An expression cloning approach was employed to identify the receptor for B-lymphocyte stimulator (Blys) and identified the tumor necrosis factor receptor superfamily member TACI as a Blys-binding protein. Expression of TACI in HEK293T cells confers on the cells the ability to bind Blys with subnanomolar affinity. Furthermore, a TACI-Fc fusion protein recognizes both the cleaved, soluble form of Blys as well as the membrane Blys present on the cell surface of a recombinant cell line. TACI mRNA is found predominantly in B-cells and correlates with Blys binding in a panel of B-cell lines. We also demonstrate that TACI interacts with a novel tumor necrosis factor homologue APRIL for which no clear in vivo role has been described. Blys and APRIL are capable of signaling through TACI to mediate NF-κB responses in HEK293 cells. We conclude that TACI is a receptor for Blys and APRIL and discuss the implications for B-cell biology.

Members of the tumor necrosis factor superfamily of cytokines play diverse roles in the regulation of cell proliferation, differentiation, and survival. Notably, several members of this family play key roles in the regulation of the immune system (1). We and others have previously identified a novel TNF-related ligand, Blys (also known as BAFF, TALL-1, thank, TNFsf20, and zTNF4) which is expressed on monocytes and related ligand, BLyS (also known as BAFF, TALL-1, THANK, BLyS has activity in vitro and in vivo (2–6). Like many members of the TNF family, Blys has activity in vitro as a 152-amino acid soluble molecule and as a 258-amino acid transmembrane form (3). However, the biological significance of these two forms and their relative contributions in vivo remain to be resolved. More recently, transgenic mice that ectopically overexpress Blys were shown to develop autoimmune-like phenotypes reminiscent of those observed in systemic lupus erythematosus (7–9). These findings suggest that Blys plays an important role in the regulation of B-cell growth and humoral immunity.

In order to understand the precise mechanism by which Blys activates B-cells, the range of cell types Blys may affect, and the potential role of Blys as a therapeutic agent or target, we have used expression cloning to identify the receptor for Blys. We have identified the orphan receptor TACI (10), previously characterized as being present on B-cells and a subset of T-cells, as the receptor for Blys and show that this receptor is capable of mediating NF-κB signaling in response to ligand binding. We also show that TACI interacts with another TNF family member, APRIL, which is closely related to Blys. Parallel work by others has recently shown that TACI and a second TNF family member, BCMA, are Blys receptors (9, 11–14).

**EXPERIMENTAL PROCEDURES**

*Cell Culture and Media—*HEK293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and transfected using LipofectAMINE Plus (Life Technologies, Gaithersburg, MD) according to the manufacturer’s protocol. For expression cloning scening, cells were attached to plates with poly-d-lysine.

*Flow Cytometry—*Cells were stained with monoclonal antibodies against BLys (HGGG2A) with biotinylated BLys as described previously (2), with recombinant TACI-Fc fusion protein or with recombinant Flag-tagged proteins which were subsequently detected by the M2 anti-Flag monoclonal antibody (Sigma). Flow cytometry was performed using a FACScan instrument and associated CellQuest software (Becton Dickinson, San Jose, CA).

*Library Preparation, Screening, and Other DNA Manipulations—*All common DNA manipulations such as restriction, ligation, and PCR were as described previously (15). Human tonsillar B-cell cDNA, size-selected for enrichment for potential cDNA clones of >1.5 kilobase, was ligated into expression vector pCMV-Sport3 (Life Technologies) to generate a library of approximately 5 million independent clones. To facilitate library screening, wells containing 150 clones in a 36-well format were generated, and plasmid DNA was purified from these using the Qiagen biorobot 9600 (Qiagen, Valencia, CA). Pools of approximately 1200 cDNAs (8 wells) were used to transfect HEK293 cells. At 40–48 h post-transfection, cells were washed once with PBS and incubated for 2 h at 37°C with 300 µL 125I-radiolabeled Blys (17–35 µCi/µg) in binding buffer (DMEM, 10% fetal bovine serum, 25 mM HEPES, pH 7.4). Subsequently, cells were washed three times with PBS, and fixed with glutaraldehyde (2.5% v/v). The bottoms of the plates were removed as described previously (16) and subjected to autoradiography. Pools that bound radiolabeled Blys were subsequently partitioned into the individual eight wells of 150 clones that composed the initial positive pool and used for transfection. 600 clones from positive wells of 150 clones were end-sequence. Full-length TACI was generated by a two-step PCR reaction using the primers ATCTGCCACCATGAGTGGCCTGGGCCGGAGC sequentially at the 5′ end together with the reverse primer GAATTTCTAGACCCCCATTATGCACCCGG. The PCR product was cut with BglII and XhoI and
TACI Is the Receptor for BLyS and APRIL

BLyS was purified as described previously (2).

