Isolation and Immuno-Phenotypic Characterization of Tumor Infiltrating Lymphocytes (TILs) Obtained from Breast Malignant Tumor Tissues of Egyptian Patients

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BACKGROUND: Isolation and phenotypic characterization of tumor infiltrating lymphocytes (TILs) in some malignant tumors have been shown. TILs possess a good prognostic value as well as a therapeutic effect in these solid tumors. Our preliminary work shed some light on a good possibility of synthesis and secretion of specific protease enzyme system with a dimeric structure above 92 kDa for the lytic activity of TILs against breast tumor cells propagated ex vivo.

PURPOSE: This work aims at first isolation, activation and immuno-phenotypic characterization of TILs derived from malignant tumor tissues of breast cancer patients. Second, to optimize the conditions for the biological therapeutic efficiency of the identified TILs sub-populations as targeted cell therapy against breast cancer.

PATIENTS and METHODS: The present work presented twelve patients with breast cancer from NCI, Cairo. T-cell isolation, activation, immunophenotyping and immunohistochemical investigations were performed. Enzymatic digestion method, mesh with pore size 355 & 45mm and flow cytometric analysis were used.

RESULTS: The results revealed that, lymphocytes infiltrating the malignant tumor tissues were mainly of the T-cell type indicated by CD45RO positive markers as shown by immunohistochemical observations. The immunophenotypic analysis of the isolated TILs obtained from breast tumor tissues specimens and activated with interleukin-2 (IL-2), showed that the ratio of CD4+/CD8+ was 0.89 which represents helper and cytotoxic sub-populations of TILs, respectively. Meanwhile, the ratio of CD4+/CD25+ was 16.03 representing the regulatory system of TILs sub-population. In the peripheral blood of patients, the percentages of the CDs positive cells were different and the ratio of CD4+/CD8+ was 1.14±0.57 whereas the ratio of CD4+/CD25+ was 18.38±5.95. After mixing the isolated TILs and the T-lymphocytes obtained from the peripheral blood, the ratio of CD4+/CD8+ increased insignificantly to 1.45±0.67. Also the ratio of CD4+/CD25+ increased rough insignificantly, to 23.64±9.83.

CONCLUSION: The isolated and identified TILs sub-populations have to be tested for their biological therapeutic efficacy first at ex-vivo level using the cr51 release assay; second at in-vivo level using experimental animal models as a sub-clinical investigation before going further to clinical study of using TILs as targeted bio-immunotherapy against human cancers.

Key Words: TILs – Immunohistochemistry – Immuno-phenotyping – Breast cancer.

INTRODUCTION

Tumor infiltrating lymphocytes (TILs) play a key role in the immunogenic reaction against tumors [1]. Although TILs are present in the tumor microenvirionment and recognize the antigenic characteristics of malignant cells, they are incapable of preventing tumor growth. However, pre-clinical data on ex-vivo studies revealed that TILs activation has the ability to kill autologous cancer cells [2].

In a suspension of fresh TILs prepared from human breast ductal adenocarcinoma, the phenotypic pattern of this preparation revealed a high score for CDs with a varying number of CD4+ and CD8+ cells. The latter surface markers were significantly different in the lymph node metastasis group and the lymph node negative one. TILs with CD8+/CD4+ ratio greater than
1 were associated with lymph node metastasis. Moreover, the ratio was also correlated with tumor size but not with hormone receptor expression [3].

Flow cytometric analysis of leukocytes from tumor tissues, axillary lymph nodes, and peripheral blood of human breast cancer patients were carried out to evaluate the ability of TILs to synthesize type 1 cytokines (TNF-α, INF-γ and IL-2) and type 2 cytokines (IL-4, IL-6, and IL-10). The analysis revealed that CD8 positive T-cells were the major leukocyte population detected in each tissue with CD4 being predominant. Moreover type 1 cytokines were the predominant type produced by T cells for each population [4].

