

Abnormally High Expression of BAFF on T Lymphocytes from Lung Cancer-associated Pleural Effusions and Its Potent Anti-tumor Effect

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Abstract In the present study, the expressions of B cell activating factor belonging to the tumor necrosis factor family (BAFF) and its receptors (BAFF-R and TACI) on T lymphocytes from malignant pleural effusion (MPE) were examined by fluorescence-activated cell sorting (FACS) analysis, and compared with those on the T lymphocytes from non-malignant pleural effusion (NMPE) and healthy controls. It was found that CD3 positive T lymphocytes (including CD4, CD8, and part of CD25 and CD69 positive cells) of MPE in lung cancer highly and consistently expressed the BAFF molecule, while high expressions of BAFF could only be found in phytohemagglutinin (PHA) or interleukin 2 (IL-2) induced T lymphocytes from NMPE or healthy controls. These results were consistent with the results from *BAFF* mRNA detection by real-time PCR. In addition, T lymphocytes from MPE expressed significantly more BAFF-R than those from NMPE or healthy controls, while the expression of TACI was increased on CD4⁺ T cells but decreased on CD8⁺ T cells when compared with controls. The Annexin/PI assay suggested that recombinant human BAFF (rhBAFF) could promote the survival rate of T lymphocytes from MPE, while the decoy receptor TACI-Fc fusion protein could promote the apoptosis rate of T lymphocytes. Cytokines in the supernatant detected by ELISA assay showed that rhBAFF could significantly upregulate the secretion of IFN- γ *in vitro*, and the IFN- γ level in the TACI-Fc-treated group resembled that of the control groups. All of these results indicated that the abnormally high expression of BAFF on T lymphocytes from MPE may play a role of anti-tumor effect.

Keywords BAFF; malignant pleural effusions; T lymphocyte; survival; cytokine

B cell activating factor belonging to the TNF family (BAFF) [1], also known as BLys [2], THANK [3], TALL-1 [4], TNFSF13B, or zTNF, is synthesized as a 285-amino acid type II membrane protein and exists in both membrane-bound and cleaved 152-amino acid soluble forms (soluble BAFF, sBAFF). It has been acknowledged that BAFF is expressed on monocytes, macrophages, monocyte-

derived dendritic cells, neutrophils, and activated T cells. BAFF can bind to three receptors, BAFF-R [5], TACI [6], and BCMA [7]. Among them, TACI and BCMA also bind to APRIL, which has 48% homology with BAFF in the extracellular region in the nucleotide level [8]. B and T lymphocytes express these receptors.

BAFF acts as an important B cell survival factor [9], and studies have shown that BAFF can bind to naive and primed/memory CD4⁺ and CD8⁺ T cells. In the presence of a suboptimal signal, it can costimulate T cell activation, proliferation, and cytokine production *in vitro* [10]. Ye *et al.* found that the binding of BAFF to BAFF-R expressed by a subset of primarily CD4⁺ T cells costimulated T cell activation and allo-proliferation [11]. Huard *et al.* reported

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that the BAFF molecule could be detected on normal activated T lymphocytes by Southern blot analysis [12]. Lavia *et al.* found that T cells infiltrating labial salivary glands expressed the BAFF molecule in patients with Sjögren's syndrome, and double immunofluorescence revealed that T cells and macrophages were the main cell types expressing BAFF [13]. Yashimoto *et al.* also reported that peripheral T cells of systemic lupus erythematosus patients treated with anti-CD3 antibody robustly expressed BAFF and subsequent release of sBAFF from these cells [14]. These data indicate that BAFF is expressed on T cells under some pathological conditions.

Lymphocytes separated from pleural effusions associated with lung cancer have been studied from its phenotype and secreted cytokines, but their characteristics still need to be deeply investigated. Duncan *et al.* reported that these T-cell antigen receptor β -chain variable gene repertoires were abnormal, and these T-cell clones were nearly equally divided between CD4 and CD8 [15]. And Sikora *et al.* proved that various immunosuppressive factors exist in the microenvironment of malignant pleural effusions, such as significantly high concentration of interleukin (IL)-10, transforming growth factor- β 1, soluble Fas ligand, and nuclear membrane proteins [16]. Takeuchi *et al.* found that in lung cancer patients, pleural mononuclear cells (MNC) incubated with IL-12 produced more γ -interferon (IFN- γ) than blood MNC. And when stimulated with both IL-12 and IL-2, pleural MNC produced more IL-10 than blood MNC. This is the first report indicating that MNC from pleural effusions of patients with lung cancer can produce both type 1 (IFN- γ) and type 2 (IL-10) cytokines following exposure to IL-2 and IL-12 [17]. All observations suggest that lymphocytes in pleural effusions due to lung cancer are in a special condition. It has not been reported whether BAFF molecules are expressed on T lymphocytes, nor whether they play a significant role in malignant pleural effusions associated with lung cancer.

