Association between αβ and γδ T-cell subsets and clinicopathological characteristics in patients with breast cancer

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Abstract. The aim of the present study was to discuss the effect of surgery on the T-lymphocyte subsets of patients with breast cancer (BC) and investigate the association between peripheral blood αβ and γδ T-cell counts and the clinicopathological characteristics of BC. The CD3$^+$, CD4$^+$, CD8$^+$ and γδ T-cell subsets in the peripheral blood of healthy volunteers and Patients with BC before and after surgery were determined using flow cytometry. The association between αβ and γδ T-cell counts in the peripheral blood and clinicopathological characteristics was analyzed by comparing the differences in the αβ and γδ T-cell counts in the peripheral blood of Patients with BC before and after surgery with those of healthy volunteers and combining with clinicopathological data. The CD3$^+$, CD4$^+$ and γδ T-cell counts in the peripheral blood of Patients with BC were lower compared with those in healthy volunteers (P=0.0077, 0.0116 and 0.0003, respectively), whereas the number of CD8$^+$ cells was higher (P=0.0241). The CD3$^+$, CD4$^+$ and γδ T-cell counts and the CD4$^+$/CD8$^+$ ratio after surgery were significantly higher compared with those before surgery (P=0.0109, 0.0031, 0.0165 and 0.018, respectively). There was no significant difference between the number of CD8$^+$ cells before and after surgery (P=0.0053), but the number of CD8$^+$ cells was higher in healthy volunteers compared with that in Patients with BC (P<0.05). Moreover, the CD3$^+$ cell number was higher in patients with TNM stage II/III compared with those with TNM stage I disease (P=0.187 and 0.022, respectively), and the peripheral blood CD4$^+$/CD8$^+$ ratio and number of γδ T cells were lower in stage III compared with stage I Patients with BC (P=0.0065 and 0.0176, respectively). Histological grading demonstrated that the CD4$^+$/CD8$^+$ ratio and number of γδ T cells in patients with stage III BC were lower compared with those with stage I BC (P=0.02 and 0.0128, respectively). The γδ T-cell count in patients with luminal A and B subtypes was significantly higher compared with that in patients with basal-like subtype (P=0.004 and 0.0104, respectively). The CD3$^+$, CD4$^+$ and γδ T-cell counts were significantly lower in patients with lymph node (LN) metastasis compared with those without LN metastasis, and the CD8$^+$ cell number was lower in patients without LN metastasis compared with that in patients with >10 LN metastases (P=0.0086, 0.0000 and 0.00468, respectively). The CD8$^+$ cell count in patients without LN metastasis was lower compared with that in patients with 4-9 and >10 LN metastases (P=0.0435 and 0.0283, respectively). Surgery affects the T-lymphocyte subpopulations in patients with BC, and αβ and γδ T-cell counts may increase following mastectomy. Therefore, measurement of peripheral blood lymphocyte subsets is crucial for understanding the immune function status of Patients with BC with differences in TNM stage, histological grade, cell subtypes and LN metastases, and may provide a basis for the application of T-cell subsets in the comprehensive treatment of BC.

Introduction

Breast cancer (BC) is the most common malignant tumor affecting women worldwide, and has the highest incidence among female malignancies. The morbidity and incidence of BC in China account for ~12.2 and 9.6% of the total cases worldwide, respectively, and they exhibiting a rapid increasing trend (1). The development and progression of BC includes the interaction of tumor cells and the microenvironment, involving gene mutations, and also the interaction of microbes and tumor cells, immune cells, extracellular matrix, and new supporting blood vessels. The complex evolution of the tumor microenvironment is also closely associated with the development and progression of BC (2). Tumor immunotherapy is a new treatment strategy in addition to surgery, radiotherapy and chemotherapy, and its mechanism of action is based on the stimulation of the body's own immune system and enhancement of the ability of the microenvironment to antagonize tumor immunity, thereby acting to control and eliminate tumor cells. It has high specificity and minimal

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damage to normal tissues, and can also stimulate immunological memory, among other functions. T lymphocytes (αβ and γδ T cells) play an important role in human immunity, and there is a relatively constant proportion of cells among the subsets, which ensures immune system stability. Tumors can disrupt the balance between αβ and γδ T cells, thereby disrupting immune function. Of note, the proportion of T-lymphocyte subsets reflect the immune status of the body (3). The CD3^{+}, CD4^{+} and CD8^{+} T-lymphocyte subsets are relatively constant, and they coordinate and restrict each other, which ensures the stability of immune function and prevents invasion by harmful pathogens. The CD4^{+}/CD8^{+} ratio reflects a dynamic balance under normal conditions (4). The proportion of CD8^{+} cells increases, while the proportions of CD3^{+}, CD4^{+} and CD19^{+} cells decrease in the peripheral blood of tumor patients (5,6). In recent years, γδ T cells have been attracting increasing attention due to their unique histological distribution and immunological characteristics, and their key anti-infection and antitumor properties. These cells can exert cytotoxic effects against malignant tumors through participating in immune surveillance and tumor cell elimination (7). The proportion of αβ and γδ T cells in the peripheral blood of patients with BC may be a dynamically changing factor and exhibits a specific association with clinical pathology. In order to further elucidate the background of the immune function of αβ and γδ T cells in patients with BC and their role in the development of BC, the aim of the present study was to determine and analyze αβ and γδ T-cell counts in the peripheral blood of patients with BC and their association with clinical phenotype, so as to provide clinical evidence supporting the use of immunotherapy for BC in the future.

