HER-2 DNA and Protein Vaccines Containing Potent Th Cell Epitopes Induce Distinct Protective and Therapeutic Antitumor Responses in HER-2 Transgenic Mice

Valéry Renard, Lene Sonderbye, Kirsten Ebbehøj, Peter Birk Rasmussen, Klaus Gregorius, Tine Gottschalk, Søren Mouritsen, Anand Gautam and Dana R. Leach

*J Immunol* 2003; 171:1588-1595; doi: 10.4049/jimmunol.171.3.1588

http://www.jimmunol.org/content/171/3/1588

---

**References**

This article cites 58 articles, 30 of which you can access for free at:

http://www.jimmunol.org/content/171/3/1588.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

Copyright © 2003 by The American Association of Immunologists All rights reserved.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.
HER-2 DNA and Protein Vaccines Containing Potent Th Cell Epitopes Induce Distinct Protective and Therapeutic Antitumor Responses in HER-2 Transgenic Mice

Valéry Renard,1 Lene Sonderbye,1 Kirsten Ebbehøj,2 Peter Birk Rasmussen, Klaus Gregorius, Tine Gottschalk, Søren Mouritsen, Anand Gautam, and Dana R. Leach3

Overexpression of the growth factor receptor HER-2 (c-erbB-2, new) has transforming potential and occurs in ~20–30% of breast and ovarian cancers. HER-2 is a self Ag, but Abs and T cells specific for HER-2 have been isolated from cancer patients, suggesting HER-2 may be a good target for active immunotherapy. We constructed rat HER-2 DNA and protein vaccines containing potent Th cell epitopes derived from tetanus toxin and studied their potency in two strains of mice transgenic for the rat HER-2 molecule. Vaccination with HER-2 DNA protected nontransgenic mice from tumor challenge, but induced only moderate protection in one of the tumor models. However, vaccination with the modified HER-2 protein resulted in almost complete protection from tumor challenge in both tumor models. This protection could be mediated by Abs alone. In addition, protein vaccination efficiently eliminated pre-established tumors in both models, even when vaccination occurred 9 days after tumor implantation. These data demonstrate the potential of HER-2-based vaccines as therapeutic agents for the treatment of cancers overexpressing HER-2. The Journal of Immunology, 2003, 171: 1588–1595.

H

ER-2 is a receptor tyrosine kinase of the epidermal growth factor receptor family. Amplification of the HER-2 gene and/or overexpression of the HER-2 protein can induce cell transformation (1) and has been demonstrated in a variety of human malignancies, including breast and ovarian cancers (2, 3), in which it is associated with tumor aggressiveness and a poor prognosis (4, 5). For these reasons, HER-2 is an attractive target for cancer immunotherapy, and a number of approaches have been investigated, including a humanized mAb against HER-2 (trastuzumab) that has been approved for clinical use in the treatment of patients with metastatic breast cancer (6, 7).

Active vaccination against HER-2 is an approach that may present advantages over passive mAb therapy by activating cytotoxic T cell responses and inducing a polyclonal Ab response, potentially leading to better activation of Ab-dependent effector functions. However, the efficacy of active vaccination might be limited by the fact that HER-2 is a self Ag with poor immunogenicity due to immunological tolerance. In contrast, specific Abs (8, 9) and CTL (10–13) have been detected in patients with HER-2-expressing mammary and ovarian cancers. Thus, tolerance to HER-2 is not absolute and may be overcome, but naturally occurring immune responses are too weak to be effective, as tumors continue to grow and metastasize in these patients. Therefore, active immunization strategies must use potent mechanisms to enhance the anti-HER-2 immune response to therapeutic levels.

A number of different approaches have been investigated in both human and animal studies in an attempt to develop an effective HER-2 vaccine. Peptides representing Th epitopes of rat HER-2 could overcome tolerance in rats and induce both T cell and Ab responses to the immunizing peptides and to the whole protein (14). Subsequently, similar studies have been conducted in human clinical trials (13). However, peptide-based vaccines might be limited by several factors, including the necessity to match multiple HLA alleles (reviewed in Ref. 15). Promising results were also obtained using DNA vaccines against HER-2 in rodent models. Different groups showed that naked DNA vaccines encoding truncated or modified rat HER-2 were capable of eliciting protective anti-rat HER-2 immunity in transgenic (16–18) and nontransgenic (19–24) models. Protein-based vaccines represent yet another alternative to peptide and DNA vaccines. Full-length HER-2 protein was not effective in one model (14); however, modified or truncated proteins have resulted in effective immune responses to HER-2 (25–27).