By the classic hybridoma technique of B lymphocytes, our laboratory obtained one hybridoma, named 4F7, which secreted monoclonal antibody (mAb) recognizing the BAFF molecule expressed on normal activated T lymphocytes from healthy volunteers [18]. The recognition profile indicates that BAFF molecules are significantly up-regulated on activated CD4⁺ and CD8⁺ T cells. In the present study this 4F7 mAb was used to explore the expression of the BAFF molecule on T lymphocytes separated from pleural effusions associated with lung cancer and controls, such as non-malignant pleural effusions and healthy volunteers. Some interesting results were obtained.

Materials and Methods

Patients with lung cancer

Twelve patients from the First Affiliated Hospital of Soochow University (Suzhou, China) with primary lung cancer were studied after obtaining their informed consent. Their mean age was 56.2 years old. Lung cancer was diagnosed by either histological examination of tissue specimens or cytological examination of sputum, pleural effusion, or specimens obtained by bronchial brushing, lymph node biopsy, or lung aspiration. Histological examinations revealed that five patients had adenocarcinoma, four had small cell carcinoma, and three had large cell carcinoma.

Control subjects

Twelve subjects were studied as controls. Of these subjects, seven were control patients (mean age 51.2 years old). They suffered from tuberculous pleurisy (three patients) and suppurative pleurisy (four patients). Examinations revealed no malignant lesions or autoimmune disease in these seven patients. The other five controls were healthy volunteers who had no sign of infection and were not taking any medication (mean age 53.2 years old). They all gave their informed consent before participating in the study.

Isolation of peripheral blood and pleural T lymphocytes

Peripheral blood MNC were isolated from heparinized venous blood diluted 2-fold with Hanks' solution in lymphocyte separation medium (Ficoll) [19]. Malignant and non-malignant pleural effusions were obtained by puncture with heparin during the diagnostic procedure and centrifuged at 150 g for 10 min at room temperature. The cell fraction was resuspended in phosphate-buffered saline (PBS) and pleural MNC were separated in lymphocyte separation medium.

Peripheral blood MNC and pleural MNC were washed twice with PBS and resuspended in complete RPMI 1640 medium to a density of 10⁶ cells/ml, then added into 6-well culture plates (2 ml/well) and incubated for 3 h at 37 °C in a humidified atmosphere with 5% CO₂. The supernatant was collected, and T lymphocytes were obtained by a human T cell enrichment column (R&D Systems, Minneapolis, USA), according to the recommended procedure provided by the manufacturer.

The phenotypes of T lymphocytes were analyzed using a flow cytometer (Coulter, Fullerton, USA). The supernatant was frozen at -80 °C for future use, in which CD3⁺ T cells exceeded 95%.

Immunophenotyping of peripheral blood and pleural lymphocytes

The immunophenotype of different lymphocyte subpopulations in different conditions was determined by flow cytometry. Fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated monoclonal antibodies (anti-CD3, -CD4, -CD8, -CD25, and -CD69) were purchased from Immunotech (Marseilles, France), and were used to detect the corresponding surface antigens, according to instructions provided by the manufacturer. Biotin-conjugated anti-BAFF mAb (named 4F7 mAb) was produced in our laboratory. Biotin-conjugated anti-BAFF-R (Cat. ab16582) was a product of Abcam (Cambridge, UK) and anti-TACI mAb (Cat. 165604) was purchased from R&D Systems. Isotype-matched antibodies (BD PharMingen, San Diego, USA) were used as controls and IgG block (Sigma, St Louis, USA) was used to avoid non-specific binding to Fc receptors.

Briefly, T lymphocytes obtained from malignant pleural effusions (MPE), non-malignant pleural effusions (NMPE), and healthy volunteers, were incubated with the relevant mAb at 4 °C for 30 min, washed twice with PBS, stained with PE-avidin (Immunotech) for 30 min at 4 °C, washed twice again, then analyzed by flow cytometry.

The cultured groups of peripheral blood lymphocytes and pleural lymphocytes were adjusted to the same density (10^6 cells/ml), and were added into 6-well culture plates (2 ml/well) at 37 °C in a humidified atmosphere with 5% CO₂. Each of the three wells was stimulated with 10 µg/ml phytohemagglutinin (PHA) (R&D Systems) for 24 and 36 h, and 500 U/ml IL-2 (R&D Systems) for 48 and 96 h. One group that was not stimulated was used as the control. The cells were collected and the phenotype of lymphocytes was tested by single- and double-staining fluorescence-activated cell sorting analysis.

Real-time polymerase chain reaction (PCR) detection of BAFF mRNA in peripheral blood and pleural lymphocytes

The total mRNA of different T lymphocyte subpopulations (5×10^6 cells/ml) in different conditions was extracted by an SV Total RNA Isolation System kit (Promega, Madison, USA). The first-strand cDNA was obtained using a reverse transcription system kit (Promega). Oligo 6.0 software (Molecular Biology Insights, Cascade, USA) was used to design primers of *BAFF* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (Table 1). *GAPDH* gene expression was determined in separate tubes as the control for the input cDNA. The mRNA levels of *BAFF*

Table 1 Sequences of primers designed for real-time polymerase chain reaction

Gene	Primer sequence
BAFF	Upper primer 5'-AATTCCGTTCTTCATTCCGCA-3'
	Lower primer 5'-CATGGCGTAGGTCTTATCAG-3'
GAPDH	Upper primer 5'-ACCACAGTCCATGCCATCAC-3'
	Lower primer 5'-GTCCAGGGGTCTTACTCCTTG-3'

