ME designed the experiments. DN, HB, AE and RB conducted the experiments and undertook the analysis. DN, HB and ME wrote the paper with input from AE.

Declaration of interests

The authors declare no competing interests

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KEY RESOURCES TABLE

REAGENTS or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse Monoclonal anti-CD4 AF700	BD Biosciences	#557922
Mouse Monoclonal anti-CD25 PE	BD Biosciences	#555432
Mouse Monoclonal anti-IFN-γ PE-Cy7	BD Biosciences	#557643
Mouse Monoclonal anti-CCR6 BV605	Biolegend	#353419
Goat Monoclonal anti-IL-17-AF 488	Biolegend	#516604
Mouse Monoclonal anti-CCL20 PE	R&D Systems	#IC360P
Mouse Monoclonal anti-FoxP3 efluor 660	ThermoFisher Scientific	#50-4777-42
Mouse Monoclonal anti-CD127 APC-efluor 780	ThermoFisher Scientific	#47-1278-42
Mouse Monoclonal anti-CD14 PE	ThermoFisher Scientific	#12-0149-42
Mouse Monoclonal anti-CD3	ThermoFisher Scientific	#16-0039-81
Reagents		
Recombinant Human MIP-3α (CCL20)	PeproTech	#300-29A
Golgi Stop	BD Biosciences	#554724
Ficoll Paque Plus	Merk	#GE17-1440-02
Phorbol 12-Myristate 13-Acetate	Merk	#P-8139
Ionomycin	Merk	#I0634
PKH-26	Merk	#CPKH26GL
FoxP3 Transcription buffer staining kit	ThermoFisher Scientific	#00-5523-00
Live/Dead Fixable Blue Dead Cell Stain Kit	ThermoFisher Scientific	#L23105
Cell Trace Violet Cell Proliferation CFSE Kit	ThermoFisher Scientific	#C34557
OneComp eBeads	ThermoFisher Scientific	#01-1111-42
Biological samples		
Peripheral blood from patients with psoriatic arthritis	University College London Hospital	N/A
Peripheral blood from healthy individuals	University College London Hospital	N/A
Software		
FACS DIVA v9.0.0	BD Bioscences	https://www.bdbiosciences.com/
FlowJo v10.7.1	BD Biosciences	https://www.flowjo.com/
Prism v8	GraphPad	https://www.graphpad.com/

