The Shaping of a Polyvalent and Highly Individual T-Cell Repertoire in the Bone Marrow of Breast Cancer Patients

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Abstract

We analyzed the T-cell repertoires from the bone marrow of 39 primary operated breast cancer patients and 11 healthy female donors for the presence and frequencies of spontaneously induced effector/memory T lymphocytes with peptide-HLA-A2-restricted reactivity against 10 breast tumor-associated antigens (TAA) and 3 normal breast tissue-associated antigens by short-term IFN-γ enzyme-linked immunospot (ELISpot) analysis. Sixty-seven percent of the patients recognized TAAs with a mean frequency of 144 TAA reactive cells per 10⁶ T cells. These patients recognized simultaneously an average of 47% of the tested TAAs. The T-cell repertoire was highly polyvalent and exhibited pronounced interindividual differences in the pattern of TAAs recognized by each patient. Strong differences of reactivity were noticed between TAAs, ranging from 100% recognition of prostate-specific antigen p141-149 to only 25% recognition of MUC1 p12-20 or Her-2/neu p369-377. In comparison with TAAs, reactivity to normal breast tissue-associated antigens was lower with respect to the proportions of responding patients (30%) and recognized antigens (27%), with a mean frequency of only 85/10⁶ T cells. Healthy individuals also contained TAA-reactive T cells but this repertoire was more restricted and the frequencies were in the same range as T cells reacting to normal breast tissue-associated antigens. Our data show a highly individual T-cell repertoire for recognition of TAAs in breast cancer patients. This has potential relevance for T-cell immune diagnostics, for tumor vaccine design, and for predicting immune responsiveness. (Cancer Res 2006; 66(16): 8258-65)

Introduction

During the last years, evidence has increased that malignant human tumors can be naturally recognized by the host’s immune system and induce spontaneous tumor antigen–specific T-cell responses, which together constitute an antitumoral T-cell memory repertoire in lymphoid tissues, such as the bone marrow (1–5). The implications of natural T-cell responses against tumor-associated antigens (TAA) for the course of human tumor diseases are currently debated. Mouse models showed the ability of spontaneously induced TAA-specific T cells to reject syngeneic tumors under circumstances of reduced immune-suppression (6). In the past, clonal expansions of T lymphocytes and pronounced tumor infiltrations by T cells have been correlated with an improved clinical prognosis of patients with multiple myeloma, colorectal carcinoma, and breast cancer (7–9). Experimental therapies of xenotransplanted human breast and ovarian carcinomas with autologous, ex vivo isolated memory T cells showed a therapeutic potential of spontaneously induced T cells in vivo (2, 10). Selective therapeutic exploitation of natural T-cell repertoires of cancer patients (e.g., by means of TAA-vaccination) requires information on the immune recognition of tumor antigens in individual patients. Whereas spontaneously induced TAA-reactive T cells have been described in patients with breast cancer (1, 2), malignant melanoma (11), multiple myeloma (3), colorectal carcinoma (12), or pancreatic carcinoma (4), the frequency of their occurrence among patients and the fine specificity and individuality of such T-cell repertoires have not been reported.

We did in this study a comparative analysis of tumor patients to identify the presence, specificity, and functional competence in IFN-γ secretion of tumor antigen–specific T lymphocytes. We decided to analyze the T-cell repertoire of breast cancer patients because breast cancer is among the most frequent tumors worldwide and a leading cause of death in women.

We focused this analysis on the bone marrow as a T-cell source because many attempts to detect functional tumor-reactive T cells in the blood of tumor patients have failed (1–3, 12, 13). The bone marrow was recently shown to be a predominant site for T-cell priming against blood borne antigens (14), for generation of T-cell memory, and for recruitment of large amounts of circulating memory T cells and antigen-pulsed dendritic cells (15–17). Recent studies showed high frequencies of TAA-specific memory T cells in the bone marrow of patients with multiple myeloma, pancreatic carcinoma, and breast cancer, whereas they showed no TAA-specific immunoreactivity in their peripheral blood (1, 3, 4).

