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Effects of Human Soluble BAFF Synthesized in Escherichia coli on $CD4^+$ and $CD8^+$ T Lymphocytes as well as NK Cells in Mice

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Summary

B cell-activating factor belonging to the TNF family (BAFF, also called BLyS, TALL-1, zTNF-4, or THANK) is an important survival factor for B lymphocytes. In this study, we injected mouse abdominal cavity with human soluble BAFF (hsBAFF, 0.01, 0.1, 0.5, 2 mg/kg body mass) synthesized in *Escherichia coli*. On the 8th day after injection, we investigated the effects of hsBAFF on immune functional activities of splenic B lymphocytes, $CD4^+$ and $CD8^+$ T lymphocytes and natural killer (NK) cells in mice. The results showed that B lymphocyte proliferation significantly increased in hsBAFF-treated groups with dosages of 0.1 mg/kg ($p<0.05$), 0.5 and 2 mg/kg ($p<0.01$). We observed a dose-dependent increase of $CD4^+$ T lymphocyte percentage and significantly higher values in 0.5 and 2 mg/kg hsBAFF-treated groups ($p<0.05$ and $p<0.001$, respectively) compared to control group, but $CD8^+$ T lymphocyte percentage remained unchanged. The ratio of $CD4^+$ to $CD8^+$ T lymphocytes rose with increasing hsBAFF dosage ($p<0.05$ for 2 mg/kg hsBAFF *vs.* control). Significantly stronger NK cell activities were found in 0.5 and 2 mg/kg hsBAFF-treated groups ($p<0.05$). The main finding of this study is that the hsBAFF can enhance immune responses in the body by increasing B lymphocyte and $CD4^+$ T lymphocyte function as well as elevating NK cell activity.

Key words

hsBAFF • B lymphocyte • $CD4^+$ T lymphocyte • $CD8^+$ T lymphocyte • NK cell

Introduction

B cell-activating factor belonging to TNF family (BAFF), also known as BLyS, TALL-1, THANK, TNFSF13B or zTNF4, is synthesized as a 285-amino acid membrane protein and exists in both membrane and cleaved 152-amino acid soluble forms (Schneider *et al.* 1999, Moore *et al.* 1999, Shu *et al*. 1999, Mukhopadhyay *et al*. 1999). BAFF promotes survival and differentiation of the B lymphocytes *in vitro* and has recently emerged as a key regulator of peripheral B lymphocyte populations *in vivo* (Moore *et al.* 1999, Shu *et al*. 1999). BAFF also stimulates the production of specific antibody responses to both T-independent (TI) and T-dependent

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(TD) antigens (Do *et al*. 2000). Studies on BAFF transgenic (BAFF-Tg) and BAFF-deficient mice have displayed a certain role that BAFF may play in therapy of autoimmunity and immunodeficiency syndromes (Groom *et al*. 2002). Such findings demonstrate that BAFF may play a critical role in regulating B lymphocyte immune responses (Harless and Cancro 2003). In addition, some studies indicate that BAFF has a wide function on T lymphocyte responses *in vitro* (Huard *et al*. 2001), but the functional changes on both $CD4^+$ and $CD8^+$ subsets *in vivo* by BAFF remain to be ambiguous. Natural killer (NK) cells are a population of large, granular lymphocytes that do not express the set of surface markers of B or T lymphocytes. These cells have been implicated to play important roles in viral immunity and in defense against tumors, and they represent the "first line of defense" in the body (Hercend and Schmidt 1988, Trinchieri 1989, Ravetch and Lanier 2000). Although the studies on BAFF have become more comprehensive, few of them described the effects on NK cells, which are also important lymphoid cells.

