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Distinct and Non-Overlapping T Cell Receptor Repertoires Expanded by DNA Vaccination in Wild-Type and HER-2 Transgenic BALB/c Mice

Simona Rolla,* Chiara Nicoló,† Silvia Malinarich,* Massimiliano Orsini,† Guido Forni,‡ Federica Cavallo,2* and Francesco Ria†

Central tolerance to tumor-associated Ags is an immune-escape mechanism that significantly limits the TCR repertoires available for tumor eradication. The repertoires expanded in wild-type BALB/c and rat-HER-2/neu (rHER-2) transgenic BALB-neuT mice following DNA immunization against rHER-2 were compared by spectratyping the variable (V)β and the joining (J)β CDR 3. Following immunization, BALB/c mice raised a strong response. Every mouse used one or more CD8+ T cell rearrangements of the Vβ9-Jβ1.2 segments characterized by distinct length of the CDR3 and specific for 63-71 or 1206-1214 rHER-2 peptides. In addition, two CD4+ T cell rearrangements recurred in >50% of mice. Instead, BALB-neuT mice displayed a limited response to rHER-2. Their repertoire is smaller and uses different rearrangements confined to CD4+ T cells. Thus, central tolerance in BALB-neuT mice acts by silencing the BALB/c mouse self-reactive repertoire and reducing the size of the CD8+ T cell component. CD8+ and CD4+ T cells from both wild-type and transgenic mice home to tumors. This definition of the T cell repertoires available is critical to the designing of immunological maneuvers able to elicit an effective immune reaction against HER-2-driven carcinogenesis. The Journal of Immunology, 2006, 177: 7626–7633.

Tumor-prone mice transgenic for oncogenes provide a significant complementary addition to the data stemming from tumors engrafted in syngeneic mice. The slowness of neoplastic progression, its relationship with the surrounding tissues, the natural occurrence of invasion and metastasis, and the presence of a long-lasting interaction between the tumor and the host immune system are the appealing features of these transgenic models (1). As these transgenic mice and their wild-type (WT)3 counterparts differ in their expression of a single gene, they provide an unique opportunity to assess how overexpression of an oncogene protein product influences the TCR repertoire (2). This comparison acquires crucial importance when evaluating the results of vaccination against tumor-associated Ags (TAA). Identification of TAA provides new targets for antitumor immunity. However, most currently defined TAA are products of self-genes overexpressed by neoplastic lesions. This poses a significant challenge because effective vaccine strategies should be able to by-pass self-tolerance. Mice engrafted with a TAA-expressing tumor are not suitable for evaluation of this critical issue in antitumor vaccination, because their TCR repertoire may be dramatically different from that of cancer-prone transgenic mice and of patients with neoplastic lesions overexpressing the target TAA.

Central tolerance to an overexpressed self-TAA involves intrathymic deletion of T cells bearing a TCR able to bind them with high affinity (3). In the thymus, an elaborated system permits the presentation of most self-epitopes by various kinds of APCs (4). Failure of this mechanism results in extended autoreactivity (5–7). A large body of observations confirms that a residual TCR repertoire exists for overexpressed self-Ag in the periphery and encompasses “low affinity” TCRs specific for dominant epitopes, and/or TCRs characterized by “high affinity” for subdominant or cryptic epitopes (8–10). Thus the natural tolerance induced by an Ag differentially expressed during early life may account for very dissimilar peripheral TCR repertoires.