Materials and methods

Study population. Between January 2017 and December 2018, 138 female patients with BC, with a mean age of 43.62±3.28 years, were included in the present study. All the patients had undergone modified radical mastectomy at the Second Affiliated Hospital of Shandong First Medical University (Tai'an, China). The patients had the following pathological types of cancer: Invasive ductal carcinoma (n=128), medullary carcinoma (n=4), invasive lobular carcinoma (n=2), cribriform carcinoma (n=2), mucinous carcinoma (n=2) and ductal carcinoma (n=1). Hematoxylin and eosin staining of tissue samples was performed. There were 89 cases with lymph node (LN) metastasis and 49 cases without LN metastasis. According to the 7th edition of TNM staging described by the Union for International Cancer Control and the American Joint Committee on Cancer (5), there were 56 cases with stage I, 55 cases with stage II and 27 cases with stage III BC. No patient received neoadjuvant therapy prior to surgery. Patients with the following conditions were excluded: i) Autoimmune diseases; ii) recent major infections; iii) use of drugs that affect the immune function of the body; and iv) serious diseases of the heart, liver, kidney, lung, or other major organ, and endocrine disorders. A total of 50 healthy volunteers were selected as the control group, and they did not use any drugs or foods known to affect the immune function of the body. The study protocol was approved by the hospital medical ethics committee, and all participants provided written informed consent.

Reagents and instruments. Fluorescence-labeled mouse anti-human monoclonal antibodies: Mouse anti-human TCR-γ/APC (0.2 mg/ml; cat. no. BD-555718); mouse anti-human CD3/FITC (0.1 mg/ml; cat. no. BD-561806); mouse anti-human CD45/PerCP-Cy™5.5 (0.1 mg/ml; cat. no. BD-564105); mouse anti-human CD4/APC (0.2 mg/ml; cat. no. BD-551980); mouse anti-human CD8/PE (0.2 mg/ml; cat. no. BD-555367); FACS hemolysin, mouse IgG1-FITC (0.5 mg/ml; cat. no. BD-550618); and mouse IgG2-PE (0.25 mg/ml; cat. no. BD-560550) were purchased from BD Biosciences. A flow cytometer (FACSCalibur) and tissue cell separator were also purchased from BD Biosciences.

Detection of αβ T cells in the serum. A total of 3 ml of peripheral blood was collected from all patients on the day prior to surgery and on postoperative day 15 for anticoagulation in anticoagulant tubes. In addition, 3 ml of venous blood was collected from the 50 healthy volunteers to use as control. The total number of lymphocytes and proportion of lymphocyte subsets in the peripheral blood, including CD3^{+} and CD4^{+} helper T lymphocytes, CD8^{+} killer T lymphocytes and the CD4^{+}/CD8^{+} T-cell ratio, were detected by FACS flow cytometry. For performing the procedure, 50 μl of anticoagulated whole blood was collected into each test tube and mixed gently, followed by the addition of 30 μl of CD3/FITC-CD8/PE-CD4/APC-CD45/PerCP-Cy™5.5 fluorescent antibodies. Following incubation in the dark at room temperature (24°C) for 20 min, 2 ml of 1:10 diluted 1X FACS lysing solution was added to each tube. The samples were vortexed, and the test tubes were incubated in the dark for 10 min at room temperature. The supernatant was discarded after centrifugation at 300 x g at 4°C for 5 min, and 2 ml PBS was added into each tube. The samples were vortexed and centrifuged 300 x g at 4°C for 5 min. Subsequently, the supernatant was removed, and 0.5 ml PBS was added to resuspend the cells for analysis in the flow cytometer.

Detection of γδ T cells in the peripheral blood by fluorescence staining. Two FACS sample tubes were taken from each sample, and 100 μl of anticoagulated whole blood was added to each sample tube. Mouse anti-CD3/FITC (20 μl) and mouse anti-human CD45/PerCP-Cy™5.5 (5.55 μl) were added into one tube as negative control. In the other tube, mouse anti-TCR-γδ/APC (5 μl), mouse anti-human CD3/FITC (20 μl), and mouse anti-human CD45/PerCP-Cy™5.5 (5.55 μl) were added as the testing sample. The mixture was uniformly mixed with mild oscillation and kept away from light for 15-30 min at room temperature (20-25°C). Then, 2 ml of FACS hemolysin was added to each tube, the contents were mixed well, and the tubes were kept away from light at room temperature for 8-12 min. Subsequently, the sample was centrifuged at 300 x g at 4°C for 5 min. The supernatant was discarded, and 2 ml PBS was added to the precipitate. After shaking and centrifuging at 300 x g at 4°C for 5 min, the supernatant was discarded and the precipitate was mixed with 1% paraformaldehyde buffer at 0.5 ml. The supernatant was stored at 2-8°C away from light and analyzed using a Nikon

Table I. Comparison of T-cell subsets in the peripheral blood of patients with breast cancer before and after surgery and healthy volunteers.