Immunohistochemistry was performed on tissue sections of primary breast tumors using monoclonal antibodies to T lymphocytes (CD3), T-helper cell (CD4) cytotoxic T cells (CD8), natural killer cells (CD56), interleukin-2 receptor (IL-2R), major histocompatibility class-1 antigen (HLA-ABC) and histocompatibility class-2 antigen (HLA-DR). All tumors stained positive for CD3, CD4 and CD8, most tumors stained positive for IL-2R, and a limited number of tumors were positive for HLA-DR [5].

Immunostaining of TILs on paraffin-embedded sections of tumor tissues of medullary carcinoma of the breast showed that TILs were quantified separately into cells infiltrating tumor nests (intra-epithelial TILs) and cells infiltrating tumor stroma (stromal TILs). Furthermore, the number of CD8 positive T-cells and CD20 positive cells (B cells) infiltrating tumor nests and tumor stroma of the breast were significantly increased in typical medullary carcinoma and in contrast to poorly differentiated infiltrating ductal carcinoma [6].

In another study using histological sections from the pre- and post-treatment surgical specimens of breast cancer patients receiving neo-adjuvant chemotherapy, Paclitaxel, revealed that the development of TILs after treatment correlates with clinical response to the applied therapy [7].

Flow cytometric analysis of TILs obtained from other types of cancer such as human cervical carcinoma showed CD8+ and CD4+ T cells. The ratio of these cell populations was likely a key to appropriate TILs function and illustrating the critical balance of cell phenotypes needed to prevent the tumor escaping from the control of the host immune response [8]. However, immunophenotypic study of papillary ovarian cancer patients revealed that depletion of CD8+ (T-cytotoxic cells) and CD56+ (natural killer cells) was observed and this might in part explain the poor clinical outcome of this disease [9].

The phenotype and cell cycle status of TILs in certain types of cancer such as hepatocellular carcinoma (HCC) were analyzed via immunohistochemistry of sections from patients undergoing surgery for the disease and via flow cytometry of peripheral blood mononuclear cells and TILs isolated from the same patients. The results revealed that less than 10% of CD8+ T-cells expressed Perforin or granzyme B and 8.7% comprised CD4+CD25+ T (reg.) cells of TILs [10].

The utility of examining human specimens for infiltration by lymphocytes as either a prognostic indicator or a means to guide treatment options for patients suffering from cancers, such as colorectal cancer patients with low and high levels of DNA microsatellite instability could be a hot research area in cancer prognosis and therapy [11]. The main mechanism by which TILs control tumor growth was postulated to be via a cytotoxic mechanism. In our previous study, a specific novel matrix metalloproteinase (MMP) was detected above 92 kDa with dimeric structure in a conditioned medium of a mixed culture of TILs and the autologous tumor cells obtained from breast tumor tissue of Egyptian patients using gelatin zymographic analysis. This indicated the ability of TILs to kill cancer cells via releasing specific protease enzyme systems [12].

The present work aims at isolating, activating and characterizing immunophenotypes of TILs originated from malignant tumor tissue of patients suffering from breast cancer obtained from NCI, Egypt. Moreover, TILs preparation could be useful for adjuvant targeted biological therapy after further studies.

**PATIENTS AND METHODS**

Tumor tissues from twelve patients with breast cancer of different pathological type;
invasive ductal carcinoma (TDC) and invasive lobular carcinoma (ILC) with different lymph node status (LN) were obtained under the supervision of surgical and pathological oncologists, at NCI, Cairo University, during the period from January 2007 to August 2007.

Patients were diagnosed according to the protocols adopted at Surgical and Medical Oncology Departments, NCI, Egypt. The patients were subjected to clinical and laboratory investigations such as liver and kidney function tests, chest X-ray, abdominal pelvic ultrasound (US), CT scanning and isotope bone scanning. The histopathological work was performed according to the protocol of Department of Pathology, NCI, Cairo University.

The patients selected for our study showed the following:
- CT was free.
- Bone scan was free.
- Abdominal US was free.
- Liver function and kidney function tests were normal.

Reagents and chemicals:
- RPMI 1640, fetal calf serum and antibiotics (5000u penicillin and 5000µg streptomycin) were purchased from Sigma, USA.
- Ficoll for separating mononuclear leukocytes solution was from biochom., KG, Leonoemstr, Berlin.