Several lines of mice transgenic for the rat-HER-2/neu (rHER-2) oncogene overexpress the transgenic rHER-2 at different times of their life and develop carcinomas whose progression mirrors that of their human counterparts (11, 12). The protein product coded by HER-2 is a member of the epidermal growth factor receptor family endowed with a potent tyrosine kinase activity. Receptor tyrosine kinases are central components of cell signaling networks and play crucial roles in physiological processes, such as embryogenesis, cell proliferation, and apoptosis. Malfunction of HER-2 is a leading cause of major human diseases, and correlates with more aggressive tumor growth, greater invasiveness, enhanced metastatic potential, and increased resistance to therapy (13). Probably the most aggressive and consistent model of rHER-2 mammary carcinogenesis is provided by female BALB/c mice transgenic for the transforming rHER-2 oncogene under the transcriptional control of a long-terminal repeat sequence from mouse mammary tumor virus (MMTV) (BALB-neuT mice, (http://cancermodels.nci.nih.gov/mmhc/index.jsp)). rHER-2 protein overexpression and the onset of
epithelial nodular side buds stemming from mammary ducts are already evident in all 4-wk-old mice, and a palpable invasive carcinoma is displayed in all their mammary glands around the 33rd week (12, 14). The gene expression signatures of this carcinoma progression neatly model the progression of human breast tumors (15).

Comprehension of the features of the T cell repertoire available for a rHER-2-specific immune response in BALB-neuT mice will provide information for a more rational design of immunological maneuvers to reverse tolerance and allow residual T cells to mount an effective immune response to rHER-2+ tumors (2). Although it has been shown that rHER-2 is expressed in the thymus of FVB mice made transgenic for the rHER-2 proto-oncogene (3), information about expression of the transforming rHER-2 in the thymus of BALB-neuT mice is lacking. However, whereas single immunization regimens trigger a strong immune reaction leading to the rejection of large established rHER-2+ tumors in WT BALB/c mice (16, 17), only combined and repeated immunizations can elicit an immune response strong enough to delay carcinoma onset in BALB-neuT mice (18–20). This raises the question of whether expression of the oncogene in the thymus actually silences the immune response or facilitates its regulation through peripheral mechanisms (17). In the latter eventuality, the TCR repertoires expanded by rHER-2 in BALB/c and BALB-neuT mice should overlap, that remaining in BALB-neuT mice being only reduced in its width and/or ability to respond.

We now compare the T cell responses elicited by DNA immunization against the extracellular and transmembrane domains of rHER-2 protein product (EC-TMrHER-2) in BALB/c mice and transgenic BALB-neuT mice. By means of a modified CDR 3-β spectratyping technique, the bulk T cell population was divided into ~2400 groups in function of the rearrangement of their variable (V)β and the joining (J)β gene segments, and the length of the TCR’s CDR3 was measured. This dissection of an Ag-specific TCR repertoire reveals Ag-driven expansions and makes it possible to follow Ag-specific T cells (21).

Present data show that in every BALB/c mouse immunized to xenogenic rHER-2, responding CD8+ cells display one or more public TCR CDR3-β arrangements. CD8+ cells carrying these rearrangements are specifically triggered by 63-71 or 1206-1214 rHER-2 peptides. Moreover, two rearrangements specific for rHER-2 and carried by CD4+ T cells recur in >50% of treated BALB/c mice. We also show that in BALB-neuT mice rHER-2 is expressed in the thymus. Consequently, the anti-rHER-2 TCR repertoire is smaller than that of BALB/c mice, and uses distinct Vβ and Jβ genes. Public and semiprivate rearrangements are distinct from those used by BALB/c mice and confined to CD4+ T cells. Once BALB/c as well as residual BALB-neuT repertoires are activated, rHER-2-specific T cells equally home to tumors. Thus central tolerance in BALB-neuT mice appears to act by mainly silencing the CD8+ T cell component of the TCR repertoire, whereas a non-overlapping CD4+ repertoire is generated and escapes tolerance induction. Natural tolerance to HER-2, as well as the pathologic and gene expression analogy between HER-2+ carcinogenesis in BALB-neuT mice and patients with HER-2+ tumors (14, 15), may lead to similar modulation of the TCR repertoire. Present results thus suggest that vaccines designed to first trigger CD4+ T cell reactivity would be the most rational attempt to circumvent tolerance and elicit a protective immune reaction against HER-2-driven carcinogenesis.