BAFF, B cell activating factor belonging to the tumor necrosis factor family; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

and *GAPDH* were measured by the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Basel, Switzerland). PCR was carried out in a 20 µl reaction mixture containing 2 µl of 10×SYBR green buffer, 0.5 µl of 5 U/µl *Taq*, 2 µl of 2.5 mM dNTP, 0.8 µl of 25 mM MgCl₂, 1 µl of both 100 µM forward and reverse primers, 11.8 µl H₂O, and 1.2 µl of cDNA. Thermal cycling conditions included the following steps: 95 °C for 5 min to activate *Taq* polymerase, then 40 cycles of a three-step PCR was carried out consisting of 15 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C. PCRs were carried out on a LightCycler (Roche Diagnostics) and samples were amplified simultaneously in duplicates in one assay run.

Viability assay

The cell density of T lymphocytes was adjusted to 5×10^5 /ml with complete RPMI 1640 medium, then added into 24-well culture plates. Each of the three wells was added separately into 150 ng/ml recombinant human BAFF (rhBAFF; Peprotech, NJ, USA), 200 µg/ml TACI-Fc (prepared in our laboratory) [20] and human IgG1 isotype as negative controls. After co-cultured for 5 d, the cells of each group were collected, and their viability was tested by Annexin V/PI kits (Boehringer Mannheim, Mannheim, Germany), according to instructions provided by the manufacturer.

Quantitative measurements of cytokines

The cultural supernatant of each group was collected, and the IL-10, IFN-γ, and sBAFF were tested using IL-10 enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems), IFN-γ ELISA kits (Biosource, San Jose, USA), and human sBAFF ELISA kits (R&D Systems) according to the manufacturer's recommendations.

Statistical analysis

The data were analyzed by one-way ANOVA with a statistical software package (SPSS version 10.0; SPSS,

Chicago, USA). Statistical significance was defined as *P* values less than 0.05. All *P* values were two-sided.

Results

Highly and consistently expressed BAFF on T lymphocytes from pleural effusions due to lung cancer

The obtained results indicated that T lymphocytes from peripheral blood and non-malignant pleural effusions highly expressed the BAFF molecule after being stimulated with

PHA and IL-2 *in vitro*, detected by 4F7 mAb (**Fig. 1**). However, lymphocytes separated from malignant pleural effusions significantly expressed BAFF without any stimulation, and PHA and IL-2 did not increase the expression rate of BAFF (data not shown). During culturing on days 1, 5, 7, and 9 *in vitro*, the phenotypes of lymphocytes from malignant pleural effusions were also analyzed. The results were interesting in that BAFF molecules were consistently expressed on almost all of the CD3⁺CD4⁺ cells and CD3⁺CD8⁺ cells, and on part of the CD25⁺ and CD69⁺ cells (**Fig. 2**). With the removal of the activating factors, the BAFF molecule could not be