Human soluble B cell-activating factor belonging to the TNF family (hsBAFF), a recombinant form of the extracelluar domain of the BAFF, is expressed in *Escherichia coli* BL21 (DE3) by the constructed recombinant plasmid pET30a(+)-hsBAFF (Cao *et al*. 2005). We have demonstrated that the final purified material was biologically active in a validated induced human B lymphocyte proliferation bioassay (Cao *et al*. 2005). The aim of this study was to further evaluate effects of hsBAFF on immune function activities of splenic B lymphocytes, $CD4^+$ and $CD8^+$ T lymphocytes and natural killer cells in mice in order to understand the role of hsBAFF on enhancing immune responses in the body.

Methods

Reagents

Anti-CD19 fluorobeads-B was obtained from One Lambda (Canoga Park, CA, USA). Mouse monoclonal antibodies (mAbs) of FITC-conjugated anti-CD4 and R-PE-conjugated anti-CD8a were from BD PharMingen (San Jose, CA, USA). RPMI 1640 Medium was from Gibco (Rockville, MD, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and paraformaldehyde were from Sigma Chemical Co. (St. Louis, MO, USA). Neonatal cattle serum (NCS) was purchased from Hangzhou Sijiqing Co. (Hangzhou, P.R. China). Other chemicals were purchased from local commercial sources and were of analytical grade quality.

Preparation of recombinant hsBAFF protein

Briefly, the constructed recombinant plasmid pET30a(+)-hsBAFF was transformed into competent *Escherichia coli* BL21 (DE3). The bacteria were cultured in a LB medium to a density of OD600 = 0.6, and then induced for the expression of the recombinant protein *via* isopropyl-β-D-thiogalacto-pyranoside (IPTG). After induction, the resulting insoluble bodies were separated from cellular debris by centrifugation and solubilized with 8 M urea. Then, a rapid and simple on-column refolding procedure was developed. This was applied and then the refolded hsBAFF was purified by an anion-exchange. The purified final product was > 98 % pure by SDS-PAGE stained with Coomassie brilliant blue R-250. The renatured protein displayed its immunoreactivity with antibodies to BAFF protein by Western blotting. The expression and *in vitro* refolding of hsBAFF resulted in the production of an active substance in a yield of 15 mg/l flask cultivation (Cao *et al*. 2005).

Animals

Forty ICR mice, half males-half females, weighing 33.54 ± 0.62 g, were obtained from Laboratory Animal Center of Nanjing Medical University, Nanjing, China. The mice were housed at room temperature (20-25 °C) in a 12 h-light and night cycle. Free access to water and food was allowed during the experimental period. All mice were randomly divided into five groups: normal control group (n=8) and four hsBAFF treatment groups (n=8 each). The mice in four hsBAFF treatment groups were given an intraperitoneal injection of hsBAFF solution which was diluted with phosphate buffered saline (PBS, pH 7.4) at dosage of 0.01, 0.1, 0.5, 2 mg/kg body weight once a day for eight days, respectively. Mice in the control group received an intraperitoneal injection of PBS at the same dose and time interval.

Isolation of splenic cells

On the 8th day post injection with hsBAFF, animals from each group were sacrificed by cervical dislocation to collect spleens under sterile conditions. Spleens were minced with dissecting scissors into pieces, followed by further grinding and filtering through sterilized nylon membranes and washed in Hank's solution (pH 7.2) by two centrifugations at 1500 rpm for

5 min after erythrocytes had been dissolved with a 0.85 % ammonium chloride solution. The isolated cells were fresh splenic cells.

Assay for B lymphocyte proliferation

B lymphocytes were isolated from splenic cell suspensions by anti-CD19 FluoroBeads, then resuspended and diluted to 1×10^5 cells/ml in RPMI 1640 medium containing 10 % NCS and 100 U/ml penicillin/streptomycin. Freshly isolated B lymphocytes were seeded at 0.1 ml/well in a flat-bottomed plate with 96 wells and incubated for 72 h at 37 °C with 5 % CO_2 humidified air. Then, 0.01 ml (5 mg/ml) of MTT reagent was added into each well and incubated for 4 h. After incubation, the incubation precipitates were dissolved with 0.1 ml of SDS. The optical density (OD) value was measured by spectrophotometry at 570 nm using an ELx800 Microplate Reader (Bio-Tek Instruments, Inc. Winooski, Vermont, USA).

