Cell lines
The Daudi Burkitt lymphoma line was obtained from ATCC (Rockville, MD). EL4 and EL4-CD20 were kind gifts from Drs. Martino Introna and J. Golay, Milano, Italy. A lymphoblastoid cell line (TM-LCL) used for feeder cells in T-cell cultures was generated by EBV-transformation of PBMC obtained from a normal volunteer. Cells were cultured at 37°C and 5% CO_2 in RPMI 1640 medium containing 2 mmol of L-glutamine, 25 mmol HEPES, and 10% fetal calf serum. Medium for cells not used in human subjects also contained 100 U/ml penicillin and 100 μg/ml streptomycin.

Complete list of Ab used in immunophenotyping experiments
All Ab used were obtained from Becton-Dickinson (BD), Pharmingen (P), Dako (D) and Beckman-Coulter (BC) as follows: CD2 FITC (BD), CD3 FITC (BD), CD3 Pacific Blue (P), CD4 PE-TR (BC), CD4 PE-Cy5 (BC), CD5 PE (BD), CD8 FITC (BD), CD8 PE-Cy5.5 (BC), CD11a PE (P), CD25 PE (BD), CD28 FITC (BC), CD44 PE (P), CD45RA FITC (BC), CD45RA Pe-Cy7 (BD), CD45RO PE (BC), CD49d PE (P), CD56 PE (BD), CD56 APC (BD), CD62L FITC (BC, P), CD94 FITC (P), CD95 FITC (Dako), CD127 Alexa-647 (P), CD134 FITC (P), CD137 PE (P), CD154 PE (P), CD195 FITC (P), CD314 PE (P), CCR6 PE (P), CCR7 PE (P), CXCR3 Alexa 488 (P), CXCR4 PE (P), CXCR5 Alexa-488 (P), IgG1 FITC (BD), IgG1 PE (BD), TCR α/β FITC (BD), and Vα24 FITC (BC). All Ab were used in simple two color combinations with the exception of two multicolor combinations as follows: CD3 Pacific Blue, CD4 PE-TR, CD8 PE Cy5.5, CD127 Alexa-647, CCR7 PE, CD62L FITC, CD45RA PE-Cy7 and CD3 Pacific Blue, CD4 PE-TR, CD8 PE-Cy5.5, CD56 APC, Vα24 FITC. Instrument performance was monitored daily using quality control beads. Assays for FoxP3 were performed using a kit from eBioscience as directed by the manufacturer.

Flow cytometry to detect anti-cTCR Ab responses
Patient sera (100 μl in dilutions of neat, 1:10, and 1:100) from baseline (negative control), 2 weeks after T-cell infusions, and 3 months after T-cell infusions were incubated with washed cTCR+ and cTCR- Jurkat T cells at 4°C for 30 minutes. Cells were then washed twice with PBS containing 2.5 mM EDTA and 1% FBS before incubation with a saturating concentration of FITC-conjugated goat IgG $F(ab')_2$ specific for human $F(ab')_2$ (Jackson Immunoresearch) (a secondary Ab designed to detect only human Ab without binding to the portions of the cTCR derived from human IgG1) for 15 minutes at 4°C in the dark. Flow cytometry was then performed using standard methodology. Data were analyzed using FlowJo software.

T-cell clonality assays
T-cell clonality was determined by PCR amplification of rearrangements at the T-cell receptor gamma (TCRγ) locus as previously described,[26] except that VγI-Jγ1/2, VγII-Jγ1/2, VγI-JγP1/2, and VγII-JγP1/2 rearrangements were amplified in a single multiplex PCR reaction and analyzed by capillary electrophoresis on an Applied Biosystems Model 3130. PCR reactions (50 μl) contained 500 ng genomic DNA (Gentra Puregene), 2.5 U AmpliTaq (Applied Biosystems), 0.55 μg TaqStart (Clontech), 200 μM each dNTP, and 10 pmol of each primer: VγI (5′ ACCAGGAGGGGAAGGCCCCACAG 3′), VγII (5′ GAAAGGAATCCGGCATTCCG-3′), FAM-Jγ1/2 (5′ 6-carboxyfluorescein-ACCTGTGACAAC(A/C)AGTGTTGTTC 3′), and HEX-JγP1/2 (5′ 6-hexachlorofluorescein-AGTTACTATGAGC(T/C)TAGTCCC 3′). PCR

amplification conditions were: denaturation at 95˚C for 15 seconds and annealing/extension at 60˚C for 1 minute (50 cycles) followed by a final extension at 72˚C for 10 min.

Vβ spectratyping was also performed by flow cytometry using standard methodology. Briefly, frozen cells were thawed, washed, and labeled with monoclonal antibodies CD8 ECD (Beckman-Coulter) and IOTest Beta Mark Kit (Beckman-Coulter) for 15 minutes at room temperature in the dark per manufacturer's instructions. The samples were then washed once, resuspended in phosphate-buffered saline containing bovine serum albumin and up to 100,000 events acquired on an LSRII (Becton-Dickinson). Data were analyzed using software developed in our laboratory (WoodList). The expression of each of the 24 T-cell receptor isoforms present in the Beta Mark Kit were determined independently on the CD8+ T-cell populations, and a threshold of 85% positivity for a single isoform or an absence of expression of all 24 isoforms outside the reference range was considered to represent a clonal expansion. Samples lacking a clonal expansion showed two or more isoforms outside the reference range and were considered oligoclonal.