Groups	Number of cases	T-cell subsets				
		CD3+	CD4+	CD8+	CD4+/CD8+	γδT
Healthy volunteers	50	68.16±7.12	36.24±4.68	31.56±3.02	1.38±0.62	3.38±2.16
Patients before surgery	138	65.24±6.36[b]	34.12±5.16[a]	32.78±3.33[a]	1.27±0.29	2.12±2.04[b]
Patients after surgery	138	67.18±6.22[c]	35.81±4.19[d]	32.61±3.19[a]	1.35±0.26[c]	2.74±2.28[c]

Data are presented as mean ± standard deviation. [a]P<0.05, [b]P<0.01 compared with the volunteer group. Patients before and after surgery [c]P<0.05, [d]P<0.01.
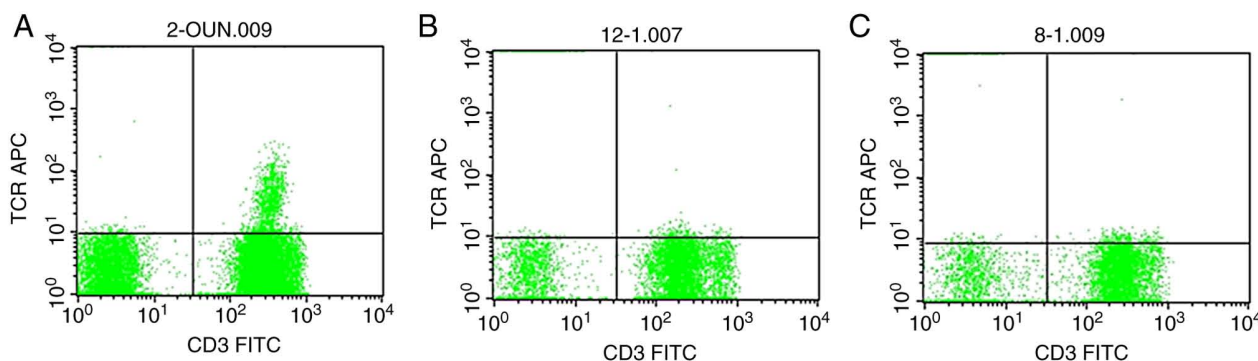

Figure 1. Screening of γδ T cells in the peripheral blood of healthy volunteers and patients with breast cancer before and after surgery. Flow cytometry was used to sort APC mouse anti-human TCR γδ antibody-positive and FITC mouse anti-human CD3 antibody-positive γδ T cells (A) from healthy volunteers, (B) from breast cancer patients before surgery and (C) from breast cancer patients after surgery. The experiment was repeated three times for each group, and relatively good results were obtained. Sample numbers: 2-0UN.009, 12-7.001 and 8-1.009. TCR, T-cell receptor; APC, antigen-presenting cell.

TE-2000S microscope (Nikon Corp.; magnification, x40) within 24 h.

Statistical analysis. The results were analyzed using SPSS 19.0 for Windows (SPSS Inc.) and are expressed as mean ± standard deviation. Statistical significance was determined with the help of the t-test used to compare pairwise sample means between groups. P<0.05 was considered to indicate statistically significant differences.

Results

Detection of serum αβ and γδ T cells in patients with BC. The numbers of CD3+, CD4+ and γδ T cells in the blood of Patients with BC prior to surgery were lower compared with those in healthy volunteers (P=0.0077, 0.0116 and 0.0003, respectively), while the ratio of CD4+/CD8+ cells did not differ significantly between the two groups (P>0.05). The number of CD8+ cells was also higher in Patients with BC compared with that in healthy volunteers (P=0.0241). The numbers of CD3+, CD4+ and γδ T cells and the CD4+/CD8+ ratio after surgery were significantly higher compared with those prior to surgery (P=0.0109, 0.0031, 0.0165 and 0.018, respectively), while the number of CD8+ cells was not significantly different from that prior to surgery (P>0.05) and was still higher compared with that in healthy volunteers (P=0.0053). The results are summarized in Table I and Figs. 1-3.

