METHODS AND COMPOSITIONS OF MATTER CONCERNING APRIL/G70, BCMA, BLYS/AGP-3, AND TACI

This invention concerns interactions among APRIL/G70, AGP-3/BLYS, BCMA, and TACI and related methods of use and compositions of matter. It has been found that (1) sAPRIL/G70 binds to the cell-surface receptors BCMA and TACI on T and B lymphoma cells, resulting in stimulation of proliferation of primary human and mouse B and T cells both in vitro and in vivo; (2) APRIL competes with AGP3’s binding to TACI and BCMA; (3) sBCMA inhibits APRIL and AGP3 binding to its receptors; (4) sBCMA ameliorates T cell dependent and T cell independent humoral immune responses in vivo; (5) sTACI inhibits APRIL and AGP3 binding to its receptors and ameliorates T cell dependent and T cell independent humoral immune response in vivo; and (6) BCMA exhibits similarity with TACI within a single cysteine rich domain located N-terminal to a potential transmembrane domain. These discoveries provides a strategy for development of therapeutics for treatment of autoimmune diseases, and cancer, for prevention of transplant rejection. Disease states and disease parameters associated with APRIL and AGP-3 may be affected by modulation of BCMA or TACI; disease states and parameters associated with TACI can be affected by modulation of APRIL; disease states and parameters can be affected by modulation of any of TACI, BCMA, APRIL, and AGP-3 by a single therapeutic agent or two or more therapeutic agents together.
METHODS AND COMPOSITIONS OF MATTER
CONCERNING APRIL/G70, BCMA, BLYS/AGP-3, AND
TACI

This application claims the benefit of U.S. Provisional Application
Serial No. 60/204,039, filed May 12, 2000 and U.S. Provisional Application
Serial No. 60/214,591, filed June 27, 2000, which are incorporated herein
by reference.

Field of the Invention

The present invention relates to proteins that are involved in
inflammation and immunomodulation, survival, or activation. The
invention further relates to proteins related to the tumor necrosis factor
(TNF)/nerve growth factor (NGF) superfamily and related nucleic acids,
expression vectors, host cells, and binding assays. The specification also
describes compositions and methods for the treatment of immune-related
and inflammatory, autoimmune and other immune-related diseases or
disorders, such as rheumatoid arthritis (RA), Crohn’s disease (CD), lupus,
and graft versus host disease (GvHD).

Background of the invention

After years of study in necrosis of tumors, tumor necrosis factors
(TNFs) α and β were finally cloned in 1984. The ensuing years witnessed
the emergence of a superfamily of TNF cytokines, including fas ligand
(FasL), CD27 ligand (CD27L), CD30 ligand (CD30L), CD40 ligand
(CD40L), TNF-related apoptosis-inducing ligand (TRAIL, also designated
AGP-1), osteoprotegerin binding protein (OPG-BP or OPG ligand), 4-1BB
ligand, LIGHT, APRIL, and TALL-1. Smith et al. (1994), Cell, 76: 959-962;
Lacey et al. (1998), Cell, 93: 165-176; Chichepotiche et al. (1997), J. Biol.
Chem., 272: 32401-32410; Mauri et al. (1998), Immunity, 8: 21-30; Hahne et
al. (1998), J. Exp. Med., 188: 1185-90; Shu et al. (1999), J. Leukocyte Biology, 65: 680-3. This family is unified by its structure, particularly at the C-terminus. In addition, most members known to date are expressed in immune compartments, although some members are also expressed in other tissues or organs, as well. Smith et al. (1994), Cell 76: 959-62. All ligand members, with the exception of LT-α, are type II transmembrane proteins, characterized by a conserved 150 amino acid region within C-terminal extracellular domain. Though restricted to only 20-25% identity, the conserved 150 amino acid domain folds into a characteristic β-pleated sheet sandwich and trimerizes. This conserved region can be proteolytically released, thus generating a soluble functional form. Banner et al. (1993), Cell, 73: 431-445.

Many members within this ligand family are expressed in lymphoid enriched tissues and play important roles in the immune system development and modulation. Smith et al. (1994). For example, TNFα is mainly synthesized by macrophages and is an important mediator for inflammatory responses and immune defenses. Tracey & Cerami (1994), Annu. Rev. Med., 45: 491-503. Fas-L, predominantly expressed in activated T cell, modulates TCR-mediated apoptosis of thymocytes.


The cognate receptors for most of the TNF ligand family members have been identified. These receptors share characteristic multiple cysteine-rich repeats within their extracellular domains, and do not possess catalytic motifs within cytoplasmic regions. Smith et al. (1994). The receptors signal through direct interactions with death domain proteins (e.g. TRADD, FADD, and RIP) or with the TRAF proteins (e.g.
TRAF2, TRAF3, TRAF5, and TRAF6), triggering divergent and 
overlapping signaling pathways, e.g., apoptosis, NF-κB activation, or JNK 
activation. Wallach et al. (1999), Annual Review of Immunology 17: 331-
67. These signaling events lead to cell death, proliferation, activation or 
differentiation. The expression profile of each receptor member varies. 
For example, TNFR1 is expressed on a broad spectrum of tissues and cells, 
whereas the cell surface receptor of OPGL is mainly restricted to the 
proteins are believed to play a role in inflammatory and immune 
processes, suggesting their usefulness in treating autoimmune and 
inflammatory disorders.

A number of research groups have recently identified TNF family 
ligands with the same or substantially similar sequence, but they have not 
identified the associated receptor. The ligand has been variously named 
neutrokine-α (WO 98/18921, published May 7, 1998), 63954 (WO 
98/27114, published June 25, 1998), TL5 (EP 869 180, published October 7, 
1998), NTN-2 (WO 98/55620 and WO 98/55621, published December 10, 
1998), TNRL1-alpha (WO 9911791, published March 11, 1999), kay ligand 
(WO99/12964, published March 18, 1999), and AGP-3 (U.S. Prov. App. 
Nos. 60/119,906, filed February 12, 1999 and 60/166,271, filed November 
18, 1999, respectively). Each of these references is hereby incorporated by 
reference. Hereinafter, this protein sequence is referred to as "AGP-3."

A recent paper has identified two previously known proteins as 
receptor was previously identified as a lymphocyte surface receptor 
named Transmembrane Activator and CAML Interactor (TACI). See WO 
Science, 278:138-140, each of which is hereby incorporated by reference in 
itself entirety. According to these references, TACI binds an intracellular
cyclophilin ligand designated CAML, which modulates the calcium signaling pathway in lymphocytes.

The second receptor identified for AGP-3 is the so-called B cell maturation protein (BCMA). The human BCMA gene was discovered by molecular analysis of a t(4;16) translocation, which characteristic of a human T cell lymphoma. Laabi et al. (1993), EMBO J, 11: 3897-3904. BCMA mRNA was reported to be found mainly in lymphoid tissues. Human BCMA cDNA encodes a 184 amino acids protein (185 residues for the mouse), and the literature reports no obvious similarity with any known protein or motif, and its function remained unknown. The protein was reported to reside in the Golgi apparatus (Gras et al. (1995), Intl. Immunol. 7: 1093-1106). Recent speculation suggested that BCMA may be a distant member of the TNFR super family. Madry et al. (1998), Intl. Immunol. 10: 1693-1702.

Summary of the Invention

It has now been found that sG70 binds to cell-surface receptors on T and B lymphoma cells resulting in stimulation of proliferation of primary human and mouse B and T cells both in vitro and in vivo.

It has now been found that BCMA and TACI are cell-surface receptors for APRIL. It has also been found that APRIL competes with AGP3's binding to TACI and BCMA. Furthermore it is shown here that sBCMA inhibits G70 and AGP3 binding to its receptors. sBCMA ameliorates T cell dependent and T cell independent humoral immune responses in vivo. In addition it has now been found that sTACI inhibits G70 and AGP3 binding to its receptors and ameliorates T cell dependent and T cell independent humoral immune responses in vivo. It has also been found that BCMA exhibits similarity with TACI within a single cysteine rich domain located N-terminal to a potential transmembrane domain. This invention concerns novel methods of use and compositions of matter that exploit these discoveries. The discoveries provides a strategy for development of therapeutics for treatment of autoimmune diseases, and cancer, for prevention of transplant rejection.

These discoveries show that activity, disease states, and disease parameters associated with APRIL and AGP-3 may be affected by modulation of BCMA. Likewise, disease states and disease parameters associated with TACI can be affected by modulation of APRIL. Further, such disease states and disease parameters can be affected by modulation of any of TACI, BCMA, APRIL and AGP-3 together. This discovery further suggests molecules and methods of treatment by which more than one of TACI, BCMA, APRIL, and AGP-3 may be modulated by a single molecule.

Description of the Figures

Figure 1 shows the sequence of human G70 (SEQ ID NOS: 1 and 2)
Start and stop codons are underlined.