Flow cytometry of T lymphocyte sorting

One hundred μl suspension samples containing freshly isolated splenic cells which were resuspended at a density of more than 2×10^6 cells/ml with PBS were incubated with mAbs of 1 μg FITC-conjugated anti-CD4 and 1 μg R-PE-conjugated anti-CD8a in the dark for 30 min at 4 □, and then washed 3 times with PBS and resuspended in 300 μl of 1 % paraformaldehyde for inactivation. The antibodies were replaced by PBS as negative controls. All samples were analyzed for detecting distributions of $CD4^+$ and $CD8^+$ subsets under a fluorescence-activated cell sorter (FACS) Vantage SE flow cytometer (Becton Dickinson, California, USA). Usually, 20 000 events for each sample were acquired.

Measurement of NK cell activity

The spleen NK cell activity was determined by the release of lactate dehydrogenase (LDH). Firstly, freshly isolated splenic cells were resuspended and diluted to 1×10^7 cells/ml in RPMI 1640 medium supplemented with 10 % NCS and 100 U/ml penicillin/streptomycin to use as NK cell suspensions. Secondly, the SP20 myeloma cells were diluted to 1×10^5 cells/ml in the same medium as target cell suspensions. Thirdly, NK cell and SP20 cell suspensions were added at 0.1 ml/well according to ratio of 1:1 in a flat-bottomed plate with 96 wells and incubated for 2 h at 37 °C with 5 % CO_2 humidified air. In the meantime, the maximum LDH release was determined from the supernatant when target SP20 cells were lysed by adding 0.1 ml Triton X-100. Spontaneous release of LDH by SP20 cells was assessed by incubating the SP20 cells in the absence of effector cells. The activity of LDH was assayed according to the enzyme-substrate reaction (Lv and Li 2002). Thereafter, the reaction was terminated by citric acid. The OD value was measured spectrophotometrically at 570 nm. Splenic NK cell activity was expressed as follows:

NK cell activity (%) = (experimental group's OD – spontaneous LDH release group's OD)/(maximum LDH release group's OD – spontaneous LDH release group's OD).

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis was performed by Student's t-test (STATISTICA, Statsoft Inc, Tulsa, USA) on a conventional personal computer.

Results

Effect of hsBAFF on splenic B cell proliferation in mice

As shown in Figure 1, proliferation of splenic B lymphocytes increased in mice after hsBAFF treatment and showed a dose-dependent effect at 0.01-2 mg/kg of hsBAFF. Significantly higher values were observed in hsBAFF-treated groups with dosages of 0.1 mg/kg ($p<0.05$), 0.5 and 2 mg/kg ($p<0.01$) compared to that in the control group.

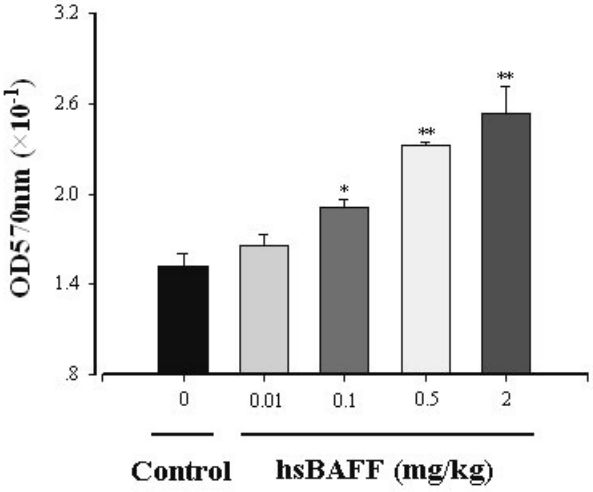

Fig. 1. Change of splenic B lymphocyte proliferation in mice after hsBAFF treatment. B lymphocytes of splenic cells in mice from each group were isolated by anti-CD19 FluoroBeads. B lymphocyte proliferation was evaluated using an MTT assay. Results are presented as mean ± S.E.M. * $p<0.05$, ** $p<0.01$ (student's t-test). hsBAFF treatment groups *vs.* control group.