Association between preoperative levels of αβ and γδ T cells in the peripheral blood and clinicopathological characteristics in patients with BC. Following TNM staging, the number of CD3+ cells in stage II/III was higher than that in stage I BC (P=0.187 and 0.022, respectively); the CD4+/CD8+ ratio and number of γδ T cells were lower in stage III patients compared with those in stage I patients (P=0.0065 and 0.0176, respectively), and there was no statistical difference between I vs. II and II vs. III (P>0.05). Histological classification showed that the CD4+/CD8+ ratio and number of γδ T cells were lower in stage III patients compared with those in stage I patients (P=0.02 and 0.0128, respectively), and there was no statistically significant difference between the other groups (P>0.05). Among the different molecular subtypes, the number of γδ T cells was significantly higher in patients with luminal A and luminal B subtype compared with that in patients with basal-like subtype (P=0.004 and 0.0104, respectively), and there was no significant difference in the number of T-cell subsets among the other subtypes (P>0.05). The number of CD3+ cells was significantly lower in patients with LN metastasis compared with that in patients without LN metastasis. The difference in the number of CD3+ cells was statistically significant when comparing patients without LN metastases with those with 1-3 LN metastases (P=0.0097), 4-9 LN metastases (P=0.0024), or >10 LN metastases (P=0.0086). The number of γδ T cells was significantly higher in patients without LN metastases compared with that in patients with LN metastases. The difference was statistically significant when
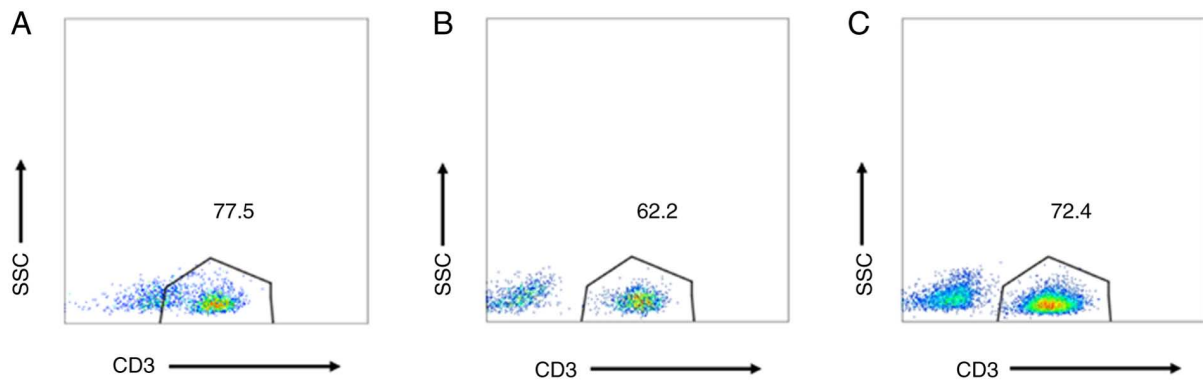
Figure 2. Screening of CD3^{+} T cells in the peripheral blood of healthy volunteers and breast cancer patients before and after surgery. CD3 antibody-positive T cells in the peripheral blood of (A) healthy volunteers; (B) preoperative breast cancer patients; (C) postoperative breast cancer patients. SSC, side scatter.
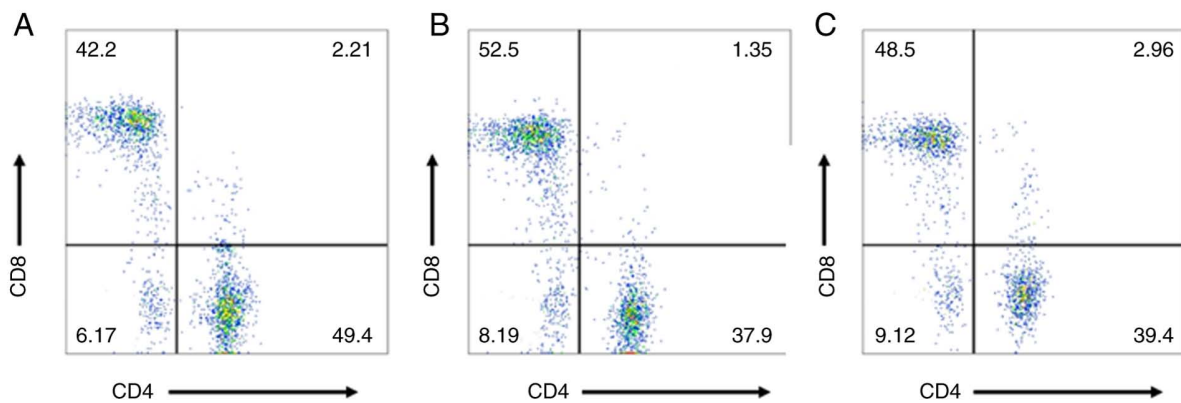

Figure 3. Screening of CD4^{+} and CD8^{+} T cells in the peripheral blood of healthy volunteers and breast cancer patients before and after surgery. CD4 and CD8 antibody-positive T cells in the peripheral blood of (A) healthy volunteers; (B) preoperative breast cancer patients; (C) postoperative breast cancer patients.

comparing patients without LN metastases with those with 1-3 LN metastases (P<0.0000), 4-9 LN metastases (P=0.0004), or >10 LN metastases (P=0.0000). The number of CD4^{+} cells was higher in patients without LN metastasis and patients with 1-3 LN metastases compared with that in patients with >10 LN metastases (P=0.00468 and 0.0494, respectively). The number of CD8^{+} cells was lower in patients without LN metastasis compared with that in patients with 4-9 or >10 LN metastases (P=0.0435 and 0.0283, respectively). There was no significant difference in the number of CD8^{+} cells among the other groups (P>0.05; Table II).