Figures 2A and 2B show the DNA and amino acid sequences of mouse APRIL/G70 (SEQ ID NOS: 3 and 4, respectively). Start and stop codons are underlined. The amino acid sequence of FLAG-tagged soluble mouse G70 (SEQ ID NO: 19) is also provided.

Figure 3 shows an alignment of human (SEQ ID NO: 22) and mouse (SEQ ID NO: 23) G70. The middle line of each row shows the consensus sequence (SEQ ID NO: 24).

Figure 4 shows that G70/APRIL is a potent stimulator for B and T cell lymphoma. Figure 4A shows dose-dependent stimulation of proliferation of Jurkat cells (human leukemic T cells), Raji (human Burkitt lymphoma) and K562 cells (human chronic myelogenous leukemia cells). The proliferation of cells was determined by incubating $3 \times 10^4$ cells/well in 100 µl medium with indicated concentration of recombinant sG70/APRIL and phosphate-buffered saline (PBS, no ligand) as a control. After 48 hours, the number of viable cells were measured by Celltiter 96 AQ proliferation assay (Promega, Madison, WI). In Figure 4B, U937 cell (monocyte-like leukemia cells), NIH/3T3 (mouse embryo cell line) and 293 (transformed human primary embryonal kidney cell line) did not respond to sG70/APRIL stimulation.

Figures 5A and 5B show FACS analysis of G70/APRIL receptor binding. G70/APRIL receptor expression was assessed on indicated cell line using anti-Flag monoclonal antibody followed by FITC-conjugated goat antibody to mouse IgG. A anti-mouse CD16/CD32 monoclonal antibody (Fc Block) was used to block non-specific binding to cells.

Figure 6 shows the effect of sG70/APRIL on human peripheral blood B cell, T cell and granulocyte proliferation. Human peripheral T cell (CD4+ and CD8+), B cells and granulocyte were purified from three different donors by using RosetteSep cocktail antibodies (Stem cell Tech.
Vancouver). Purified cells were cultured in tissue culture-treated plastic wells (Becton-Dickinson, Lincoln park, NJ) for 6 Days in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine and 2-ME (50uM) in the present different concentration of sG70/APRIL. For the B cell proliferation assay, plastic wells were coated with purified mouse anti-human Ig M monoclonal antibody (3 μg/ml, Pharmingen, San Diego, CA). The positive control for T cell stimulation is IL-2.

Figure 7 shows the effect of G70/APRIL on murine T-and B-cell proliferation in vitro. T-and B-cells from the spleens of C57B1 mice were purified by selection through a murine T-cell and B-cell enrichment columns. 1x10^6 cells per well were cultured in the absence or presence of various G70/APRIL for 48 hours, pulsed during the last 18 hours with 0.5 μCi ^3H thymidine and harvested to count the incorporated radioactivity.

Figure 8 shows the effect of G70/APRIL on murine T cell proliferation costimulated through anti-CD28 antibody. T-cells from the spleens of C57B1 mice were purified by selection through a murine T-cell enrichment column. 1x10^6 T-cells per well were treated with G70/APRIL in the absence or presence of subliminal concentration of anti-CD28 antibody (0.9 μg/ml) for 48 hours, pulsed during the last 18 hours with 0.5 μCi ^3H thymidine and harvested to count the incorporated radioactivity.

Figure 9 shows the effect of G70/APRIL on murine T cell proliferation costimulated though anti-CD3 antibody. T-cells from the spleens of C57B1 mice were purified by selection through a murine T-cell enrichment column. 1x10^6 T-cells per well were treated with G70/APRIL in the absence or presence of subliminal concentration of anti-CD3 antibody (0.9 μg/ml) for 48 hours, pulsed during the last 18 hours with 0.5 μCi ^3H thymidine and harvested to count the incorporated radioactivity.
Table 1 shows FACS analysis of spleen (Table 1A), and mesenteric lymph nodes (Table 1B) after in vivo systemic administration of TNF family members. Several members of TNF family have been tested in vivo, each group have 5 mice (BDF-1, 8 weeks of age, Dose: 1 mg/kg/day 0.2 ml for 5 days). Spleen, thymus and mesenteric lymph nodes from three mice of each group have been isolated for FACS analysis using a panel of T cell and B cell surface mark antibodies. Results of FACS analysis have been summarized as following tables.

Figures 10A and 10B show the sequence of human BCMA (SEQ ID NO: 5). BCMA's extracellular domain (SEQ ID NO: 6) extends from aa 1 to aa 51 and is identified by arrows. The cysteine-rich consensus region (SEQ ID NO: 7, described further hereinafter) is shown in boldface. The transmembrane region (SEQ ID NO: 8) is underlined. huBCMA-Fc (SEQ ID NO: 9). mBCMA-Fc (SEQ ID NO: 10).

Figure 11 shows an alignment of human BCMA amino acid sequence and murine BCMA amino acid sequence (SEQ ID NO: 11). The human sequence is shown on the top line, the murine on the bottom line in each row. The human-murine consensus sequence (SEQ ID NO: 12) appears as the middle line of each row. A "+" in the consensus sequence indicates a conservative substitution. The cysteine-rich portion of the consensus sequence (SEQ ID NO: 13) appears in boldface.

Figures 12A and 12B show the sequence of hTACI (SEQ ID NO: 14). TACI's extracellular domain (SEQ ID NO: 15) extends from aa 1 to aa 166. The cysteine-rich consensus region (SEQ ID NO: 16) is shown in boldface, and the transmembrane region (SEQ ID NO: 17) is underlined. hTACI-Fc (SEQ ID NO: 18).

Figure 13 shows an alignment of cysteine rich extracellular regions of human TACI and human BCMA. The BCMA cysteine rich consensus region (SEQ ID NO: 20) appears as the top line, the TACI cysteine rich.
consensus region (SEQ ID NO: 21) appears as the bottom line of each row. Conserved amino acid residues are indicated by a vertical bar (|). Related amino acid residues are indicated with a colon (:).

Figures 14A, 14B and 14C show soluble mouse G70/APRIL binding to 293 cells expressing the BCMA gene. Human 293 cells transfected with the pmBCMA and pcDNA3 vectors were incubated with G70/APRIL-Flag, followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. A. 293 cells transfected with pcDNA3 vector only. B. 293 cells transfected with antisense pmBCMA vector. C. 293 cells transfected with sense pmBCMA vector.

Table 2 shows BIACore analysis of the stoichiometric binding kinetics of APRIL and AGP-3 to BCMA and TACI. Flag-APRIL specifically binds to murine and human BCMA with affinities of 0.25 nM and 0.29 nM, respectively, and to human TACI with an affinity of 1.48 nM.

Also a longer version of Flag-tagged APRIL (aa 50-240) binds to BCMA and TACI with high affinity similar to that of Fc-AGP-3 (Table 2). In separate experiments, we determined that neither APRIL nor AGP-3 bind to OPG and also that TNFα, OPGL, LIGHT, TWEAK, and TRAIL do not bind to BCMA or TACI. Hence, APRIL and AGP-3 specifically bind to both BCMA and TACI with high affinity.

Figure 15 shows G70/APRIL binding to 293 cells expressing the hTACI gene. Human 293 cells transfected with the phTACI and pcDNA3 vectors were incubated with G70/APRIL-Flag, followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. In Figure 15A, 293 cells were transfected with phTACI vector. In Figure 15B, 293 cells were transfected with pcDNA3 vector only.

Figure 16 shows G70/APRIL completely blocks AGP3 binding to its receptor. Mouse B lymphoma cells A20 were stained with AGP3-Fc or plus 10 fold excess G70/APRIL, CD40 ligand, TRAIL ligand and Tweak.
After washing 3 times, cells were incubated with FITC-conjugated goat anti-human IgG-Fc secondary antibody. In Figure 16A, 10 fold G70/APRIL completely blocked AGP3 binding to A20 cells. In Figures 16B,C and D, 10 fold CD40 ligand, Tweak and TRAIL do not have that effect on AGP3 binding.

Figure 17 shows that soluble TACI receptor (sTACI) binding competes with G70/APRIL binding to A20 cells. A20 cells were incubated with G70/APRIL or at same time plus 10 fold soluble TACI, TRAIL R2 and TRAIL R3 receptor, followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. In Figure 17A, soluble TACI receptor partially competed in binding G70/APRIL binding to A20 cells. In Figures 17B and C, soluble TRAIL R2 and TRAIL R3 receptors did not interfere with G70/APRIL binding.

Figure 18 shows that soluble human BCMA-Fc receptor fusion protein (shBCMA-Fc) and soluble human TACI-Fc receptor fusion protein (shTACI-Fc) completely blocks soluble human AGP-3-Fc receptor fusion protein (shAGP3-Fc) binding to A20 cells. A20 cells were incubated shAGP3-Fc or at same time plus 10 fold shBCMA-Fc or shTACI-Fc followed by FITC-conjugated anti-Flag antibody staining for FACS analysis.