We describe the results of short-term IFN-γ enzyme-linked immunospot (ELISpot) analyses with ex vivo isolated bone marrow T cells from 39 primary operated breast cancer patients and 11 healthy female donors tested for the presence and frequencies of spontaneously induced effector/memory T lymphocytes with reactivity against altogether 17 defined, HLA-A2-restricted nonameric peptides derived from 10 different TAAs and 3 non-malignancy-associated antigens. We show that breast cancer induces a highly individual shift of the T-cell memory repertoire towards recognition of TAAs. Our screening results lead to the recommendation of four peptides from four different TAAs for future screenings to identify immune-responsive breast cancer patients.

Materials and Methods

Patients. Bone marrow samples were obtained from 39 patients with primary breast cancer and from 11 healthy women. Informed consent was...
obtained from all participants. The protocol was approved by the Ethical Committee of the University of Heidelberg (Heidelberg, Germany). Bone marrow samples were aspirated from each anterior iliac crest immediately after surgery while the patients were still anesthetized (1). Bone marrow of healthy donors was obtained using local anesthesia. Heparanized bone marrow was subjected to Ficoll gradient centrifugation (Pharmacia, Uppsala, Sweden) and cells at the interphase were collected.

Antigens. As test tumor antigens, we used 14 different HLA-A0201-restricted nonameric peptides derived from 10 different tumor antigens [lactadherin (BA46; refs. 18, 19), Bel-2 (20), carcinoembryonic antigen (CEA; refs. 21, 22), cyclin D1 (23, 24), Her-2/neu (25, 26), MAGE-2 (27, 28), MUC1 (29, 30), prostate-specific antigen (PSA; refs. 31, 32), p53 (33, 34), and heparanase (ref. 35, and Sommerfeldt et al. manuscript in revision)] expressed in breast cancer cells. All peptides have previously been described as immunogenic T-cell targets. The tumor antigens and amino acid sequences of respective peptides are summarized in Table 1. In addition to TAAs, we used HLA-A0201-restricted nonameric peptides derived from 3 non-malignancy-associated antigens keratin 18 (36), collagen-IV (37), and collagen α1 (38) expressed by epithelial cells and stromal cells in normal breast tissue (summarized in Table 2). As negative control antigens, we used HIV-1-derived peptide SLYNTVATL (1) and insulin-derived peptide HLVEA1YVL (ref. 2; Table 2). In some cases, dendritic cells were pulsed overnight with lysates from autologous breast tumors (test antigen) or autologous PBMCs (negative control antigen) as source of respective control antigen. Cell lysates were generated from freshly excised and mechanically dissection breast tumor biopsies or from PBMCs by five cycles of freezing and thawing, followed by filtration through a 0.3-μm filter as previously described (1).

IFN-γ ELISPOT assay. ELISPOT assays were done as described (1) with modifications. Briefly, T cells were cultured for 7 days in RPMI medium containing 10% AB serum, 100 units/mL interleukin (IL)-2, and 60 units/mL IL-4. Afterwards, T cells were transferred into cytokine-free medium for 12 hours and subsequently separated from contaminating cells by anti-CD19, anti-CD15, and anti-CD56 monoclonal antibody–conjugated magnetic beads (Dynal, Hamburg, Germany; ref. 1). Dendritic cells were generated from peripheral blood samples were cultured for 7 days in serum-free X-VIVO 20 containing 50 ng/mL recombinant human granulocyte-macrophage colony-stimulating factor and 1,000 units/mL IL-4. Dendritic cells were enriched using anti-CD3 and anti-CD19-coupled magnetic beads and pulsed for 2 hours with test or control peptides. IFN-producing T lymphocytes were determined as follows: (spots in test wells – spots in control wells)/T-cell numbers per well. Each test group consisted of three to five wells. We included ELISPOT assays with a minimum mean of 10 spots per control well and excluded ELISPOTs exceeding 200 spots per control well. Low background spot numbers were not correlated with a higher probability to achieve positive results.

Statistical evaluation. ELISPOT results and differences between test groups were analyzed using Student t test (P < 0.05). Differences in proportions of responders between test groups (Fig. 1A) were analyzed with χ2 test.