Materials and Methods

Mice

Specific pathogen-free virgin WT BALB/c and rHER-2 transgenic BALB-neuT (H-2b) females (Charles River Laboratories) were treated in accord-
2 μl of PCR product as a template, run-off reactions were performed with the following internal fluorescent J$_{β}$ primers: 1.1, ACTTGAGTCTGTTCCCTT; TACC; 1.2, AAAGCCTGTTCCCTGAGCAAG; 1.3, CTCTTCTC CAAATAAGGC; 1.4, GACAGGTTGGGTCATG; 1.5, GAAGCTCTTCTCCAAAAAGCC; 1.6, TCACAGTTAGCCGGTGGT CGTC; 2.1, GTAGTGCGTGGTCCTGAGG; 2.2, CACGACT GTCAAGTTTGGC; 2.3, GTTCCTGGGCAATATACGGC; 2.4, GT GGCGGGACCAATGATACAG; 2.5, GTCCCTGGCCCAAAGTACCGG; 2.7, CTAAAACCGTGACGGTC. The run-off products were denatured in formamide and analyzed on an Applied Biosystems 3100 Prism using Gene-scan 2.0 software (Applied Biosystem). Data are reported as rate of stimulation index (RSI): normalized peak area from stimulated cells/normalized peak area of non-stimulated cells. The ratio of peak areas from stimulated and control cells was expanded in a hHER-2-driven manner when RSI is > 2 (26).

Immunofluorescence separation of CD4$^+$ and CD8$^+$ T cells

LN pools from four immunized mice were cultured in vitro for 3 days with or without 3T3-NKB cells. CD4$^+$ and CD8$^+$ populations were enriched from stimulated cells by immunoaffinity magnetic sorting using MACS beads conjugated with anti-CD8 or anti-CD4 Ab (Miltenyi Biotec) according to the manufacturer’s instructions. Total, negatively selected, and positively selected cells were collected, and total RNA was isolated for TCR repertoire analysis.

Results

Transgenic BALB-neuT mice express rHER-2 in the thymus

It has been shown that FVB mice transgenic for rHER-2 proto-oncogene under the MMTV long-terminal repeat promoter express the mRNA of the transgene in their thymus (3). BALB-neuT mice are transgenic for the rHER-2 transforming oncogene under the same promoter as the FVB proto-oncogene. To assess whether the functional tolerance of BALB-neuT mice is associated with thymic expression of rHER-2, we examined the presence of mRNA encoding for rHER-2 in thymuses from 2-day- and 4-week-old BALB-neuT and control BALB/c mice. A significant amount of specific mRNA was detectable in the thymuses from 2-day-old BALB-neuT mice. The intensity of the band further increases in 4-week-old thymuses. As expected, age-matched BALB/c mice are negative at both time points (Fig. 1).

BALB/c and BALB-neuT mice expand distinct TCR repertoires following EC-TM$^{new}$ plasmid electroporation