Figure 19A shows shBCMA-Fc completely blocks shAGP3-Fc binding to A20 cells. A20 cells were incubated with shAGP3-Fc with or without 10 fold soluble hBCMA-Fc followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. Figure 19B shows that shBCMA-Fc blocks soluble murine APRIL (smAPRIL) binding to A20 cells. A20 cells were incubated with smAPRIL with or without 10 fold shBCMA-Fc followed by FITC-conjugated anti-Flag antibody staining for FACS analysis.
Figure 20 shows serum levels of anti-KLH IgG and IgM and anti-Pneumovax IgM in mice treated with TACI-Fc or BCMA-Fc fusion proteins or non-fused Fc as a control. p values refer to the comparison with the Fc-treated group. n = 7. See Materials and Methods hereinafter.

Detailed Description of the Invention

Definition of Terms

The terms used throughout this specification are defined as follows, unless otherwise limited in specific instances.

The term "comprising" means that a compound may include additional amino acids on either or both of the N- or C- termini of the given sequence. Of course, these additional amino acids should not significantly interfere with the activity of the compound.

"AGP-3 activity" refers to modulation of cell growth, survival, or activation resulting from binding by natural human AGP-3 to TACI or BCMA, particularly in B cells. Conversely, "AGP-3 antagonist activity" refers to activity in opposition to AGP-3 activity, as would result, for example, by inhibition of binding of AGP-3 to TACI or BCMA. Such activity can be determined, for example, by such assays as described in "Biological activity of AGP-3" in the Materials & Methods of PCT/US00/03653, which is hereby incorporated by reference. Additional assays by which AGP-3 activity may be identified appear in the references WO 98/18921 (May 7, 1998); WO 98/27114 (June 25, 1998); EP 869 180 (October 7, 1998); WO 98/55620 and WO 98/55621 (December 10, 1998); WO 99/11791 (March 11, 1999); WO99/12964 (March 18, 1999); and Gross et al. (2000), Nature 404: 995-9. Any of the assays described therein may be modified as needed by methods known to persons having ordinary skill in the art.

"APRIL activity" refers to modulation of cell growth, survival, or activation resulting from binding of natural human APRIL to TACI or
BCMA, particularly in T cells. Conversely, "APRIL antagonist activity" refers to activity in opposition to APRIL activity, as would result, for example, by inhibition of binding of APRIL to TACI or BCMA. Such activity can be determined, for example, by such assays as described in the Materials & Methods hereinafter. Additional assays by which APRIL activity may be identified appear in the references WO 99/00518 (June 26, 1997); WO 99/11791 (Sept. 5, 1997); WO 99/12965 (Sept. 12, 1997); EP 911 633 (Oct. 8, 1997); EP 919 620 (Nov. 26, 1997); WO 99/28462 (Dec. 3, 1997); WO 99/33980 (Dec. 30, 1997); WO 99/35170 (Jan. 5, 1998); and Hahne et al. (1998), J. Exp. Med. 188: 1185-90. Any of the assays described therein and herein may be modified as needed by methods known to persons having ordinary skill in the art.

"BCMA activity" refers to modulation of cell growth, survival, or activation resulting from binding by natural human APRIL or natural human AGP-3 to BCMA. Conversely, "BCMA antagonist activity" refers to activity in opposition to BCMA activity, as would result, for example, by inhibition of binding of AGP-3 or APRIL to BCMA. Such activity can be determined, for example, by such assays as described in the Materials & Methods hereinafter. Additional assays by which BCMA activity may be identified appear in the references WO 99/00518 (June 26, 1997); WO 99/11791 (Sept. 5, 1997); WO 99/12965 (Sept. 12, 1997); EP 911 633 (Oct. 8, 1997); EP 919 620 (Nov. 26, 1997); WO 99/28462 (Dec. 3, 1997); WO 99/33980 (Dec. 30, 1997); WO 99/35170 (Jan. 5, 1998); Hahne et al. (1998), J. Exp. Med. 188: 1185-90; WO 98/18921 (May 7, 1998); WO 98/27114 (June 25, 1998); EP 869 180 (October 7, 1998); WO 98/55620 and WO 98/55621 (December 10, 1998); WO 99/11791 (March 11, 1999); WO99/12964 (March 18, 1999); and Gross et al. (2000), Nature 404: 995-9. Any of the assays described therein and herein may be modified as needed by methods known to persons having ordinary skill in the art.
"TACI activity" refers to modulation of cell growth, survival, or activation resulting from binding by natural human AGP-3 or natural human APRIL to TACI. Conversely, "TACI antagonist activity" refers to activity in opposition to TACI activity, as would result, for example, by inhibition of binding of AGP-3 or APRIL to TACI. Such activity can be determined, for example, by such assays as described in the Materials & Methods of PCT/US00/03653, WO 98/18921 (May 7, 1998), WO 98/27114 (June 25, 1998), EP 869 180 (October 7, 1998), WO 98/55620 and WO 98/55621 (December 10, 1998), WO 99/11791 (March 11, 1999), WO99/12964 (March 18, 1999), WO 98/39361 (September 11, 1998), von Bulow & Bram (1997), Science, 278:138-140, and Gross et al. (2000), Nature 404: 995-9. Any of the assays described therein may be modified as needed by methods known to persons having ordinary skill in the art.

The term "specific binding partner" refers to any molecule that preferentially binds to a protein of interest, regardless of the antagonistic or agonistic activity of the molecule toward the protein of interest. Exemplary specific binding partners include antibodies, solubilized receptors, peptides, modified peptides as described hereinafter, and the like.

The term "vehicle" refers to a molecule that prevents degradation and/or increases half-life, reduces toxicity, reduces immunogenicity, or increases biological activity of a therapeutic protein. Exemplary vehicles include an Fc domain (which is preferred) as well as a linear polymer (e.g., polyethylene glycol (PEG), polylysine, dextran, etc.); a branched-chain polymer (see, for example, U.S. Patent No. 4,289,872 to Denkenwalter et al., issued September 15, 1981; 5,229,490 to Tam, issued July 20, 1993; WO 93/21259 by Frechet et al., published 28 October 1993); a lipid; a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide; or
any natural or synthetic protein, polypeptide or peptide that binds to a salvage receptor. Vehicles are further described hereinafter.

The term “native Fc” refers to molecule or sequence comprising the sequence of a non-antigen-binding fragment resulting from digestion of whole antibody, whether in monomeric or multimeric form. The original immunoglobulin source of the native Fc is preferably of human origin and may be any of the immunoglobulins, although IgG1 and IgG2 are preferred. Native Fc’s are made up of monomeric polypeptides that may be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (e.g., IgG, IgA, IgE) or subclass (e.g., IgG1, IgG2, IgG3, IgA1, IgGA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG (see Ellison et al. (1982), Nucleic Acids Res. 10: 4071-9). The term “native Fc” as used herein is generic to the monomeric, dimeric, and multimeric forms.

The term “Fc variant” refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn. International applications WO 97/34631 (published 25 September 1997) and WO 96/32478 describe exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated by reference. Thus, the term “Fc variant” comprises a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises sites that may be removed because they provide structural features or biological activity that are not required for the fusion molecules of the present invention. Thus, the term “Fc variant” comprises a molecule or sequence that lacks one or more native Fc sites or residues that affect or are involved in (1) disulfide bond formation, (2) incompatibility with a selected host cell (3) N-terminal heterogeneity upon
expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC). Fc variants are described in further detail in WO 00/24782, published May 4, 2000, which is hereby incorporated by reference in its entirety.

The term “Fc domain” encompasses native Fc and Fc variant molecules and sequences as defined above. As with Fc variants and native Fc’s, the term “Fc domain” includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

The term “multimer” as applied to Fc domains or molecules comprising Fc domains refers to molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

The term “dimer” as applied to Fc domains or molecules comprising Fc domains refers to molecules having two polypeptide chains associated covalently or non-covalently.

The terms “derivatizing” and “derivative” or “derivatized” comprise processes and resulting compounds respectively in which (1) the compound has a cyclic portion; for example, cross-linking between cysteinyl residues within the compound; (2) the compound is cross-linked or has a cross-linking site; for example, the compound has a cysteinyl residue and thus forms cross-linked dimers in culture or in vivo; (3) one or more peptidyl linkage is replaced by a non-peptidyl linkage; (4) the N-terminus is replaced by -NRR¹, NRC(O)R¹, -NRC(O)OR¹, -NRS(O)₂R¹, -
NHC(O)NHR, a succinimide group, or substituted or unsubstituted benzyloxy carbonyl-NH-, wherein R and R¹ and the ring substituents are as defined hereinafter; (5) the C-terminus is replaced by -C(O)R² or -NR³R⁴ wherein R², R³ and R⁴ are as defined hereinafter; and (6) compounds in which individual amino acid moieties are modified through treatment with agents capable of reacting with selected side chains or terminal residues. Derivatives are further described hereinafter.