Discussion

BC occurs and progresses via pathological mechanisms such as immune escape and immunosuppression. The proportion and distribution of T lymphocytes in the peripheral blood and tumor microenvironment are closely associated with tumor immune stability, immune clearance, or immune escape. Moreover, the relative stability, mutual coordination and mutual restriction of T-lymphocyte subsets in healthy individuals ensures the stability of immune function in the body and helps prevent invasion by pathogens. Changes in the number and ratio of T-lymphocyte subsets may disrupt the immune system and normal immune function of the body, thus leading to lesions such as tumor and immune-related conditions. CD3^{+} T cells are the main active

cells in cellular immunity, representing the proportion of mature lymphocytes among the total T cells. CD4^{+} T cells are helper T cells, whereas CD8^{+} T cells exert both cytotoxic and immunosuppressive effects, and the CD4^{+}/CD8^{+} ratio reflects the status of cellular immunity (8). The results of the present study demonstrated that, due to the presence of BC target cells, the CD3^{+}, CD4^{+} and γδ T-cell counts and the CD4^{+}/CD8^{+} ratio in the peripheral blood of the patients prior to surgery were significantly lower compared with those in healthy volunteers. This indicates that the tumor cells can elicit immune function-related changes in T-lymphocyte subsets, recruiting more CD8^{+} cells to exert cytotoxic effects, and reducing the number of CD3^{+}, CD4^{+} and γδ T cells in the circulation. In the present study patients with BC generally exhibit decreased immune function, as well as changes in the number and function of LNs that perform cellular immune functions.

Based on the different T-cell receptors (TCRs), T cells are mainly divided into αβ and γδ T cells; αβ T-cell surface receptors usually express CD4^{+} and CD8^{+}. The recognition of antigens mainly relies on APCs. Based on the histocompatibility complex (MHC) molecules on the cell surface, most αβ T cells can differentiate into both cytotoxic T and helper T cells. CD8^{+} cells are referred to as cytotoxic T cells, as their biological function is to directly kill the labeled target cells, and these cells are the main effectors of the antitumor immune response. CD4^{+} cells are characterized as helper
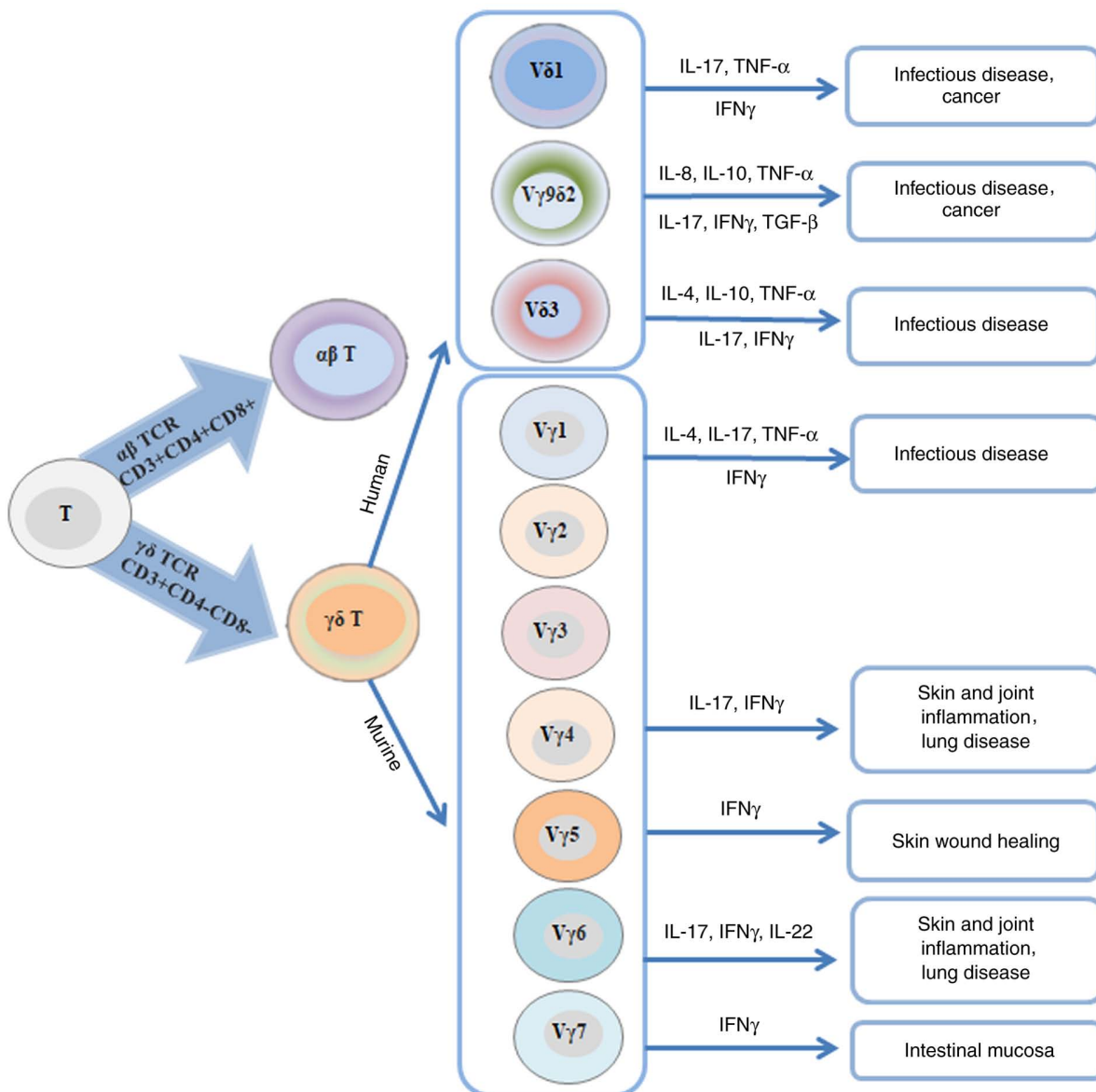
Figure 4. Classification and characteristics of human and mouse γδ T-cell subsets. Human γδ T cells are differentiated into three subtypes, namely Vδ1, Vγ9δ2 and Vδ3, and they mainly secrete TNF-α, IFN-γ, TGF-β, IL-4, IL-8, IL-10 and IL-17. In mice, Vγ1δ, Vγ4δ, Vγ5δ, Vγ6δ and Vγ7δ T cells mainly produce IL-4, IL-17, IL-22 and IFN-γ. TCR, T-cell receptor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor.