Figure 1
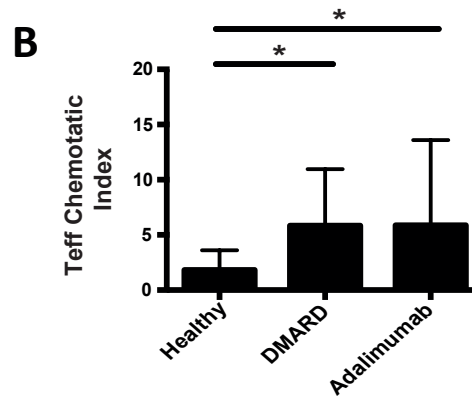
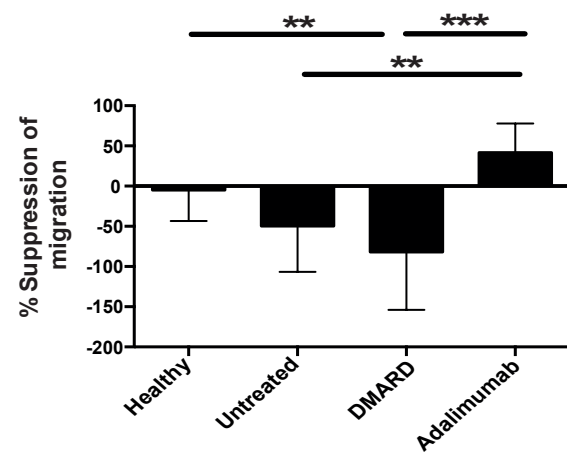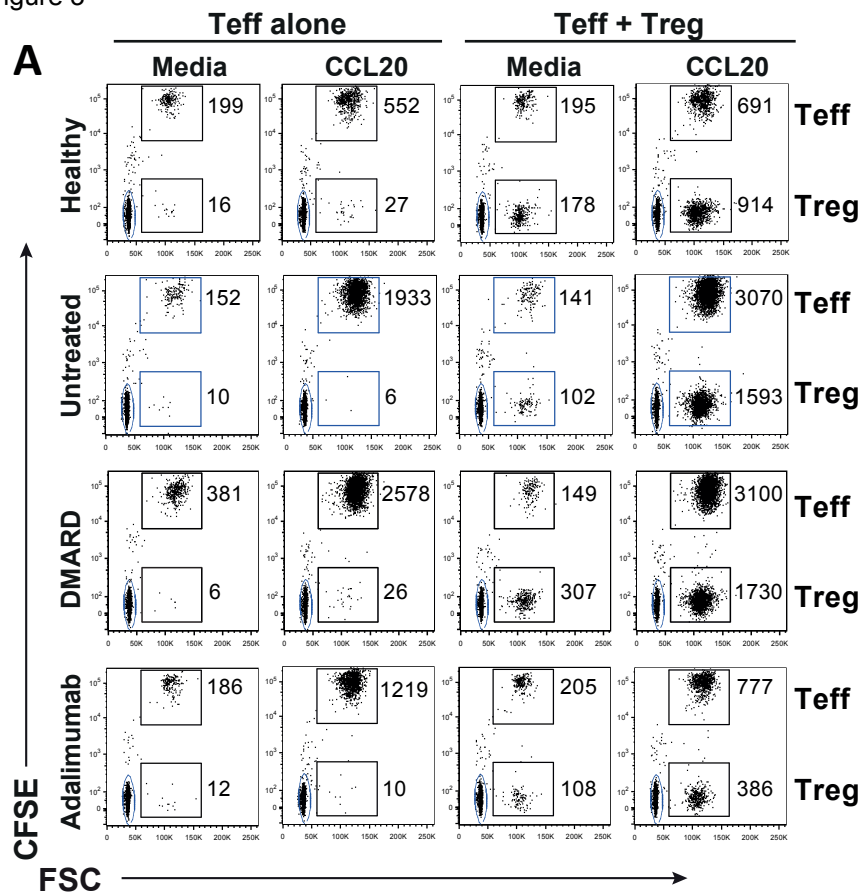Figure 1
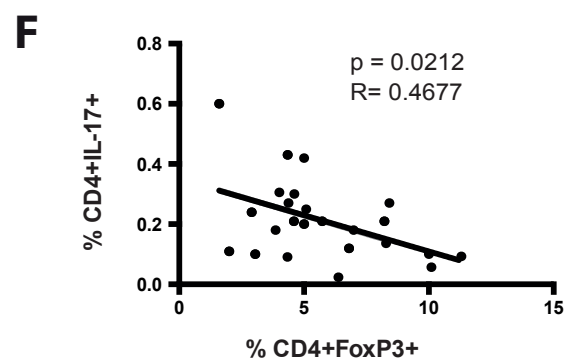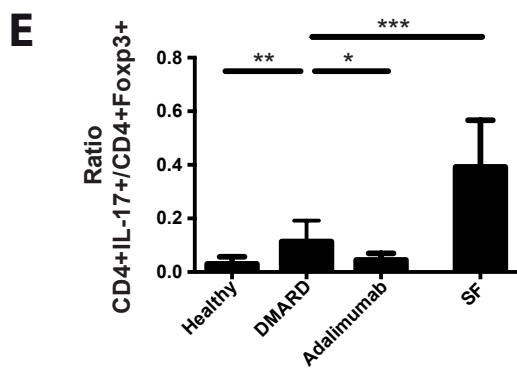