Results

Spontaneous T-cell responses in breast cancer patients are highly individual. Ten TAAs frequently overexpressed in breast carcinomas were selected for the study (Table 1). Additionally, three antigens regularly expressed by stromal cells (collagen I and IV) and epithelial cells (keratin 18) in normal as well as malignant breast tissue were chosen as normal breast tissue–associated antigens for comparison (Table 2). From these antigens, 17 well-characterized, HLA-A2-restricted nonapeptides were selected for detection of antigen-reactive T cells (Tables 1 and 2).

We included HLA-A0201+ primary breast cancer patients at the time point of the first diagnosis. The bone marrow was always taken during primary breast tumor resection. The patients did not receive any kind of tumor-related treatment before bone marrow aspiration. Median age of the tumor patients was 65 years (mean, 60.7 ± 12.8 years) ranging from 32 to 82 years. Twenty-one (54%) patients had T1 tumors, 13 (33%) T2, and 5 (13%) T3 tumors. The lymph node status was 0 in 18 patients (46%), 1 in 14 (36%), and 2 in 7 patients (18%). Two patients had detectable hepatic metastases at the time point of analysis; in all other 37 patients, metastases were not detected.

Autologous dendritic cells loaded with respective peptides were used for stimulation of ex vivo isolated purified bone marrow T cells and tested in 40-hour IFN-γ ELISPOT-assays. T cells stimulated similarly with irrelevant HLA-A0201-restricted control peptides derived from HIV and insulin served for determination of the unspecific background. Primary data from one representative patient are shown in Fig. 1A. Antigen-specific responses, defined by a significantly increased spot number in respective test antigen triplicate wells in comparison with both negative control antigen triplicates, are indicated by asterisks. Frequencies of TAA-reactive T cells were calculated by subtracting mean background values from mean test values in case of specific responses. Figure 1B shows the T-cell frequencies of nine patients tested for the same six TAAs. A similar pattern of reactivity was found in only two of these nine patients (patients 26 and 27). Thus, spontaneous T-cell responses in breast cancer patients are highly individual.

**Table 1. Tumor antigens and HLA-A0201-restricted peptides**

<table>
<thead>
<tr>
<th>TAA (reference)</th>
<th>Peptide position in AA sequence</th>
<th>Peptide sequence (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactadherin (BA46; ref. 18)</td>
<td>97-105</td>
<td>GLQHWVPVL (19)</td>
</tr>
<tr>
<td>Lactadherin (BA46; ref. 18)</td>
<td>131-139</td>
<td>NLLRMWVT (19)</td>
</tr>
<tr>
<td>Lactadherin (BA46; ref. 18)</td>
<td>194-202</td>
<td>NLFETPVEA (19)</td>
</tr>
<tr>
<td>Bel-2 (20)</td>
<td>218-226</td>
<td>KTTLSSALV (20)</td>
</tr>
<tr>
<td>CEA (21)</td>
<td>694-702</td>
<td>GVLVGVALV (22)</td>
</tr>
<tr>
<td>Cyclin D1 (23)</td>
<td>101-109</td>
<td>LLGATCMVF (24)</td>
</tr>
<tr>
<td>Her-2 (25)</td>
<td>369-377</td>
<td>KIFGSLAFL (26)</td>
</tr>
<tr>
<td>MAGE-2 (27)</td>
<td>662-670</td>
<td>KMEVHLHIFL (28)</td>
</tr>
<tr>
<td>Mucin 1 (ref. 29)</td>
<td>12-20</td>
<td>LLLTLTVLTV (30)</td>
</tr>
<tr>
<td>PSA (31)</td>
<td>141-149</td>
<td>FLTPKLLCQCV (32)</td>
</tr>
<tr>
<td>p53 (33)</td>
<td>149-157</td>
<td>STPPGTRV (34)</td>
</tr>
<tr>
<td>Heparanase (35)</td>
<td>8-16</td>
<td>ALPPPLMLL (35)</td>
</tr>
<tr>
<td>Heparanase (35)</td>
<td>16-24</td>
<td>LLLGPGFL (35)</td>
</tr>
<tr>
<td>Heparanase (35)</td>
<td>183-191</td>
<td>DLIFGLNAL (35)</td>
</tr>
</tbody>
</table>

*Sommerfeldt et al., manuscript in press.
To assess the strength of spontaneous antitumor T-cell responses, we compared the frequencies of 15 patients that responded against more than one TAA-derived peptide (Fig. 1C). Interestingly, these frequencies were intraindividual rather homogeneous but showed strong interindividual differences. Whereas some patients responded weakly to all antigens, others exerted strong T-cell responses against the majority of tested antigens. Strong interindividual differences in reactivity against TAAs were also detected in a group of 17 samples that were tested for reactivity against autologous tumor cell lysates (using autologous PBMC lysate as negative control antigen; Fig. 1C). Ten (59%) of them reacted against autologous TAAs. The mean frequencies of tumor cell–reactive T cells exceeded those of T cells that reacted against single peptides.