We previously reported that vaccination with pcDNA3 vector coding for the EC-TM$^{new}$ domains elicits a strong anti-rHER-2 immune response in WT BALB/c mice and a significant but much less effective response in transgenic BALB-neuT mice (16, 22). To assess the repertoires expanded by vaccination, both BALB/c and BALB-neuT mice received EC-TM$^{new}$ plasmids electroporated into the tibial muscle at weeks 10 and 12 (23). LN cells were collected 14 days after the last immunization. LN cells (5 × 10^6) were cultured with 5 × 10^5 rHER-2$^+$ 3T3-NKB cells for 3 days. The 288 V$β$-J$β$ primer combinations were used to perform the first CDR3 length fragment analysis on a pool of cDNA from four BALB/c or BALB-neuT mice. Each CDR3-J$β$ profile can be depicted as a function of the CDR3 length. Each peak represents a 3-bp difference in the product of recombination corresponding to one amino acid residue. In non-vaccinated control BALB/c and BALB-neuT mice, most peak patterns display a Gaussian distribution. After in vitro coculture with 3T3-NKB cells, Ag-driven perturbation of this distribution was observed in 13 spectra from immunized BALB/c mice and 9 spectra from immunized BALB-neuT mice (Fig. 2A). The BALB-neuT mice repertoire is thus less than that of the BALB/c mice. We next examined the distribution of V$β$ (Fig. 2B) and J$β$ (Fig. 2C) usage. The repertoires used by the responding do not overlap. BALB/c mice preferentially use V$β$ genes belonging to families 5, 18, and 20, especially family 5 (present in 5/13 spectra), whereas BALB-neuT mice do not display any bias in their V$β$ usage. No obvious bias in J$β$ usage is observed in BALB/c mice, whereas BALB-neuT mice preferentially use J$β$ of the 2nd (J$β$2) family and therefore the second diversity region. This observation suggests that amino acid residues encoded by this region play a role in rHER-2 recognition in BALB-neuT mice. Interestingly, whereas T cells using TCRs obtained through V$β$ gene family recombination constitute up to 30% of the total in BALB/c mice and thus form a substantial component of any Ag-specific T cell response displayed by this strain (29), the response to EC-TM$^{new}$ immunization in both BALB/c and BALB-neuT mice does not use this family.

TCR repertoires expanded in BALB/c and BALB-neuT are mutually exclusive

The majority of Ag-specific TCR rearrangements are private for each mouse. However, we (30) and others (21, 31) have used the immunoscope technique to detect public or semiprivate TCR rearrangements in several Ag-specific responses. TCRs using the same V$β$-J$β$ rearrangement are considered public when expanded in all mice, semiprivate when used by almost 50% of mice. The expansion of each rearrangement was evaluated as RSI. To determine whether shared TCRs are also present in the response to EC-TM$^{new}$ plasmid immunization, usage of candidate rearrangements chosen on peak expansion with a RSI greater than 4 (not shown) was individually evaluated on cDNA from 18 BALB/c and 16 BALB-neuT mice. Public and semiprivate rHER-2-specific rearrangements are detected in both groups (Fig. 3). All EC-TM$^{new}$ plasmid immunized BALB/c mice show an Ag-driven expansion of T cells carrying one or more rearrangements involving V$β$9-J$β$1.2 and identified by 98-, 101-, or 104-bp length. In addition, >50% of mice display Ag-driven expansions of rearrangements V$β$18-J$β$1.6 and V$β$20-J$β$2.7 (138 bp and 113 bp) (Fig. 3, first row). By contrast, none of the BALB-neuT mice uses any of these rearrangements. Instead they use TCRs carrying a rearrangement of V$β$13-J$β$2.3 genes of 121-bp length, and one derived from V$β$11-J$β$2.7 genes (either 120 or 123 bp) (Fig. 3, second row). Conversely, none of the BALB/c mice uses these V$β$-J$β$ rearrangements.

To assess the specificity of these public and semiprivate rearrangements, LN cells from EC-TM$^{new}$ immunized BALB-neuT and BALB/c mice (five for each strain) were cocultured with 3T3 BALB cells. Results showed that none of these rearrangements was expanded by 3T3 BALB cells, which lack rHER-2 (Fig. 3, third and fourth rows). Moreover, LN cells from BALB-neuT and BALB/c mice immunized with empty plasmid failed to expand any of these rearrangements after coculture with 3T3-NKB cells (eight...
mice per group, not shown). Taken together these results support the notion that the identified CDR3-β/H9252 regions belong to TCRs that specifically recognize rHER-2-derived epitopes.

Overall, we examined the use of eight rearrangements specific for EC-TMneu plasmid in 16 + 18 mice. These data show that TCR repertoires used by BALB/c and transgenic BALB-neuT mice are distinct and do not overlap. This is in agreement with the result of our CDR3 length fragment analysis on a pool of cDNA deriving from four BALB/c or four BALB-neuT mice (Fig. 2).