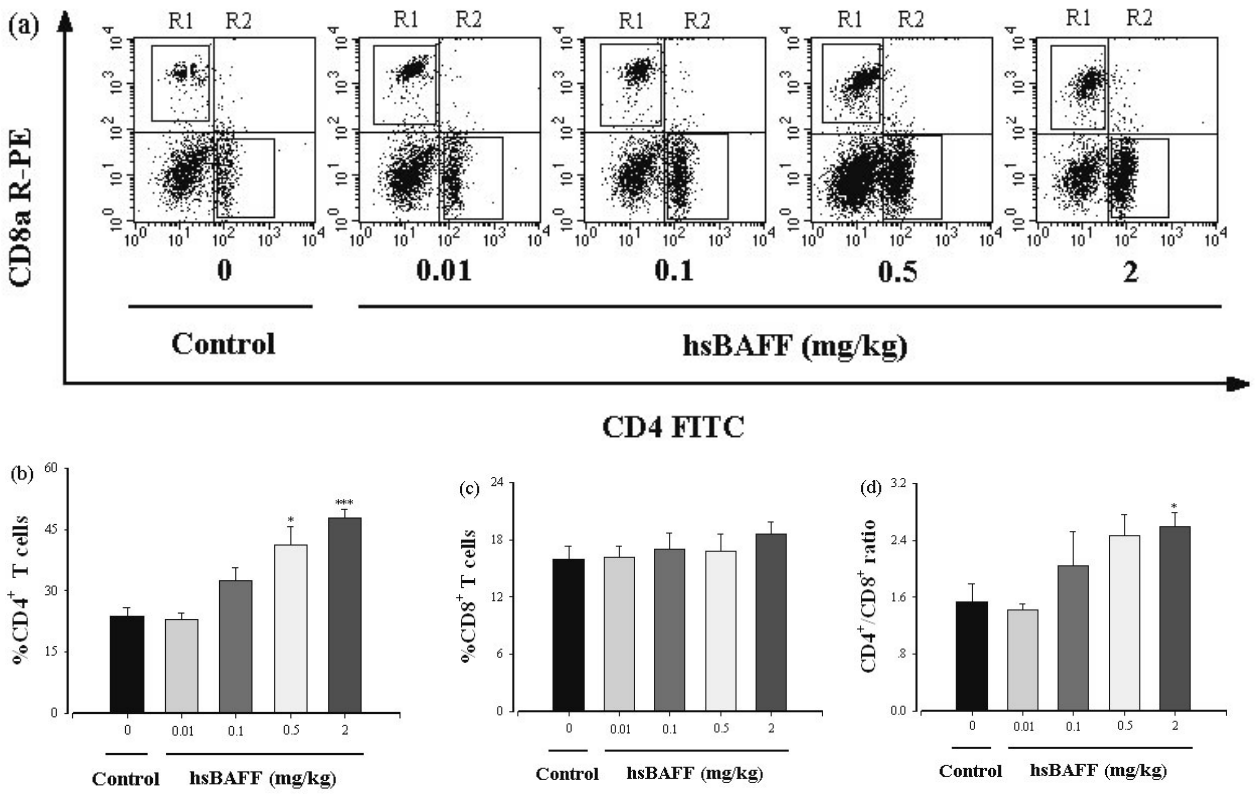

Fig. 2. Changes of splenic CD4$^+$ and CD8$^+$ subsets in mice after hsBAFF treatment. (a): T lymphocytes of splenic cells in mice from each group were sorted with two mAbs of FITC-conjugated anti-CD4 and R-PE-conjugated anti-CD8a and analyzed using flow cytometry. Gates R1 (CD8$^+$) and R2 (CD4$^+$) were indicated in each plot. (b): Changes of CD4$^+$ T lymphocytes (%). (c)**:** Changes of CD8$^+$ T cells (%). (d): Changes of ratio of CD4$^+$ to CD8$^+$ T lymphocytes. Results are presented as mean ± S.E.M. * $p<0.05$, *** $p<0.001$ (Student's t-test). hsBAFF treatment groups *vs.* control group.

Effects of hsBAFF on CD4$^+$ and CD8$^+$ T lymphocytes in mice

To understand the immunity of T lymphocytes in mice after hsBAFF treatment, two-color mAbs, which are able to distinguish both CD4$^+$ and CD8$^+$ T lymphocytes, were used to detect distributions of CD4$^+$ and CD8$^+$ subsets under a flow cytometry. As demonstrated in Figure 2a, cells gated R1 appeared to be CD8$^+$ T lymphocytes and those gated R2 were CD4$^+$ T lymphocytes. On the 8th day after hsBAFF injection, a dose-dependent increase of CD4$^+$ T lymphocytes (%) and significantly higher changes in 0.5 and 2 mg/kg hsBAFF-treated groups ($p<0.05$ and $p<0.001$, respectively) were observed compared to that in the control group (Fig. 2b), but the CD8$^+$ T lymphocytes (%) remained unchanged (Fig. 2c). The ratio of CD4$^+$ to CD8$^+$ T lymphocytes rose with the increased hsBAFF dosage and was significantly higher in the 2 mg/kg hsBAFF group than in the control group ($p<0.05$, Fig. 2d).