**TAA-reactive memory T cells are enriched in breast cancer patients compared with healthy donors.** Bone marrow samples of 39 breast cancer patients and 11 healthy female donors were analyzed using a mean number of six or seven simultaneously tested TAAs per patient and healthy donor, respectively. Sixty-seven percent (26 of 39) of patients, subsequently designated as “responder patients,” revealed a spontaneous T-cell reactivity against at least one TAA-derived peptide (Fig. 2A). In contrast, normal breast tissue–associated antigens were recognized by significantly fewer patients (30%; $P < 0.003$).

Similar to patients, a majority (9 of 11, 82%) of healthy donors (subsequently designated as “healthy responders”) reacted against at least one TAA-derived peptide. However, in contrast to patients, the majority of healthy donors similarly recognized normal breast tissue–associated antigens (5 of 9, 56%; Fig. 1A).

Although a similar proportion of patients and healthy donors recognized at least one TAA, responder patients recognized more different TAA-derived peptides (median, 46% of tested antigens) than healthy donors (median, 23% of tested antigens; Fig. 2B).

Thus, breast cancer patients spontaneously induce selective T-cell responses against a broad pattern of breast cancer–associated antigens to a significantly greater extent than healthy donors.

### Table 2. Normal breast tissue–associated antigens, control antigens, and HLA-A0201-restricted peptides

<table>
<thead>
<tr>
<th>Normal breast tissue–associated antigens and control antigens (reference)</th>
<th>Peptide position</th>
<th>Peptide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin 18 (36)</td>
<td>365-377</td>
<td>ALLNIKVKL</td>
</tr>
<tr>
<td>Collagen IV (37)</td>
<td>18-26</td>
<td>SLWQPVEEA</td>
</tr>
<tr>
<td>Collagen α1 (38)</td>
<td>253-261</td>
<td>RGLPGTGAEL</td>
</tr>
<tr>
<td>HIV (1)</td>
<td>77-85</td>
<td>SLYNTVATL</td>
</tr>
<tr>
<td>Insulin (2)</td>
<td>34-42</td>
<td>HLVEALYLYV</td>
</tr>
</tbody>
</table>

**Figure 1.** Individuality of T-cell responses against tumor antigens in breast cancer patients. A, primary data of an exemplary IFN-γ ELISpot assay. Bone marrow T cells of patient 27 were stimulated with peptides derived from negative control antigen HIV or insulin (white columns), the normal breast tissue–associated autoantigen keratin 18 (gray columns), or the respective test tumor antigens (black columns). Columns, mean spot numbers of three wells per antigen; bars, SD. *, $P < 0.05$, significant difference between spot numbers in test wells compared with pooled spot numbers of negative control antigens (HIV and insulin).

B, pattern of reactivity against different tumor antigens in nine patients. Gray columns, frequencies of T cells significantly reactive against the respective antigen. Nonsignificant results were considered negative (0). n.t., not tested. C, frequencies of T cells reactive against different tumor peptides (P 3-39) or against autologous tumor cell lysate (TU-L, P 40-56) from individual breast cancer patients. All patients with reactivity against at least two different peptides or against tumor cell lysate are shown. Full lines, mean frequencies of peptide-reactive T cells for each patient. *, significant difference between frequencies of T cells reactive against tumor lysate or tumor peptides.
T-cell repertoire in the bone marrow of cancer patients

Figure 2. Spontaneous T-cell reactivity against tumor antigens in patients and healthy donors. A, proportion of patients (Pat) and healthy donors (HD) among all tested individuals that exerted significant T-cell reactivity against at least one peptide derived from TAs or normal breast tissue–associated autoantigens (NAA). n.s., not significant. B, proportion of TAs recognized by T cells from individual responder patients (●) or healthy responders (○) among all TAs tested on the respective individual. Each circle represents one individual. Horizontal lines, median values of patient and healthy donor groups. *, significant difference between the groups of responder patients and healthy responders.