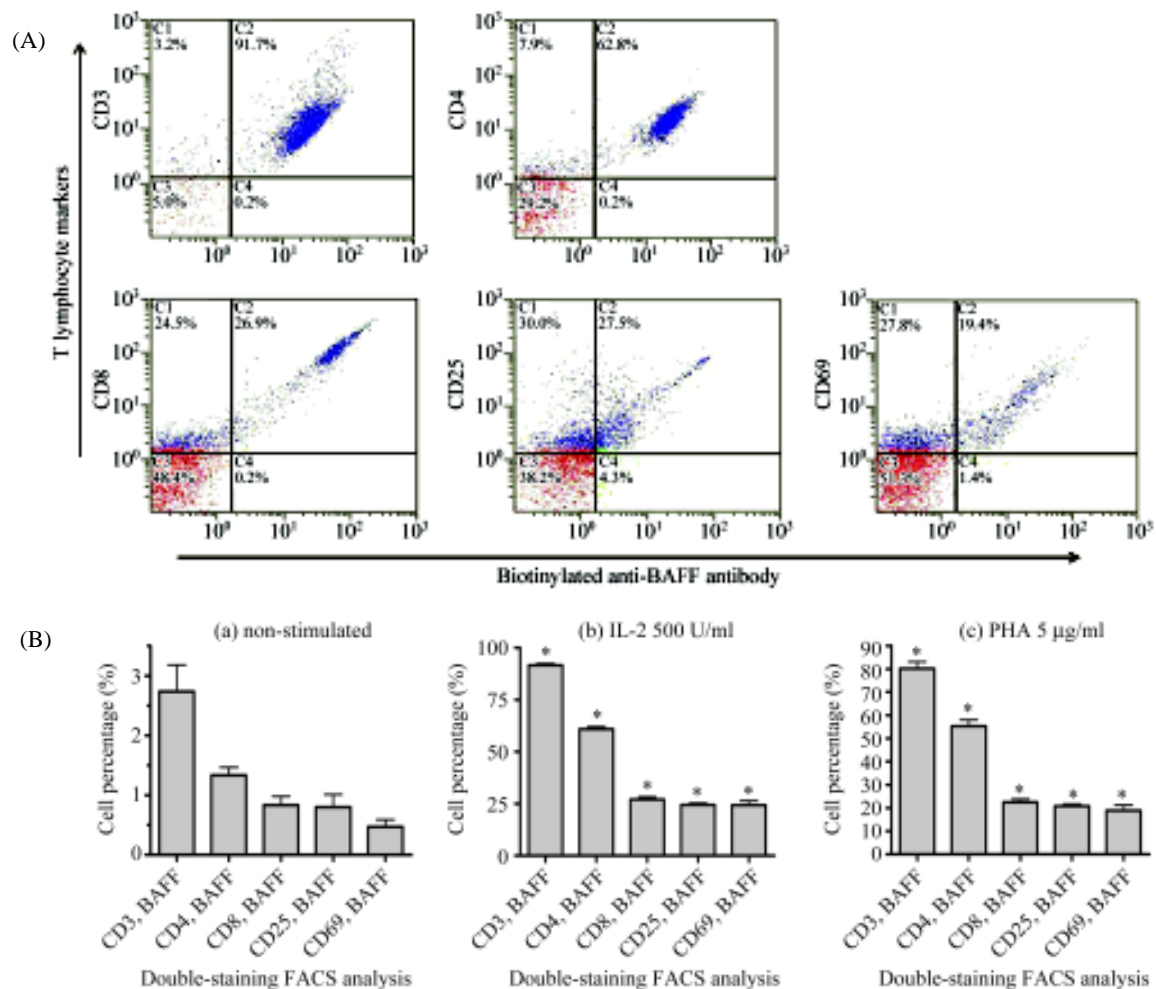


Fig. 1 Expression of B cell activating factor belonging to the tumor necrosis factor family (BAFF) on T lymphocytes derived from non-malignant pleural effusions and healthy volunteers

(A) Two-color staining was carried out on T lymphocytes using fluorescein isothiocyanate-coupled antibodies specific for CD3, CD4, CD8, CD25, and CD69. Presence of BAFF on the cell surface was detected with biotinylated anti-BAFF antibody followed by phycoerythrin-conjugated streptavidin. T lymphocytes from twelve donors were analyzed for BAFF expression. A representative experiment is shown in which T cells were stimulated with interleukin-2 (IL-2; 500 U/ml). (B) BAFF could not be detected on the non-stimulated T lymphocytes (a). However, after T cells were stimulated with IL-2 (500 U/ml) (b) and phytohemagglutinin (PHA; 5 µg/ml) (c) for 48 h, the expression of BAFF was highly regulated. Data are expressed as mean±SE. **P*<0.05, IL-2-treated group versus non-stimulated group; PHA-treated group versus non-stimulated group. FACS, fluorescence-activated cell sorting analysis.