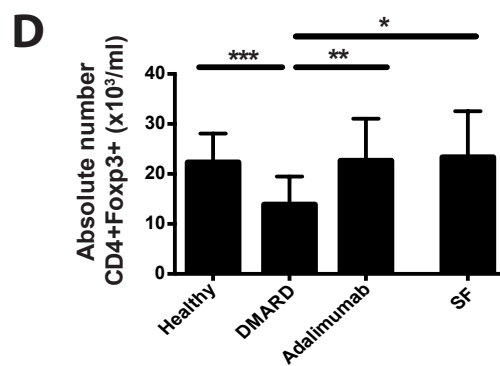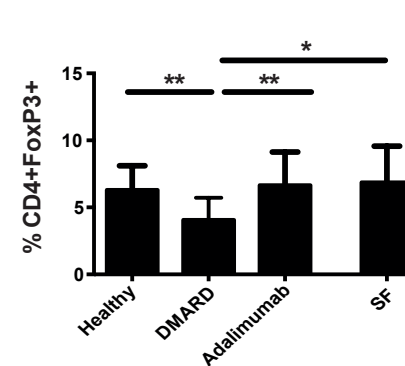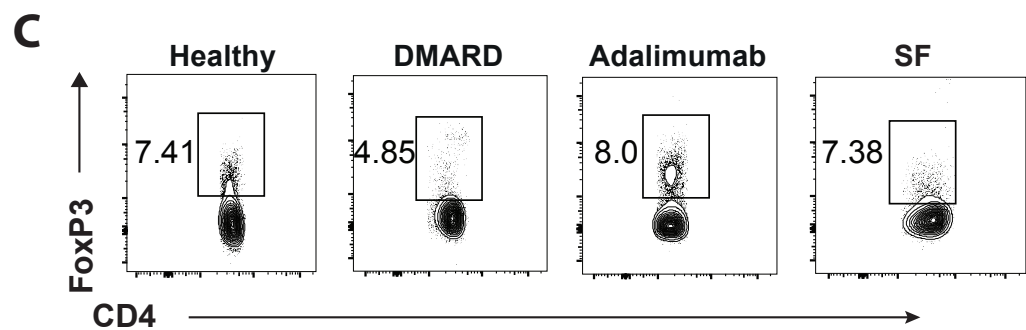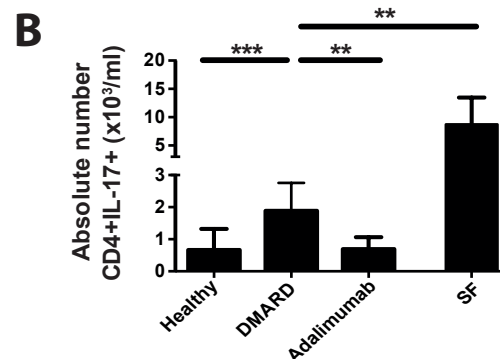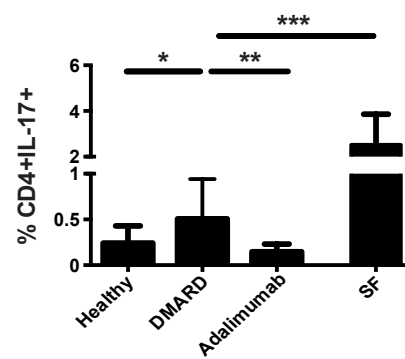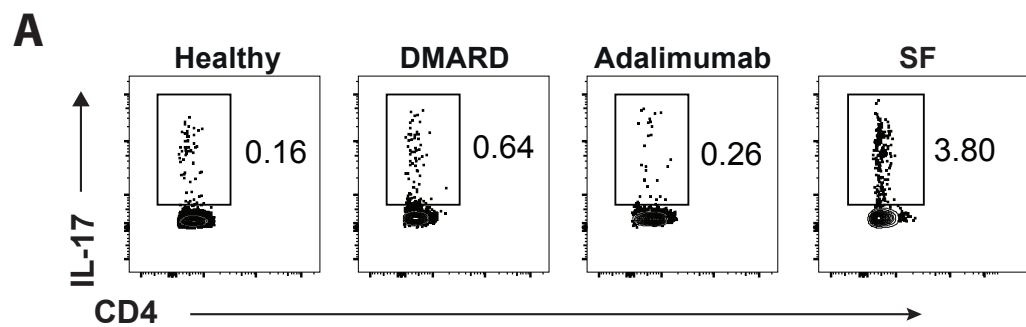
Figure 2

