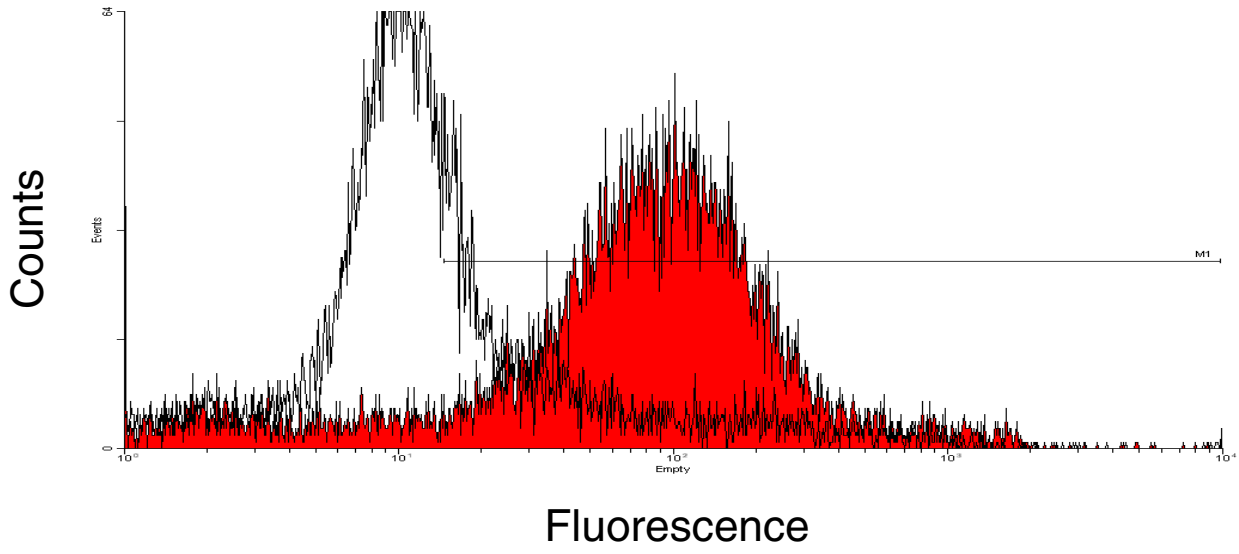
Figure 1



Flow cytometric analysis of A2L2 cells with serum from mice vaccinated with virus-like replicon particles (VRP)-*neu* or VRP-hemagglutinin (HA). Serum was collected 2 weeks after a single vaccination of Balb/c mice with 10^6 IU VRP-*neu* (filled curve) or 10^6 IU VRP-HA (open curve). The primary serum was diluted with PBS (1:100), and FITC-labeled goat anti-mouse IgG diluted in PBS (1:1000) was used as the secondary antibody.

tive with 66.3 cells, the parental cell line from which A2L2 was derived by transfection with *neu* (data not shown).

Protection from tumor challenge in a mammary fat pad prevention model following vaccination with VRP-neu

Groups of mice ($n = 7$ per group) were vaccinated subcutaneously with 10^5 IU or 10^6 IU VRP-*neu* or with 10^6 IU VRP-HA three times at 14-day intervals. Two weeks after the final vaccination, the mice were challenged with 2.5×10^4 A2L2 cells injected into a mammary fat pad. Five weeks after tumor challenge, the largest tumor dimension was measured and the mice were killed. If a tumor was present, its mass was determined. All of the mice vaccinated with VRP-HA had a measurable tumor, whereas only one mouse in each group vaccinated with 10^6 IU VRP-*neu* or 10^5 IU VRP-*neu* had a measurable tumor (Fig. 2a,2b). These findings clearly demonstrate that vaccination three times with either 10^5 IU or 10^6 IU VRP-*neu* protected mice from challenge with A2L2 cells. VRP-HA failed to provide protection for any of the mice, and therefore the protective effect was specific for the vaccine containing the gene for HER2/*neu*.

Determination of the minimal effective vaccine dose in two tumor prevention models

Because vaccination three times with 10^5 IU VRP-*neu* prevented tumor growth in a mammary fat pad, we next determined the minimum number of VRP-*neu* particles and the minimum number of vaccinations necessary to significantly inhibit tumor growth. In the mammary fat pad prevention model, vaccination twice with 10^5 IU VRP-*neu* or vaccination three times with 10^4 IU VRP-*neu* completely prevented tumor growth in many mice and significantly reduced the tumor mass in the entire group compared with the tumor mass of the mice vaccinated three times with VRP-HA (Fig. 3a). Identical results were obtained in the experimental lung metastasis prevention model, in which mice were injected with A2L2 cells intravenously in the tail vein after vaccination (Fig. 3b). These results demonstrate that, in both tumor models, vaccination three times with 10^4 IU VRP-*neu* or twice with 10^5 IU VRP-*neu* significantly reduced the tumor mass and lung metastasis. In addition, several mice in each vaccinated group were tumor free in mammary tissue or lungs.

Vaccination of MMTV-c-neu transgenic mice

MMTV-c-*neu* transgenic mice contain the activated rat *neu* gene under the control of the MMTV promoter and spontaneously develop *neu*$^+$ breast tumors within 110–120 days [45]. Without intervention, all of the mice die of breast cancer. We vaccinated groups of mice ($n = 10$ per group) three times at 14-day intervals with 10^6 IU VRP-*neu* or 10^6 IU VRP-HA and determined the effect on survival. Eight of the 10 mice vaccinated with VRP-HA were killed by 140 days owing to moribundity, and the remaining two mice were killed on day 195 (Fig. 4). None of the mice vaccinated with VRP-*neu* showed any sign of illness at 240 days, and breast tumors were not evident on palpation. The mice in the VRP-*neu*-vaccinated group were killed at this time, and gross pathologic examination of the breasts fol-