The TCR repertoire residual after tolerance induction is deemed to sustain low avidity responses (32). This result may rest on a selective depletion of high-affinity TCRs and/or a shift of specificity toward epitopes displaying low affinity for the restriction element. When the ability of cells from immunized BALB/c and BALB-neuT mice to expand in response to graded numbers of 3T3-NKB cells was compared, it was evident that most rearrangements belong to T cells that expand effectively at comparable low doses of stimulating Ag (data not shown).

**BALB/c public CD8+ TCR repertoires are specific for two epitopes of rHER-2**

Because all EC-TMneu plasmid immunized BALB/c mice show an expansion of T cells carrying the Vβ9-Jβ1.2 rearrangement, we looked to see whether it was expressed by CD4+ or CD8+ T cells. LN cells recovered from 3 days’ coculture with 3T3-NKB were separated into CD4+ and CD8+ T cells by immunoaffinity and then analyzed by immunoscope to look for shared rearrangements. It was found that T cells carrying the public Vβ9-Jβ1.2 repertoires are EC-TMneu plasmid-driven CD8+ cells (Table I). Their epitope specificity was determined by assessing the ability of peptides of rHER-2 predicted to bind to H-2Kd glycoproteins with high affinity [(p63-71 (TYVPANASL), p400-407 (VFETLEEI), p425-432 (VFQNLRII), p1206-1214 (ASPPHPSPA)] to expand in vitro CD8+ cells carrying Vβ9-Jβ1.2 rearrangements. The BALB/c mouse immunodominant peptide p63-71 (25) selectively expands (RSI6) cells carrying the 98 and 104 Vβ9-Jβ1.2 rearrangements (Fig. 4A). Intriguingly, T cells carrying the Vβ9-Jβ1.2 rearrangement of 101 bases length are expanded by peptide p1206-1214 (RSI2) (Fig. 4C). This peptide belongs to the intracellular domain of rHER-2 and is not encoded by the EC-TMneu plasmid used for immunization. Nevertheless, the response directed against it is consistently observed (Fig. 3, and S. Rolla, unpublished data). This observation suggests that an early spread of the immune response to other epitopes of HER-2 occurs, possibly through presentation of self-peptides and priming of potentially self-reactive T cells. LN cells from immunized BALB-neuT
mice fail to expand any Vβ-Jβ1.2 rearrangement, even when stimulated in vitro with the peptides (Fig. 4, B and D).

**BALB/c mice expand semiprivate CD4⁺ T cell repertoires**

Immunized BALB/c mice expand T cells expressing two additional semiprivate TCR rearrangements. In ~55% of these mice, expanded T cells expressed the 138-bp Vβ18-Jβ1.6 rearrangement, whereas 66% expressed the 113-bp Vβ20-Jβ2.7 rearrangement (Fig. 3). Both rearrangements are carried by CD4⁺ T cells (Table I). Unfortunately, no sustained expansion of T cells carrying either rearrangement was observed following stimulation in CD8⁺ and in CD4⁺ enriched cells.

**Expanded BALB-neuT public TCR repertoires are expressed by CD4⁺ T cells**

All immunized BALB-neuT mice expand T cells expressing the 121-bp Vβ13-Jβ2.3 and these expanded T cells frequently carry the 120-bp or (preferably) the 123-bp Vβ11-Jβ2.7 rearrangement. These rearrangements characterize a T cell repertoire used solely by BALB-neuT mice and carried by CD4⁺ cells (Table I). LN cells were then restimulated with the previously predicted four peptides for I-A⁺ and I-E⁺ restriction elements, but no expansion of T cells carrying BALB-neuT rearrangements was observed (unpublished data).