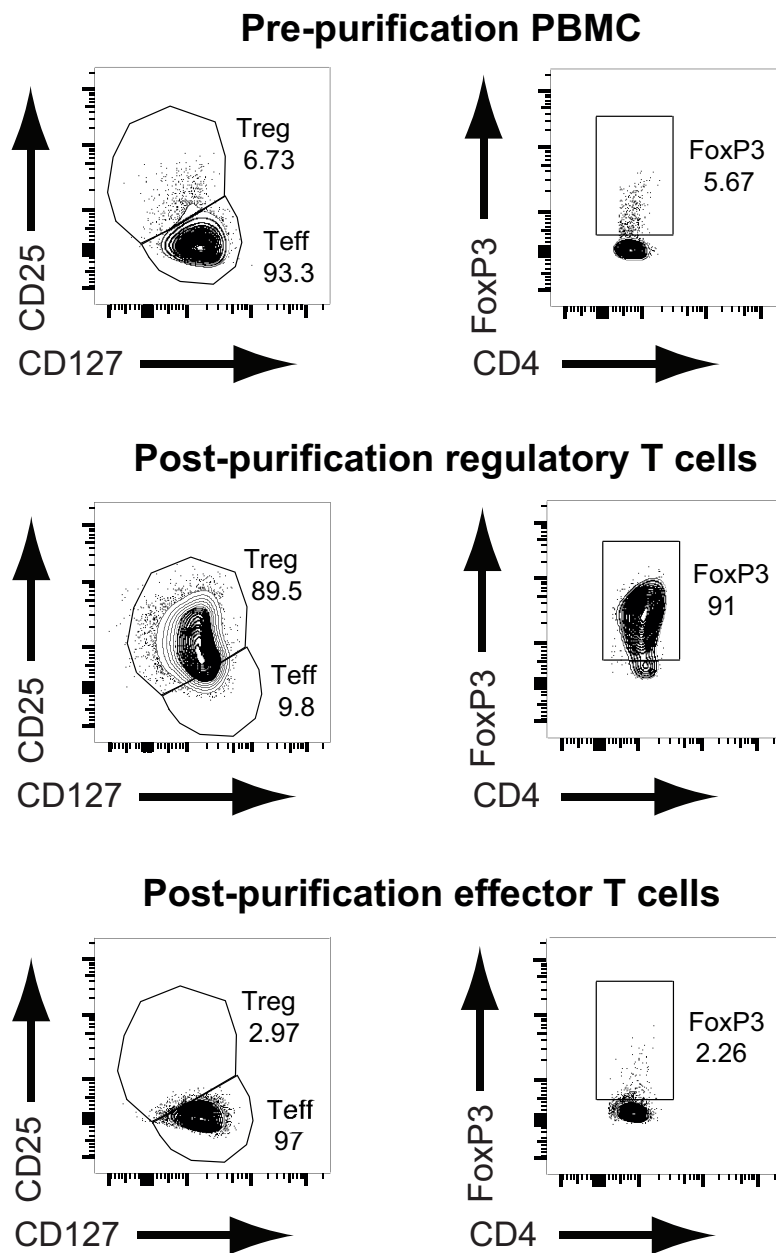Figure 2
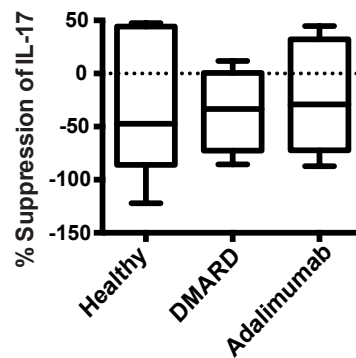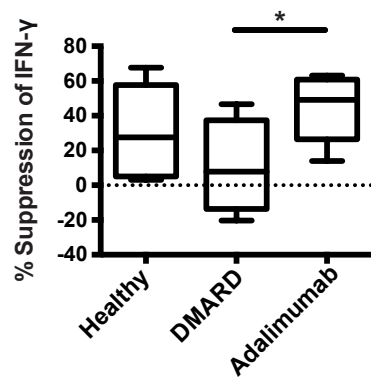