TAAs. Whereas patients showed a significant difference in reactivity against TAAs versus normal breast tissue–associated antigens, this was not the case in healthy individuals, who also reacted more scarcely to TAAs.

Calculated frequencies of all tests done in the study are shown in Fig. 3A. Reactivity against TAAs was found in 33% of all tests done with T cells from cancer patients but only in 21% of tests done with T cells from healthy individuals. Reactivity against normal breast tissue–associated antigens was detected in 24% of all tests with T cells from cancer patients and in 38% of healthy individuals. Similarly, mean frequencies of TAA-reactive T cells in all tested breast cancer patients were significantly higher than in healthy donors (110/10⁶ T cells and 49/10⁶ T cells, respectively) and also exceeded those of normal breast tissue–associated antigen–reactive T cells (71/10⁶ T cells; Fig. 3B). Moreover, responder patients contained higher frequencies of TAA-reactive T cells in comparison with healthy responders and in comparison with T cells reactive against normal breast tissue–associated antigen (Fig. 3C). The mean frequency of T cells reactive against TAAs in all positive tests was higher in breast cancer patients (338/10⁶ T cells and 204/10⁶ T cells, respectively; P = 0.09) than in healthy donors (Fig. 3D).

The relative proportions of T cells reactive against TAAs or normal breast tissue–associated antigens showed major differences between healthy individuals and breast cancer patients: whereas T cells from healthy donors predominantly reacted against normal breast tissue–associated antigens, breast cancer patients exerted predominant T-cell responses against TAAs and responses against normal breast tissue–associated antigens were decreased (Figs. 2A and 3).

The data suggest that spontaneous T-cell responses against TAAs lead to higher frequencies of antigen-reactive memory T cells in breast cancer patients than in healthy individuals. Accordingly, the distribution of T-cell frequencies from bone marrow samples shows a particularly large proportion of medium- to high-frequency T-cell responses (>250 reactive T cells/10⁶ T cells) against breast TAAs in cancer patients compared with healthy donors or with normal breast tissue–associated antigens (Fig. 3E).

A selection of tumor antigens suitable for screening of spontaneous T-cell responses in breast cancer patients. Figure 4A shows a comparison of all results obtained with TAA-derived peptides in responder cancer patients and responder healthy individuals. Eleven of 14 selected TAA peptides (position 1-11) were recognized more frequently by cells from cancer patients than by cells from healthy donors, whereas three peptides (position 12-14) were equally or even more frequently recognized by T cells from healthy individuals. There was one TAA, PSA (p141), which was recognized by 100% of tested responder patients. This was, however, also recognized by half of the healthy individuals. Altogether, a TAA reactivity by a majority of responder patients was directed against lactadherin (BA, pp 131, 194), heparanase (p183), PSA (p141), Bcl-2 (p218), and cyclin D1 (p101). In contrast, the broadly used tumor antigens MUC1 (p12) and Her-2/neu (p369) were recognized by only 25% of the patients. The different TAAs varied strongly in their capacity to induce T-cell responses in the tested patients.

Figure 4B shows the frequency of T cells reactive against each of the selected test antigens. These were quite similar in tumor patients, ranging from 154/10⁶ T cells (e.g., Her-2-250) to 363/10⁶ T cells (BA27). Interestingly, there was one peptide derived from lactadherin (BA104), which was recognized at particularly high frequencies of epitope-specific T cells in breast cancer patients (mean, 698/10⁶ T cells).

To facilitate future screenings of spontaneous T-cell responses in breast cancer patients, we suggest a selection of a few TAA peptides that are recognized by a high proportion of responder patients and by as few as possible healthy individuals. Our screening results recommend lactadherin (p131), heparanase (p183), MUC1 (p12), and Her-2/neu (p369). A combination of three to four of these peptides revealed no reactivity in healthy individuals but covered 91% of respectively tested responder patients (Table 3).