The pivotal role of Th cells in the initiation of the immune response is well documented (28), and the inability to control tumor outgrowth may be due to inadequate activation of tumor-specific Th cells, leading to poor tumor-specific immunity. We hypothesized that tolerance to HER-2 might limit the availability of high avidity CD4+ T cells necessary to initiate effective anti-HER-2 immune responses. Therefore, to bypass HER-2-specific CD4+ T cell tolerance, we constructed HER-2 DNA and protein molecules containing promiscuous Th cell epitopes (29) from tetanus toxin (Th chimeric molecules). Insertion of foreign helper epitopes was designed to overcome tolerance to HER-2 by providing exogenous T cell help to HER-2-specific B and T lymphocytes. We have previously shown that this approach could effectively bypass the immune tolerance toward the highly conserved ubiquitin protein in mice (30), the proinflammatory cytokine TNF-α, leading to the endogenous production of therapeutic anti-TNF-α Abs (31, 32) and toward IL-5, involved in allergy and asthma (33).

We evaluated the immunogenic and therapeutic effects of Th chimeric DNA and protein vaccines against transplantable HER-2-positive tumors in two different rat HER-2 transgenic models as well as wild-type mice. Both of the transgenic models exhibited
functional tolerance to HER-2 as compared with wild-type parental strains. Protein vaccination elicited high titer Ab responses and was very effective in both transgenic models, whereas DNA vaccination had a significant effect in only one model. Furthermore, the inclusion of Th epitopes increased the immunogenicity of the vaccines. These results suggest that vaccination against HER-2 is potentially an effective means of cancer therapy, but that the efficacy of such vaccinations may depend on the nature of the tumor itself, its susceptibility to different host effector mechanisms, and the robustness of the immune response.

Materials and Methods

**Rat HER-2 DNA constructions**

Truncations of the wild-type rat HER-2 cDNA were made by PCR of the native cDNA sequence (EMBL accession number I21129; SwissProt accession number P05944) using standard molecular biology techniques. The activating T to A mutation at aa 661 was incorporated to make the constructs identical with the rat HER-2 gene product in the transgenic mice (34). The resulting truncated products, rHER2T2A, rHER2MA6, and rHER2T5, included the native signal sequence and were cloned into the pcDNA3.1 mammalian expression vector (Invitrogen, Groningen, The Netherlands).

Th chimeric vaccine molecules were made from the truncated wild-type sequences by insertion of sequences coding for the promiscuous tetanus toxoid Th cell epitopes P2 (QYIKANSKFIGITEL) and/or P30 (FNNFTVS-WFLRVPKVSAHLLE) (29) by sequence overlap extension PCR. The P30 toxin was inserted into the native sequence, whereas the P2 epitope replaced the native sequence at the insertion point. DNA was purified using Qiagen (Valencia, CA) EndoFree Giga-prep plasmid purification kits, according to the manufacturer’s instructions (Merck Eurotrol, Albertslund, Denmark), and resuspended at 1 mg/ml in sterile 0.9% saline for injection.

**Expression and purification of rat HER-2 proteins**

Two of the constructs, rHER2T5 and rHER2T5-D (minus leader sequences), were cloned into the pM/T/BiP/V5-HisA expression vector (Invitrogen) for production of the recombinant proteins rM5 and rM5-D. The native stop codon was included to avoid expression of the V5-HisA fusion. Drosophila melanogaster S2 cells (American Type Culture Collection, ATCC, Manassas, VA) were transfected using Drosophila Expression System, according to manufacturer’s instructions (Invitrogen). Polyclonal stable lines were established and grown in large scale flasks for protein production. The supernatants were concentrated by diafiltration, and the rM5 and rM5-D proteins were purified by immunoaffinity chromatography using anti/V5 (Invitrogen, Carlsbad, CA) specific for rat HER-2. The column fractions containing HER-2 protein were then dialyzed and freeze-dried. The proteins were characterized by SDS-PAGE, Coomassie staining, silver staining, Western blotting, N-terminal sequencing, and amino acid analysis. All rat HER-2 proteins were ≥95% pure. The rat HER-2 proteins were reconstituted in 50 mM NH4HCO3 + 0.3% pluronic F68 (Sigma-Aldrich, Vallensbæk Strand, Denmark) at pH 7.8 before injection.