Protein Purification—BLyS was purified as described previously (2). APRIL was purified by capture on cation exchange resin (HS-50 Fores) and the trimeric form of APRIL isolated by size exclusion chromatography. Full-length TACI(M1-Q159)-Fc or truncated TACI(M31-Q159)-Fc were purified from transiently transfected HEK293T cells in 100 μl volumes of 50 g/ml BLyS or soluble APRIL. The flow cell surface was regenerated by disassociation of the protein using 0.1 M glycine-HCl, pH 3.5. To further enrich for the dimeric form, eluted with 0.1M citrate buffer, pH 3.5. To further enrich for the dimeric form, proteins were subjected to NH2-terminal sequence analysis to verify their NH 2-terminal sequence using a model ABI-494 sequencer (PerkinElmer Life Sciences user bulletin number 2, 1997) using a 18 S ribosomal RNA probe as endogenous reference. Expression levels are shown relative to expression levels in B-cells.

Protein Purification—BLyS was purified as described previously (2). APRIL was purified by capture on cation exchange resin (HS-50 Fores) and the trimeric form of APRIL isolated by size exclusion chromatography. Full-length TACI(M1-Q159)-Fc or truncated TACI(M31-Q159)-Fc were purified from transiently transfected culture supernatants by capture on Protein A HyperD resin (Life Technologies) and eluted with 0.1M citrate buffer, pH 3.5. To further enrich for the dimeric form, proteins were subjected to NH2-terminal sequence analysis to verify their NH 2-terminal sequence using a model ABI-494 sequencer (PerkinElmer Life Sciences user bulletin number 2, 1997) using a 18 S ribosomal RNA probe as endogenous reference. Expression levels are shown relative to expression levels in B-cells.

Protein Purification—BLyS was purified as described previously (2). APRIL was purified by capture on cation exchange resin (HS-50 Fores) and the trimeric form of APRIL isolated by size exclusion chromatography. Full-length TACI(M1-Q159)-Fc or truncated TACI(M31-Q159)-Fc were purified from transiently transfected culture supernatants by capture on Protein A HyperD resin (Life Technologies) and eluted with 0.1M citrate buffer, pH 3.5. To further enrich for the dimeric form, proteins were subjected to NH2-terminal sequence analysis to verify their NH 2-terminal sequence using a model ABI-494 sequencer (PerkinElmer Life Sciences user bulletin number 2, 1997) using a 18 S ribosomal RNA probe as endogenous reference. Expression levels are shown relative to expression levels in B-cells.

Protein Purification—BLyS was purified as described previously (2). APRIL was purified by capture on cation exchange resin (HS-50 Fores) and the trimeric form of APRIL isolated by size exclusion chromatography. Full-length TACI(M1-Q159)-Fc or truncated TACI(M31-Q159)-Fc were purified from transiently transfected culture supernatants by capture on Protein A HyperD resin (Life Technologies) and eluted with 0.1M citrate buffer, pH 3.5. To further enrich for the dimeric form, proteins were subjected to NH2-terminal sequence analysis to verify their NH 2-terminal sequence using a model ABI-494 sequencer (PerkinElmer Life Sciences user bulletin number 2, 1997) using a 18 S ribosomal RNA probe as endogenous reference. Expression levels are shown relative to expression levels in B-cells.