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**Table 1.** Expression of the identified TCR rearrangements on CD8⁺ or CD4⁺ T cells and their localization at the tumor site

<table>
<thead>
<tr>
<th>Mice</th>
<th>Recurrent TCR Rearrangements</th>
<th>Coreceptor Expression⁺</th>
<th>Presence at the Tumor Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>Vβ9-Jβ1.2 (98)</td>
<td>CD8⁺</td>
<td>Yes⁺</td>
</tr>
<tr>
<td></td>
<td>Vβ9-Jβ1.2 (101)</td>
<td>CD8⁺</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Vβ9-Jβ1.2 (104)</td>
<td>CD8⁺</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Vβ18-Jβ1.6 (138)</td>
<td>CD4⁺</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Vβ20-Jβ2.7 (113)</td>
<td>CD4⁺</td>
<td>Yes</td>
</tr>
<tr>
<td>BALB-neuT</td>
<td>Vβ11-Jβ2.7 (120)</td>
<td>CD4⁺</td>
<td>Yes⁺</td>
</tr>
<tr>
<td></td>
<td>Vβ11-Jβ2.7 (123)</td>
<td>CD4⁺</td>
<td>Yes⁺</td>
</tr>
<tr>
<td></td>
<td>Vβ13-Jβ2.3 (121)</td>
<td>CD4⁺</td>
<td>Yes⁺</td>
</tr>
</tbody>
</table>

⁺ Expression of TCR rearrangements in LN cells separated by immunofluorescence magnetic sorting following restimulation in CD8⁺ and in CD4⁺ enriched cells.

⁺⁺ Expression of the BALB/c TCR rearrangement by T lymphocytes infiltrating TUBO cells transplanted into immunized BALB/c mice detecting using Vβ-Jβ CDR3 spectratyping.

⁺⁺⁺ Expression of the BALB-neuT TCR rearrangement by T lymphocytes infiltrating HER-2⁺ neoplastic lesion in the mammary gland of immunized BALB-neuT mice.
FIGURE 4. Peptides predicted to bind H-2Kd stimulate CD8+ cells carrying Vβ9-Jβ1.2 rearrangements in BALB/c mice, but not in BALB-neuT mice. The immunodominant peptide p63-71 selectively expands (RSI = 6) the cells carrying the Vβ9-Jβ1.2 rearrangement of 98 and 104 bases length, whereas intracellular p1206-1214 peptide expands (RSI = 2) CD8+ cells carrying the Vβ9-Jβ1.2 rearrangement of 101 bases length. Empty profiles: CDR3 distribution in stimulated LN cells from BALB/c or BALB-neuT mice; shaded profiles: unperturbed Gaussian CDR3 distribution in non-stimulated LN cells from BALB/c or BALB-neuT mice (controls). Dotted vertical lines show the peak of 101 bases length. The profiles are expressed as indicated in Fig. 2.

CD8+ and CD4+ T cells infiltrating tumor during rejection express the identified TCR repertoires

Rejection of rHER-2+ tumors following EC-TMnew plasmid immunization in BALB/c mice is mainly mediated by Abs and CTL (16), and almost completely Ab-mediated in BALB-neuT mice (33). Whereas in both cases histochemistry shows that CD4+ and CD8+ cells infiltrate the tumors (22, 23), direct evidence of rHER-2 specificity of the T cells within the tumor mass is lacking.

To assess the expression of the TCR rearrangements by T cells infiltrating tumors during immuno-mediated rejection, BALB/c mice were challenged with TUBO cells and immunized with EC-TMnew plasmid electroporation when the growing tumor reached 4-mm mean diameter. Tumors were excised 21 days after vaccination, total RNA and cDNA were prepared, and immunoscope analysis of the TCR repertoire identified was performed. Results show that infiltrating T cells express Vβ9-Jβ1.2, Vβ18-Jβ1.6, and Vβ20-Jβ2.7 rearrangements. Intriguingly, among CD8+ T cells carrying the Vβ9-Jβ1.2, only those specific for the immunodominant p63-71 peptide (i.e., T cells carrying the 98- and 104-bp Vβ9-Jβ1.2) are detected within the tumor mass. Therefore, whereas CD8+ cells specific for rHER-2 are directly involved in the eradication of rHER-2+ cells, self-reactive CD8+ T cells do not participate. Moreover, following EC-TMnew plasmid electroporation, CD4+ T cells expressing the Vβ11-Jβ2.7 and Vβ13-Jβ2.3 public rearrangements infiltrate mammary lesions in both 22- and 42-week-old BALB-neuT mice (Table I).