Enzymes and cytokines:
- Collagenase-Hyaluronidase and DNase were from MP Biomedicals, France.
- Interleukin-2 human (IL-2) was from Roche Diagnostic GMbH.
- Monoclonal antibodies for CD4, CD8 and CD25 for flow cytometric analysis were from Trinity Medical Co., Netherland and Invitrogen Co. U.S.A. Mesh metal with pore size of 355mm and 45µm were Egyptian-industry No. 355 and 45 respectively

Preparation and Isolation of TILs:

The cells were isolated according to the protocol of Cochet et al., 1998 with some modification [13]. The cells were subjected to activation using IL-2 (200U/ml).

Immuo-phenotypic characterization of TILs:

A- Flow cytometric analysis was done using flow cytometry of FACS (Becton Dickinson, San Jose, CA) according to a protocol used in Clinical Pathology Department, NCI, Cairo University [14].

B- Immunohistochemical technique was performed using formalin-fixed, paraffin-embedded sections with antibodies CD3 and CD45RO (Dako, Denmark) markers via an avidin-biotin-peroxidase complex method according to a protocol used at the Pathology Department, NCI, Egypt [15].

Cell counting and viability tests:

Our cell suspensions of lymphocytes obtained from peripheral blood (PB) as well as malignant tumor tissues were subjected to cell viability test using trypan blue (0.05%) and cell counting using hemocytometer. For PB, the cell viability was 95-98% and the cell count was 0.5-1.0 million cells/ml. Whereas for TILs, the viability was 90-95% and the cell count was 0.25-0.5 million cells/ml.

The lymphocytes obtained from the PB as well as malignant tumor tissues were mixed with a ratio of 1:1 by volume.

Statistical analysis:

The SPSS version 15 was used in data analysis. Data were expressed as mean ± SD of mean. The data were analyzed using repeated measure analysis of variance (ANOVA) and the unpaired t-test. Correlations were calculated using Pearson's correlation coefficient. p-value less than 0.05 was considered significant.

RESULTS

Morphological observation:

The microscopic observation of TILs showed that two types of cell populations were found to be abundant: type 1 adherent (Fig. 1) and type 2 non-adherent (Fig. 2) TILs. In the former, one could observe that biomatrix was synthesized where TILs were able to adhere. Furthermore, both cell types proliferated in the presence of IL-2 in the culture medium supplemented with fetal calf serum and antibiotics (penicillin and streptomycin). As for the primary culture of breast tumor specimen without IL-2 led to the release of tumor cells that occurred in clumps or separate adherent tumor cells.
Pathological examination:
Pathological feature of the studied tumor tissues showed mainly invasive ductal carcinoma grade 2 and 3 with positive and negative lymph nodes (Table 1). The tissue sections showed marked lymphocyte infiltration (Fig. 3).

Table (1): Clinicopathological data and immunophenotyping results of the investigated patients with breast cancer.

<table>
<thead>
<tr>
<th>Serial</th>
<th>Age</th>
<th>LN status*</th>
<th>Tumor size</th>
<th>Grade</th>
<th>Immunophenotyping results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PB</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>P</td>
<td>&lt;5cm</td>
<td>II</td>
<td>35.8</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>P</td>
<td>&gt;5cm</td>
<td>II</td>
<td>50.5</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>P</td>
<td>&gt;5cm</td>
<td>III</td>
<td>65.3</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>N</td>
<td>&gt;5cm</td>
<td>II</td>
<td>35.4</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>P</td>
<td>&lt;5cm</td>
<td>II</td>
<td>37.2</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>P</td>
<td>&gt;5cm</td>
<td>III</td>
<td>48.1</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>N</td>
<td>&gt;5cm</td>
<td>II</td>
<td>25.5</td>
</tr>
<tr>
<td>8</td>
<td>52</td>
<td>P</td>
<td>&lt;5cm</td>
<td>III</td>
<td>36.3</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>N</td>
<td>&lt;5cm</td>
<td>III</td>
<td>35.2</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>P</td>
<td>&gt;5cm</td>
<td>III</td>
<td>26.3</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>N</td>
<td>&gt;5cm</td>
<td>II</td>
<td>25.4</td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>P</td>
<td>&lt;5cm</td>
<td>II</td>
<td>24.8</td>
</tr>
</tbody>
</table>

P = Positive.  N = Negative.  No. 12 cases.