Discussion

We here provide a comparative analysis of the spontaneously generated repertoire of functional T cells with specificity for common tumor antigens (TAs) and autoantigens (normal breast tissue–associated antigens) in the bone marrow of a large number of breast cancer patients and healthy donors. The analysis was based on antigen-derived, HLA-A*0201-restricted peptides. Therefore, the evaluation most likely covered mainly HLA-A2-restricted CD8 T cells. Accordingly, our interpretation of the data is restricted to HLA-A2-mediated T-cell responses. Because the short-term stimulation period during the ELISPOT assay precluded IFN-γ secretion by naïve T cells, we exclusively assessed effector/memory T-cell responses.

As tumor antigens and autoantigens, we evaluated breast TAAs from which HLA-A*0201-derived peptides had been already described at the start of our study, in 2001. Moreover, we added one new TAA, heparanase (35), an enzyme important for tumor invasion that recently has attracted a lot of attention and of which we characterized several HLA-A*0201-restricted peptide CD8 T-cell epitopes.4 We detected TAA-reactive T cells in the bone marrow of 67% of the tested patients. This is in striking accordance to a previous

3 C. Choi, P. Beckhove, and V. Schirrmacher, unpublished observation.
4 Manuscript in press.
report by Feuerer et al. (1) showing that a proportion of 67% of breast cancer patients contained T cells reactive against autologous breast tumor lysates in their bone marrow. We here show that such reactivity can be ascribed to the recognition of defined peptides derived from breast cancer–associated TAAs. The majority of responder patients recognized a broad pattern of the test antigens. Thus, spontaneous T-cell responses against tumor cells were polyclonal. Besides, the patterns of recognized antigens strongly differed interindividually. Because we did not quantify the expression of the respective antigens in the patients’ tumors, it remains unclear if the individual recognition patterns were caused by differences in the antigenic profiles of the tumors or by peculiarities of the individuals’ immune system. Interestingly, the individual frequencies of T cells with different specificities were very similar whereas they strongly differed between the patients. This points to interindividual differences of the responsiveness of immune systems rather than to a strong contribution of the expression rate of single tumor antigens.

One third of breast cancer patients did not secrete IFN-γ in response to any TAA tested. It seems unlikely that this lack of response was due to a mistaken choice of TAAs because most responder patients recognized simultaneously ~50% of the test antigens and we observed the same proportion (40%) of non-responding breast cancer patients when we tested against lysates of autologous tumor cells containing the whole spectrum of autologous TAAs. Thus, a subgroup of patients did not contain tumor-reactive T cells in their bone marrow or they responded by secretion of cytokines other than IFN-γ. Interestingly, TAA reactivity was not correlated to tumor stage or age of the patients (data not shown). A diagnostic distinction between patients containing or lacking a TAA-reactive T-cell repertoire may be valuable for purposes of inclusion into future immunotherapeutic studies, for immune monitoring prior and during vaccination protocols, or for assessment of T-cell effect on clinical prognosis. This may be facilitated by using a combination of a few peptides, such as BA131, HPA183, MUC112, and Her-2Δ9, which in this study were recognized by T cells from >90% of the responder patients and were not recognized by T cells from healthy individuals.

Our findings show that the T-cell repertoire of breast cancer patients is naturally selected for recognition of a broad variety of TAAs, including those antigens that are commonly used in vaccination strategies. It is therefore likely that such vaccination not only induces a priming of naïve T cells but also a reactivation of antigen-specific memory T cells. Given that an activation of
resident memory T cells after several rounds of proliferation leads to their mobilization into the peripheral blood, it may well be that the detection in the blood of antigen-specific T cells shortly after vaccination resembles such a mobilization rather than a priming event. This should be taken into consideration in case of immune monitoring of vaccination effects.

