

Effects of OK-432 on maturation of murine bone marrow-derived dendritic cells (DCs). Comparison of the mean fluorescence intensities (MFIs) of cell surface markers, murine major histocompatibility complex class II and CCR7 on OK-432-stimulated DCs and immature DCs. Data are shown as means \pm SE. *, P<0.05. **, P<0.001.



Dendritic cells (DCs) with or without OK-432 stimulation were incubated with fluorescein isothiocyanate (FITC)-dextran for 30 minutes and the uptakes were examined using flow cytometry. DCs were also stained with anti-CD11c antibody. Representative results are shown as histograms and median fluorescence intensities. The open area represents immature DCs and the filled area represents OK-432-stimulated DCs. The experiments were performed six times and the results were confirmed. The data are presented as the mean ± SE. **, P<0.001.



Kinetics of transferred dendritic cells (DCs) were evaluated using immunohistochemistry. Each organ was stained with anti-GFP antibodies and GFP-positive cells were counted at 3 days after RFA treatment, followed by the injection of immature or OK-432-stimulated DCs. Data are shown as the mean \pm SE. Bar, 50 μ m; †not detected; arrowheads, GFP positive cells; LN, lymph node.

Depletion antibodies (i.p. injection)



In vivo CD4/CD8 depletion was performed by means of i.p. injection of GK1.5 or 2.43 monoclonal antibodies. To confirm the depletion of T-cell subsets, peripheral blood lymphocytes were stained with anti-CD4 antibodies (Ab) and ant-CD8 Ab, and analyzed using flow cytometry at 3 days and 7 days after i.p. injection of 200 µg monoclonal Abs.



In vivo treatment model is described above. RFA was applied to a tumor on one side, when subcutaneous tumors grew up to about 5 mm in diameter, followed by injection of 1×10^7 DCs into the treated tumor with RFA. Untreated tumor on the opposite side was observed for 10 days. For in vivo CD4/CD8 depletion, 200 µg of the monoclonal antibodies, clone GK1.5 or clone 2.43 were i.p. injected 1 d before and 3d after RFA treatment.



In the CD4/CD8 depletion studies, the draining lymph nodes were harvested at 3 days after radiofrequency ablation (RFA), and their activations and antigenspecificities were evaluated. The mean fluorescent intensities of CD69 in CD8positive cells from the CD4-depleted mice and CD4-positive cells from the CD8depleted mice were estimated. Two mice were used in each group. Data are presented as the mean ± SE.