**Mice, cell lines, and tumor challenges**

BALB/c and FVB/N mice were obtained from M&B A/S (Ry, Denmark). Mouse mammary tumor virus (MMTV)-c-neu F1 hybrids were bred in our animal facility. Female mice 6–10 weeks old were used for all tumor experiments. The transplantable tumor cell line Hmr.jr was derived in our laboratory from a spontaneous breast tumor in BALB/c mice (34). The resulting transplanted tumors, rHER2T2A, rHER2MA6, and rHER2T5, included the native signal sequence and were cloned into the pcDNA3.1 mammalian expression vector (Invitrogen, Groningen, The Netherlands).

DNA immunizations were performed by intradermal injection of 50 µg DNA in 50 µl into two shaved sites on the back of anesthetized mice for a total dose of 100 µg DNA. Unless otherwise stated, mice were immunized a total of five times, at weeks 0, 1, 2, 4, and 6. Proteins for immunization were emulsified 1:1 (v/v) in IFA (Sigma-Aldrich), or mixed 1:1 with adjuvons (Superfos, Vedbaek, Denmark). Mice were injected s.c. in the neck region with 12.5 µg of protein in a total volume of 100 µl.

**In vivo depletion of CD4 and CD8 T cells**

Anti-CD4 (GK1.5)- and anti-CD8 (2.43)-producing hybridomas were obtained from ATCC and injected in nude mice to produce ascites. The concentration of specific Ab in the ascites was determined by radial immunodiffusion (The Binding Site, Birmingham, U.K.). Groups of mice were injected with 500 µg GK1.5, 2.43, or both, 1 week before challenge with tumor cells. Control mice received 1 mg of irrelevant rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). A single injection (250 µg) of depletion or control Abs was given 1 week later. T cell depletion was confirmed by flow cytometric analysis of PBMCs using fluorochrome-conjugated anti-CD4 and anti-CD8 Abs (BD Pharmingen, San Diego, CA) specific for different epitopes than the depleting Abs. Depletion of greater than 95% of specific T cell subsets was confirmed.

**Ab titer determination**

Sera from vaccinated mice were tested for HER-2-specific Abs by ELISA. Ninety-six-well Maxisorb plates (Nunc, Life Technologies, Taastrop, Denmark) were coated with 100 µl rat HER2 protein in carbonate buffer, pH 9.6, at a concentration of 0.5 µg/ml, incubated for 1 h, washed with PBS + 0.5 M NaCl and 1% Triton X-100, and then blocked for 1 h with washing buffer plus 1% BSA. Partially purified proteins (dialyzed supernatant) from D. melanogaster S2 cells transfected with an empty vector were coated at a concentration of 2 µg/ml for a specificity control. Standard (pooled sera from mice immunized with rTS-D in IFA) and diluted serum samples were added in duplicate and incubated at room temperature for 30 min. After washing, secondary Ab (HRP-conjugated rabbit anti-mouse IgG (DAKO, Glostrup, Denmark)) was added at a dilution of 1:1000 and incubated for 30 min. The plates were then washed, and o-phenylenediamine substrate (Sigma-Aldrich) was added. The reaction was stopped with 2N H2SO4, and the OD was measured in a Dynex MRX ELISA plate reader at 490 nm. The standard was given an arbitrary concentration of 1 × 107 U/ml, and the standard curve and serum Ab concentrations were calculated using Revelation Quicklink software (Dynex Technologies, Chantilly, VA).