Effect of hsBAFF on NK cell activity in mice

Figure 3 showed that NK cell activities in 0.01 mg/kg hsBAFF-treated mice were similar to those in control animals, but a dose-dependent increase of stronger NK activities with the elevation of hsBAFF dosages was found and significantly higher activities ($p<0.05$) occurred in 0.5 and 2 mg/kg hsBAFF groups compared to the control group.

Discussion

In this study, we showed that hsBAFF synthesized in *Escherichia coli* increased proliferation of splenic B lymphocytes *in vivo* with a dose-dependent effect at 0.01-2 mg/kg ($p<0.05$ for 0.1 mg/kg hsBAFF *vs.* control; $p<0.01$ for 0.5 and 2 mg/kg hsBAFF *vs.* control). The finding is consistent with previous reports that soluble forms of BAFF are biologically active in promoting proliferation of B lymphocytes *in vitro* and *in vivo* (Moore *et al.* 1999, Shu *et al.* 1999), indicating that administration of hsBAFF to mice results in an elevated B lymphocyte function. Recently, some data suggest that BAFF deficiency or overexpression create a state of immune imbalance that leads to a breakdown in

tolerance and autoimmunity (Baker 2004, Liu *et al.* 2003). BAFF gene knockout further reveals that BAFF is required for normal B lymphocyte development (Thompson *et al.* 2000, Rahman *et al.* 2003). Taken together, such results demonstrate that BAFF plays a critical role in regulating immune responses (Shu *et al.* 1999, Harless and Cancro 2003).