Memory T cells of breast cancer patients predominantly recognized antigens that are selectively expressed in malignant breast epithelial cells (e.g., lactadherin, heparanase, Bcl-2, cyclin D-1, p53, PSA, and CEA) whereas autoantigens constitutively expressed in nonmalignant breast epithelial cells (cytokeratin 18) or in breast tissue stromal cells (collagen I and IV) were recognized less frequently and by lower numbers of T cells. Thus, breast carcinoma cells seem to be more immunogenic than nonmalignant cells (epithelial cells and stromal cells) from the same tissue. Three aspects might contribute to this finding. First, on dissemination or cell death, tumor cells release molecules such as the matrix-degrading enzyme heparanase and heat shock proteins that activate antigen-presenting cells. Second, due to the high turnover rate of tumor cells, tumor cell death is a more frequent event in tumors than stromal cell death. Therefore, large amounts of tumor cell–derived antigens are provided to the immune system subsequently triggering respective T-cell responses. Third, autoantigens constitutively expressed in most tissues in the absence of danger signals favor the development of regulatory mechanisms to prevent autoimmunity. In contrast, many TAAs are expressed in immune-privileged sites (cancer testis antigens), at low amounts in small organs (PSA), or reexpressed after a lifelong interval (CEA) and may have therefore not induced efficient regulatory mechanisms. In addition, danger signals may be provided by cellular stress responses and by proinflammatory cytokines in the tumor microenvironment.

Memory T cells are generated and stored in secondary lymphoid organs, such as lymph nodes, spleen, and the bone marrow (39).

Figure 4. Differential recognition of distinct tumor antigens by patients' and healthy donors' T cells. A, immunogenicity of tested tumor antigens (black and white columns) and normal breast tissue–associated autoantigens (gray columns) presented as proportions of positive tests among all tests done with the respective peptides and T cells from responder patients (black and dark gray columns) or healthy responders (white and light gray columns). Horizontal gray lines, net difference between immunogenicity in patients and healthy individuals. N, number of tests done. B, frequencies of T cells from patients (black and dark gray columns) and healthy donors (white and light gray columns) reactive against specified tumor antigens (black and white columns) or pooled normal breast tissue–associated autoantigens (gray columns). Columns, mean; bars, SE. The calculation of frequencies is based on positive results only and a minimum of two positive results per antigen. Horizontal gray lines, net difference between mean frequencies in patients and healthy individuals. 0, respective TAA peptides were not recognized at all. n.n, only one positive result was detected.
Others are selectively recruited to the bone marrow (15–17). Therefore, the bone marrow seems to be more suitable for a screening of the whole memory T-cell repertoire. It was recently shown that dendritic cells transport antigens from peripheral sites to the bone marrow where they interact with resident memory T cells (17). This leads to a pronounced memory T-cell proliferation and might result in a constant shaping of the memory T-cell repertoire. In tumor patients, dendritic cells carrying TAs from the tumor site or soluble blood-borne TAA may similarly affect the memory T-cell repertoire in favor of tumor antigen–specific T cells competing for niches with T cells of other specificities.

Unexpectedly, our analysis revealed that the bone marrow of healthy individuals harbors a repertoire of autoreactive T cells. Recognition of a broad variety of autoantigens, among them TAs, may be a regular event. Such latent autoreactivity may contribute to tumor immune surveillance, a hypothetical feature of the immune system proposed by some tumor immunologists. In contrast to tumor patients, healthy donors recognized common autoantigens in a similar way as they did tumor-associated (auto) antigens. Our data suggest that the T-cell repertoire of breast cancer patients is shaped for recognition of TAs whereas the T-cell repertoire of healthy individuals does not differentiate between common autoantigens and tumor-associated autoantigens with respect to T-cell frequency, number of recognized antigens, or proportion of responding individuals. Thus, the generation of the memory T-cell repertoire seems to be a dynamic and balanced process that involves enlargement of the pool in response to a set of presented antigens at the expense of irrelevant T cells. The T-cell memory repertoire might therefore represent the pattern of immunogenic antigens of a tumor against which autologous spontaneous immune responses could be generated during a certain time period.

We conclude that breast tumors modulate the memory T-cell repertoire of at least two thirds of all patients in favor of polyvalent tumor antigen recognition and probably at the expense of autoantigen recognition. The T-cell repertoires vary between individual patients with respect to the patterns of recognized antigens and the resulting T-cell frequencies. We suggest that the bone marrow is a suitable organ for the assessment of the TAA-specific T-cell repertoire. A selection of frequently and selectively recognized TAs may facilitate the screening of high numbers of breast cancer patients before and after immunotherapeutic interventions.

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