**Passive transfer**

Sera from MMTV-c-neu mice vaccinated with rM5 or rM5-D protein were pooled, and the HER-2-specific Ab concentration was determined by ELISA using a standard curve from a reference sera. A total of 100–200 µl of the pooled sera was injected into the peritoneum of naïve MMTV-c-neu mice 1 day before s.c. challenge with 6 × 105 Hmr.jr tumor cells. Control animals were injected with 500 µg nonspecific mouse IgG (Jackson ImmunoResearch Laboratories).

**Results**

MTTV-c-neu and F1 transgenic mice exhibit tolerance to HER-2

Many preclinical studies have suggested that DNA-based immunization can induce protective and therapeutic immune responses, including Abs and CTL (reviewed in Ref. 36), against the HER-2 oncogene (16–22). However, the tolerance to HER-2 in these various models has not always been well documented, or has been disputed (37). We therefore compared the in vivo efficacy of HER-2 DNA vaccines in two transgenic mouse models (MTTV-c-neu and MMTV-c-neu × BALB/c F1 hybrids) that express an activated form of rat HER-2 under the MMTV promoter (34) and their respective parental strains FVB/N and BALB/c.

Wild-type and transgenic mice were immunized, as described in Materials and Methods, with a total of nine different rat HER-2 DNA vaccines representing two truncated wild-type sequences and one of the synthetic HER-2 vaccines described above. The animals were immunized three times, at weeks 3, 4, and 6. Anti-HER-2 Abs were measured by ELISA in serum samples collected 1 week after the third immunization. The results of these experiments are summarized in Fig. 1. The data show that the DNA vaccines induced anti-HER-2 Abs in a consistent manner, and that the levels of Abs were highest in F1 hybrid mice that expressed the truncated rat HER-2 cDNA.

**Conclusion**

These results demonstrate that DNA vaccines can induce protective and therapeutic immune responses against HER-2. The data also show that the DNA vaccines were effective in both wild-type and transgenic mice, and that the levels of Abs were highest in F1 hybrid mice that expressed the truncated rat HER-2 cDNA. This suggests that DNA vaccines may be a promising new approach for the treatment of HER-2-positive breast cancer.

---

*a Abbreviation used in this paper: MMTV, mouse mammary tumor virus.*
seven Th chimeric molecules (Table I). The mice were then challenged with the appropriate syngeneic transplantable tumor (Hm-ref for FVB/N and MMTV-c-neu mice; HTH-K for BALB/c and F₁ hybrids). Tumor cells expressing the foreign rat HER-2 molecule grew progressively in naive nontransgenic animals, showing that the rat HER-2 molecule did not constitute a highly immunogenic tumor Ag, as previously described (20). Following immunization, significant differences in tumor protection were observed between the two wild-type strains as well as between wild-type and transgenic mice (Table II). DNA vaccination protected between 50 and 80% of FVB/N and BALB/c mice from tumor challenge. In contrast, DNA vaccination had little effect in MMTV-c-neu mice, with only sporadic protection observed. There was a modest effect in F₁ mice with an overall protection rate of 21%. The best results in F₁ mice were obtained with selected Th chimeric DNA vaccines; however, these constructs had no significant effect in paired experiments in MMTV-c-neu mice, with only sporadic protection observed. There was a modest effect in F₁ mice with an overall protection rate of 21%. The best results in F₁ mice were obtained with selected Th chimeric DNA vaccines; however, these constructs had no significant effect in paired experiments in MMTV-c-neu mice (Fig. 1). Taken together, these results indicate that mice transgenic for rat HER-2 exhibit some level of tolerance, with MMTV-c-neu mice consistently more tolerant than F₁ mice, and are less responsive than wild-type mice to HER-2 DNA vaccination.

**Protein vaccination induces protective antitumor immune responses in both HER-2-specific tumor models**

Naked DNA vaccines are generally associated with relatively weak Ab responses. Therefore, we next tested HER-2 protein vaccines, which we anticipated would result in much higher Ab responses, to see whether these would provide tumor protection in one or both of the animal models. Three immunizations with the wild-type rM5 or the Th chimeric rM5-D protein in adjuvans resulted in high Ab titers in both MMTV-c-neu and F₁ mice and no significant reactivity toward host insect cell proteins (Fig. 2, A and B).