Protein Purification—BLyS was purified as described previously (2). APRIL was purified by capture on cation exchange resin (HS-50 Fores) and the trimeric form of APRIL isolated by size exclusion chromatography. Full-length TACI(M1-Q159)-Fc or truncated TACI(M31-Q159)-Fc were purified from transiently transfected culture supernatants by capture on Protein A HyperD resin (Life Technologies) and eluted with 0.1M citrate buffer, pH 3.5. To further enrich for the dimeric form, proteins were subjected to NH2-terminal sequence analysis to verify their NH 2-terminal sequence using a model ABI-494 sequencer (PerkinElmer Life Sciences user bulletin number 2, 1997) using a 18 S ribosomal RNA probe as endogenous reference. Expression levels are shown relative to expression levels in B-cells.
**TACI Mediates Binding to Soluble and Membrane BLyS—** We confirmed binding of BLyS to HEK293T transfected with TACI by FACS analysis using biotinylated BLyS; transient transfection of TACI results in a population of cells which bind BLyS in contrast to vector-transfected HEK293T cells. (Fig. 2A). The TNF-related ligand LIGHT/HVEM-L (21, 22) failed to bind to the TACI-transfected cells (Fig. 2C). To further explore the TACI-BLyS interaction we created a TACI-Fc fusion protein in which the extracellular domain of TACI is fused to the Fc domain of human IgG1. This fusion protein was used to effectively compete for soluble biotinylated BLyS binding to the B-cell-derived line IM-9 (Fig. 3). Because TACI-transfected cells bind soluble biotinylated and radiolabeled BLyS and TACI-Fc competes with IM-9 cells for binding of soluble BLyS, we conclude that TACI is able to interact with the soluble form of BLyS.

To determine if TACI is capable of binding membrane-bound BLyS, a recombinant cell line that expresses cell surface BLyS was generated. HEK293F cells were stably transfected with BLyS and demonstrated to express cell surface BLyS by FACS analysis using a monoclonal antibody derived against BLyS (Fig. 4, A–D). These cells were then tested for their ability to bind TACI-Fc protein using FACS analysis. As demonstrated in Fig. 4, TACI-Fc specifically binds the HEK293-BLyS stable cell line, but not a HEK293-vector cell line. Furthermore, the binding to the HEK293-BLyS cells is inhibited by the addition of soluble BLyS into the binding reaction (not shown). We conclude that TACI is able to bind membrane-bound BLyS.

**Binding Specificity—** TACI-Fc fusion protein was employed to assess the specificity of the interaction between TACI, BLyS, and other members of the TNF ligand family. We used a panel of 4 conditioned media containing Flag-epitope tagged proteins APRIL (23), LIGHT (21), FasL (24), and BLyS to assess interaction specificity. To evaluate the level of Flag-tagged protein present in the conditioned media, we used anti-Flag antibodies to immunoprecipitate the tagged proteins (Fig. 5A). An equivalent aliquot of the conditioned media was also subject to immunoprecipitation with TACI-Fc (Fig. 5B) or with beads alone (not shown). Immunoprecipitates were detected by Western analysis using anti-Flag antibody. We find that TACI-Fc effectively immunoprecipitates all of the Flag-BLyS present in the conditioned medium (Fig. 5, A and B, lane 3) but does not precipitate either LIGHT or FasL. We also find that TACI-Fc immunoprecipitates approximately 20% of the APRIL present in the conditioned medium, suggesting that TACI-Fc interacts with APRIL (Fig. 5B, lane 1). Interaction between APRIL and TACI-Fc has also been observed with BIAcore analysis (next section).

**BIAcore Analysis—** We assessed the ability of TACI-Fc to bind BLyS by BIAcore analysis. TACI-Fc was bound to a BIAcore chip and different concentrations of human BLyS were allowed to flow over the chip. There was significant binding to the flow cell of ~1000 and 600 relative units at 5 and 2.5 μg/ml BLyS, respectively. This binding was specific because BLyS binding to TACI-Fc was competed with increasing concentrations of cold competitor. Either full-length TACI or truncated TACI (Fig. 4, A and D, respectively) or with beads alone (not shown). Immunoprecipitates were detected by Western analysis using anti-Flag antibody. We find that TACI-Fc effectively immunoprecipitates all of the Flag-BLyS present in the conditioned medium (Fig. 5, A and B, lane 3) but does not precipitate either LIGHT or FasL. We also find that TACI-Fc immunoprecipitates approximately 20% of the APRIL present in the conditioned medium, suggesting that TACI-Fc interacts with APRIL (Fig. 5B, lane 1). Interaction between APRIL and TACI-Fc has also been observed with BIAcore analysis (next section).

**TACI is the Receptor for BLyS and APRIL**
APRIL, respectively. Thus, the on-rate is faster for BLyS compared with APRIL, whereas, the off-rate for BLyS is faster than that of APRIL. The calculated $K_d$ values for the binding of APRIL and BLyS to TACI-Fc were 6.4 and 0.16 nM, respectively. Overall BLyS has a ~25-fold higher binding constant than APRIL.

