Preparation and validation of rabbit polyclonal anti–human CD83 (RA83)

RA83 was prepared from serum of rabbits immunized with CD83-Ig, a recombinant fusion protein consisting of the extra cellular domain of human CD83 fused to human IgG1-Fc (Hock, B.D., M. Kato, J.L. McKenzie, and D.N.J. Hart. 2001. *Int. Immunol.* 13:959–967). Total IgG was prepared from rabbit serum by Protein A affinity chromatography, which was then depleted of anti–human IgG-Fc immunoreactivity by passage through immobilized human IgG (Intragam P, a human IgG preparation for i.v. injection [CSL Ltd], immobilized on a 1-ml NHS-activated HiTrap column [Amersham]), as previously described (Munster, D.J., K.P.A. MacDonald, M. Kato, and D.N.J. Hart. 2004. *Int. Immunol.* 16:33–42). To ensure specificity for CD83 of the RA83 used in the present study, an additional purification step was added in which specific anti–human CD83 immunoreactive IgG was affinity isolated on CD83-Ig immobilized on a 1ml NHS-activated HiTrap column. This final antigen affinity isolation step increased the antibody potency by >25-fold (see ELISA results in the next paragraph and Fig. S7). Negative control rabbit IgG (RAneg) was prepared from serum from non-immunized rabbits by Protein A affinity chromatography followed by passage through a column of immobilized human IgG, as described earlier in the paragraph.

Both RA83 and RAneg were pure IgG by SDS-PAGE. RA83, but not RAneg, was strongly immunoreactive by ELISA with CD83-Ig but not with human IgG (Fig. S7). RA83, but not RAneg, stained stable human full-length CD83-transfected FDCP1 cells but not untransfected FDCP1 cells (Fig. S8) or mouse CD83-transfected cells (not depicted). RA83, but not RAneg, blocked staining of the human CD83 transfectant above with the Hb15a mAb for human CD83 (Beckman Coulter; Fig. S8). There were no obvious differences between RA83 and RAneg in staining fresh human PBMC. Both antibodies stained monocytes as a result of nonspecific Fc receptor binding but not B cells or T cells. Overnight cultured PBMC had a small population of RA83+ CD14-CD19/20- cells (activated DC) and a small subset of RA83+ B cells (not depicted), as was previously observed with Hb15a (Hock, B.D., D.B. Fearnley, A. Boyce, A.D. McLellan, R.V. Sorg, K.L. Summers, and D.N. Hart. 1999. *Tissue Antigens.* 53:320–334).