**Although vaccination with the Th chimeric rM5-D protein consistently resulted in higher Ab titers than with the rM5 protein, both vaccines were able to induce nearly complete tumor protection in MMTV-c-neu mice (Fig. 2C) with only 1 of 20 vaccinated mice developing a tumor. Slightly less protection was observed in F₁ mice challenged with HTH-K tumor cells (Fig. 2D). There was significant reduction in average tumor size, but 6 of 9 mice (66.7%) immunized with rM5 and 3 of 8 mice (37.5%) immunized with rM5-D developed small, slow growing tumors.**

We next reduced the number of vaccinations to more accurately assess whether the addition of the P30 helper epitope in rM5-D enhanced the immunogenicity of the protein. After only one immunization, minimal Ab titers were detected in MMTV-c-neu mice vaccinated with rM5-D, but not rM5, protein was clearly more effective in priming anti-HER-2 responses, as anti-HER-2-specific Ab titers could be measured after tumor challenge only in rM5-D-immunized mice.

**Table I. Translation products of the rat HER-2 DNA constructs indicating the position of inserted tetanus toxin epitopes P2 and P30**

<table>
<thead>
<tr>
<th>Construct Name</th>
<th>Position of P2 (aa)</th>
<th>Position of P30 (aa)</th>
<th>Length (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rHER2TA2 wild type</td>
<td>NA</td>
<td>NA</td>
<td>818</td>
</tr>
<tr>
<td>rHER2TA2-6Y</td>
<td>348–362</td>
<td>733–753</td>
<td>818</td>
</tr>
<tr>
<td>rHER2TA2-6E</td>
<td>348–362</td>
<td>28–48</td>
<td>818</td>
</tr>
<tr>
<td>rHER2MA6-C</td>
<td>676–690</td>
<td>655–675</td>
<td>702</td>
</tr>
<tr>
<td>rHER2TA5 wild type</td>
<td>NA</td>
<td>NA</td>
<td>665</td>
</tr>
<tr>
<td>rHER2TA5-D</td>
<td>NA</td>
<td>655–675</td>
<td>687</td>
</tr>
<tr>
<td>rHER2TA5-4D</td>
<td>233–247</td>
<td>655–675</td>
<td>687</td>
</tr>
<tr>
<td>rHER2TA5-6D</td>
<td>348–362</td>
<td>655–675</td>
<td>687</td>
</tr>
<tr>
<td>rHER2TA5-8D</td>
<td>488–502</td>
<td>655–675</td>
<td>687</td>
</tr>
</tbody>
</table>

a The overall length and positions of the epitopes are relative to the start methionine, and thus include the native leader sequence of 23 aa (NA, not applicable).

b rHER2TA5 and rHER2TA5-D correspond to the protein sequence of rM5 and rM5-D molecules, respectively.

**Table II. Tumor protection induced by DNA vaccination with wild-type and HER-2 transgenic animals**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Tumor Bearing</th>
<th>Tumor Free</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB/N</td>
<td>33</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>BALB/c</td>
<td>10</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>MMTV-c-neu</td>
<td>193</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>F₁ hybrids</td>
<td>159</td>
<td>43</td>
<td>21</td>
</tr>
</tbody>
</table>

Data summarize different experiments in which mice were vaccinated with HER-2 or HER-2/Th chimeric DNA vaccines, as described in Materials and Methods. Total numbers of tumor-bearing mice and tumor-free mice at the termination of the experiment are indicated.
FIGURE 2. Immune responses in protein-vaccinated HER-2 transgenic mice. Ab responses in MMTV-c-neu (A) and F1 (B) mice vaccinated three times with 12.5 μg protein in adjuvants. The figures show the titration curves of anti-HER-2 sera against HER-2 protein (circles) or S2 insect cell proteins (triangles). Ab titers induced by rM5 and rM5-D protein vaccines were significantly different in MMTV-c-neu \((p = 6.25 \times 10^{-5})\) and in F1 \((p = 1.57 \times 10^{-3})\) mice. C and D. Protective antitumor immunity induced by protein vaccination in MMTV-c-neu mice \((n = 10)\) (C) and F1 hybrids \((n = 8)\) (D). Three weeks following the last immunization, MMTV-c-neu and F1 hybrid mice were challenged s.c. with \(1 \times 10^6\) Hmr.jr and \(2 \times 10^5\) HTH-K tumor cells, respectively. Data are representative of three separate experiments.