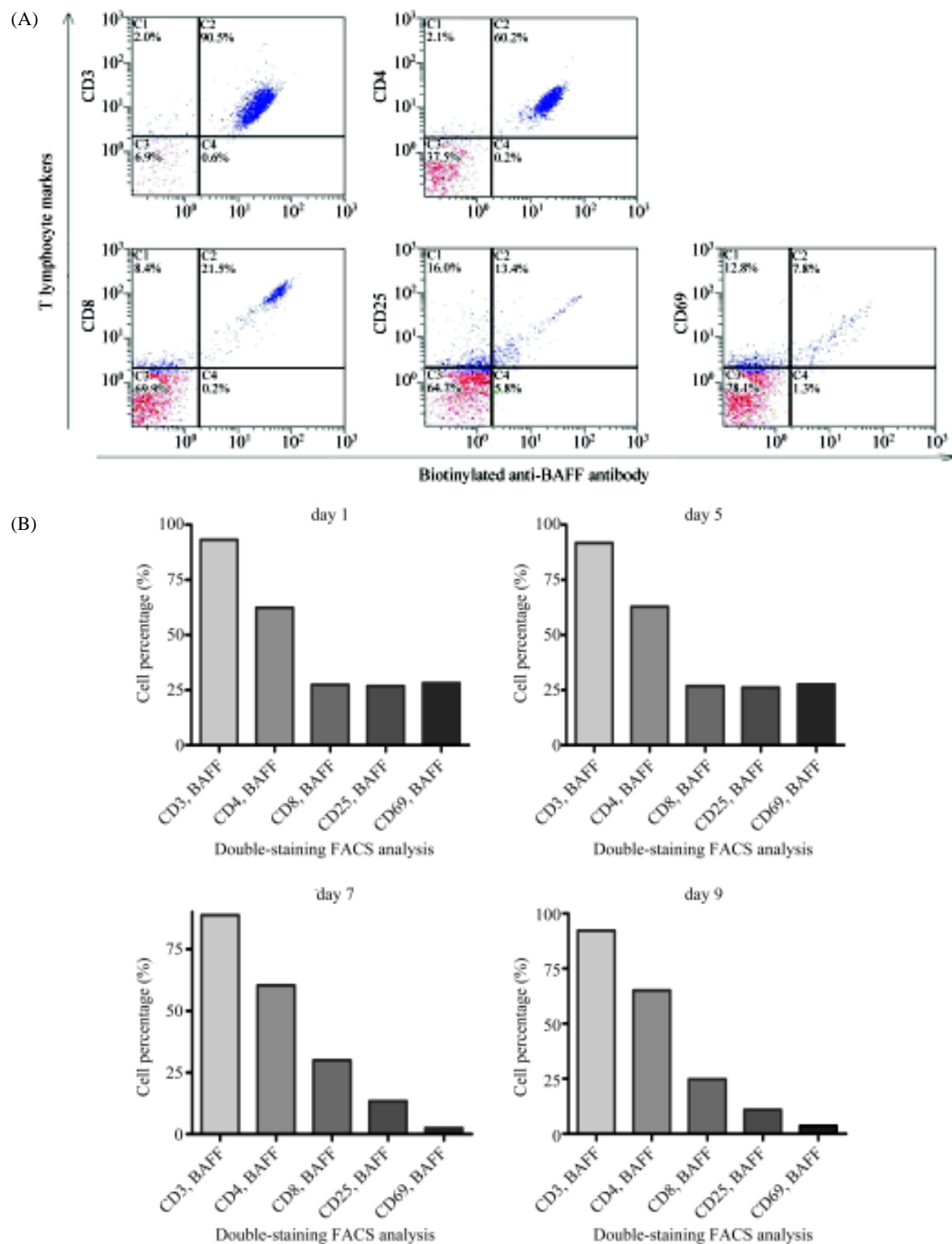


Fig. 2 Expression of B cell activating factor belonging to the tumor necrosis factor family (BAFF) on T lymphocytes from malignant pleural effusions (MPE) cultured *in vitro*

(A) Two-color staining was carried out on T lymphocytes using fluorescein isothiocyanate-coupled antibodies specific for CD3, CD4, CD8, CD25, and CD69. Presence of BAFF on the cell surface was detected with biotinylated anti-BAFF antibody followed by phycoerythrin-conjugated streptavidin. T lymphocytes from 12 donors were analyzed for BAFF expression. A representative experiment is shown in which T cells were cultured *in vitro* for 7 d. (B) BAFF could be detected on T lymphocytes from pleural effusion at day 1, 5, 7, and 9 after culture *in vitro*. The expression rates were consistent on CD3, CD4, and CD8 positive cells. T lymphocytes from MPE of 12 patients were analyzed and data are presented as mean \pm SE. FACS, fluorescence-activated cell sorting analysis.

detected on the control groups 3 d later (data not shown). The difference in BAFF expression on normal T lymphocytes between the PHA-treated group, IL-2-treated group, and the control were statistically significant ($P < 0.01$). There was no significant difference in BAFF expression between treated normal T lymphocytes and T lymphocytes from malignant pleural effusions.

Moreover, the real-time PCR results showed that the BAFF mRNA level increased in the T lymphocytes of peripheral blood and non-malignant pleural effusion after stimulation by PHA and IL-2. In the T lymphocytes from pleural effusion, the BAFF mRNA level was high during the whole 9 d of culture *in vitro* (Fig. 3), and PHA and IL-2 did not increase the expression rate of BAFF.

BAFF-R and TACI expression patterns differ from control groups

Our flow cytometry analysis results suggested that the expression of BAFF receptors on pleural lymphocytes varied compared with control groups. BAFF-R was more highly expressed on lymphocytes from malignant pleural effusions, as shown in Fig. 4 and Table 2, and the BAFF-R expression rate on T lymphocytes from MPE, NMPE, and normal peripheral blood was $20.1\% \pm 1.2\%$, $4.6\% \pm$

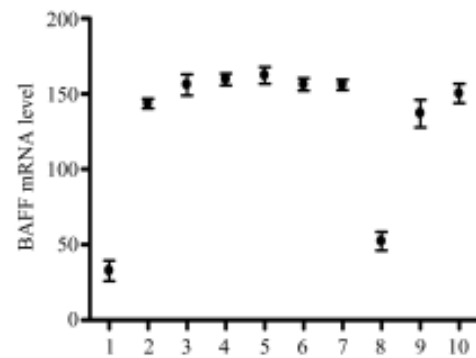


Fig. 3 Detection of B cell activating factor belonging to the tumor necrosis factor family (BAFF) mRNA in T lymphocytes from peripheral blood of healthy volunteers, patients with non-malignant pleural effusions (NMPE), and malignant pleural effusions (MPE)

1, T cells from peripheral blood without any stimulator; 2, T cells from peripheral blood with PHA (5 µg/ml) stimulation for 48 h; 3, T cells from peripheral blood with IL-2 (500 U/ml) stimulation for 48 h; 4, T cells from MPE cultured *in vitro* at day 1; 5, T cells from MPE cultured *in vitro* at day 5; 6, T cells from MPE cultured *in vitro* at day 7; 7, T cells from MPE cultured *in vitro* at day 9; 8, T cells from NMPE without any stimulator; 9, T cells from NMPE stimulated with PHA (5 µg/ml) for 48 h; 10, T cells from NMPE stimulated with IL-2 (500 U/ml) for 48 h. These experiments were repeated three times, and data are expressed as mean±SE.

