

Supplementary Appendix

Flow cytometry and cell count

Treg subpopulations were gated as shown in supplementary figure 1. These settings were applied for subsequent CD4⁺ T cells analysis of 100,000 events. CD4⁺ T-cell count was determined using a standard flow cytometry technique with a TruCOUNT tube (BD Biosciences). The absolute numbers of each subpopulation were calculated based on CD4⁺ T-cell counts and their frequencies. Data acquired by FACS-Calibur (BD Biosciences) were analysed using FlowJo software (Tree Star, Ashland, OR, USA).

Cells for FoxP3 and cytotoxic T lymphocyte antigen (CTLA)-4 staining were prepared for intracellular flow cytometry with a “Fix and Perm” kit (e-Bioscience) following the manufacturer’s instructions. After permeabilisation and washing, cells were labelled with anti-Foxp3-PE (e-Bioscience) and anti-CTLA-4-APC (e-Bioscience). Corresponding isotype controls were used as staining controls (BD Biosciences and e-Bioscience). Intracellular cytokine elaborations were determined in PBMC sand stimulated for 5 h with phorbol myristate acetate (PMA; 5 ng/ml) and calcium ionomycin (500 ng/ml) in the presence of GolgiStop. Cells were fixed, permeabilised, and stained with Anti-hIFN- γ -FITC (BD Biosciences) and anti-hIL-17A-Percp-cy5.5 (BD Biosciences).

Cell culture and *in vitro* suppression assay

For *in vitro* suppression assay of different Treg subsets, CD4⁺CD25⁻ cells were purified by labelling with anti-human CD25-PE antibody (eBioscience) and anti-PE microbeads (Miltenyi Biotech, BergischGladbach, Germany) from healthy controls as responder cells. Carboxyfluorescein succinimidyl ester (CFSE)-labelled CD4⁺CD25⁻ responder cells (5×10^4) were cocultured with 1×10^4 unlabelled FrI (CD25⁺⁺CD45RA⁺,

rTreg), FrII(CD25⁺⁺⁺CD45RA⁻, aTreg) or Fr III (CD25⁺⁺CD45RA⁻) cells, respectively, in anti-CD3 (5 g/ml, OKT3 mAb; eBioscience) coated plates in the presence of soluble anti-CD28 (5 µg/ml; eBioscience) for 72–96 h. Proliferation of CFSE-labelled cells was assessed by flow cytometry and the percentage suppression was determined based on the percentage of dividing of CFSE-labelled cells as compared with the percentage of dividing CFSE-labelled cells when cultured alone.