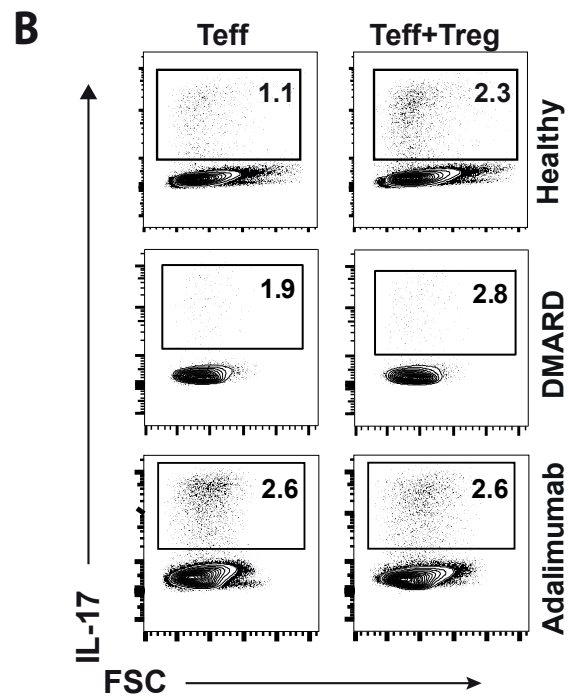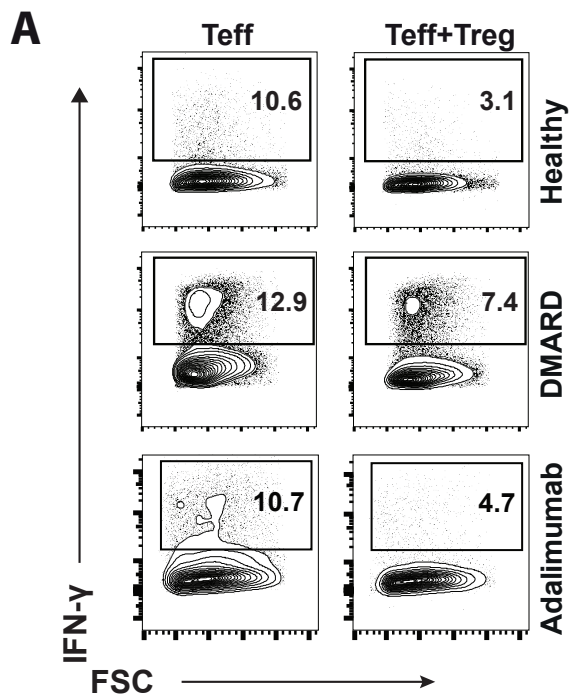
Figure 2