T cells, which mainly regulate or stimulate other lymphocytes to exert their immune effects. γδ T cells are cells that often do not express CD4+ or CD8+, recognize antigens mainly in a non-MHC-restricted manner, and induce an adaptive immune response by secreting a variety of cytokines. This cell type is considered to be an unconventional T cell that serves as a link between innate and adaptive immunity. Due to its immune characteristics, it plays a key role in anti-infection and anti-tumor responses, can produce effective cytotoxicity against malignant tumors, and has the functions of immune surveillance and tumor cell elimination (9). In the peripheral blood, αβ T cells account for ~95%, while γδ T cells account for only 5% of the total CD3+ cells. Based on the differences between the γ and δ chains, γδ T cells are also divided into several subgroups, and different subsets have specific tissue distribution among different species. As shown in Fig. 4, the majority of γδ T cells are distributed in human epithelial tissues, while

Vγ9Vδ2 TCR subsets are mainly expressed in peripheral blood lymphocyte repository. Generally, 50-75% of γδ T lymphocytes in the peripheral blood express the Vδ2 chain and co-express the Vγ9 chain. These cells are referred to as Vγ9Vδ2T cells, Vγ9Vδ2T cells are only found in human and non-human primates, and 1-10% of T cells are found in the peripheral blood of healthy individuals. It was previously reported that early-stage BC is associated with low expression of Vg9Vd2+ T lymphocytes in the circulation (10). Human γδ T cells can mediate antitumor immunity through different pathways, such as secretion of pro-apoptotic molecules and pro-inflammatory cytokines, and cell-cell contact-dependent cleavage through the NK transduction pathway or TCR-dependent pathways. Activated γδ T cells can secrete several cytokines that act on tumor cells or their microenvironment, such as interferon-γ, tumor necrosis factor-α, interleukin (IL)-2, perforin, granzyme B and Fas/FasL. As shown in Fig. 5, γδ T cells also negatively

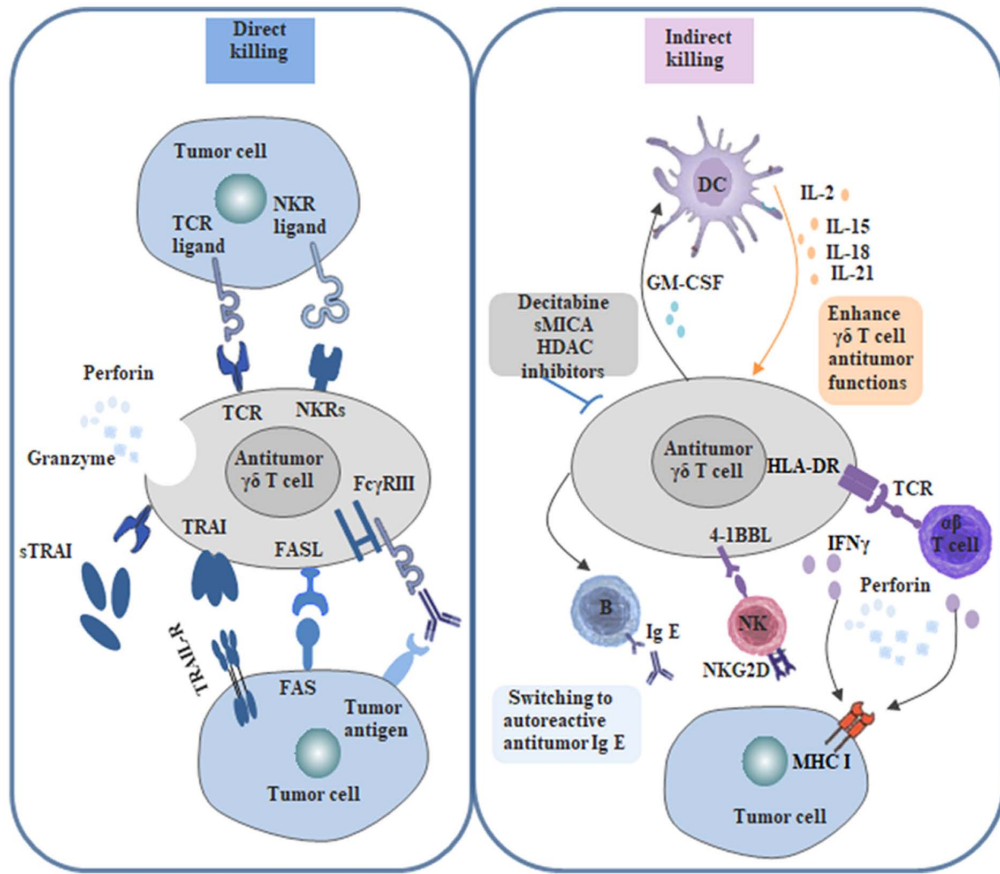Figure 5. Regulatory mechanism of tumor cell killing by γδ T cells. γδ T cells directly recognize tumor-killing cells through the TCR and NKR. γδ T cells can mediate tumor cell killing by expressing TRAIL, FASL or release of cytolytic granules. γδ T cells induce antitumor immunity through IFN-γ production and antigen-presenting cell function to induce αβ T-cell activation, whereas expression of 4-1BBL can stimulate NK cells. In addition, γδ T cells induce antibody class switching in B cells, which contributes to the protective humoral response. γδ T cells can also modulate DC infiltration through GM-CSF. TCR, T-cell receptor; NK, natural killer; NKR, natural killer cell receptor; IFN, interferon; DC, dendritic cell; 4-1BBL, 4-1BB ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor.