Discussion

In the present study, we used Vβ-Jβ spectratyping to compare the TCR repertoire used by WT BALB/c mice and rHER-2 transgenic BALB-neuT mice in response to EC-TMnew plasmid electroporation. BALB-neuT mice express rHER-2 in the thymus and respond to electroporation by expanding a smaller repertoire and using different Vβ and Jβ regions compared with WT BALB/c mice. BALB/c mice expand public CD8+ and CD4+ cell repertoires, whereas that of BALB-neuT mice comprises CD4+ cells only. By contrast, no differences in ability to infiltrate rHER-2+ tumors were displayed by these repertoires.

Starting from the 4th week of age, BALB-neuT mice overexpress rHER-2 protein in the mammary gland and undergo a devastating expansion of mammary lesions leading to multiple carcinomas (12, 14). Early EC-TMnew plasmid electroporation elicits a protective immune response that controls the progression of initial mammary lesions. Induction of this response fully rests on CD4+ cells (34). Protection is due to IFN-γ-producing T cells and a high production of anti-rHER-2 IgG2a Abs (19, 33), whereas a significant CD8+ T cell-mediated cytotoxic response was never found (22, 23, 27).

The observation that WT BALB/c mice respond to EC-TMnew plasmid electroporation with a larger repertoire than BALB-neuT is not surprising, because rHER-2 is a protein with several xenogeneic determinants in BALB/c mice (22, 25). By contrast, the mechanisms leading all BALB-neuT mice to expand a set of TCRs specific for rHER-2 never used by BALB/c mice are less obvious. Two mechanisms may be postulated. Recruitment of TCR repertoire is critically determined by both a competition between T cells to access the APCs (35, 36) and precursor frequency (37, 38). Thus, it may be suggested that the TCRs belonging to the repertoire activated by immunization in BALB/c mice (namely those carrying the 138-bp Vβ18-Jβ1.6, 113-bp Vβ18-Jβ1.6, and 113-bp Vβ20-Jβ2.7 rearrangements) have a high precursor frequency that prevents activation of other, low frequency T cells. Once this repertoire is deleted by induction of tolerance in BALB-neuT mice, a cryptic repertoire (exemplified by Vβ13-Jβ2.3 recombination of 121 bp and Vβ11-Jβ2.7 rearrangement of 120 or 123 bp) can access the APCs to be primed.