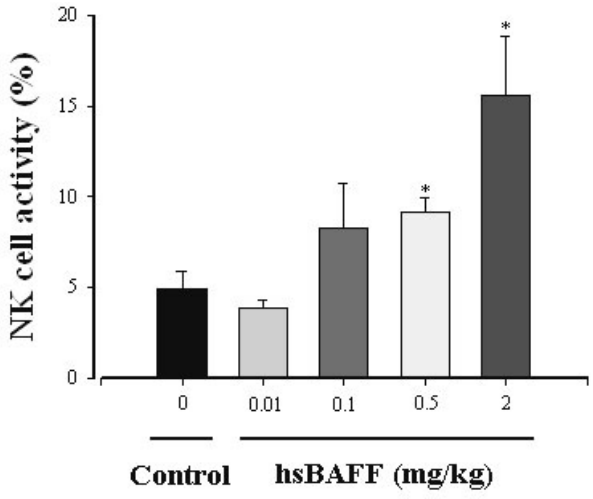

Fig. 3. Change of splenic NK cell activity in mice after hsBAFF treatment. Splenic cells in mice from each group were diluted to 1×10^7 cells/ml to use as NK cell suspensions. The SP20 myeloma cells were diluted to 1×10^5 cells/ml as target cell suspensions. NK cell and SP20 cell suspensions were added together according to ratio of 1:1 and incubated for 2 h. NK cell activity was determined via the release of lactate dehydrogenase (LDH). Results are presented as mean ± S.E.M. * $p<0.05$ (Student's t-test). hsBAFF treatment groups *vs.* control group.

During an early period after BAFF discovery, many investigators considered that BAFF had no effect on T lymphocytes but only on B lymphocytes. However, recent studies have revealed that BAFF is able to regulate T lymphocyte activation (von Bulow and Bram 1997, Xia *et al.* 2000, Huard *et al.* 2001). Xia *et al.* (2000) found that both $CD4^+$ and $CD8^+$ T lymphocytes of BAFF transgenic mice are activated in the spleen, although the total number of splenic T lymphocytes shows no increase. We observed a dose-dependent increase of the percentages of $CD4^+$ T lymphocytes ($p<0.05$ for 0.5 mg/kg hsBAFF *vs.* control; $p<0.001$ for 2 mg/kg hsBAFF *vs.* control) in hsBAFF-treated mice (0.1-2 mg/kg), but the $CD8^+$ T lymphocyte percentages remained almost unchanged. Taking these findings into account, hsBAFF may induce and increase immune function of helper T lymphocytes (TH cells). T lymphocytes displaying $CD4^+$generally function as TH cells, whereas those displaying $CD8^+$ generally function as cytotoxic T lymphocytes (TC cells) (Kuby 1994). Proper lymphocyte homeostasis is critical for normal immune function and is maintained by a complex series of cellular interactions and the action of secreted cytokines (Zamorano *et al.* 2001). The ratio of TH to TC cells in the body is approximately stable and can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T lymphocytes. We showed that the ratio of $CD4^+$ to $CD8^+$ T lymphocytes rose with an increasing dosage from 0.1 to 2 mg/kg ($p<0.05$ for 2 mg/kg hsBAFF *vs.* control) in hsBAFF-treated mice, suggesting that hsBAFF plays an important action in upregulating TH cell immune responses. The $CD4^+$ T lymphocytes can polarize towards helper T 1 (TH1) or helper T 2 (TH2) cells, which produce different sets of cytokines for instance interferon-γ (IFN-γ) or interleukin-4 (IL-4), IL-5, and IL-13, respectively. They provide protection against intracellular or extracellular pathogens (Li *et al.* 2000). The secreted cytokines play an important role in activating B lymphocytes, TC cells, macrophages, and various other cells that participate in the immune response (Mills and Cambier 2003). The $CD8^+$ T lymphocytes differentiate into TC cells capable of killing virus-infected cells. Ossendorp *et al.* (2000) clarified that $CD4^+$ T lymphocytes generated by an immunity reaction can enhance the antitumor efficacy induced by $CD8^+$ T lymphocytes. Increasing the number and improving the quality of $CD4^+$ T lymphocytes are vital in tumor therapy. Therefore, the elevation in $CD4^+$ T lymphocytes investigated in our study suggested that hsBAFF can enhance immune responses in the body by increasing $CD4^+$ T lymphocyte function.