In order to show that the interaction of TACI with APRIL was not an artifactual result of using a TACI-Fc fusion protein, we determined if TACI present on the surface of cells could bind APRIL. We find that TACI-transfected cells are capable of binding Flag-tagged-APRIL or BLyS but not Flag-tagged Fas ligand (Fig. 7, A–F). We also find that APRIL competes for radiolabeled BLyS binding to TACI-transfected cells (Fig. 7G). In agreement with the BIACore analysis, the relative affinity of TACI for APRIL was again found to be some 10–20-fold lower than for BLyS. We also observe interaction of TACI-Fc with membrane-bound APRIL on cells transiently transfected with a full-length APRIL construct (not shown).

**TACI Expression Correlates with BLyS Binding Capacity**—TACI was previously characterized as being present on B-cells and a subset of activated T-cells (10). We extended this analysis initially by Northern analysis on a series of tissues. In agreement with previous observations, we find that TACI is expressed primarily in immune tissues with weaker signals pres-
TACI Is the Receptor for BLyS and APRIL

FIG. 5. TACI interacts with BLyS and APRIL. Conditioned medium from HEK293 cells transiently transfected with FLAG epitope-tagged versions of APRIL, LIGHT, BLyS, or FasL were immunoprecipitated with anti-FLAG epitope antibodies (A) or with TACI-Fc fusion protein (B). Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis and Western analysis with anti-FLAG epitope monoclonal antibody. A cross-reacting band that co-migrates with FasL is present, however, FasL is clearly visible above this. Molecular masses in kDa are indicated.

FIG. 6. TACI interaction by BIAcore analysis. A, sensorgram of binding of BLyS to TACI-Fc. The sensorgram shows the on- and off-rate region of the BIAcore sensorgram of a series of different concentrations (range = 1.5–100 nM) of BLyS. $K_d$ value was calculated using the BIAevaluation kinetic software program. B, sensorgram of binding of APRIL to TACI-Fc. Sensorgram shows the on- and off-rate region of the BIAcore sensorgram of a series of different concentrations (range = 1.5–196 nM) of APRIL.

DISCUSSION

Using an expression cloning protocol we have identified the previously described TNFR family member TACI as a BLyS-binding protein that is present predominantly on B-cells. We present several lines of evidence to suggest that TACI is the BLyS receptor; however, the high affinity of the interaction between BLyS and TACI ($K_d$ of 0.1 nM), which is typical of physiological receptor-ligand interactions, is probably the most compelling. Additionally, there is good concordance between the TACI expression profile and the BLyS-binding potential of cells. Notably, among different B-cell lines, Daudi cells (which do not bind BLyS) do not express TACI. Significantly, we show that TACI is able to interact with both soluble BLyS and the membrane-bound form of BLyS present on a recombinant cell line, an observation that suggests that TACI is able to mediate signal transduction of both forms of BLyS to B-cells. Previous work has shown that BLyS present on the surface of cells is competent to act as a co-stimulator of B-cells (3); we show here that this activity is probably mediated, at least in part, by TACI.

In parallel work, others have recently shown that TACI as well as another TNFR family member, BCMA (26), act as BLyS-binding proteins (9, 11–14). Importantly, we show here that the interaction between TACI and BLyS is not exclusive and that TACI is capable of interacting with the TNF-related ligand APRIL, as judged by four independent in vitro methods. The interaction between TACI and APRIL was found to be some 10–20-fold lower in affinity than that seen for the BLyS-TACI interaction. However, the affinity was still in the nanomolar range (6 nM) and is similar to affinities of other receptor-ligand interactions in the TNF/TNFR superfamily. The interaction of a TNFR superfamily member with multiple TNF-
like ligands is not unprecedented; the receptor HVEM was shown to interact with the ligands LIGHT and lymphotoxin-α (21). The in vivo role of APRIL remains elusive; a role as a growth promoting factor has been shown, and a role in tumor cell growth has been suggested (23), while other work has suggested a role in apoptosis (27). However, the expression of APRIL in peripheral blood lymphocytes (23), monocytes, and macrophages (TALL-2 (4)) and its interaction with TACI demonstrated here, make a role in regulation of immune function seem likely.

