10.1148/radiol.15142849

Appendix E1

Cell Preparations and Activation

Cells were grown in Roswell Park Memorial Institute 1640 medium supplemented with penicillin (100 IU/mL)-streptomycin (100 μg/mL), 2 mmol/L L-glutamine (all from Life Technologies, Grand Island, NY), 10% fetal calf serum (Gemini Bio-Products, West Sacramento, Calif), and 50 μmol/L 2-mercaptoethanol (Sigma-Aldrich, St Louis, Mo). Dendritic cells (DCs) and natural killer (NK) cells were differentiated from the bone marrow of wild-type mice by using granulocyte-macrophage colony-stimulating factor (20 ng/mL, Peprotech; Rocky Hill, NJ) and 25 nmol/L human interleukin-15 (Peprotech), respectively. The NK culture consisted of more than 95% NK cells ($NK1.1^+$, $CD3^-$) after depletion of $CD3^+$ cells with anti-CD3 magnetic beads (Miltenyi, Auburn, Calif). DCs were activated by a lipopolysaccharide (5ng/mL, Sigma-Aldrich) overnight on day 5 with or without ovalubumin (OVA) peptide (SIINFEKL; 1 μg/mL, AnaSpec, Fremont, Calif) and used on the following day. Naïve cytotoxic T cells (CTLs) were purified from the spleen by using anti-CD8 magnetic beads (Miltenyi). Wild-type CTLs were activated by plate-coated anti-CD3 antibody (10 μg/mL) and anti-CD28 antibody (5 μg/mL) (eBiociences, San Diego, Calif). In some experiments, activated CTLs were transferred to an antibody-free culture supplemented with human interleukin-15 (0.3 nmol/L) on day 3. Splenocytes from OT-1 mice were activated with OVA peptide (1 μg/mL) for 3 days, washed with phosphate-buffered saline, and further cultured with mouse interleukin-2 (1 nmol/L, Peprotech) for 2 days (>99% of the cells were CTLs).

Appendix E2

^{89}Zr-Oxalate Production

Zirconium 89 (^{89}Zr) was produced by using the nuclear reaction $Y(p,2n)^{89}Zr$ and an in-house GE PETtrace beamline (GE Healthcare, Piscataway, NJ) (30), with modifications to a previously described method (31). In summary, yttrium metal mesh (200 mg, American Elements, Los Angeles, Calif) target cups were bombarded with 13 MeV protons in a GE PETtrace imager. The irradiated target metal was dissolved with 6 N hydrochloric acid (2 mL, Fisher Scientific, Pittsburgh, Pa), and 10 mol/L hydrogen peroxide (0.1 mL, Sigma-Aldrich) at 100°C for 1 hour. After dilution with water, the ^{89}Zr solution was absorbed onto a hydroxamate resin column prepared as previously described (31). The column was washed with 2 N hydrochloric acid followed by water, and ^{89}Zr was eluted as oxalate with 1 mol/L oxalic acid (Sigma-Aldrich) for a radiochemical yield of greater than 96% (<0.2% ^{89}Zr at end of bombardment).

Appendix E3

High-performance Liquid Chromatography and Chloroform Extraction Analyses of ^{89}Zr-Oxine Complex

High-performance liquid chromatography was performed by using a Beckman Gold high-performance liquid chromatography system equipped with a Model 126 programmable solvent module, Model 168 variable wavelength detector, β-Ram Model 4 radioisotope detector, and Beckman System Gold remote interface module SS420× and with use of 32 Karat software (Beckman Coulter, Brea, Calif). Analyses were performed by using a Waters StyraGel HT 1 (7.8 × 250 mm, 5 μm) column. Tetrahydrofuran solvent (Sigma-Aldrich) was used at a 0.8 mL/min flow rate. Chloroform (Mallinckrodt, St. Louis, Mo) extraction of the synthesized ^{89}Zr-oxine complex was performed, and radioactivity before the extraction and activities in the extracted chloroform phase and the aqueous phase were measured by using a dose calibrator (Capintec, Ramsey, NJ) or a gamma counter (WIZARD2 Automatic Gamma Counter, Perkin Elmer, Waltham, Mass).

Appendix E4

Biodistribution Study

Wild-type mice were injected with 5×10^6 DCs labeled with ^{89}Zr-oxine complex at the equivalent radioactive dose used for imaging (444 kBq). Mice were euthanized by means of carbon dioxide inhalation either 1 day or 7 days after cell transfer. Whole-body weight was measured, and blood and various organs were harvested, weighed, and assessed for radioactivity with a gamma counter. Radioactive dose associated with blood or each organ per total injected dose (percentage of injected dose was equal to radioactivity of organ divided by total injected dose times 100) and injected dose per gram of tissue normalized to a 20-g mouse were calculated. Percentage of injected dose per gram was equal to radioactivity of the organ divided by total injected dose divided by weight of the organ times body weight divided by 20 times 100 ($n = 5$).