FIGURE 3. Antitumor immunity in MMTV-c-neu and F1 transgenic mice after a single HER-2 protein vaccination. A and B. Average Ab titer \((n = 10)\) in vaccinated mice before and after tumor challenge (calculated as described in Materials and Methods). C and D. Average tumor size in vaccinated mice challenged 3 wk later with \(1 \times 10^6\) Hmr.jr (MMTV-c-neu mice) or \(2 \times 10^5\) HTH-K (F1 mice) tumor cells injected s.c. The difference in the protection induced by a single immunization with rM5 vs rM5-D protein was significant \((p = 0.04\) by Student’s t test\) and reproduced in three separate experiments.
87.5% (7 of 8) F1 mice. Thus, a single vaccination with Th chimeric protein resulted in significant tumor protection in HER-2 transgenic mice, which appeared to correlate with the ability to prime HER-2-specific Ab responses.

rM5-D protein vaccine treats established tumors

To test the therapeutic efficacy of the vaccine in a situation that more closely resembles the clinical setting, we allowed tumors to establish before vaccinating with HER-2 proteins. HER-2 transgenic mice were first challenged with tumor cells (day 0) and then treated by a single injection of rM5-D or rM5 protein formulated in IFA. Animals were treated either at the same time as tumor challenge, or up to 9 days later, giving the tumors time to firmly establish in the host. Treatment with rM5-D protein had a profound effect on tumor growth at all treatment points (Fig. 4, A and C). Again, tumor incidence was more dramatically reduced in MMTV-c-neu than in F1 mice, because 87.5% (35 of 40) of MMTV-c-neu mice remained tumor free 34 days after tumor challenge, vs 21% (5 of 24) of F1 mice.

In contrast, treatment with rM5 protein vaccine had much less effect on tumor growth (Fig. 4, B and D). Although the average tumor growth rate was decreased in some instances, all MMTV-c-neu mice and 92% (22 of 24) of F1 mice treated with rM5 protein developed tumors.

Effector T cells are not required for rM5-D protein vaccine-mediated tumor rejection

We next performed in vivo T cell depletion experiments to determine the role of specific T cell subsets in tumor rejection. Groups of rM5-D protein-immunized MMTV-c-neu mice and F1 hybrids were treated with control Abs (rat IgG) or Abs to deplete CD4+ T cells and/or CD8+ T cells, and then challenged with the appropriate tumor cells. Mock-vaccinated control animals developed progressively growing tumors (Fig. 5, A and B). In contrast, MMTV-c-neu and F1 hybrid mice vaccinated with rM5-D protein rejected transplantable tumors regardless of their T cell depletion status.
studied the immunological and therapeutic effects of HER-2 DNA vaccines against transplantable HER-2-positive tumors in two HER-2 transgenic mouse models. We also compared the responses in the nontransgenic parental mouse strains to assess whether there was tolerance to HER-2 in the transgenic mice. DNA vaccination resulted in 50–80% protection in wild-type mice challenged with HER-2-expressing tumor cells, demonstrating the ability of the HER-2 DNA vaccines to induce a protective immune response in nontolerant mice, as has been reported for other xenogenic HER-2 DNA vaccines against HER-2-expressing tumors (16–22, 24, 43).

In contrast, none of the vaccines had a significant effect in MMTV-c-neu mice. These results strongly suggest a functional tolerance to rat HER-2 in these mice and are in contrast to previous findings (37). Some Th chimeric DNA vaccines had a modest, but significant protective effect in F1 mice. The difference in responses between MMTV-c-neu and F1 mice may be due to differences in tolerance (F1 mice are heterozygous for the rat HER-2 transgene), or to inherent differences between Hmr.jr and HTH-K susceptibility to immune effector mechanisms.