RA83, but not RAneg, maximally inhibited allogeneic MLC proliferation at 1 µg/ml (Fig. S9). We have previously reported (Munster, D.J., K.P.A. MacDonald, M. Kato, and D.N.J. Hart. 2004. *Int. Immunol.* 16:33–42), but not shown the data, that the inhibitory effect of RA83 is specific for CD83 by blocking it with CD83-Ig but not with human IgG. This data is shown in Fig. S10.
Figure S1. Liver histology in hu-SCID mouse with GVHD. Periportal liver apoptotic bodies (short arrow) and lymphocytic infiltration (long arrow) shown in Hematoxylin & Eosin–stained formalin fixed section of liver. Bar, 20 μm.
Figure S2. Human leukocyte engraftment in hu-SCID mice. Flow cytometry dot plots show human CD45+ leukocytes in peritoneal cavity, spleen, and bone marrow 9 d after i.p. injection of $5 \times 10^6$ PBMC into a conditioned SCID mouse. GVHD score at time of sacrifice = 5.25 (severe GVHD). Human leukocyte engraftment levels for this animal were 98.2% for peritoneal cavity (mouse CD45+ = 1.7%), 76.2% for spleen (mouse CD45+ = 3.8%), and 47.1% for bone marrow (mouse CD45+ = 34.1%).
Figure S3. Clinical GVHD in antibody-treated hu-SCID mice. Mean and 1 SEM percent weight changes and GVHD scores for RA83, alemtuzumab (Campath-1H), and RAneg negative control antibody-treated hu-SCID mice for up to 25 d after transplant. Mice were sacrificed when GVHD score reached 5.
Figure S4. Human leukocytes were found in spleen 30 d after transplant in hu-SCID mice treated with 25 µg RA83 per mouse. Human CD45+ cells = 0.33% of total live gated cells in this example and mouse CD45+ cells = 56.9%. Circulating human IFN-γ and IL-5 concentrations at day 30 were significantly higher in RA83-treated mice compared to alemtuzumab (Campath-1H)-treated mice (Mann-Whitney U test, P = 0.001 and P < 0.05, respectively; horizontal lines show median values), which is consistent with higher engraftment in RA83-treated hu-SCID mice. No Tx mice did not receive a human PBMC graft.
Figure S5. Detection of human CMV-specific CD8+ T cells in hu-SCID mice. A conditioned SCID mouse was injected with $5 \times 10^6$ human PBMC from a CMV+ HLA-A*0201+ donor and treated with 125 µg RA83 per mouse. After 10 d, cells from spleen, peritoneal cavity, and bone marrow were pooled and analyzed for CMVp65 pentamer-positive human CD8+ T cells by flow cytometric staining. Human CD45+ mouse CD45+ leukocytes were gated and CMVp65 pentamer-positive CD8+ T cells within were enumerated and expressed as a percentage of human CD45+ leukocytes.
RA83 does not substantially affect in vitro induction of antileukemic cell line activity by allogeneic PBMC. 0.5 × 10^6/ml irradiated (30Gy) U937 (AML) or K562 (CML) cells were cultured with 10^6/ml PBMC and 5 µg/ml RA83, RAneg, or Nil antibody. On day 6, 50% of the medium was replaced with fresh medium and 20 U/ml IL-2. On day 14, fresh medium, antibodies, irradiated leukemic cells, and irradiated (30Gy) autologous PBMC were added. On day 19, 50% of the medium was again replaced with fresh medium and IL-2. On day 22, the cells were harvested and tested in a 4-h ^51Cr release assay using ^51Cr-labeled U937 and K562 cells as targets.
Figure S7. Sandwich ELISA of anti-CD83 preparations. Shown is nonreactivity of RAneg, >25-fold greater potency of the antigen affinity-purified RA83 antibody used in the work described in this paper compared to the two-step purified preparation, and minimal residual cross-reactivity of RA83 with human IgG (300-fold excess Intragam was added to the RA83-containing test sample). Maxisorb ELISA plate wells (Thermo Fisher Scientific) were coated with 2 µg/ml CD83-Ig for capture. After blocking with fish gelatin, diluted antibody-containing samples were added, incubated for 90 min, washed, and bound rabbit Ig was detected with HRP-conjugated sheep anti-rabbit Ig (Silenus Laboratories; <0.2% cross-reactivity with human Ig) and OPD substrate color development.
Figure S8. RA83 recognizes human CD83 expressed on the cell surface. FDCP1 cells expressing full-length human CD83 were stained with RA83 or RAneg. Bound rabbit Ig was detected with FITC-conjugated goat anti–rabbit Ig (top). Prior incubation of human CD83-transfected FDCP1 cells with RA83 blocked subsequent binding of FITC-conjugated Hb15a mAb for human CD83 (bottom).
Figure S10. The inhibitory effect of RA83 is mediated specifically via CD83. 10 µg/ml CD83-Ig (diagonal lines), but not 10 µg/ml of human IgG (vertical lines), abrogates the inhibitory effect of 5 µg/ml RA83 but has no effect by itself (Nil) or with RAneg, on alloproliferation in MLC ([3H]thymidine incorporation; histogram shows mean CPM ±SEM). Horizontal lines show proliferation in the presence of RA83, RAneg, or Nil antibody in the absence of CD83-Ig and human IgG.

Figure S9. RA83 inhibits proliferation in allogeneic two-way MLC. PBMC from two donors were cultured together, each at 10^5 cells/well in a 96-well plate, with different concentrations of RA83 or RAneg. On day 5, each well received 1 µCi of [3H]thymidine. The cells were harvested 18 h later and incorporated ^3H quantitated by scintillation counting (CPM). Error bars (±SEM) show technical reproducibility and were each calculated from five replicate wells.