It has been suggested that TACI, as soluble receptor, may prove useful as a therapeutic agent to antagonize the function of BLyS in autoimmune diseases such as systemic lupus erythematosus (28). Indeed, it has been shown that administration of TACI-Fc soluble receptor results in reduction of proteinuria and prolongation of lifespan in an animal model of systemic lupus erythematosus (9) or inhibition of primary immune responses and germinal center disruption in normal mice (13, 11). The interaction between APRIL and TACI demonstrated here has implications for any potential therapeutic utility based on soluble TACI receptor. Since we show that TACI is not specific for BLyS but also binds with high affinity to APRIL, TACI-based therapeutics will almost certainly also antagonize the function of APRIL. The outcome of the previous in vivo studies with TACI-Fc could be the result of TACI-Fc blocking APRIL-mediated B-cell effects, or BLyS- and APRIL-mediated effects, rather than simply BLyS-mediated effects. While this manuscript was in revision, parallel work was published by others that also demonstrates interaction of TACI and APRIL (29); furthermore, this paper also demonstrates the interaction between APRIL and the alternate BLyS receptor BCMA. Clearly, further work will be required to unravel the relative contributions of BLyS, APRIL, TACI, and BCMA interactions in B-cell regulation.

We show here that TACI mRNA expression profile is predominantly B-cell specific. In contrast, TACI was initially characterized as being present in B-cells and activated T-cells (10). Other cells, such as dendritic cells and monocytes, have much lower levels of TACI. Furthermore, TACI expression is not inducible in these cells by immunomodulatory agents, such as

![Figure 7. Interaction of APRIL with membrane-bound TACI. A, FACS analysis. Cells were transiently co-transfected with vector (A, C, and E) or TACI (B, D, and F), and either Flag-Fas ligand (A and B), Flag-APRIL (C and D), or Flag-BLyS (E and F) expression constructs. Twenty-four hours post-transfection, cells were analyzed by FACS for binding of the M2 anti-Flag antibody. H, APRIL competes with BLyS for binding to membrane-bound TACI. Cells were transiently transfected with TACI and 0.3 nM radio-labeled BLyS bound in the presence of unlabeled BLyS (filled squares) or APRIL (open circles). The calculated EC₅₀ values for cold BLyS and APRIL are 0.33 and 11.8 nM, respectively.](image)
interferon γ and interleukin-10. In agreement with the previous report on TACI, we observe weak expression of TACI on T-cells. However, at the mRNA level this expression is at least an order of magnitude lower than that seen on B-cells. In agreement with these findings, we are able to observe weak binding of BLyS to a purified population of stimulated T-cells. This binding, however, is less than 5% of the binding observed on B-cells (not shown). Thus if BLyS plays a role in T-cell regulation as suggested by others (5), it seems unlikely that this role will be a major one. However, we cannot exclude that under normal physiological conditions the interaction of BLyS with T-cells is required for some form of cross-talk between these two compartments of the immune system.

The clone we originally isolated as the BLyS-binding protein represented an amino-terminal truncation of the published TACI sequence. Since TACI is a Type 3 transmembrane protein, it contains no amino-terminal signal sequence; an amino-terminal truncation will not, therefore, interfere with the topological signals present in the internal signal anchor, and the protein will be transported to the cell surface. Surprisingly, this truncated version of TACI is biologically active and has an affinity which is only 5-fold lower than compared with full-length TACI. This bioactive deletion may prove useful in delineating structure-function relationships between TACI and BLyS or APRIL binding. It may also represent some fraction of the naturally translated protein that is found in vivo.

TACI was initially identified as an orphan receptor in FIG. 8. Determination of TACI messenger RNA levels by real time quantitative reverse transcriptase-PCR. A, TACI mRNA levels in B-cells and primary hematopoietic cells. RNA samples from the following primary hematopoietic cells were used: B-cells, two donors A and B, dendritic cells, dendritic cell treated with interferon (IFN)–γ (100 units/ml), dendritic cells treated with interleukin (IL)-10 (100 ng/ml), dendritic cells treated with lipopolysaccharide, 100 ng/ml, natural killer cells (NK), natural killer cells treated with interleukin-2 and interleukin-12 (100 units/ml), monocytes treated with interferon–γ (100 units/ml), monocytes treated with IL-10 (100 ng/ml), T cells treated with PHA (50 ng/ml), T-cells treated with PHA and IL-2 (100 units/ml), CD4 + T-cells, Th1 T-cells, Th1 T-cells treated with mAbCD3, T-cells, T-cells treated with phorbol 12-myristate 13-acetate and ionomycin, T-cells cord blood, T-cells from cord blood treated with phorbol 12-myristate 13-acetate (PMA) and ionomycin. Expression levels are shown relative to mRNA levels in B-cells donor A. B, TACI mRNA levels in B-cell lines. RNA samples from the following B-cell lines were analyzed: ARH-77, Daudi, Normalwa, IM-9, RAJI, REH RPMI-8226. Expression levels are compared with TACI mRNA levels in B-cells from donor A and B and shown relative to expression levels in donor A.