In the present report, we showed that NK cell activity in 0.01 mg/kg hsBAFF-treated mice was similar to that in control animals, but we found a dose-dependent increase of stronger NK activities together with the elevation of hsBAFF dosages ($p<0.05$ for 0.5 and 2 mg/kg hsBAFF *vs.* control), indicating that hsBAFF is able to enhance the immunity of the body by increasing NK cell activity. This fact may be relevant to the large elevation of $CD4^+$ T lymphocyte percentage in mice after hsBAFF treatment. Some literary data have shown a number of interactions between T lymphocytes and NK cells, but it is still not clear how T lymphocytes influence the NK cell response *in vivo* and whether they play a role in the activity of NK cells. Biron *et al.* (1990) showed that several T lymphocyte products, most notably IL-2 and IFN-γ, modulate NK cell activity. IL-2 activates the

cytotoxic potential of NK cells, stimulates their proliferation *in vitro* and *in vivo*, and serves as a chemoattractant for already activated NK cells. IFN-γ produced by activated T lymphocytes and NK cells suppresses the proliferation of cancer cells and activates TC cells and macrophages (Tannenbaum and Hamilton 2000, Ikeda *et al.* 2002). Such findings support our suggestion that hsBAFF may indirectly enhance NK cell activity by increasing the immune responses of $CD4^+$ T lymphocytes. The exact mechanisms still remain to be explored.

In conclusion, our study shows that the hsBAFF synthesized in *Escherichia coli* can enhance immune responses in the body by increasing the functions of B lymphocytes and also of $CD4^+$ T lymphocytes by elevating NK cell activity.

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References

BAKER KP: BLyS – an essential survival factor for B cells: basic biology, links to pathology and therapeutic target. *Autoimmun Rev* **3**: 368-375, 2004.

BIRON CA, YOUNG HA, KASAIAN MT: Interleukin 2-induced proliferation of murine natural killer cells in vivo. *J Exp Med* **171**: 173-188, 1990.

CAO P, MEI JJ, DIAO ZY, ZHANG SQ: Expression, refolding, and characterization of human soluble BAFF synthesized in Escherichia coli. *Protein Expr Purif* **41**: 199-206, 2005.

DO RK, HATADA E, LEE H, TOURIGNY MR, HILBERT D, CHEN-KIANG S: Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. *J Exp Med* **192**: 953-964, 2000.

GROOM J, KALLED SL, CUTLER AH, OLSON C, WOODCOCK SA, SCHNEIDER P, TSCHOPP J, CACHERO TG, BATTEN M, WHEWAY J, MAURI D, CAVILL D, GORDON TP, MACKAY CR, MACKAY F: Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J Clin Invest* **109**: 59-68, 2002.

HARLESS SS, CANCRO MP: Integrating B cell homeostasis and selection with BLyS. *Arch Immunol Ther Exp* **51**: 209–218, 2003.

HERCEND T, SCHMIDT RE: Characteristics and uses of natural killer cells. *Immunol Today* **9**: 291-293, 1988.

HUARD B, SCHNEIDER P, MAURI D, TSCHOPP J, FRENCH LE: T cell costimulation by the TNF ligand BAFF. *J Immunol* **167**: 6225-6231, 2001.

IKEDA H, OLD LJ, SCHREIBER RD: The roles of IFN gamma in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev* **13**: 95-109, 2002.

KUBY J: *Immunology*. WH Freeman, New York, 1994.

LI NL, ZHANG DQ, ZHOU KY, CARTMAN A, LEROUX JY, POOLE AR, ZHANG YP: Isolation and characteristics of autoreactive T cells specific to aggrecan G1 domain from rheumatoid arthritis patients. *Cell Res* **10**: 39-49, 2000.

LIU Y, HONG X, KAPPLER J, JIANG L, ZHANG R, XU L, PAN CH, MARTIN WE, MURPHY RC, SHU HB, DAI S, ZHANG G: Ligand-receptor binding revealed by the TNF family member TALL-1. *Nature* **423**: 49-56, 2003.