T cell maturation in the thymus also involves positive selection of cells bearing TCRs that interact with self-MHC molecules. As in negative selection, promotion of proliferation also depends on presentation of self-epitopes, which shape the overall TCR repertoire that reaches the periphery (39). Thus, the presence of the rHER-2 protein in the thymus may allow the positive selection of distinct TCRs with respect to mouse HER-2. Here we show that following EC-TMnew plasmid electroporation, the immune response of these mice rests on the expansion of Vβ11-Jβ2.7 and Vβ13-Jβ2.3 rHER-2-specific CD4+ cells, whereas no CD8+ public T cells can be detected. Differences in thymic selection of CD8+ and CD4+ cells may be responsible for this selective expansion of self-reactive CD4+ cells. CD8+ and CD4+ cells have different sensitivity to epitope density on the surface of APC, because CD8+ cells can be activated by a 100-fold lower number of peptide/MHC complexes with respect to CD4+ cells (40–42). This sensitivity may result in distinct outcomes of negative selection in the thymus, despite equivalent presentation of self-Ag derived epitopes. Although it is well established that autoreactive thymocytes are subject to deletion during their development, not all autoreactive CD4+ T cells are eliminated from the peripheral T cell repertoire (43). The generation of CD4+CD25+ T cells with immunoregulatory properties is one of the ways in which potentially autoreactive CD4+ cells are controlled (44). Generation of these regulatory cells is typically accompanied by the development of clonally related CD25+ T cells bearing an identical TCR specificiy (45). These CD4+CD25+ cells are both more proliferative than their CD25− counterparts and unable to suppress T cell proliferation. They may be the CD4+ cells expanded after EC-TMnew electroporation in BALB-neuT mice. Expression of rHER-2 in the thymus of BALB-neuT mice and subsequent positive selection are the reasons why these TCR rearrangements are not found in BALB/c mice. Indeed, anti-rHER-2 reactive CD4+ T cell repertoires of BALB-neuT and BALB/c mice are mutually exclusive, rather than one being a subgroup of the other.
The rHER-2 specific CD4+ T cell repertoire of BALB-neuT mice is potentially still able to prevent tumor development. However, T cells expressing a self-reactive repertoire need a strong “stimulus” to become effective (23). Presence of regulatory mechanisms that down-regulate the immune response to TAA in the periphery has been recognized in rHER-2 transgenic mice and human patients (46, 47). These mechanisms include negative T reggyulatory cells (2), immature myeloid cells (48), regulation of endothelial adhesiveness (49), and creation of an anti-inflammatory environment at the tumor site (50). Here we show that central tolerance contributes to impairment of immune responsiveness in BALB-neuT mice through a quantitative reduction of the T cell repertoire available. Such a reduction may make the anti-tumor response more easily down-regulated by specific and nonspecific control mechanisms. However, we also show that a potentially effective anti-tumor T cell repertoire is always available, even in this strongly tolerogenic context. Promoting the expansion of this repertoire may provide a significant tool for development of effective anti-tumor immune responses. These results provide a clue to the effects on the T cell repertoire of overexpression of an oncogene product.

In BALB/c mice, EC-TM neu plasmids elicit a strong immune response leading to the coordinate activation of multiple reaction mechanisms. Induction of this response rests on CD4+ cells, whereas a strong anti-rHER-2 Ab production, a significant CD8+ T cell-mediated cytotoxic response, and the release of cytokines by activated CD4+ T cells that orchestrate the recruitment of granulocytes and macrophages are the prominent effector mechanisms. This response not only fully protects against a lethal challenge of rHER-2 tumors, but is also strong enough to lead to the eradication of large, established rHER-2 tumor masses (16). Present data show that EC-TM neu plasmids elicit the expansion of Vβ18-Jβ2,6, 138 bp and Vβ20-Jβ2,7, 113 bp CD4+ cells and Vβ9-Jβ1,2, 98 and 104 bp CD8+ cells in BALB/c mice. We also consistently observe the recruitment of CD8+ cells carrying a 101-bp Vβ9-Jβ1,2 rearrangement specific for an intracellular HER-2 peptide. This suggests that an early spread of the immune response to other epitopes of rHER-2 occurs, possibly through metastatic dissemination. In conclusion, our findings show that expression of rHER-2 has a dramatic impact on the TCR repertoire that reaches the periphery. The T cells that escape tolerance are reduced, specially CD8+, although the individual ability of the cells of this residual repertoire to respond to rHER-2 is similar to that of WT mice. Although one important effector branch of T cell reactivity is severely impaired, immunization protocols that overcome suppressive regulatory mechanisms can still activate a potentially effective CD4+ T cell reactivity in BALB-neuT mice. The many analogies in the carcinogenesis progression and gene expression between BALB-neuT mice and patients with mammary lesions (14, 15) suggest that the natural and pathological HER-2 overexpression may result in a comparable modulation of the TCR repertoire. In this case, vaccination strategies aimed at the activation of IFN-γ-releasing CD4+ Th cells recognizing cryptic epitopes may provide more effective control of the neoplastic lesions overexpressing the HER-2 oncoantigens.

Disclosures
The authors have no financial conflict of interest.

References