Figure 3

Figure 3
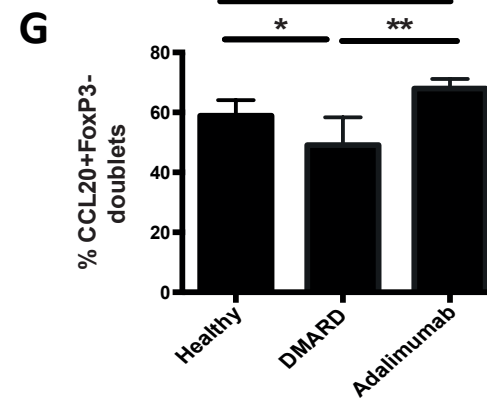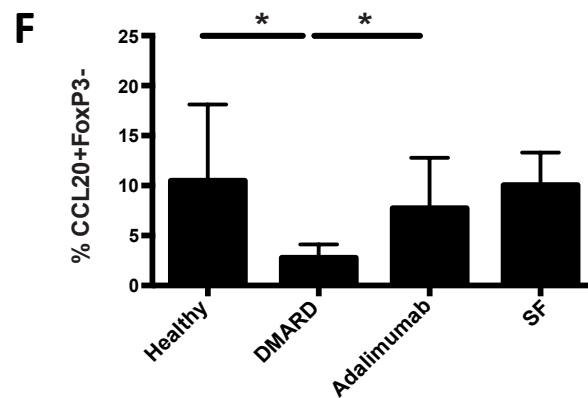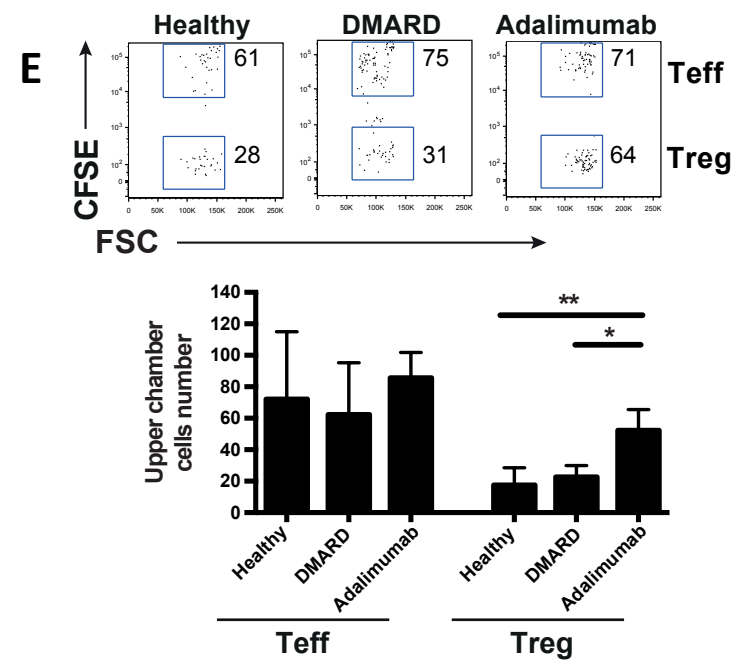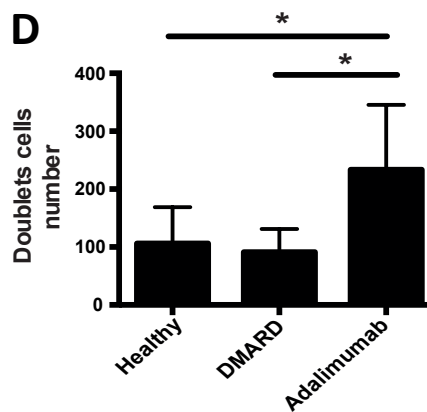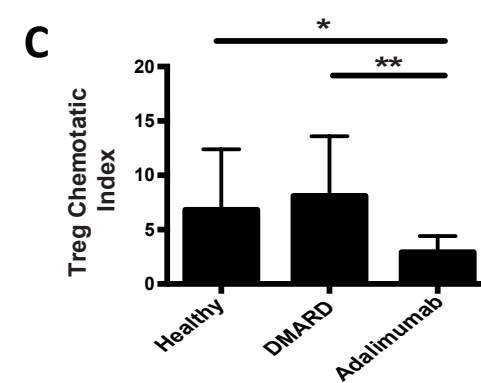
Table S1. Demographic and clinical characteristics of the study subjects, related to Figures 1-3