regulate the function of tumor cell killing. This is mainly associated with the production of IL-17, which can stimulate tumor cell proliferation and induce angiogenesis. Other negative regulatory IL-7-mediated effects of γδ T cells on tumor cell killing include inhibition of maturation of dendritic cells, inhibition of T-cell response through PDL1 expression, and inhibition of IL-17-producing γδ T cells through reactive oxygen species generation by neutrophils (11). As shown in Fig. 6, although the number of γδ T cells is small *in vivo*, the γδ T cell population may markedly enlarge under phosphate stimulation both *in vitro* and *in vivo*, which plays an important role in antitumor immunity (12).

Flow cytometry was used to detect αβ and γδ T-cell subsets in the peripheral blood of Patients with BC and healthy controls. It has been established that the normal population size of cell subsets is crucial for clinical diagnosis and disease determination. The reference values for detecting normal human αβ and γδ T-cell subsets are as follows: CD3+, 61-85%; CD4+, 28-58%; CD8+, 19-48%; CD4+/CD8+ ratio, 1-2; and γδ, 1-5%. The results revealed that the CD3+, CD4+ and γδ T-cell counts and the CD4+/CD8+ ratio were lower in Patients with BC prior to surgery compared with those in healthy volunteers, while the CD8+ cell number was higher compared with that in healthy subjects. It may be suggested that, upon the occurrence of BC, the body may

recruit more CD8+ cells and trigger an immune response. BC cell antigens lead to depletion of CD4+ and γδ T cells, as well as to a decrease in the number of CD3+ cells among total lymphocytes. The γδ, CD3+ and CD4+ T-cell counts and the CD4+/CD8+ ratio in the peripheral blood of patients with BC were higher after surgery compared with preoperative levels, but were still lower compared with those in healthy controls. The number of CD8+ cells was lower compared with that before surgery, but slightly higher compared with that of controls, although the difference was not statistically significant. It is hypothesized that BC cells may secrete substances that inhibit the proliferation of γδ T cells, and the load of tumor cells is reduced after surgical tumor resection, which can improve the cellular immune status of the body and enhance the cellular immune function of the patients. The occurrence and development of tumors will also cause changes in the T-lymphocyte subsets in the body. The present study demonstrated that the number of CD3+ cells in patients with TNM stage II and III was higher compared with that in patients with stage I disease, whereas the CD4+/CD8+ ratio and T-cell counts in stage III patients were lower compared with those in stage I patients. The histological grading revealed that the CD4+/CD8+ ratio and the number of T cells in patients with grade III tumors were lower compared with those in patients with grade I tumors. Among different molecular subtypes, the
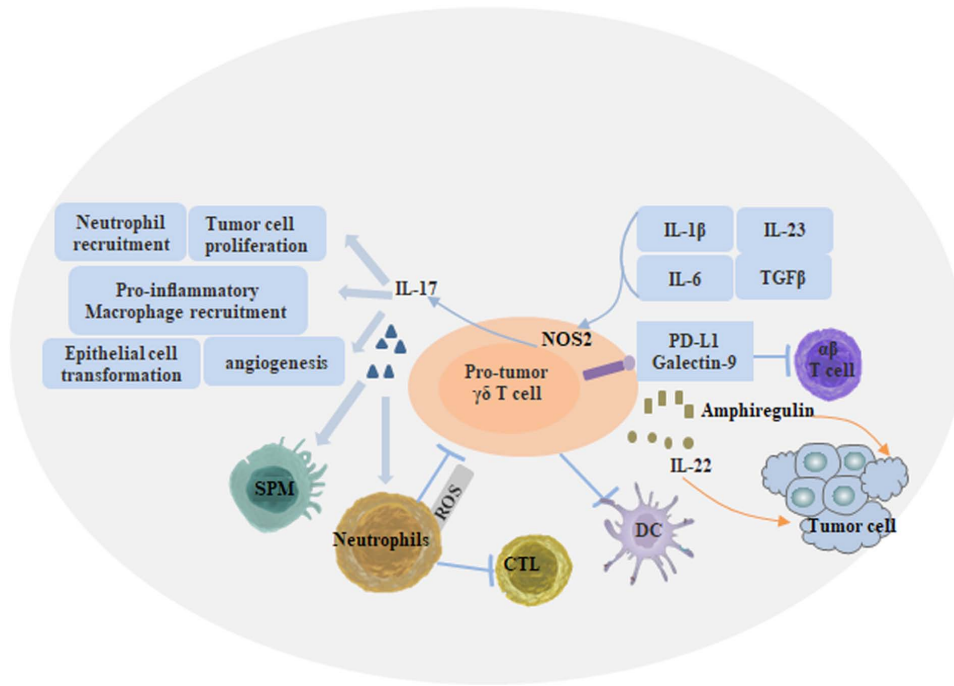
Figure 6. Mechanism of negative regulation of tumor cell killing by γδ T cells. The negative regulation of tumor cell killing by γδ T cells is primarily associated with the production of IL-17, which has multiple functions, including stimulating tumor cell proliferation and inducing angiogenesis. Other negative regulation of tumor killing by IL-7 mediated by γδ T cells includes inhibition of DC maturation; suppression of T-cell responses through PDL1 expression; and inhibition of IL-17-producing γδ T cells through ROS production by neutrophils. DC, dendritic cell; IL, interleukin; ROS, reactive oxygen species; TGF, transforming growth factor; SPM, specialized proresolving mediator.

