SUPPLEMENTAL DATA

MATERIAL AND METHODS FOR SUPPLEMENTAL DIGITAL CONTENT

Dogs

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center. The study was performed in accordance with the principles outlined in the Guide for Laboratory Animal of Sciences, National Research Council. The kennels were certified by the American Association for Accreditation of Laboratory Animal Care.

Mixed leukocyte cultures (MLC)

Peripheral blood mononuclear cells (PBMCs) from the respective dogs were cocultured in in round-bottom 96-well plates for 6 days at 37°C in a humidified 5% CO_2 air atmosphere. 1×10^5 responder cells/well were cocultered with 1×10^5 irradiated (2200 cGy) stimulator cells/well. On day 3, concanavalin A (Con A, 4 mcg) was added to responder cell triplicates used as positive control. On day 6, cultures were pulsed with 1 μCi of ^{3}H-Thymidine for 18 hours before harvesting. ^{3}H-Thymidine uptake was measured as the mean counts/minute of the 3 replicates using a β-scintillation counter (Packard BioScience Company, Meriden, CT).

Natural killer (NK) cell cytoxicity assay

Effector cells were PBMCs prepared by Ficoll-Hypaque density-gradient centrifugation (density, 1.074) and targets were cells from a canine thyroid adenocarcinoma (CTAC) cell line. Effector/target ratios of 30:1, 15:1 and 7.5:1 in triplicate wells were used. The percentage of cytotoxicity (% specific lysis) was calculated using the mean value of triplicate cultures:

$$\%\ \text{specific lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \text{ x } 100$$

Maximum release was determined in wells with target cells and 2% Triton X. Spontaneous release was determined in wells with target cells and medium alone.

Chimerism analysis

Genomic DNA of the cells of interest was extracted, and PCR was performed under conditions described previously.[41] To quantify mixed hematopoietic chimerism, digitalized PCR gel pictures were obtained using the storage phosphor imaging technique and evaluated with an image analyzing software (ImageQuant, Molecular Dynamics, Sunnyvale, CA).[42] The percentage of donor origin DNA was calculated as %D= (volume integration/density of donor specific band)/(volume integration\density of recipient specific band) × 100. This technique enables to detect between 2.5% to 97.5% donor cell chimerism.[1]

Monoclonal antibodies (mAbs) for flow cytometry

MAbs against canine CD3 (CA17.6B3,IgG_{2b}),CD4 (CA13.1.E4, IgG_1), CD8 (CA9.JD3, IgG_{2a}),[43] and TCR$\alpha\beta$ (CA15.9D5, IgG_1) were used for flow cytometry. The anti-CD3, CD4, and CD8 mAbs were kindly provided by Dr. Peter Moore (School of Veterinary Medicine, University of California, Davis). Additionally we used antibodies against canine CD44 (S5, IgG_1)[44] and canine myeloid cells (DM5, IgG_1).[45] As isotype control, we used mAb 31A (IgG_1) directed at the mouse Thy-1 receptor which does not cross-react with canine cells.[46] All mAbs were produced and purified at the Biologics Production Facilities of the Fred Hutchinson Cancer Research Center (Seattle, WA). In addition, the commercially available antibodies Goat $F(ab')_2$ anti-Mouse Ig's Fluorescein conjugate (Biosource Camarillo, CA) and anti-human CD14 (DAKO Corporation, Carpenteria, CA) crossreacting with canine CD14[47] were used. The mAbs were fluorescein conjugated according to standard protocols.

Detection of apoptosis by Annexin V (Ax)/PI staining

After overnight incubation at 37 °C in 5% humidified atmosphere, cells were harvested, lysed, washed with PBS and incubated with Annexin V-FITC (Pharmingen, San Diego, CA) and propidium iodide (PI) according to the manufacturer's manual. Cells were analyzed by flow cytometry by means of CellQuest Analysis software (Becton Dickinson, Mountain View, CA). A minimum of 10 000 events were counted per sample. Cells positive for Annexin V but negative for PI are in early apoptosis, cells double positive for Annexin V and PI are in late apoptosis. Results are reported as a percentage of annexin V- FITC positive cells.