The mechanism of limited tumor protection in DNA-vaccinated F1 mice, and the reasons for the lack of protection in MMTV-c-neu mice are not known. We were able to measure HER-2-specific Abs in DNA-vaccinated F1 and MMTV-c-neu mice, but the titers were >1000-fold lower than those in protein-vaccinated mice and did not correlate with tumor protection (data not shown). Given the apparent susceptibility of the tumors to Ab-mediated rejection, it is possible that DNA vaccines simply failed to generate sufficient Ab titers in tolerant animals. In contrast, others have reported no correlation between Ab titers and protection from tumor challenge in DNA-vaccinated mice, and in those models protection was attributed to T cells (16, 20, 22).

In contrast to DNA vaccines, in which Ab responses appeared negligible, HER-2-protein vaccines elicited high HER-2-specific Ab titers and efficiently prevented transplantable tumor growth in both MMTV-c-neu and F1 hybrid mice. In addition, vaccination with the rM5-D protein was much more efficient than the rM5 HER-2 vaccine in eradicating pre-established tumors, even when the treatment was delayed by up to 9 days postchallenge, demonstrating the enhancing effect of the inserted tetanus toxin epitopes.

Our results also showed that the protein vaccine-mediated anti-tumor effect was consistently more pronounced in MMTV-c-neu than in F1 mice, both in terms of tumor incidence and tumor growth rate, which conflicted with the DNA vaccines data. It was particularly clear after only one vaccination with rM5-D, in which MMTV-c-neu mice developed higher Ab titers than F1 mice and were accompanied by greater tumor rejection. These data may indicate a lower degree of B cell tolerance in MMTV-c-neu than in F1 mice or inherent differences in the susceptibility of Hmr.jr cells to Ab-mediated rejection.

T cell depletion experiments together with passive transfer of Abs demonstrated that Abs alone could protect MMTV-c-neu mice from tumor development. An effective therapeutic role for HER-2-specific Abs was previously suggested by Dakappagari et al. (26), who developed a HER-2 B cell epitope vaccine designed to induce an Ab response to HER-2. They showed that the vaccine could significantly slow the development of spontaneous tumors in transgenic mice, demonstrating that HER-2-specific Abs can be effective in tumor growth control. We were also able to see complete protection against spontaneous mammary carcinomas in rM5-D protein-vaccinated MMTV-c-neu mice for up to 1 year (data not shown). The mechanisms by which polyclonal anti-HER-2 Abs induced by vaccination affect tumor growth need to be elucidated. A variety of mechanisms has been suggested for mAbs, such as down-regulation of cell surface expression by tumor cells
(44, 45), alteration of HER-2 tyrosine kinase activity (44–47), and interference with HER-2 heterodimer (46–48) or homodimer formation (49). Vaccine-induced Abs against HER-2 may induce any combination of these proposed effects as well as in vivo mechanisms such as Ab-dependent cellular cytotoxicity and complement activation.

The remarkable therapeutic efficacy of HER-2-specific Abs that we observed in HER-2 transgenic animal models is of particular interest for the development of HER-2-specific vaccines for clinical use. Many of the present approaches targeting HER-2 are designed to favor the development of a cellular response using DNA vaccines (16–22), cell-based vaccines (37, 50–56), or polysaccharide complexes (57). Our data would suggest that different tumors might be susceptible to different immune effector mechanisms. Indeed, other investigators have shown that the combination of CTL and Ab responses was necessary for efficient tumor rejection (19, 51). It is very likely that such a diversity of tumor responsiveness exists in humans. In clinical situations, vaccination protocols that can elicit cellular and humoral responses would be expected to be more efficient in tumor therapy. In that view, the Th chimeric approach represents a flexible way of administering both DNA as well as protein vaccines, to elicit the desired immune responses in cancer patients. In addition, DNA and protein can be used in a prime/boost regimen to enhance both cellular and humoral immune responses, as has been shown for an HIV vaccine (58).

Taken together, our results demonstrate that the Th chimeric approach represents a potentially useful method for active immunotherapy of HER-2-positive tumors in human cancer patients.

Acknowledgments
We acknowledge the excellent technical assistance of the following individuals, without whom this work would not have been possible: Lone M. Igel, Heidi Winter Jensen, Annika Anthonssen, Gitte Juhl Funch, and Helene Pedersen.

References