66 patients with psoriatic arthritis (PsA) were recruited fulfilling the CASPAR criteria for PsA. 38 healthy controls were also recruited. PB: peripheral blood, SF: synovial fluid, DMARD: disease-modifying anti-rheumatic drugs, PASI: Psoriasis activity and severity index, CRP: C-reactive protein, DAS28-ESR: Disease Activity Score in 28 joints. The mean and standard deviation are provided.

| Parameter	Healthy Controls	All PsA	Paired PB/SF
Total sample number	38	66	4
Male	11	34	4
Female	27	32	0
Age	34.07 ± 8.8	52.83 ± 14.2	39.7 ± 21.08
Treatment			
Untreated		7	
DMARD (Mtx/SSZ)		25	4
Adalimumab		34	
Untreated patients			
PASI		4.2 ± 3.52	
CRP		9.6 ± 6.44	
DAS 28 (ESR)		3.6 ± 1.22	
DMARD patients			
PASI		4.1 ± 6.34	2.45 ± 0.63
CRP		9.07 ± 6.11	81.6 ± 110.5
DAS 28 (ESR)		3.5 ± 1.49	3.9 ± 0.09
Adalimumab patients			
PASI		2.4 ± 5.49	
CRP		1.9 ± 1.94	
DAS 28 (ESR)		2.3 ± 1.04	

Figure S1. Purification of effector T cells and Treg for the in vitro suppression assays, **related to Figure 2 and Figure 3**

CD4 Treg (CD25^{high}CD127^{low}), CD4 effector T cells (Teff) (CD25$^-$CD127$^+$) and autologous CD14$^+$ monocytes were purified from freshly isolated PBMC of healthy controls, DMARD and adalimumab treated PsA patients, using a FACS Aria Cell Sorter. Cells were stained for expression of CD4, CD25, CD127, and FoxP3 following isolation to check purity. Data shows pre- and post- FACS Aria™ sorted purified effector T cells (Teff) and Treg based on CD25/CD127 and FoxP3 expression measured by flow cytometry.

Pre-purification PBMC

Post-purification regulatory T cells

Post-purification effector T cells

Figure S2. Gating strategy for effector T cells and Treg in chemotaxis assay, related to Figure 3

