Supplementary Materials and Methods

Cytokine Analysis

A total of 10^6 $CD8^+$ Ts lines were plated in 1 mL complete medium and stimulated with αCD3/CD28 activation beads at a 1:1 ratio. After 72 hours, supernatant was collected and stored at −20°C. IL-2, IL-4, IL-5, IL-10, tumor necrosis factor α, and IFN-γ levels were determined by Cytometric Bead Array. IL-17 levels were measured using enzyme-linked immunosorbent assay (eBioscience, San Diego, CA). Levels of biologically active TGF-β were determined using a Tgf-$\beta^{-/-}$ mouse embryonic fibroblast cell line stably transfected with a reporter plasmid consisting of TGF-β−responsive Smad-binding elements coupled to a secreted alkaline phosphatase (SEAP) reporter gene. Assays for reporter gene activity were assessed using the Great EscAPe SEAP Reporter System 3 (Clontech, Mountain View, CA).[2]

Surface and Intracellular Staining

$CD8^+$ Ts lines were stained by incubation for 30 minutes on ice with fluorochrome-conjugated antibodies against CD8α (RPA-T8; eBioscience), CD3 (SK7; eBioscience), CD4 (RPA-T4; eBioscience), integrin β7 (FIB504; eBioscience), integrin α4 (9F10; eBioscience), CD8β (2ST8.5H7; BD Bioscience), CD101 (BB27; eBioscience), CD56 (301040; R&D Systems), CD122 (27302; R&D Systems), CD25 (M-A251; BD Bioscience), CD103 (Ber-ACT8; BD Bioscience), CD28 (CD28.2; BD Bioscience), ICOS (DX29; BD Bioscience), CTLA4 (BN13; BD Bioscience), CD16 (3G8; BD Bioscience), PD1 (eBioJ105; eBioscience) and isotype controls (rat immunoglobulin [Ig]G2a eBRG2a; eBioscience), mouse IgG1 (MOPC-21; BD Bioscience), mouse IgG2a (MOPC-173; BD Bioscience), and mouse IgG2b (MPC-11; BD Bioscience) in phosphate-buffered saline. The FoxP3 staining buffer set (eBioscience) was used for intracellular staining of FoxP3 (236A/E7; eBioscience), perforin (δG9; BD Bioscience), and granzyme B (GB11; BD Bioscience). Cells were fixed and made permeable for 30 minutes at room temperature and were stained for 30 minutes on ice with antibodies in permeabilization buffer. FACSCalibur (BD Bioscience) and FlowJo software (Tree Star, Ashland, OR) were used for flow cytometry and analysis.

Microarray Data Analysis

After background subtraction, intensity values were scaled to the median average intensity of the entire sample set using the average normalization function available in GenomeStudio V2009.1 software (Illumina). GeneSpring GX version 7.3.1 (Agilent Technologies) was used for all downstream analyses. Intensity values were floored to 10, and the intensity of each probe in each sample was normalized to the median intensity of this probe across all samples.

Statistical Methods for Microarray Results

For microarray data processing, background subtracted data was obtained using GenomeStudio software (Illumina). Background subtracted data was normalized by rescaling the difference in overall intensity to the median average intensity for all samples across multiple arrays and chips. Following this, expression values less than 10 were set to 10 and log (base 2) transformed. Finally, all the probes were filtered to match the criteria; at least one in all sample detection *P* values was less than .01. A total of 24,977 probes were selected for analysis.

Cross-correlation analysis was conducted for each microarray probe individually to measure the strength of the associations between gene expression and suppressor activity. Spearman rank correlation coefficient (ρ) was calculated to account for non-normal distribution. A false discovery rate of 0.10 was used to adjust for multiple testing. The range of ρ for all probes was from −0.672 to 0.622. There was one probe that had a statistically significant correlation between gene expression and suppressor activity using a false discovery rate of 0.10.

Statistical analyses of microarrays were performed using SAS software (version 9.2; Cary, NC) and JMP/Genomics (version 4.0; Cary, NC).

Tissue Explant Cultures

Explant cultures from surgical specimens were performed following a modification as previously described.[6] Briefly, intestinal mucosa (2-cm segments) were washed in phosphate-buffered saline, standardized by weight, and cultured in 24-well culture plates in serum-free RPMI 1640 medium supplemented with penicillin, streptomycin, glutamine, amphotericin B, a protease inhibitor cocktail (Thermo Scientific), and a phosphatase inhibitor cocktail (Thermo Scientific). The volume of the media was adjusted to the tissue weight. After 24 hours, supernatants were collected and stored at −20°C.

Supplementary References

1. Allez M, Brimnes J, Dotan I, et al. Expansion of CD8+ T cells with regulatory function after interaction with intestinal epithelial cells. Gastroenterology 2002;123:1516–1526.
2. Pessah M, Prunier C, Marais J, et al. c-Jun interacts with the corepressor TG-interacting factor (TGIF) to suppress Smad2 transcriptional activity. Proc Natl Acad Sci U S A 2001;98:6198–6203.
3. Lachmann A, Xu H, Krishnan J, et al. ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. Bioinformatics 2010;26:2438–2444.
4. Berger SI, Posner JM, Ma'ayan A. Genes2Networks: connecting lists of gene symbols using mammalian protein interactions databases. BMC Bioinformatics 2007;8:372.
5. Lachmann A, Ma'ayan A. KEA: kinase enrichment analysis. Bioinformatics 2009;25:684–686.
6. Burke JP, Watson RW, Mulsow JJ, et al. Endoglin negatively regulates transforming growth factor beta1-induced profibrotic responses in intestinal fibroblasts. Br J Surg 2010;97:892–901.