The term "peptide" refers to molecules of 2 to 40 amino acids, with molecules of 3 to 20 amino acids preferred and those of 6 to 15 amino acids most preferred. Exemplary peptides may be randomly generated by any of the methods cited above, carried in a peptide library (e.g., a phage display library), or derived by digestion of proteins.

The term "randomized" as used to refer to peptide sequences refers to fully random sequences (e.g., selected by phage display methods) and sequences in which one or more residues of a naturally occurring molecule is replaced by an amino acid residue not appearing in that position in the naturally occurring molecule. Exemplary methods for identifying peptide sequences include phage display, E. coli display, ribosome display, yeast-based screening, RNA-peptide screening, chemical screening, rational design, protein structural analysis, and the like. Randomized peptides and methods of generating them appear in WO 00/24782, published May 4, 2000, which is hereby incorporated by reference in its entirety.

The term "pharmacologically active" means that a substance so described is determined to have activity that affects a medical parameter (e.g., T cell proliferation) or disease state (e.g., cancer, autoimmune disorders). Thus, pharmacologically active compounds comprise agonistic or mimetic and antagonistic compounds as defined below.
The terms "-mimetic" and "agonist" refer to a molecule having biological activity comparable to a protein (e.g., APRIL, AGP-3) that interacts with a protein of interest. These terms further include molecules that indirectly mimic the activity of a protein of interest, such as by potentiating the effects of the natural ligand of the protein of interest.

The terms "antagonist" or "inhibitor" refer to a molecule that blocks or in some way interferes with the biological activity of the associated protein of interest, or has biological activity comparable to a known antagonist or inhibitor of the associated protein of interest.

Additionally, physiologically acceptable salts of the compounds of this invention are also encompassed herein. By "physiologically acceptable salts" is meant any salts that are known or later discovered to be pharmaceutically acceptable. Some specific examples are: acetate; trifluoroacetate; hydrohalides, such as hydrochloride and hydrobromide; sulfate; citrate; tartrate; glycolate; and oxalate.

**Methods of Treatment**

The present invention concerns a method of inhibiting T cell proliferation in a mammal, which comprises administering a therapeutic agent comprising:

- a. a specific binding partner for TACI, wherein the specific binding partner has TACI antagonist activity;
- b. a specific binding partner for BCMA, wherein the specific binding partner has BCMA antagonist activity;
- c. both a and b; or
- d. a specific binding partner for TACI and BCMA, wherein the specific binding partner has TACI antagonist activity, BCMA antagonist activity or both.
The present invention also concerns a method of inhibiting APRIL activity in a mammal, which comprises administering a therapeutic agent comprising a through d above.

The invention also concerns a method of inhibiting TACI activity, BCMA activity, or both in a mammal, which comprises administering a specific binding partner for APRIL. This method may further comprise administering a specific binding partner for AGP-3.

Some indications benefit from an increase in the immune response. Accordingly, the invention further relates to a method of increasing T cell proliferation in a mammal, which comprises administering a therapeutic agent comprising:

a. a specific binding partner for TACI, wherein the specific binding partner has TACI agonist activity;

b. a specific binding partner for BCMA, wherein the specific binding partner has BCMA agonist activity;

c. both a and b; or

d. a specific binding partner for TACI and BCMA, wherein the specific binding partner has TACI agonist activity, BCMA agonist activity or both.

The invention also concerns a method of increasing APRIL activity in a mammal, which comprises administering a therapeutic agent comprising a through d above.

The inventors contemplate carrying out the foregoing methods of treatment with any of several different types of molecules, including small molecules, antibodies, and engineered peptides and fusion molecules described hereinafter. These molecules may also be used in assays to identify cells and tissues that express AGP-3, TACI, APRIL, or BCMA. The invention further concerns nucleic acids, vectors, and host cells useful in preparing such molecules.
The invention further concerns methods of identifying compounds that are useful in the aforementioned methods of use. Such compounds include nucleic acids, peptides, proteins, carbohydrates, lipids or small molecular weight organic molecules and may act either as agonists or antagonists of BCMA, TACI, AGP-3 or APRIL-protein activity.

AGP-3, APRIL, BCMA, and TACI are believed to play a role in regulation of immune function. Accordingly, these molecules, their soluble forms, and agonists and antagonists thereof may be useful for the diagnosis and/or treatment of inflammation and immune function diseases. Indications for antagonists include, but are not limited to the following:

- infections such as bacterial, fungal, protozoan and viral infections, especially HIV-1 or HIV-2;
- diarrhoea;
- psoriasis;
- inflammation;
- allergies;
- atopic dermatitis;
- respiratory allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung disease, hypersensitivity pneumonitis, eosinophilic pneumonia (e.g. Loeﬄer's syndrome, chronic eosinophilic pneumonia, interstitial lung disease (ILD), such as idiopathic pulmonary fibrosis or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, Sjögren's syndrome, polymyositis or dermatomyositis);
- systemic anaphylaxis or hypersensitivity responses;
- drug allergy;
- insect sting allergy;
• inflammatory bowel disease, such as Crohn's disease and ulcerative colitis;
• spondyloarthritis;
• scleroderma;
• psoriasis;
• inflammatory dermatosis such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, vasculitis (e.g. necrotizing, cutaneous and hypersensitivity vasculitis), eosinophilic myositis and eosinophilic fasciitis;
• autoimmune diseases such as rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, juvenile onset diabetes, glomerulonephritis, autoimmune thyroiditis and Behcet's disease;
• graft rejection, including allograft rejection or graft-versus-host disease;
• cancers with leukocyte infiltration of the skin or organs;
• reperfusion injury;
• atherosclerosis;
• certain haematologic malignancies;
• shock, including septic shock and endotoxic shock.

Agonists can be used for treating:
• immunosuppression e.g. in AIDS patients or individuals undergoing radiation therapy, chemotherapy, therapy for autoimmune disease or other drug therapy, and immunosuppression due congenital deficiency in receptor function or other causes; and
• infectious diseases such as parasitic diseases, including helminth infections, such as nematodes (round worms).

Compositions of matter
Any number of molecules may serve as specific binding partners within the present invention. Of particular interest are antibodies, peptides, and Fc-peptide fusion molecules.

**Antibodies.**

The invention also provides for an antibody or antigen binding domain thereof, or a fragment, variant, or derivative thereof, which binds to an epitope on any of the target molecules (APRIL, AGP-3, TACI, or BCMA) and has partial or complete agonist or antagonist activity. Preferably, the target molecule is mammalian, more preferably human, and may be in soluble or cell surface associated forms, or fragments, derivatives and variants thereof.

A number of methods for antibody generation are known in the art. All such methods are useful in generating molecules useful in accordance with the present invention. Conventionally, an antibody may be prepared by immunizing an animal with the target molecule (e.g., murine or human BCMA or TACI) or with an immunogenic fragment, derivative or variant thereof. In addition, an animal may be immunized with cells transfected with a vector containing a nucleic acid molecule encoding the target molecule such that the target molecule is expressed and associated with the surface of the transfected cells. Alternatively, specific binding partners that are antibodies may be obtained by screening a library comprising antibody or antigen binding domain sequences for binding to the target molecule. Such a library is conveniently prepared in bacteriophage as protein or peptide fusions to a bacteriophage coat protein which are expressed on the surface of assembled phage particles and the encoding DNA sequences contained within the phage particles (so-called "phage display library"). In one example, a phage display library contains DNA sequences encoding human antibodies, such as variable light and heavy
chains. Sequences binding to the target molecule may be further evolved by multiple rounds of mutagenesis and screening.

Specific binding partners that are antibodies or antigen binding domains may be tetrameric glycoproteins similar to native antibodies, or they may be single chain antibodies; for example, Fv, Fab, Fab′ or F(ab)′ fragments, bispecific antibodies, heteroantibodies, or other fragments, variants, or derivatives thereof, which are capable of binding the target molecule and partially or completely neutralize the target molecule activity. Antibodies or antigen binding domains may be produced in hybridoma cell lines (antibody-producing cells such as spleen cells fused to mouse myeloma cells, for example) or may be produced in heterologous cell lines transfected with nucleic acid molecules encoding said antibody or antigen binding domain.

Antibodies of the invention include polyclonal monospecific polyclonal, monoclonal, recombinant, chimeric, humanized, fully human, single chain and/or bispecific antibodies. Antibody fragments include those portions of an antibody that bind to an epitope on a target molecule. Examples of such fragments include Fab F(ab′), F(ab′), Fv, and sFv fragments. The antibodies may be generated by enzymatic cleavage of full-length antibodies or by recombinant DNA techniques, such as expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. An antigen is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen can have one or more epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective
manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens.