FIG. 9. TACI mediates signal transduction in HEK293 cells. HEK293 (1 x 10⁵) were transiently transfected with an NF-κB-SEAP reporter plasmid together with the indicated concentration of TACI expression vector. Expression plasmids directing expression of soluble BLyS or soluble APRIL were also co-transfected where indicated at 20 ng/ml. 18 h post-transfection, supernatants were collected and alkaline phosphatase levels determined.

two-hybrid screen using the signal transduction component CAML as bait and was shown to mediate activation of NF-κB, NFAT, and AP-1 in the Jurkat T-cell line (10). We confirm here that TACI is capable of activating the NF-κB pathway in
HEK293 cells. At the highest levels of TACI transfected, the level of NF-κB activation by TACI is comparable to levels observed with RANK (not shown) (17, 30), a TNF receptor gand stimulation. which TRAFs are specifically recruited to TACI following li-

ments (11). Further work will be required in order to delineate with regions of TACI in a yeast 2-hybrid system and in un-

stimulated HEK293 cells expressing epitope-tagged com-

ponents (11). Further work will be required in order to delineate which TRAFs are specifically recruited to TACI following li-

gand stimulation.

Acknowledgments—We thank Drs. Jian Ni and Yangu Shi for the Flag tagged-TNF ligand expression constructs. Roberto Patarca and Tim Beardsley are thanked for critical reading of the manuscript.

REFERENCES

1. Wallach, D., Bigda, J., and Engelmann, H. (1999) in The Cytokine Network and Immune Functions (Theze, J., ed) pp. 51–84, Oxford University Press, Oxford, Oxford, Oxford. 1999

2. Moore, P. A., Belvedere, O., Orr, A., Pieri, K., LaFleur, D. W., Feng, P., Soppet, D., Charters, M., Gentz, R., Parmelee, D., Chaires, J., Emery, J. G., Deen, K., Eichman, C., Chabot-Fletcher, M., Truneh, A., and Young, P. R. (1998) J. Biol. Chem. 273, 27548–27556

3. Schneider, P., MacKay, F., Steiner, V., Hofmann, K., Bodmer, J. L., Holler, N., Ambrose, C., Lawton, P., Bixler, S., Acha-Orbea, H., Valmori, D., Romero, P., Werner-F惙re, C., RH, Z., Browning, J., L., and Tschopp, J. (1999) J. Exp. Med. 189, 1747–1756

4. Shu, H. B., Hu, W. H., and Johnson, H. (1999) J. Leukocyte Biol. 65, 680–683

5. Tribouley, C., Wallroth, M., Chan, V., Paliard, X., Fang, E., Lamson, G., Pot, D., Escobedo, J., and Williams, L. T. (1999) Biol. Chem. 380, 1443–1447

6. Mukhopadhyay, A., Ni, J., Zhai, Y., Yu, G.-L., and Aggarwal, B. B. (1999) J. Biol. Chem. 274, 15978–15981

7. Khare, S. D., Sarosi, I., Xio, X. Z., McCabe, S., Miner, K., Solovyev, I., Hawkins, N., Guo, J., Stolina, M., Yu, G., Wang, J., Delaney, J., Meng, S.-Y., Boyle, W. J., and Hsu, H. (2000) J. Exp. Med. 192, 137–143

8. Bateman, A., Birney, E., Darbin, R., Eddy, S. R., Howe, K. L., and Sonnhammer, E. L. (2000) Nucleic Acids Res. 28, 263–266

9. Ware, C. F., Santuc, S., and Glass, A. (1998) in The Cytokine Handbook (Thompson, A., ed) 3rd Ed., pp. 549–592, Academic Press, London

10. von Bulow, G.-U., and Bram, R. J. (1997) Science 278, 138–141

11. Schneider, P., MacKay, F., Steiner, V., Hofmann, K., Bodmer, J. L., Holler, N., Tschopp, J., and Browning, J. L. (1999) Nature 390, 175–179

12. Anderson, D. M., Marakovsky, E., Billingsley, W. L., Dougall, W. C., Tometsko, M. E., Roux, E. R., Frohn, H. P., Cost, M., and Ware, C. F. (1999) Nature 390, 1094–1098

13. Pacholczyk, T., Blakely, R. D., and Amara, S. G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9156–9161

14. Tim Beardsley are thanked for critical reading of the manuscript.