(**A**) Peripheral blood was taken from healthy controls, DMARD and adalimumab treated PsA patients (PBMC). Treg and effector T cells were isolated; effector T cells labelled with CFSE while Treg were left unlabelled. Effector T cells (150,000) with or without Treg (50,000) were cultured in the upper chamber of a transwell. Plots show migrated cells after 3 hours incubation towards media alone or CCL20 (50 ng/ml). Migrated cells were harvested and a set number of beads were added to each tube. The number of migrated cells was analysed using flow cytometry. (**B**) Ratio of effector T cell migration in the absence of Treg: Treg migration, represented as mean +/- SD from Healthy controls n=9, DMARD n=5, adalimumab n=9. (**C**) Forward and side scatter FACS plots showing numbers of single and doublet cells, harvested from the upper transwell after 3 hours of incubation of effector T cells and Treg with CCL20 in the lower chamber, from a healthy control, DMARD and adalimumab treated patient. Healthy individuals n=5, DMARD n=4, adalimumab n=5. $p < 0.05$*, $p < 0.01$**.
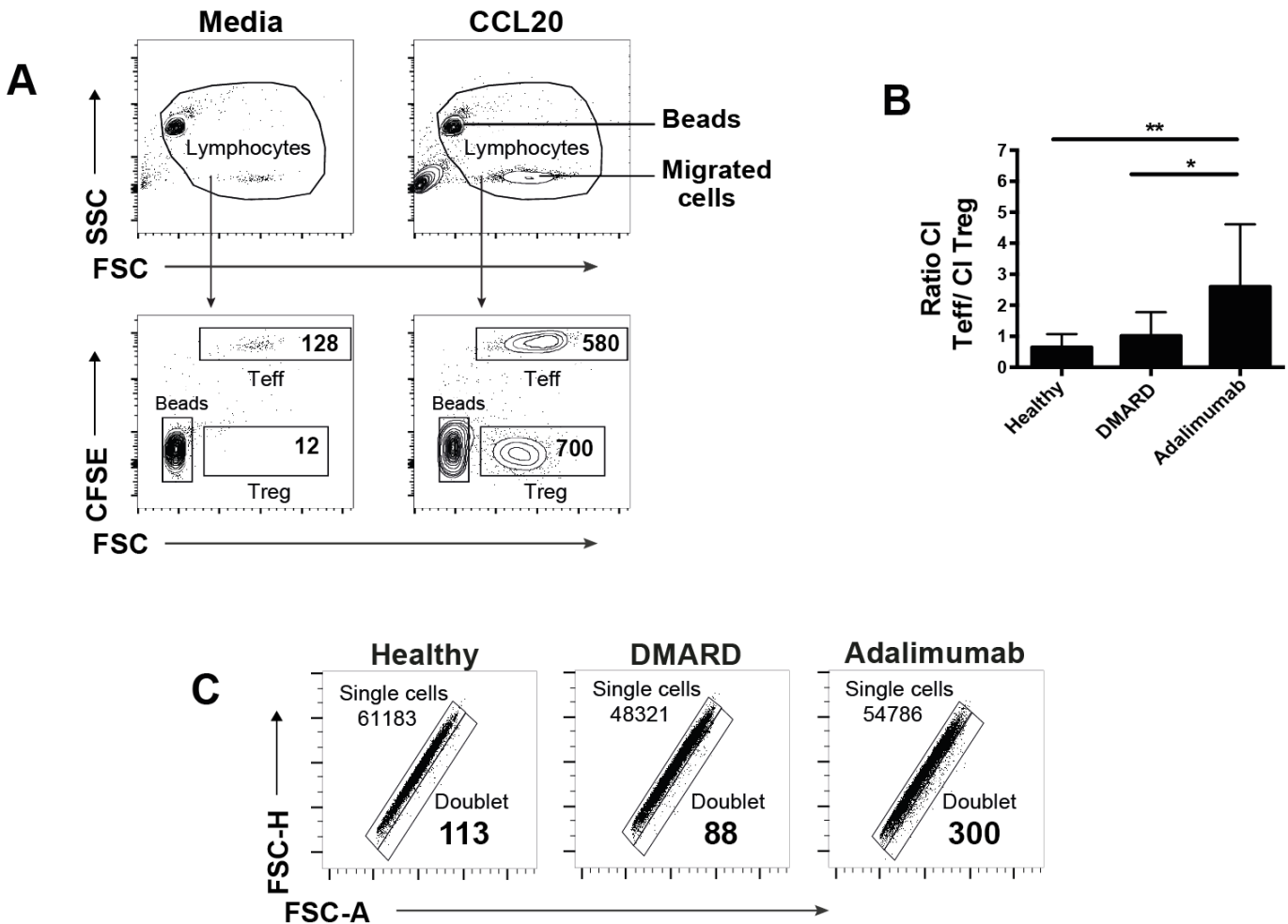
Figure S3. Percentage and expression of CCR6 T effector cells and Treg in peripheral blood, related to Figure 3F-G

Peripheral blood was taken from untreated, DMARD and adalimumab treated PsA patients and the expression of CD4, CCR6 and FoxP3 measured by flow cytometry. (**A**) Percentage of CCR6$^+$FoxP3$^-$ effector T cells (**B**) and CCR6+FoxP3$^+$ Treg. CCR6 MFI expression on (**C**) CD4+ T effector cells and (**D**) Treg. The mean and standard deviation are provided. Untreated n=6, DMARD n=12, adalimumab n=21. Unpaired t-tests were used to determine significance. p<0.05*, p<0.01**