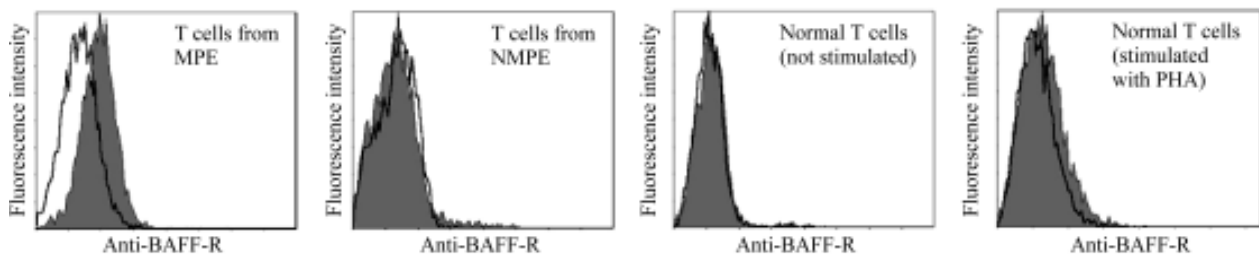


Fig. 4 Expression of B cell activating factor belonging to the tumor necrosis factor family (BAFF) receptor (BAFF-R) on T lymphocytes from malignant pleural effusions (MPE), non-malignant pleural effusions (NMPE), and healthy volunteers

The presence of BAFF-R was analyzed by fluorescence-activated cell sorting analysis. Gray histogram represented that related cells were incubated with biotin-conjugated anti-BAFF-R; open histogram represented that isotype and fluorochrome controls. PHA, phytohemagglutinin.

Table 2 Expression of B cell activating factor belonging to the tumor necrosis factor family (BAFF) receptor (BAFF-R) on T lymphocytes from malignant pleural effusion (MPE), non-malignant pleural effusion (NMPE), and healthy volunteers

	CD3 ⁺ BAFF-R ⁺	CD4 ⁺ BAFF-R ⁺	CD8 ⁺ BAFF-R ⁺	BAFF-R ⁺
T cells from MPE	15.8%±1.3% ↑	7.2%±0.8% ↑	8.5%±2.1% ↑	20.1%±1.2% ↑
T cells from NMPE	4.1%±0.5%	2.2%±0.2%	1.4%±0.7%	4.6%±0.3%
Normal T cells (not stimulated)	0.8%±1.2%	0.6%±0.5%	0.6%±1.3%	3.1%±0.8%
Normal T cells (stimulated with PHA for 24 h)	2.3%±0.9%	2.7%±1.5%	1.3%±0.3%	5.3%±1.2%

Data shown are mean±SD. ↑, significantly high compared to the other groups; PHA, phytohemagglutinin.

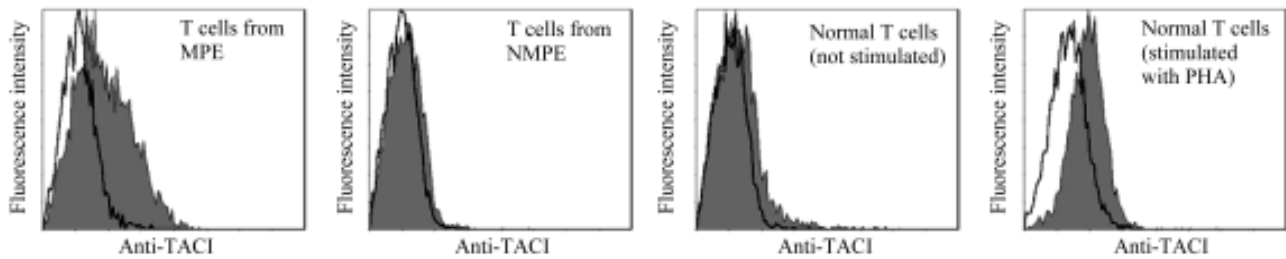


Fig. 5 Expression of TAC1 receptor on T lymphocytes from malignant pleural effusion (MPE), non-malignant pleural effusion (NMPE), and healthy volunteers

The presence of TAC1 was analyzed by fluorescence-activated cell sorting analysis. Gray histogram represented that related cells were incubated with biotin-conjugated anti-BAFF-R; open histogram represented that isotype and fluorochrome controls. PHA, phytohemagglutinin

Table 3 Expression of TAC1 receptor on T lymphocytes from malignant pleural effusion (MPE), non-malignant pleural effusion (NMPE), and healthy volunteers

	CD3 ⁺ TAC1 ⁺	CD4 ⁺ TAC1 ⁺	CD8 ⁺ TAC1 ⁺	TAC1 ⁺
T cells from MPE)	24.4%±0.5% ↑	13.3%±2.3% ↑	10.8%±3.8% ↓	32.2%±0.1% ↑
T cells from NMPE	9.3%±1.2%	4.5%±2.1%	3.6%±1.2%	12.6%±3.1%
Normal T cells (not stimulated)	1.4%±1.2%	0.6%±0.9%	0.8%±1.1%	10.9%±2.1%
Normal T cells (stimulated with PHA for 24h)	29.7%±2.1% ↑	5.8%±1.5%	23.9%±2.5% ↑	32.0%±0.7% ↑

Data shown are mean fluorescence values. ↑, significant high compared to controls; ↓, significant low compared to controls; PHA, phytohemagglutinin.