Polyclonal antibodies directed toward a target molecule generally are raised in animals (e.g., rabbits or mice) by multiple subcutaneous or intraperitoneal injections of the target molecule and an adjuvant. In accordance with the invention, it may be useful to conjugate the target molecule, or a variant, fragment, or derivative thereof to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-target antibody titer.

Monoclonal antibodies (mAbs) contain a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. A hybridoma producing a monoclonal antibody of the present invention may be cultivated in vitro, in situ, or in vivo. Production of high titers in vivo or in situ is a preferred method of production.

Monoclonal antibodies directed toward the target molecule are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include hybridoma methods of Kohler et al., *Nature* 256, 495-497 (1975), and the human B-cell hybridoma method, Kozbor, J. *Immunol.* 133, 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Harlow and Lane, *Antibodies:
A Laboratory Manual, Cold Spring Harbor Laboratory (1988); the contents of which references are incorporated entirely herein by reference.

Preferred specific binding partners include monoclonal antibodies which will inhibit partially or completely the binding of the human target molecule to its cognate ligand or receptor or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol., 92:589-601 (1983). Each of these references is incorporated herein by reference in its entirety.

Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with target polypeptides.

Chimeric antibodies are molecules in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine monoclonal antibodies have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric monoclonal antibodies are used.


A chimeric monoclonal antibody of the invention may be used as a therapeutic agent. In such a chimeric antibody, a portion of the heavy and/or light chain is identical with or homologous to corresponding sequence in antibodies derived from a particular species or belonging to one particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci.*, 81, 6851-6855 (1985).

As used herein, the term "chimeric antibody" includes monovalent, divalent or polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer (HL) formed by a chimeric H chain associated through disulfide bridges with a chimeric L chain. A divalent chimeric antibody is tetramer (H\textsubscript{2}L\textsubscript{2}) formed by two HL dimers associated through at least one disulfide bridge. A polyvalent chimeric antibody can also be produced, for example, by employing a C\textsubscript{\textepsilon} region that aggregates (e.g., from an IgM H chain, or \mu chain).

Murine and chimeric antibodies, fragments and regions of the present invention may comprise individual heavy (H) and/or light (L) immunoglobulin chains. A chimeric H chain comprises an antigen binding region derived from the H chain of a non-human antibody specific for the target molecule, which is linked to at least a portion of a human H chain C region (C\textsubscript{\textepsilon}H\textsubscript{3}), such as CH\textsubscript{1} or CH\textsubscript{2}.

A chimeric L chain according to the present invention comprises an antigen binding region derived from the L chain of a non-human antibody
specific for the target molecule, linked to at least a portion of a human L chain C region (C\(_2\)).

Specific binding partners, such as antibodies, fragments, or derivatives, having chimeric H chains and L chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to known method steps, e.g., according to Ausubel et al., eds. Current Protocols in Molecular Biology, Wiley Interscience, N.Y. (1993), and Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988). The contents of these references are incorporated entirely herein by reference. With this approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

As an example, the antigen binding region of the specific binding partner (such as a chimeric antibody) of the present invention is preferably derived from a non-human antibody specific for the human analog of the target molecule. Preferred sources for the DNA encoding such a non-human antibody include cell lines which produce antibodies, such as hybrid cell lines commonly known as hybridomas.

The invention also provides for fragments, variants and derivatives, and fusions of anti-target antibodies, wherein the terms "fragments", "variants", "derivatives" and "fusions" are defined herein. The invention encompasses fragments, variants, derivatives, and fusions of anti-target antibodies which are functionally similar to the unmodified antibody, that is, they retain at least one of the activities of the unmodified antibody. In
addition to the modifications set forth above, also included is the addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins. The fragments, variants, derivatives and fusions of the antibodies can be produced from any of the hosts of this invention.

Suitable fragments include, for example, Fab, Fab’, F(ab’)2, Fv and scFv. These fragments lack the Fc fragment of an intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody. See Wahl et al., J. Nucl. Med., 24:316-325 (1983). These fragments are produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab’)2 fragments). The identification of these antigen binding regions and/or epitopes recognized by monoclonal antibodies of the present invention provides the information necessary to generate additional monoclonal antibodies with similar binding characteristics and therapeutic or diagnostic utility that parallel the embodiments of this invention.

Variants of specific binding partners are also provided. In one embodiment, variants of antibodies and antigen binding domains comprise changes in light and/or heavy chain amino acid sequences that are naturally occurring or are introduced by in vitro engineering of native sequences using recombinant DNA techniques. Naturally occurring variants include "somatic" variants which are generated in vivo in the corresponding germ line nucleotide sequences during the generation of an antibody response to a foreign antigen.

Variants of antibodies and antigen binding domains are also prepared by mutagenesis techniques known in the art. In one example, amino acid changes may be introduced at random throughout an antibody coding region and the resulting variants may be screened for a desired
activity, such as binding affinity for the target molecule. Alternatively, amino acid changes may be introduced in selected regions of an antibody, such as in the light and/or heavy chain CDRs, and framework regions, and the resulting antibodies may be screened for binding to the target molecule or some other activity. Amino acid changes encompass one or more amino acid substitutions in a CDR, ranging from a single amino acid difference to the introduction of all possible permutations of amino acids within a given CDR, such as CDR3. In another method, the contribution of each residue within a CDR to target binding may be assessed by substituting at least one residue within the CDR with alanine (Lewis et al. (1995), Mol. Immunol. 32: 1065-72). Residues which are not optimal for binding to the target molecule may then be changed in order to determine a more optimum sequence. Also encompassed are variants generated by insertion of amino acids to increase the size of a CDR, such as CDR3. For example, most light chain CDR3 sequences are nine amino acids in length. Light chain CDR3 sequences in an antibody which are shorter than nine residues may be optimized for binding to the target molecule by insertion of appropriate amino acids to increase the length of the CDR.

In one embodiment, antibody or antigen binding domain variants comprise one or more amino acid changes in one or more of the heavy or light chain CDR1, CDR2 or CDR3 and optionally one or more of the heavy or light chain framework regions FR1, FR2 or FR3. Amino acid changes comprise substitutions, deletions and/or insertions of amino acid residues.

Variants may also be prepared by "chain shuffling" of either light or heavy chains. Marks et al. (1992), Biotechnology 10: 779-83. Typically, a single light (or heavy) chain is combined with a library having a repertoire of heavy (or light) chains and the resulting population is screened for a desired activity, such as binding to the target molecule. This technique
permits screening of a greater sample of different heavy (or light) chains in combination with a single light (or heavy) chain than is possible with libraries comprising repertoires of both heavy and light chains.

The specific binding partners of the invention can be bispecific.

Bispecific specific binding partners of this invention can be of several configurations. For example, bispecific antibodies resemble single antibodies (or antibody fragments) but have two different antigen binding sites (variable regions). Bispecific antibodies can be produced by chemical techniques (see e.g., Kranz et al., *Proc. Natl. Acad. Sci. USA*, 78:5807 (1981)), by "polydoma" techniques (see U.S. Pat. No. 4,474,893 to Reading) or by recombinant DNA techniques. For example, a bispecific antibody in accordance with this invention may bind to APRIL and AGP-3. As another example, a bispecific antibody may bind to TACI and BCMA.

The specific binding partners of the invention may also be heteroantibodies. Heteroantibodies are two or more antibodies, or antibody binding fragments (Fab) linked together, each antibody or fragment having a different specificity.

The invention also relates to "humanized" antibodies. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into a human antibody from a source which is non-human. In general, non-human residues will be present in CDRs. Humanization can be performed following methods known in the art (Jones et al., *Nature* 321, 522-525 (1986); Riechmann et al., *Nature*, 332, 323-327 (1988); Verhoeyen et al., *Science* 239, 1534-1536 (1988)), by substituting rodent complementarily-determining regions (CDRs) for the corresponding regions of a human antibody.

The specific binding partners of the invention, including chimeric, CDR-grafted, and humanized antibodies can be produced by recombinant
methods known in the art. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein and known in the art. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells.

Fully human antibodies may be produced by expression of recombinant DNA transfected into host cells or by expression in hybridoma cells as described above.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. To do so, antibody-specific messenger RNA molecules are extracted from immune system cells taken from an immunized animal, and transcribed into complementary DNA (cDNA). The cDNA is then cloned into a bacterial expression system. One example of such a technique suitable for the practice of this invention uses a bacteriophage lambda vector system having a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional Fab fragments for those which bind the antigen. Such target molecule specific binding partners (Fab fragments with specificity for the target molecule) are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Also within the scope of the invention are techniques developed for the production of chimeric antibodies by splicing the genes from a mouse antibody molecule of appropriate antigen-specificity together with genes from a human antibody molecule of appropriate biological activity, such as the ability to activate human complement and mediate ADCC.

