[18,19]. The replicon RNA contained the replication/transcription genes of the parent virus, but the structural protein genes were replaced by the gene for rat *neu*. From this plasmid construct, the replicon RNA is synthesized in the nucleus of the host cell and is transported to the cytoplasm for replication and transcription. The *neu* gene product is produced at high levels in the cytosol. Since the structural protein genes from the parent virus are not encoded by the replicon, progeny infectious Sindbis virions are not generated [20-22].

Alternatives to Alphavirus replicon plasmid vaccines [17,19,23-25] are Alphavirus-based virus-like replicon particles (VRP). As already mentioned, the replicon RNA does not contain the structural genes from the parent virus. It is therefore a single-cycle, propagation-defective RNA and replicates only within the cell into which it is introduced. To generate VRP from an attenuated strain of the Alphavirus Venezuelan equine encephalitis virus (VEE), the replicon RNA is packaged into particles by co-transfecting the replicon RNA and two separate helper RNAs, which together encode the full complement of VEE structural proteins [26]. Although the VRP can infect target cells in culture or *in vivo*, and can express the foreign gene to a very high level, the VRP are defective since they lack critical portions of the VEE genome – they lack the VEE structural protein genes necessary to produce infectious virus particles capable of spreading to other cells [21,27].

Several reports have demonstrated that VRP are extremely effective vaccine vectors [28-39]. The VEE VRP vaccine vectors are particularly attractive because the VEE envelope glycoproteins target the VRP to cells of lymphoid tissue [40], because they can be administered multiple times [39], because they induce both cellular and humoral immune responses, and because pre-existing immunity to VEE in humans should not be problematic since the incidence of VEE infection is low.

In the current study, we sought to determine whether vaccination with VEE-derived VRP containing the gene for HER2/*neu* would inhibit tumor growth in prevention models in which HER2/*neu*-expressing tumor cells had been injected either into a mammary fat pad or intravenously. We also sought to determine whether vaccination could prevent spontaneous tumorigenesis in HER2/*neu* transgenic mice. VRP-*neu* vaccination induced antigen-specific $CD8^+$ T-cell and IgG responses that corresponded with the lack of tumor growth in both tumor models. In light of the clinical benefit of trastuzumab, a safe and effective vaccine that can induce cellular and humoral immunity, VRP-*neu* warrants clinical evaluation.

Materials and methods

Tumor cell line and reagents

The A2L2 cell line that expresses high levels of rat HER2/*neu* has been previously described in detail [17]. The A2L2 cell line has consistently expressed high levels of HER2/*neu* for more than 5 years and consistently induces tumors in Balb/c mice when injected into a mammary fat pad or intravenously. The A2L2 cell line was maintained in Eagle's MEM containing 5% FCS, sodium pyruvate, nonessential amino acids, L-glutamine, and vitamins (GIBCO, Carlsbad, CA, USA). The monolayer cultures were subdivided at approximately 75% confluence by treatment for 1–3 min with 0.25% trypsin and 0.02% EDTA at 37°C.

Virus-like replicon particles

The pSV2-*neu* plasmid containing the gene for rat HER2/*neu* was provided by Dr Mien-Chie Hung (The University of Texas MD Anderson Cancer Center, Houston, TX, USA). VEE VRP were constructed at AlphaVax, Inc. (Research Triangle Park, NC, USA) according to the published procedure [26]. Control VRP containing the gene for A/PR/8/34 influenza hemagglutinin (HA) were also prepared following the same procedure. VRP preparations were screened in a sensitive *in vitro* Vero cell cytopathic effect assay for the detection of replication competent virus. Prior to their use in these studies, the preparations were shown to be devoid of detectable replication competent virus.

Flow cytometry

A2L2 cells were incubated for 1 hour at 37°C with either immune serum or control serum diluted in PBS. FITC-labeled goat anti-mouse IgG diluted 1:1000 in PBS was added to the cells and incubation was continued for 1 hour at 37°C. The cells were washed three times in PBS and were analyzed by flow cytometry using an EPICS Profile Analyzer (Beckman Coulter, Inc., Fullerton, CA, USA).

Mice

Female Balb/c mice, aged 6–8 weeks, were obtained from the Frederick Cancer Research Facility (Frederick, MD, USA). Mice were allowed to acclimate for at least 1 week before use. Six-week-old MMTV-c-*neu* transgenic mice were obtained from Charles River Laboratories Inc. (Wilmington, MA, USA). This strain of mice is called the 'Oncomouse' by Charles River Laboratories due to the spontaneous generation of breast cancer. These mice are of the FVB/N strain, and the transgene is the 'activated' rat c-*neu* oncogene preceded by the MMTV promoter [41].

Vaccination with VRP

Mice were vaccinated subcutaneously in the hind foot pad with VRP suspended in 50 μl normal saline. Repeat vaccinations, when administered, were performed using alternate hind feet. Tail vein blood was removed and tumor challenge performed 2 weeks after the final vaccination.