Immunohistochemical study:
The immunohistochemical staining using CD45RO and CD3 as markers of T-cell differentiation showed that, 33.3% of patients (4 out of 12 cases) revealed marked infiltration of T-cell population, 50% showed moderate infiltration (6 out of 12) and only 16.7% (2 cases only) showed minimal infiltration of T-cell population into the tumor microenvironment (Figs. 4, 5).

Flow cytometric analysis:
The immunophenotypic analysis of the isolated TILs obtained from breast tumor tissues specimens showed different types of cell populations identified by markers of differentiation such as CD8+ T-cell subpopulation that accounted for 35.61±10.23% of the CD3+ cells as well as CD4+ and CD25+ cell sub-populations which represented 29.65±11.56% and 1.95±0.52% of the CD3+ cell population, respectively. The ratio of CD4+/CD8+ was 0.89 that represents helper and cytotoxic sub-populations of TILs, respectively. Meanwhile the ratio of CD4+/CD25+ was 16.03 representing the regulatory system of TILs sub-population (Table 2).

In the peripheral blood of patients, the percentages of the CDs positive cells were different. TILs with CD4+ were accounted for 37.15±12.22% and CD8+ cells percentage was 34.21±4.93% while CD25+ cells comprised 2.06±0.36%. The ratio of CD4+/CD8+ was more than in the TILs but insignificantly (1.14±0.57) while the ratio of CD4+/CD25+ was more than in the TILs but also insignificantly (18.38±5.95).

After mixing the isolated TILs and the T-lymphocytes taken from the peripheral blood, the percentage of CD4+ cells significantly increased to 46.75±14.35% whereas the percentages of both CD8+ and CD25+ cells insignificantly changed. They accounted for 36.43±13.80% and 2.16±0.69%, respectively. The ratio of CD4+/CD8+ increased but insignificantly to 1.45±0.67% and the ratio of CD4+/CD25+ also increased insignificantly to 23.64±9.83%.
Correlation between the immunophenotyping results and the clinicopathological data:

The age of the patients did not correlate to any of the clinicopathological data (Table 3A). Table (3B) shows the association between the tumor size, the LN status and the tumor grades with the percentage of the different subpopulations of T-lymphocytes. The percentage of CD4+ cells after mixing was significantly higher in patients with grade III (57.12±12.42%) than those with grade II (41.15±12.67%, p=0.028). No association of the percentage of CD4+ cells with the tumor size, LN status and grades was detected.

Table (2): The percentage of CD4, CD8 and CD25 positive T-lymphocytes in the TILs, peripheral blood and after their mixing as evaluated by repeated measures ANOVA in breast cancer patients.

<table>
<thead>
<tr>
<th></th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD25+</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PB</td>
<td>TILs</td>
<td>PB + TILs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>CD8</td>
<td>CD25</td>
<td>CD4+/CD8+</td>
</tr>
<tr>
<td>PB</td>
<td>37.15±12.22</td>
<td>34.21±4.93</td>
<td>2.06±0.36</td>
<td>1.14±0.57</td>
</tr>
<tr>
<td>TILs</td>
<td>29.65±11.56</td>
<td>35.61±10.23</td>
<td>1.95±0.52</td>
<td>0.89±0.45</td>
</tr>
<tr>
<td>PB + TILs</td>
<td>46.47±14.35</td>
<td>36.43±13.80</td>
<td>2.16±0.69</td>
<td>1.45±0.67</td>
</tr>
</tbody>
</table>

*p-value 0.001 0.898 0.63 0.12 0.06

PB = Peripheral blood. TILs = Tumor infiltrating lymphocytes.
Data are expressed as mean ± SD. The different letters indicate significant difference according to LSD post hoc test.

Table (3-A): Change to association between the immunophenotyping results and the age of the patients with breast cancer.