Table II. Comparison of T-cell subsets in the peripheral blood of patients with breast cancer in different pathological groups.

Groups	Number of cases	T-cell subsets				
		CD3^{+}	CD4^{+}	CD8^{+}	CD4^{+}/CD8^{+}	γδT
TNM stage	138					
I	56	66.86±6.36	34.32±4.16	31.52±5.82	1.39±0.45	2.92±1.54
II	55	65.24±5.27[a]	33.27±5.02	31.63±4.82	1.29±0.39	2.46±1.39
III	27	64.35±4.76[a]	32.72±5.35	32.51±6.05	1.12±0.32[a]	2.12±1.08[a]
Histological grade	138					
I	48	66.29±7.32	35.02±5.13	32.26±4.86	1.48±0.75	2.76±1.12
II	59	66.02±6.63	34.62±5.08	32.56±6.01	1.39±0.69	2.56±1.18
III	31	64.74±7.16	33.19±4.86	33.06±5.18	1.09±0.65[b]	2.12±1.04[b]
Molecular subtype	138					
Luminal A	38	66.24±5.36	35.12±4.12	31.87±4.78	1.38±0.68	2.36±1.12[c]
Luminal B	32	67.12±6.33	35.18±5.08	31.76±4.89	1.36±1.05	2.32±1.18[c]
ERBB2[+]	28	65.15±4.63	34.82±5.13	32.86±6.12	1.37±0.82	2.02±1.14
Basal-like	31	64.29±4.55	33.11±4.76	33.59±5.82	1.28±0.66	1.52±1.22
Special type	9					
Endocrine responsive	5	67.06±1.31	34.78±5.06	31.96±2.16	1.35±0.45	1.41±1.06
Endocrine non-responsive	4	66.72±1.36	34.56±5.18	32.16±3.42	1.33±0.65	1.39±1.13
No lymph node metastasis	49	67.36±3.28	36.36±5.25	31.28±4.39	1.37±0.69	3.29±1.22
Lymph node metastases	89					
1-3	52	65.61±3.38[e,f]	36.15±5.12[f]	31.34±5.38	1.42±0.85	2.01±1.74[e]
4-9	25	64.52±4.35[e]	35.19±5.34	31.66±5.81[d]	1.39±0.76	2.12±1.43[e]
≥10	12	64.23±4.65[e]	33.11±5.67[d]	33.52±4.82[d]	1.22±1.06	1.39±1.75[e]

Data are presented as mean ± standard deviation. TNM staging, [a]P<0.05 compared with stage I; histological grade, [b]P<0.05 compared with grade I; molecular subtype group, [c]P<0.05 compared with basal-like type; lymph node condition, [d]P<0.05, [e]P<0.01 compared with non-lymph node metastasis group; and >10 lymph node metastases [f]P<0.05.

number of T cells in patients with luminal A and luminal B subtypes was significantly higher compared with that in patients with basal-like subtype. The numbers of CD3$^+$, CD4$^+$ and $\gamma\delta$ T cells were significantly lower in patients with LN metastasis compared with those in patients without LN metastasis. This indicates that there is an overall decrease in immune function in Patients with BC, which is manifested by a change in the number and function of lymphocytes performing cellular immune functions, which is one of the important causes of BC development and progression. There is a need for more in-depth basic and clinical research on expanding the peripheral blood $\gamma\delta$ T-cell population and enhancing its tumor cell killing ability, in order to improve the immune function and quality of life of Patients with BC. Therefore, measurement of peripheral blood lymphocyte subsets may help elucidate the immune function status of Patients with BC with different molecular subtypes, TNM stage and histological types, and may prove to be of great value for the diagnosis of BC, as well as for monitoring therapeutic efficacy and prognosis.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Conceptualization, XQL and MZ; methodology, XQL, MZ, XLL and CW; writing-original draft preparation, XQL, MZ and XLL; writing-review and editing, XQL and MZ; supervision, XQL. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of the Second Affiliated Hospital of Shandong First Medical University (Tai'an, China). The procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Patient consent for publication

Not applicable.

Competing interests

All the authors declare that they have no competing interests.

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