0.3%, and 3.1%±0.8%, respectively. The statistical analysis indicated that the difference in BAFF-R expression between T cells from MPE and control groups was significant ($P<0.05$). Peripheral T lymphocytes stimulated with PHA highly expressed more TAC1 than naive T lymphocytes, and most of them were CD8⁺ T cells. TAC1 could also be found on the membrane of T lymphocytes from MPE similar to the activated lymphocytes in control groups, as shown in **Fig. 5** and **Table 3**. However, the expression pattern of TAC1 between the two groups was different, in that the rate increased on CD4⁺ T cells but decreased on CD8⁺ T cells.

Survival rate increased by rhBAFF but blocked by TAC1-Fc fusion protein of T cells from malignant pleural effusions

According to the characteristics of apoptotic cells and live cells, the data about the apoptotic cells (FITC⁺PI⁻ cells) and live cells (FITC⁻PI⁻ cells) were analyzed, and the results indicated that rhBAFF could promote the survival rate of T lymphocytes from MPE. There was a statistical significance between the rhBAFF-treated group and the IgG1 isotype group (rhBAFF 150 ng/ml $P<0.01$, and 75 ng/ml group $P<0.05$, respectively). The decoy

receptor TAC1-Fc fusion protein could promote apoptosis of T lymphocytes; this effect was significant in the TAC1-Fc 200 μg/ml group, compared to the IgG1 isotype group ($P<0.05$) (**Fig. 6**).

IFN-γ level increased in the rhBAFF-treated group when T lymphocytes cultured *in vitro*

In malignant pleural effusions, the level of IL-10 and sBAFF was low, and IFN-γ could not be detected (data not shown). But when the lymphocytes from MPE were cultured *in vitro*, the IL-10 level in the supernatant became lower, whereas IFN-γ increased, and the sBAFF level changed irregularly (data not shown). The IFN-γ level in the TAC1-Fc-treated group resembled that of the controlled groups, but became significantly high in the rhBAFF-treated group ($P<0.05$, compared to the IgG1 isotype group) (**Fig. 7**).

Discussion

In the present study, our results suggested that T lymphocytes from MPE robustly expressed the BAFF molecule when cultured *in vitro* without any stimulators. BAFF

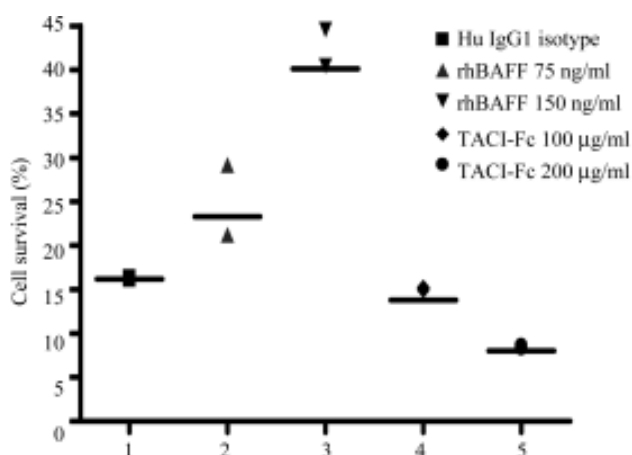


Fig. 6 Effects of B cell activating factor belonging to the tumor necrosis factor family (BAFF) on the viability of T lymphocytes from malignant pleural effusions (MPE)

1, Hu IgG1 isotype; 2, rhBAFF 75 ng/ml; 3, rhBAFF 150 ng/ml; 4, TACI-Fc 100 µg/ml; 5, TACI-Fc 200 µg/ml. The recombinant human BAFF (rhBAFF)-treated group could significantly survive the T lymphocytes from MPE ($P < 0.05$) compared to the control group. The TACI-Fc fusion protein could increase the death of T cells; this effect was significant in the TACI-Fc (200 µg/ml) group. These experiments were repeated three times, and data are expressed as the mean \pm SE. Hu IgG1, human immunoglobulin G1.