Nature, 312:604 (1984)). One example is the replacement of a Fc region with that of a different isotype. Specific binding partners such as antibodies produced by this technique are within the scope of the invention.

In a preferred embodiment of the invention, the antibodies are fully human antibodies. Thus encompassed by the invention are antibodies that bind target molecules and are encoded by nucleic acid sequences which are naturally occurring somatic variants of human germline immunoglobulin nucleic acid sequence, and fragments, synthetic variants, derivatives and fusions thereof. Such antibodies may be produced by any method known in the art. Exemplary methods include immunization with a target antigen (any target polypeptide capable of eliciting an immune response, and optionally conjugated to a carrier) of transgenic animals (e.g., mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. See, for example, Jakobovits et al., Proc. Natl. Acad. Sci., 90, 2551-2555 (1993); Jakobovits et al., Nature, 362, 255-258 (1993); Bruggermann et al., Year in Immunol., 7, 33 (1993).

Alternatively, human antibodies may be generated through the in vitro screening of phage display antibody libraries. See Hoogenboom et al., J. Mol. Biol., 227, 381 (1991); Marks et al., J. Mol. Biol., 222, 581 (1991), incorporated herein by reference. Various antibody-containing phage display libraries have been described and may be readily prepared by one skilled in the art. Libraries may contain a diversity of human antibody sequences, such as human Fab, Fv, and scFv fragments, that may be screened against an appropriate target. As described further below, phage display libraries may comprise peptides or proteins other than antibodies which may be screened to identify specific binding partners of the target molecule.
An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the monoclonal antibody with the monoclonal antibody to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. Pat. No. 4,699,880, which is herein entirely incorporated by reference. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original monoclonal antibody which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

**Peptides and Peptide fusion molecules.**

The patent application WO 00/24782, published May 4, 2000, mentioned previously herein describes in detail various peptide generation techniques. That patent application further describes various derivatives and fusion molecules.

In particular, a peptide used as a specific binding partner may be comprised within a molecule of the formula

\[(X^i)_a-P^i-(X^o)_b\]

wherein:

- \(F^i\) is a vehicle;
- \(X^i\) and \(X^o\) are each independently selected from \(-(L^i)_c-P^i\), \(-(L^i)_c-P^i-(L^o)_d-P^d-(L^o)_e-P^e\), and \(-(L^i)_c-P^i-(L^o)_d-P^d-(L^o)_e-P^e\);
- \(P^i\), \(P^d\), \(P^e\), and \(P^o\) are each independently peptide sequences, wherein
at least one is a specific binding partner;

L', L^2, L^3, and L^4 are each independently linkers; and

a, b, c, d, e, and f are each independently 0 or 1, provided that at
least one of a and b is 1.

5 Preferably, such a molecule comprises a structure of the formulae

X^1-P^1

or

F^2-X^2.

A more preferred molecule comprises a structure of the formula

10 F^1-(L^1)_c-P^1.

or a structure of the formula

F^1-(L^1)_c-P^1-(L^3)_c-P^2

wherein P^1 and/or P^2 is a specific binding partner for TACI or BCMA.
Such molecules facilitate modulation of both TACI and BCMA; for

15 example, one of P^1 and P^2 is a specific binding partner for TACI and the
other is a specific binding partner for BCMA. Conversely, in a ligand
inhibitor, one of P^1 and P^2 is a specific binding partner for APRIL and the
other is a specific binding partner for AGP-3.

For all of these molecules, the preferred vehicle is an Fc domain.

20 Among Fc domains, IgG Fc, particularly IgG1, are preferred.

The Fc domains, linkers, and processes of preparation of the
foregoing molecules is described in WO 00/24782, published May 4, 2000.

Soluble receptor fragments

Another class of specific binding partners are soluble receptor

fragments. Of particular interest are the fragments identified in the

figures:

a. the extracellular region of TACI (SEQ ID NO: 15).

b. the extracellular region of BCMA (SEQ ID NO: 6).

c. the consensus region of TACI (SEQ ID NO: 16).
d. the consensus region of BCMA (SEQ ID NO: 7).

e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13).

These molecules have the heretofore unrecognized advantage of binding both APRIL and AGP-3. Like the aforementioned peptides, these specific binding partners may also be covalently linked to a vehicle, preferably an Fc domain.

Muteins

Additional useful peptide sequences may result from conservative and/or non-conservative modifications of the amino acid sequences of the aforementioned antibodies, peptides, Fc-fusion peptides, and receptor fragments.

Conservative modifications will produce molecules having functional and chemical characteristics similar to those of the molecule from which such modifications are made. In contrast, substantial modifications in the functional and/or chemical characteristics of the molecules may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the size of the molecule.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (see, for example,

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the molecule sequence, or to increase or decrease the affinity of the molecules described herein. Exemplary amino acid substitutions are set forth in Table 3.
Table 3—Amino Acid Substitutions

<table>
<thead>
<tr>
<th>Original Residues</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>Val, Leu, Ile</td>
<td>Val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>Lys, Gln, Asn</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>Gln</td>
<td>Gln</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>Glu</td>
<td>Glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>Ser, Ala</td>
<td>Ser</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>Asn</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>Asp</td>
<td>Asp</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>Pro, Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>His (H)</td>
<td>Asn, Gln, Lys, Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>Leu, Val, Met, Ala, Phe, Norleucine</td>
<td>Leu</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>Norleucine, Ile, Val, Met, Ala, Phe</td>
<td>Ile</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>Arg, 1,4 Diaminobutyric Acid, Gln, Asn</td>
<td>Arg</td>
</tr>
<tr>
<td>Met (M)</td>
<td>Leu, Phe, Ile</td>
<td>Leu</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>Leu, Val, Ile, Ala, Tyr</td>
<td>Leu</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>Ala</td>
<td>Gly</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>Thr, Ala, Cys</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>Ser</td>
<td>Ser</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>Tyr, Phe</td>
<td>Tyr</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>Trp, Phe, Thr, Ser</td>
<td>Phe</td>
</tr>
<tr>
<td>Val (V)</td>
<td>Ile, Met, Leu, Phe, Ala, Norleucine</td>
<td>Leu</td>
</tr>
</tbody>
</table>
In certain embodiments, conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

As noted in the foregoing section "Definition of Terms," naturally occurring residues may be divided into classes based on common sidechain properties that may be useful for modifications of sequence. For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the molecule that are homologous with non-human orthologs, or into the non-homologous regions of the molecule. In addition, one may also make modifications using P or G for the purpose of influencing chain orientation.

In making such modifications, the hydrophobic index of amino acids may be considered. Each amino acid has been assigned a hydrophobic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., J. Mol. Biol., 157: 105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydrophobic index or score and still retain a similar biological activity. In making changes based upon the hydrophobic index, the substitution of amino acids whose hydrophobic indices are within ±2 is preferred, those which
are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in the foregoing sequences using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a molecule to similar molecules. With such a comparison, one can identify residues and portions of the molecules that are conserved.
among similar polypeptides. It will be appreciated that changes in areas of a molecule that are not conserved relative to such similar molecules would be less likely to adversely affect the biological activity and/or structure of the molecule. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the molecule structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar molecules that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a molecule that correspond to amino acid residues that are important for activity or structure in similar molecules. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of the molecules.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polymolecules. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a molecule with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such data could be used to
gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moul J., Curr. Op. in Biotech., 7(4): 422-427 (1996), Chou et al., Biochemistry, 13(2): 222-245 (1974); Chou et al., Biochemistry, 11(2): 211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47: 45-148 (1978); Chou et al., Ann. Rev. Biochem., 47: 251-276 and Chou et al., Biophys. J., 26: 367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide’s or protein’s structure. See Holm et al., Nucl. Acid. Res., 27(1): 244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3): 369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

253: 164-170 (1991); Gribskov et al., Meth. Enzym., 183: 146-159 (1990);
"evolutionary linkage" (See Home, supra, and Brenner, supra).

Production of specific binding partners

When the specific binding partner to be prepared is a proteinaceous
specific binding partner, such as an antibody or an antigen binding
domain or an Fc-peptide fusion molecule, various biological or chemical
methods for producing said partner are available.

Biological methods are preferable for producing sufficient
quantities of a specific binding partner for therapeutic use. Standard
recombinant DNA techniques are particularly useful for the production of
antibodies and antigen binding domains of the invention. Exemplary
expression vectors, host cells and methods for recovery of the expressed
product are described below.