<table>
<thead>
<tr>
<th>Age</th>
<th>PB</th>
<th>TILs</th>
<th>PB + TILs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4</td>
<td>CD8</td>
<td>CD25</td>
</tr>
<tr>
<td>&lt;5cm (n=5)</td>
<td>33.86±</td>
<td>36.36±</td>
<td>2.31±</td>
</tr>
<tr>
<td>&gt;5cm (n=7)</td>
<td>5.12</td>
<td>3.18</td>
<td>0.25</td>
</tr>
<tr>
<td>LN status:</td>
<td>15.52</td>
<td>5.36</td>
<td>0.34</td>
</tr>
<tr>
<td>Positive (n=8)</td>
<td>39.50±</td>
<td>32.67±</td>
<td>1.88±</td>
</tr>
<tr>
<td>Negative (n=4)</td>
<td>13.47</td>
<td>5.46</td>
<td>0.38</td>
</tr>
<tr>
<td>Grade:</td>
<td>14.37</td>
<td>4.47</td>
<td>0.28</td>
</tr>
<tr>
<td>II (n=8)</td>
<td>30.37±</td>
<td>33.82±</td>
<td>1.88±</td>
</tr>
<tr>
<td>III (n=4)</td>
<td>5.68</td>
<td>4.38</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table (3-B): Change to association between the immunophenotyping results and the clinicopathological data of the patients.

<table>
<thead>
<tr>
<th>Tumor size:</th>
<th>PB</th>
<th>TILs</th>
<th>PB + TILs</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5cm (n=5)</td>
<td>CD4</td>
<td>CD8</td>
<td>CD25</td>
</tr>
<tr>
<td>&gt;5cm (n=7)</td>
<td>33.86±</td>
<td>36.36±</td>
<td>2.31±</td>
</tr>
<tr>
<td>LN status:</td>
<td>5.12</td>
<td>3.18</td>
<td>0.25</td>
</tr>
<tr>
<td>Positive (n=8)</td>
<td>39.50±</td>
<td>32.67±</td>
<td>1.88±</td>
</tr>
<tr>
<td>Negative (n=4)</td>
<td>13.47</td>
<td>5.46</td>
<td>0.38</td>
</tr>
<tr>
<td>Grade:</td>
<td>14.37</td>
<td>4.47</td>
<td>0.28</td>
</tr>
<tr>
<td>II (n=8)</td>
<td>30.37±</td>
<td>33.82±</td>
<td>1.88±</td>
</tr>
<tr>
<td>III (n=4)</td>
<td>5.68</td>
<td>4.38</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*: Significant (p=0.028). LN = Lymph node.
DISCUSSION

The interplay between tumor development and the host immune system as well as the impact of the immune system on the tumor metastatic potentials is incompletely defined. However, the identification of TILs was reported and represented great hope in cancer treatment and control [16].

There are some studies that threw a light on the immunophenotyping as well as immunohistochemistry of the T-cells infiltrating the tumor microenvironment for some types of solid tumors such as breast, ovarian, cervical, liver and colorectal cancers [4,5,6,8,10,11].

The present work stressed on patients suffering from breast cancer. In this preliminary work, we isolated TILs that originated from tumor
tissues of those patients and we did immunophenotyping using flow cytometry and confirmed by immunohistochemistry so as to identify the percentages of TIL sub-populations mainly CD8+, CD4+ and CD25+. Our results revealed that the CD4+/CD8+ ratio was 0.89, meanwhile Leong et al. [14] showed a 1.86 ratio of CD4+/CD8+ T cells that originated from tumor specimens of patients with infiltrating ductal carcinoma of the breast. Furthermore, our results regarding CD4+/CD25+ ratio of TILs was 16.03, almost close to what has shown by Fumiko et al. [17] who reported that patients with gastric & esophageal cancers showed 14.2 and 19.8 values as CD4+/CD25+ ratios, respectively. In spite of the fact that these are different types of cancers, the results obtained in our work and supported with others could explain the poor immune response of TILs toward tumor growth and their incapability of preventing cancer growth. The new point in our work is the observed changes obtained in these ratios after mixing of the TILs and lymphocytes obtained from peripheral blood. We showed that the CD4+/CD8+ ratio was increased but insignificantly to 1.45 and the CD4+/CD25+ ratio was also increased insignificantly also to 23.64. The increase in these ratios may be due to a decrease of the percentage of CD25+ cells as suppressor regulatory T-cells as well as an increase in the percentage of the CD4+ helper cells. Therefore TILs are in need for activators of their function as well as an increase of the percentage of CDs as cytotoxic T cells and decrease of the percentage of CD25 as suppressor regulatory T-cells.

