Supplemental Methods

Animals

Rhesus macaques (*Macaca mulatta*; 5~8kg, male) were obtained from AlphaGenesis, Inc. (Yemassee, SC). The animals were housed in Yerkes National Primate Research Center (YNPRC; Atlanta, GA) and all experiments described herein were performed in compliance with the Emory IACUC. The donor-recipient pairs were chosen to maximize genetic disparity at MHC class II with complete MHC I mismatch based on 454 deep sequencing analyses (University of Wisconsin-Madison, WI).

Allosensitization by skin transplantation

Full-thickness abdominal skin (~1 inch diameter) from the donor was transplanted on the right dorsal area of the recipient (allograft) while full-thickness abdominal skin from the recipient transplanted on the left dorsal area of the recipient (autograft). Both grafts were sutured in a simple interrupted manner, covered with a tegaderm with plastic adhesive bandage and a protective primate jacket (Lomir Biomedical, Malone, NY, USA) for 7 days. Graft survival was evaluated by visual inspection. Necrosis of more than 7~80% of the transplanted skin surface was considered as rejection. Donor specific alloantibody (DSA) measured by T and B cell flow crossmatch gradually decreased over time. Once DSA levels stabilized, four animals received bortezomib (1.33mg/m^2; Selleck Chemicals LLC, Houston Tx) I.V. twice weekly. Blood, bone marrow and peripheral or mesenteric lymph nodes were procured and used for flow cytometry.

Detection of donor-specific antibodies (DSA)

Alloantibody production was assessed by flow cytometry crossmatch of donor peripheral blood mononuclear cells (PBMC) with serially collected recipient serum samples. Donor PBMC (3×10^5 cells) were incubated with diluted recipient serum, washed and subsequently stained with FITC-labeled anti-monkey IgG (KPL, Gaithersburg, MD, USA), PE-labeled anti-CD20 (2H7) and PerCP-Cy5.5-labeled anti-CD3 (SP34-2) (both BD Bioscience, San Diego, CA, USA). DSA positivity was defined by a two fold increase of mean fluorescence intensity (MFI) from pre-transplant levels (MFI ratio ≥2). For analysis T cell crossmatches (representing MHC class I antibodies) were favored.

Polychromatic flow cytometric analysis

Cells were stained with Live/Dead Fixable Aqua dead cell stain kit (Life Technologies) and then with the following antibodies: monoclonal antibodies (mAb) against human CD3 PerCP-Cy5.5 (SP34-2), CD3 Alexa Fluor ®700 (SP34-2), CD4 PerCP-Cy5.5 (L200), CD8 Pacific Blue (RPA-T8), CD8 APC-Cy7 (RPA-T8), IgG Alexa Fluor ®700 (G18-145), CD56 FITC (B159) (all BD Bioscience, San Jose, CA, USA); CD279 (PD-1) APC (eBio105 (J105)), CXCR5 PE (MU5UBEE), CD28 PE-Cy7 (CD28.2), CD27 PE-Cy7 (O323), CD95 eFluor®450 (DX2) (all eBioscience, San Diego, CA, USA); CD20 APC-Cy7 (2H7), IgM Pacific Blue (MHM-88), CD278 (ICOS) PE-Cy7 (C398.4A) (all Biolegend, San Diego, CA, USA); CD159a (NKG2a) PE (Z199) (Beckman Coulter, Indianapolis, IN, USA); CD14 Pacific Orange (TüK4), CD25 mb APC (CD25-3G10) (both Life Technologies, Grand Island, NY, USA); CD25 PE (4E3) (Miltenyi Biotec, San Diego, CA, USA); CD3 V500 (SP34-2) (BD Horizon, San Diego, CA, USA). Cells were fixed in Fix/Perm solution (ebioscience) and then stained for intracellular Ki-67 PE (B56) and anti-human FOXP3 Pacific Blue (206D) (Biolegend). Samples were collected with a LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software vX (Tree Star, Ashland, OR, USA).

ELISA and ELISPOT analysis

The concentration of serum IL-6, Il-21 and BAFF level was determined by enzyme linked immunosorbent assay (ELISA) using a Due set kits for human IL-6, IL-21 and BAFF purchased from R&D systems (Minneapolis, MN). The cytokine level for each time point was an average of measurements of duplicate. Total IgG secreting Antibody Secreting Cells (ASCs) were detected using goat anti-human Ig (Caltag). Bone marrow cells (2×10^5, 1×10^5, 5×10^4, 2.5×10^4, 1.25×10^5, and 6.25×10^3 per well) were incubated in supplemented RPMI medium for 24 h in ELISPOT plates. After washing the plates, ASC was detected with anti-huIgG-biotin (Caltag) and avidin-D-HRP (Vector Laboratories) and developed with AEC substrate (Sigma) before analysis on an ELISPOT reader (Cellular Technologies Ltd.).

Immunohistochemistry and quantitative image analysis (QIA)

In situ germinal center staining procedure and QIA were performed as described in our previous studies (13). We performed immunostaining human Ki67 (clone MM1, Vector, Burlingame, CA), CD20 (Thermo scientific, Rockford, IL), and human CD3 (clone CD3-12, AbD serotec, Raleigh, NC) with antibodies. Appropriate secondary antibodies (Jackson ImmunoResearch, West Grove, PA) and nucleus dye (Hoechst 33342, Invitrogen, Carlsbad, CA) were used. All images were acquired with an Axio Imager Z1 microscope (Zeiss) using 20x objectives. Mean fluorescence

intensities of Ki67, CD3 and CD20 were analyzed using AxioVs40 V4.8.1.0 program (Zeiss) and Image J1.43u (NIH).

Statistical analysis

Standard statistical methods were used to calculate mean and standard deviation. Log-rank test was used for graft survival. Otherwise, a Student's t test was used. A p-value less than 0.05 was considered to be statistically significant.