Supporting Information

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SI Text

Antibodies. The following antibodies were used for flow cytometry and confocal microscopy: FITC-CTLA-4 (48815) from R&D Systems; PECy7-CD25 (BC96), PECy5-CD127 (eBioDR5), Alexa-Fluor-488-Foxp3 (PCH101), FITC-IL-2 (MQ1–17H12), Alexa-Fluor-488- IL-17A (64CAP17) from eBioscience; APC-, PE- or FITC-CD4 (RPA-T4), Biotin- or PE-CTLA-4 (BNI3.1), Biotin-CD80 (L307.4), Biotin-CD28 (CD28.2), FITC, APC- IFN-γ (K3) from BD Bioscience; and goat anti-rabbit Alexa Fluor-647 from Invitrogen. For T lymphocyte stimulation, CD3 (HIT3a), CD28 (CD28.2), CTLA-4 (BNI3) from BD Bioscience; CTLA-4 F(ab′)$_2$ (ANC152.2/8H5) from Ancell, and goat F(ab′)$_2$ anti-mouse antibodies from Sigma were used. Dynabeads M-280 Tosylactivated (Dynal Biotech) were coated with anti-CD3, anti-CD28 antibodies (10 μg/mL each) following the manufacturer's instructions. For protein phosphorylation analysis, Alexa-Fluor-488-phospho-Y142 CD3ζ (K25–407.69), PE-phospho-T202/Y204-ERK 1/2 (20a), and PE-phospho-Y505 Lck (4/LCKY5054G10) from BD Biosciences and Alexa Fluor-488-Ser 473 Akt (193H12) from Cell Signaling were used. For Western blot analysis, HRP-anti-phosphotyrosine (4G10) from Upstate, anti-β-actin (Sigma), and rabbit phosho-Ser-473 Akt antibodies from Santa Cruz were used.
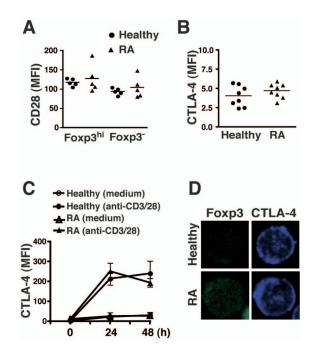
Fig. S1. Expression of CD28 in Treg and Tresp and CTLA-4 expression and kinetics in Tresp from RA patients and healthy controls. (*A*) PBMC from RA patients and healthy individuals were surface stained for CD4 and CD28, followed by intracellular staining with Foxp3. Stained cells were analyzed by flow cytometry. CD28 expression (MFI) is shown in the Foxp3^{hi} and Foxp3$^-$ population. (*B*) PBMC from RA patients and healthy individuals were surface stained for CD4 followed by intracellular staining for Foxp3 and CTLA-4. The MFI of CTLA-4 expression is indicated in the Foxp3$^-$ population. (C) Purified Tresp (CD4$^+$CD25$^-$CD127$^+$) were stimulated with anti-CD3 (1μg/mL)/CD28 (2μg/mL) antibodies for up to 48 h, and the levels of CTLA-4 were evaluated at different time points by intracellular staining and flow cytometry. Results represent the mean $\pm$ SE of 6 RA patients and 6 healthy controls. (*D*) Confocal microscopy of Tresp from a healthy control and an RA patient activated overnight with anti-CD3 (1μg/mL)/CD28 (2μg/mL) and stained for CTLA-4 and Foxp3. No induction of Foxp3 was observed. Images are representative of at least 25 cells from 6 healthy controls and 6 RA patients. (Magnifcation: $\times$63.)

Table S1. Drug treatments at time of study

Patient	Arthritis form	DMARD	NSAID
1	RA	LEF	−
2	RA	Gold	+
3	RA	−	+
4	RA	−	
5	RA	MTX	−
6	RA	MTX	−
7	RA	MTX	+
8	RA	LEF/Pred	−
9	RA	Pred	−
10	RA	−	+
11	PsA	−	+
12	PsA	MTX	+
13	PsA	−	+
14	PsA	AZA	−
15	PsA	LEF	−
16	PsA	MTX/SLZ	−
17	PsA	MTX	−
18	PsA	−	+
19	PsA	SLZ	+
20	PsA	−	−

DMARD, disease-modifying antirheumatic drugs; NSAID, nonsteroidal antiinflammatory drug; MTX, methotrexate; LEF, leflunomide; Pred, prednisolone; SLZ, sulphasalazine; AZA, Azathioprine.