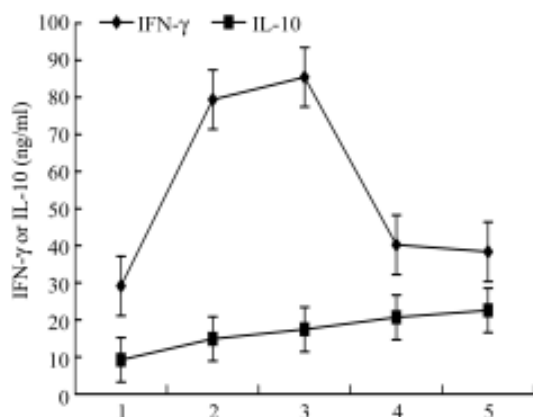


Fig. 7 Enzyme-linked immunosorbent assay analysis of the cytokine concentration in the supernatant of T lymphocytes from malignant pleural effusions (MPE)

1, Human immunoglobulin G1 isotype group; 2, recombinant human BAFF (rhBAFF) 75 ng/ml; 3, rhBAFF 150 ng/ml; 4, TACI-Fc fusion protein group 100 µg/ml; 5, TACI-Fc fusion protein group 200 µg/ml. During *in vitro* culture, T lymphocytes from MPE could produce more γ -interferon (IFN- γ) after stimulated with rhBAFF, while the production of interleukin (IL)-10 did not vary. These experiments were repeated three times, and data are expressed as the mean \pm SE.

could not be detected on the normal naive T lymphocytes and T lymphocytes from NMPE, and T lymphocytes from controls highly expressed BAFF when pulsed with IL-2

or PHA.

The receptors of BAFF expressed on T cells were also analyzed. The results showed that T cells from MPE might express one or more BAFF receptors. BAFF can bind to BAFF-R with higher affinity than to TACI or BCMA. BAFF-R mRNA could be detected in the human spleen, lymph nodes, PBLs, and thymus, and in the mouse spleen, thymus, lung, and testis by Northern blot analysis [20,21]. BAFF-R expression was observed on peripheral B cells, and memory and naive B cells similarly expressed BAFF-R [22]. CD38⁺ plasma cells isolated *in vitro* expressed BAFF-R at a low level [23]. However, the expression of BAFF-R on peripheral B lymphocytes was down-regulated after B cells were activated. Yan *et al.* reported that CD4⁺ and CD8⁺ T cells also expressed the BAFF-R molecule, and the expression was down-regulated after T cells were activated [21]. Ng *et al.* confirmed that one subset of mouse T cells and human activated peripheral T cells expressed BAFF-R [24]. Our results indicated that T lymphocytes from MPE highly expressed BAFF-R, including CD4⁺ and CD8⁺ T cells, compared to the T cells from NMPE and healthy volunteers.

TACI, another receptor of BAFF with relatively high affinity, was also analyzed in the present study. Our results suggested that the expression rate of TACI on the T lymphocytes from MPE increased, and this effect is very similar to that of normal T lymphocytes stimulated by PHA for 24 h. These results were different from those of Ng *et al.* [24], possibly because of different stimulators.

Some previous experiments had suggested the possibility that BAFF is a soluble mediator. Nardelli *et al.* reported that soluble BAFF is produced by enzymatic processing of the membrane-bound protein by conducting experiments with cells transfected with a mutant BAFF cDNA [25], as suggested in Schneider's previous report [1]. Because the BAFF sequence does not contain a predicted signal peptide, a possible mechanism for the release of BAFF from the cells is the proteolytic cleavage of the membrane-bound protein.

However, results of other experiments showed that there is no correlation between the levels of cell surface-associated BAFF and the concentration of sBAFF found in the cultures, and our results were in agreement with theirs.

Our real-time reverse transcription-PCR results suggested that, in the T lymphocytes of the experimental groups whose surface BAFF markedly increased, the level of BAFF mRNA was also high, compared with the BAFF mRNA level in the T cells of controlled groups. So it was considered that the marked increase of surface BAFF in T lymphocytes from MPE represented endogenously-pro-

duced BAFF. In the microenvironment of pleural effusions, T lymphocytes robustly expressed the BAFF molecule, and its receptor patterns also changed. So had some biological effect taken place when the BAFF molecule bound to their receptors by cell-cell contact? In *in vitro* assay, this phenomenon was observed, in that T lymphocytes could maintain good viability for a long period if their close contact was not be disturbed.

To elucidate whether BAFF and its receptors' interaction took part in the survival effect, the viability assay was conducted. The results suggested that rhBAFF could survive the T lymphocytes isolated from malignant pleural effusions in a dose-dependent way, and its decoy receptor, TACI-Fc, can weakly promote the apoptosis rate of T lymphocytes from MPE *in vitro*. Combined with the expression feature of BAFF and its two receptors, BAFF-R and TACI, the results indicated that the BAFF signal might play some physiological role, through cell-cell contact.

It has been recognized that BAFF regulates the function of T lymphocytes, particularly during T cell-mediated pathogenic reactions. BAFF is highly up-regulated in astrocytes in multiple sclerosis plaques [26] and is also produced by fibroblast-like synoviocytes from rheumatoid arthritis patients [27]. Both of these autoimmune diseases have a strong T cell association. In the present study, T lymphocytes were in MPE, where the host cells were in the vicinity of cancer cells. Lymphoid infiltration in tumor tissues is thought to be the host immune response against tumor cells. The induction of the cellular immune response is critically beneficial for cancer immunotherapy, and type 1 cytokines (IL-12 and IFN- γ) are involved in the induction of the cellular immune response. The concentrations of cytokines in the supernatants suggested that IFN- γ was produced more in the rhBAFF-treated groups than in other groups. All of these observations suggested that BAFF expressed on pleural lymphocytes might play a positive function against tumor cells, and the function should be further studied.

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