A nucleic acid molecule encoding an antibody or antigen binding
domain is inserted into an appropriate expression vector using standard
ligation techniques. The vector is typically selected to be functional in the
particular host cell employed (i.e., the vector is compatible with the host
cell machinery such that amplification of the gene and/or expression of
the gene can occur). A nucleic acid molecule encoding an antibody may
be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems)
and/or eukaryotic host cells. Selection of the host cell will depend in part
on whether an antibody is to be post-translationally modified (e.g.,
glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian
host cells are preferable. For a review of expression vectors, see Meth.
(1990)

Typically, expression vectors used in any host cells will contain one
or more of the following components: a promoter, one or more enhancer
sequences, an origin of replication, a transcriptional termination sequence, a
complete intron sequence containing a donor and acceptor splice site, a
leader sequence for secretion, a ribosome binding site, a polyadenylation
sequence, a polylinker region for inserting the nucleic acid encoding the
polypeptide to be expressed, and a selectable marker element. Each of
these sequences is discussed in more detail below.

The vector components may be homologous (i.e., from the same
species and/or strain as the host cell), heterologous (i.e., from a species
other than the host cell species or strain), hybrid (i.e., a combination of
different sequences from more than one source), synthetic, or native
sequences which normally function to regulate immunoglobulin
expression. As such, a source of vector components may be any
prokaryotic or eukaryotic organism, any vertebrate or invertebrate
organism, or any plant, provided that the components are functional in,
and can be activated by, the host cell machinery.

An origin of replication is selected based upon the type of host cell
being used for expression. For example, the origin of replication from the
plasmid pBR322 (Product No. 303-3s, New England Biolabs, Beverly, MA)
is suitable for most Gram-negative bacteria while various origins from
SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV) or
papillomaviruses (such as HPV or BPV) are useful for cloning vectors in
mammalian cells. Generally, the origin of replication component is not
needed for mammalian expression vectors (for example, the SV40 origin is
often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the
end of a polypeptide coding regions and serves to terminate transcription.
Usually, a transcription termination sequence in prokaryotic cells is a G-C
rich fragment followed by a poly T sequence. While the sequence is easily
cloned from a library or even purchased commercially as part of a vector,
it can also be readily synthesized using methods for nucleic acid synthesis such as those described above.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the marker present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection partner in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes an antibody. As a result, increased quantities of an antibody are synthesized from the amplified DNA.

A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically
located 3' to the promoter and 5' to the coding sequence of the polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (i.e., having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth above and used in a prokaryotic vector.

A leader, or signal, sequence is used to direct secretion of a polypeptide. A signal sequence may be positioned within or directly at the 5' end of a polypeptide coding region. Many signal sequences have been identified and may be selected based upon the host cell used for expression. In the present invention, a signal sequence may be homologous (naturally occurring) or heterologous to a nucleic acid sequence encoding an antibody or antigen binding domain. A heterologous signal sequence selected should be one that is recognized and processed, i.e., cleaved, by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process a native immunoglobulin signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, a native immunoglobulin signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In most cases, secretion of an antibody or antigen binding domain from a host cell will result in the removal of the signal peptide from the antibody. Thus the mature antibody will lack any leader or signal sequence.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may
alter the peptidase cleavage site of a particular signal peptide, or add
prosequences, which also may affect glycosylation. The final protein
product may have, in the -1 position (relative to the first amino acid of the
mature protein) one or more additional amino acids incident to
expression, which may not have been totally removed. For example, the
final protein product may have one or two amino acid found in the
peptidase cleavage site, attached to the N-terminus. Alternatively, use of
some enzyme cleavage sites may result in a slightly truncated form of the
desired polypeptide, if the enzyme cuts at such area within the mature
polypeptide.

The expression vectors of the present invention will typically
contain a promoter that is recognized by the host organism and operably
linked to a nucleic acid molecule encoding an antibody or antigen binding
domain. Either a native or heterologous promoter may be used depending
the host cell used for expression and the yield of protein desired.

Promoters suitable for use with prokaryotic hosts include the beta-
lactamase and lactose promoter systems; alkaline phosphatase, a
tryptophan (trp) promoter system; and hybrid promoters such as the tac
promoter. Other known bacterial promoters are also suitable. Their
sequences have been published, thereby enabling one skilled in the art to
ligate them to the desired DNA sequence(s), using linkers or adapters as
needed to supply any required restriction sites.

Suitable promoters for use with yeast hosts are also well known in
the art. Yeast enhancers are advantageously used with yeast promoters.

Suitable promoters for use with mammalian host cells are well known and
include those obtained from the genomes of viruses such as polyoma
virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine
papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus,
hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other
suitable mammalian promoters include heterologous mammalian promoters, e.g., heat-shock promoters and the actin promoter.

Additional promoters which may be used for expressing the specific binding partners of the invention include, but are not limited to:

which is active in the liver (Kelsey et al. (1987), *Genes and Devel.*, 1: 161-171); the beta-globin gene control region which is active in myeloid cells (Mogram et al. (1985), *Nature*, 315: 338-340; Kollias et al. (1986), *Cell*, 46: 89-94); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al. (1987), *Cell*, 48: 703-712); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani (1985), *Nature*, 314: 283-286); and the gonadotropin releasing hormone gene control region which is active in the hypothalamus (Mason et al. (1986), *Science*, 234: 1372-8).

An enhancer sequence may be inserted into the vector to increase transcription in eucaryotic host cells. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide coding region, it is typically located at a site 5' from the promoter.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional possible vectors include, but are not limited to, cosmids, plasmids or modified viruses, but the vector system must be compatible
with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript\textsuperscript{\textregistered} plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPOTM TA Cloning\textsuperscript{\textregistered} Kit, PCR2.1\textsuperscript{\textregistered} plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, or other known techniques.

Host cells of the invention may be prokaryotic host cells (such as E. coli) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). Prokaryotic host cells such as E. coli produce unglycosylated protein; for example, unglycosylated shBCMA and unglycosylated shTACI, which may possess advantages over the glycosylated eukaryotic molecules. The host cell, when cultured under appropriate conditions, expresses an antibody or antigen binding domain of the invention which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). Selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), Manassas, VA. Examples include mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR- cells (Urlaub et al. (1980), Proc. Natl. Acad. Sci. USA 97, 4216-20), human embryonic kidney (HEK)
293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the American Type Culture Collection, Manassas, VA). Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, ATCC No. 33694) DH5α, DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of *Pseudomonas* spp., *B. subtilis*, other *Bacillus* spp., *Streptomyces* spp., and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerevisae*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for
example in Kitts et al. (1993), *Biotechniques*, 14: 810-7, Lucklow (1993),
4566-79. Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

Transformation or transfection of a nucleic acid molecule encoding
a specific binding partner into a selected host cell may be accomplished by
well known methods including methods such as calcium chloride,
electroporation, microinjection, lipofection or the DEAE-dextran method.
The method selected will in part be a function of the type of host cell to be
used. These methods and other suitable methods are well known to the
skilled artisan, and are set forth, for example, in Sambrook et al., *supra*.

One may also use transgenic animals to express glycosylated
specific binding partners, such as antibodies and antigen binding domain.
For example, one may use a transgenic milk-producing animal (a cow or
goat, for example) and obtain glycosylated binding partners in the animal
milk. Alternatively, one may use plants to produce glycosylated specific
binding partners.

Host cells comprising (as by transformation or transfection) an
expression vector encoding a specific binding partner of the target
molecule may be cultured using standard media well known to the skilled
artisan. The media will usually contain all nutrients necessary for the
growth and survival of the cells. Suitable media for culturing *E. coli* cells
are for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable
media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of
which may be supplemented with serum and/or growth factors as
required by the particular cell line being cultured. A suitable medium for
insect cultures is Grace’s medium supplemented with yeastolate,
lactalbumin hydrolysate, and/or fetal calf serum as necessary.

Typically, an antibiotic or other compound useful for selective
growth of transfected or transformed cells is added as a supplement to the
media. The compound to be used will be dictated by the selectable
marker element present on the plasmid with which the host cell was
transformed. For example, where the selectable marker element is
kanamycin resistance, the compound added to the culture medium will be
kanamycin. Other compounds for selective growth include ampicillin,
tetracycline and neomycin.

The amount of an antibody or antigen binding domain produced
by a host cell can be evaluated using standard methods known in the art.
Such methods include, without limitation, Western blot analysis, SDS-
polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis,
HPLC separation, immunoprecipitation, and/or activity assays.

Purification of a specific binding partner that has been secreted into
the cell media can be accomplished using a variety of techniques
including affinity, immunoaffinity or ion exchange chromatography,
molecular sieve chromatography, preparative gel electrophoresis or
isoelectric focusing, chromatofocusing, and high pressure liquid
chromatography. For example, antibodies comprising a Fc region may be
conveniently purified by affinity chromatography with Protein A, which
selectively binds the Fc region. Modified forms of an antibody or antigen
binding domain may be prepared with affinity tags, such as hexahistidine
or other small peptide such as FLAG (Eastman Kodak Co., New Haven,
CT) or myc (Invitrogen) at either its carboxyl or amino terminus and
purified by a one-step affinity column. For example, polyhistidine binds
with great affinity and specificity to nickel, thus an affinity column of
nickel (such as the Qiagen® nickel columns) can be used for purification
of polyhistidine-tagged specific binding partners. See for example,
Ausubel et al., eds. (1993), Current Protocols in Molecular Biology, Section
10.11.8, John Wiley & Sons, New York. In some instances, more than one
purification step may be required.
Specific binding partners of the invention which are expressed in procaryotic host cells may be present in soluble form either in the periplasmic space or in the cytoplasm or in an insoluble form as part of intracellular inclusion bodies. Specific binding partners can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

Soluble forms of an antibody or antigen binding domain present either in the cytoplasm or released from the periplasmic space may be further purified using methods known in the art, for example Fab fragments are released from the bacterial periplasmic space by osmotic shock techniques.