LV CL, LI Y: Experimental technique for cytotoxicity. In: *Experimental Technique for Immunology*. ZH LIU, CL LV (eds), Scientific Press, Beijing, 2002, pp 114-117 (in Chinese).

MILLS DM, CAMBIER JC: B lymphocyte activation during cognate interactions with $CD4^+$ T lymphocytes: molecular dynamics and immunologic consequences. *Semin Immunol* **15**: 325-329, 2003.

MOORE PA, BELVEDERE O, ORR A, PIERI K, LAFLEUR DW, FENG P, SOPPET D, CHARTERS M, GENTZ R, PARMELEE D, Li Y, GALPERINA O, GIRI J, ROSCHKE V, NARDELLI B, CARRELL J, SOSNOVTSEVA S, GREENFIELD W, RUBEN SM, OLSEN HS, FIKES J, HILBERT DM: BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* **285**: 260-263, 1999.

MUKHOPADHYAY A, NI J, ZHAI YK, YU GL, AGGARWAL BB: Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptosis, nuclear factor-kappa B, and c-Jun NH2-terminal kinase. *J Biol Chem* **274**: 15978-15981, 1999.

OSSENDORP F, TOES RE, OFFRINGA R, VAN DER BURG SH, MELIEF CJ: Importance of $CD4^+$ T helper cell responses in tumor immunity. *Immunol Lett* **74**: 75-79, 2000.

RAHMAN ZS, RAO SP, KALLED SL, MANSER T: Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. *J Exp Med* **198**: 1157-1169, 2003.

RAVETCH JV, LANIER LL: Immune inhibitory receptors. *Science* **290**: 84-89, 2000.

SCHNEIDER P, MACKAY F, STEINER V, HOFMANN K, BODMER JL, HOLLER N, AMBROSE C, LAWTON P, BIXLER S, ACHA-ORBEA H, VALMORI D, ROMERO P, WERNER-FAVRE C, ZUBLER RH, BROWNING JL, TSCHOPP J: BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* **189**: 1747-1756, 1999.

SHU HB, HU WH, JOHNSON H: TALL-1 is a novel member of the TNF family that is down-regulated by mitogens. *J Leukoc Biol* **65**: 680-683, 1999.

TANNENBAUM CS, HAMILTON TA: Immune-inflammatory mechanisms in IFNγ-mediated anti-tumor activity. *Semin Cancer Biol* **10**: 113-123, 2000.

THOMPSON JS, SCHNEIDER P, KALLED SL, WANG L, LEFEVRE EA, CACHERO TG, MACKAY F, BIXLER SA, ZAFARI M, LIU ZY, WOODCOCK SA, QIAN F, BATTEN M, MADRY C, RICHARD Y, BENJAMIN CD, BROWNING JL, TSAPIS A, TSCHOPP J, AMBROSE C: BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. *J Exp Med* **192**: 129-135, 2000.

TRINCHIERI G: Biology of natural killer cells. *Adv Immunol* **47**: 187-376, 1989.

VON BULOW GU, BRAM RJ: NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. *Science* **278**: 138-141, 1997.

XIA XZ, TREANOR J, SENALDI G, KHARE SD, BOONE T, KELLEY M, THEILL LE, COLOMBERO A, SOLOVYEV I, LEE F, MCCABE S, ELLIOTT R, MINER K, HAWKINS N, GUO J, STOLINA M, YU G, WANG J, DELANEY J, MENG SY, BOYLE WJ, HSU H: TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. *J Exp Med* **192**: 137-143, 2000.

ZAMORANO J, KELLY AE, AUSTRIAN J, WANG HY, KEEGAN AD: Costimulation of resting B lymphocytes alters the IL-4-activated IRS2 signaling pathway in a STAT6 independent manner: implications for cell survival and proliferation. *Cell Res* **11**: 44-54, 2001.

Reprint requests

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