In our previous study regarding the mechanism of action of TILs, the results illustrated the cytolytic activity of TILs against autologous cancer cells of breast tumor that could be due to releasing a specific matrix-metalloproteinase with dimeric structure above 92 KDa [12]. This represents one mode of action of TILs that is possibly performed mainly by CD8+ with the help of CD4+ and regulated by CD25+ T-cells.

Our subpopulation of CD4+/CD25+ T-cells represents T-regulatory system that inhibits the cytotoxic activity of CD8+ cells and this was found also in patients with both non-small cell lung cancer and ovarian cancer [18]. Many studies have reported that cell-mediated immunity in cancer-bearing hosts is suppressed by many factors [19]. First: Deficient antigen presentation by down regulation of MHC class I on tumor cells; Second: Decreased or lost expression of T-cell epitopes on tumor cells, Third: Immunosuppressive factors derived from tumor cells, fourth: T-cell dysfunction in cancer-bearing hosts, including down-regulation of T-cell signaling molecules or increased induction of T-cell apoptosis; and Fifth: The increased prevalence of T-regulatory subpopulations could be included. There is no clear evidence for the mechanisms of induction of T-regulatory subpopulation (CD4+/CD25+) of TILs, however, there is a possibility of releasing cancer derived factors that could induce expansion of this specific cell sub-population. Our results concerning the T-regulatory sub-population (CD25+), helper sub-population (CD4+) and cytotoxic cell population (CD8+) can be confirmed by the previously mentioned facts.

This is in addition to our finding regarding the cytotoxic T-lymphocytes (CTLs), specifically CD4+ CD8+ subpopulations, that remain limited in their effect on the regression of the established tumors due to escape mechanisms by which tumor cells release factors such as Fas-Fas ligand interaction, oxidative metabolites and immunosuppressive cytokines that can be predicted to rapidly shut off the effector functions of CTLs. These mechanisms of action were confirmed by the work of other researchers [20,21].

In our study, the expression of both effector and regulatory markers on TIL subpopulations was determined using a panel of monoclonal antibodies. Our flow cytometric analysis revealed that CD8+ cells were predominant in TILs (35.61±2.95%) followed by CD4+ T-cells (29.65±3.33%) whereas CD25+ T-cells showed only 1.95±0.15%. On the other hand, Leong et al. [15] results revealed the following values: 23.4±2.1% and 12.6±1.7% for CD8+ and CD4+ TILs subpopulation respectively. Indeed, the differences between our findings and those of Leong et al., may be due to the difference in the population of patients since our Egyptian patients are quite different in their immune response. Regarding the CD4+/CD8+ ratio, our finding (0.89) was in disagreement with what has been found by Leong et al., that was 1.8. As a matter of fact CD4+/CD8+ ratio plays a crucial role in the efficiency of the biological activity of TILs, in another way the more you have CD8+ than CD4+ the more you may find high activity of TILs population since the former is a cytotoxic T-cells and the latter is a helper.
one. Therefore, our TIL sub-population could have therapeutic potency better than the previously mentioned one. Moreover CD25+ T-cell subpopulation plays critical role in regulation of CD8+ CD4+ T-cell interaction.

Conclusion:

Our work showed that, a good yield of TILs isolated and activated by IL-2 from breast tumor tissues of our Egyptian patients was performed. These TILs preparation showed the prevalence of CD8+ as cytotoxic T-cells and the presence of both CD4+ as helper cell and CD25+ as a regulatory one. These cell preparations can be used as a targeted biological therapy against human cancers such as breast cancer. However, further work is strongly recommended at the subclinical level using experimental animal models before going up to the clinical stage in human cancer. The therapeutic usefulness of TILs can be illustrated by isolation, culturing and activating them. Thus, they can be injected back into the patients from whom they were originated as targeted biological adjuvant cell therapy in the management of patients suffering from breast cancer.

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