If an antibody or antigen binding domain has formed inclusion bodies, they can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with chaotropic partner such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing partner such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The soluble specific binding partner can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate a solublized antibody or antigen binding domain, isolation may be accomplished using standard methods such as those set forth below and in Marston et al. (1990), Meth. Enz., 182: 264-75.

In some cases, an antibody or antigen binding domain may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating
disulfide linkages, can be used to restore biological activity. Such
methods include exposing the solubilized polypeptide to a pH usually
above 7 and in the presence of a particular concentration of a chaotrope.
The selection of chaotrope is very similar to the choices used for inclusion
body solubilization, but usually the chaotrope is used at a lower
concentration and is not necessarily the same as chaotropes used for the
solubilization. In most cases the refolding/oxidation solution will also
contain a reducing partner or the reducing partner plus its oxidized form
in a specific ratio to generate a particular redox potential allowing for
disulfide shuffling to occur in the formation of the protein’s cysteine
bridge(s). Some of the commonly used redox couples include
cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride,
dithiothreitol(DTT)/dithiane DTT, and 2-mercaptoethanol(bME)/dithio-
b(NE). In many instances, a cosolvent may be used or may be needed to
increase the efficiency of the refolding and the more common repartners
used for this purpose include glycerol, polyethylene glycol of various
molecular weights, arginine and the like.

Specific binding partners of the invention may also be prepared by
chemical synthesis methods (such as solid phase peptide synthesis) using
techniques known in the art such as those set forth by Merrifield et al.
Sci. USA, 82: 5132; and Stewart and Young (1984), Solid Phase Peptide
Synthesis, Pierce Chemical Co., Rockford, IL. Such polypeptides may be
synthesized with or without a methionine on the amino terminus.

Chemically synthesized antibodies and antigen binding domains may be
oxidized using methods set forth in these references to form disulfide
bridges. Antibodies so prepared will retain at least one biological activity
associated with a native or recombinantly produced antibody or antigen
binding domain.
The invention will now be further described by specific experimental examples. These examples are meant to be illustrative rather than limiting.

**Working Examples**

5 **Materials and Methods**

**Isolation of BCMA and TACI cDNA**

Mouse and human BCMA cDNA were isolated by PCR using the mouse BCMA sense primer

\[ 5\prime\text{-CACAATACCTGTGGCCCTCTTAAGAG-3}\prime \]

(SEQ ID NO: 25),

and antisense primer

\[ 5\prime\text{-TGGTAAACGGTCATCCTAACGACATC-3}\prime \]

(SEQ ID NO: 26),

the human BCMA sense primer

\[ 5\prime\text{-TTACCTGTCCCTCAGGCTGTTCT-3}\prime \]

(SEQ ID NO: 27),

and antisense primer

\[ 5\prime\text{-CATAGAAAACCAAGGAAGTTTCTACC-3}\prime \]

(SEQ ID NO: 28).

10 For isolation of human TACI cDNA, the sense primer

\[ 5\prime\text{-AGCATCCTGAGTAATGAGTGGCCTGG-3}\prime \]

(SEQ ID NO: 29)

and antisense primer

\[ 5\prime\text{-GTGATGACGACCTACAGCTGCACGTGGG-3}\prime \]

(SEQ ID NO: 30)

were used. Poly (A)+ RNA from the mouse B lymphoma cell line –A20 and human lymph Node were reverse –transcribed and cDNA were synthesized by using the Smart RACE cDNA amplification Kit (Clontech, Palo Alto, California). The full-length cDNA of mouse and human BCMA
genes as well as human TACI gene were cloned into pcDNA3 vector for mammalian cell expression (Invitrogen, Carlsbad, California).

**Recombinant proteins**

Soluble murine APRIL-Flag protein was generated by fusing Flag sequence in frame to the N-terminus of APRIL amino acid 101-239. Soluble mAPRIL-Flag protein was expressed in *E. coli* and the refolded protein was affinity-purified by anti-Flag M2 antibody column. Fc-tagged AGP3 protein was generated by fusing OPG signal peptide followed by human IgG·1 Fc in frame to the N-terminus of AGP3 amino acid 128-285. The protein was expressed in baculovirus and purified with protein A sepharose column. Soluble TACI protein (amino acid 1-165) and BCMA protein (amino acid 4-55) followed by human IgG·γ1 Fc in frame was expressed in *E. coli*. The inclusion bodies formed were solubilized. The refolded protein was purified by cation exchange chromatography.

**In vivo study**

B6 mice (6-8 weeks old) were purchased from Charles River Laboratories and murine APRIL-Flag and other TNF proteins were injected i.p. of 1 mg/kg/day for 5 days. On day 7, cells from mouse spleens and mesenteric lymph nodes were collected and B and T cell activation and differentiation was analyzed by FACS using specific monoclonal antibodies staining.

**Cell lines and proliferation assays**

293 human kidney epithelial cells, Raji Burkitt lymphoma, human T lymphoblastoma Jurkat cells and A20, mouse B lymphoma cell line were purchased from the American Type Culture Collection (Rockville, Maryland). Raji, Jurkat and A20 cells were maintained in a complete medium of RPMI-1640 (Life Technologies) supplemented with 10%fetal bovine serum (HyClone, Logan, Utah) and 25 mM HEPES. 293 cells were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies) with
10% fetal bovine serum. The proliferation of cells were determined by incubating 5x10^4 cells/well in 100 μL medium with the indicated concentration of APRIL-flag protein using the celltiter 96 AQ proliferation assay (Promega Corp., Madison, WI) following the manufacturer’s instructions. Alternatively, cells were pulsed for 18 h with ^3H thymidine (0.5 μCi/well), after harvesting cells, ^3H thymidine incorporation was monitored by liquid scintillation counting.

**Transfection and Flow cytometric analysis**

For 293 cell expressing BCMA and TACI receptor, 2x10^6 293 cells were plated into 6 well plate, cells were transfected with lipofectAMINE 2000 following the manufacturer’s procedure (Life Technologies), 48 h after transfection, cells were collected and incubated at 4 C with 1 μg/ml APRIL-Flag ligand or Blys (AGP3)-Fc ligand for 60 min, after washing 3 times with PBS (containing 2% FBS), cells were stained with FITC-conjugated secondary antibody for 30 min, then washed 3 times with PBS and fluorescence was analyzed by FACS scanner (Becton Dickinson, Mountain View, California).

**Determination of the binding affinities of APRIL and TALL-1 for BCMA and TACI**

Biomolecular interaction analysis (BIA) was performed using a BIACORE 2000 (Biacore AB, Uppsala, Sweden). The receptors, BCMA-Fc and TACI-Fc (2 μg/ml in 10 mM sodium acetate, pH 4.5), were immobilized on Sensor Chip CM5 using the BIACORE standard amine coupling procedure. An immobilization level of approximately 120 RU’s was achieved. The analytes, Flag-APRIL and Fc-AGP-3 were diluted between 100 nM-0.01 nM in running buffer (10 mM HEPES, 0.5 M NaCl, 3 mM EDTA, 0.005% Tween 20, 2mg/ml CM dextran, pH 6.8). The analytes were injected over an immobilized receptor surface for 2 minutes at 50 μl/min and allowed to dissociate for 10 minutes. Bound protein was
removed by a 1 minute injection of 50 mM HCl. Binding affinities were determined using a 1:1 Langmuir model (BIA Evaluation software Version 3.1.2, BIACORE).

**T cell co-stimulation assay**

T cells from the spleens of C57 BI/6 mice were purified by negative selection through a murine T cell enrichment column (R&D Systems). T cells (1x10^6 per well) were cultured in the absence or presence of various APRIL-Flag protein for 48 hr. Alternatively, 96 wells plates were precoated with subliminal quantities of anti-CD3 antibody, T cells were treated with APRIL-Flag protein for 72 hr, pulsed during the last 18 hr with 1 μCi of ^3^H thymidine and harvested to count the incorporation radioactivity.

**B cell proliferation and Ig secretion**

Mouse B cell were negatively selected from spleens by mouse B cell recovery column (Cedarlane, Hornby, Ontario Canada). 1x10^6/ml were seeded in 96-well flat bottom tissue culture plates in medium (RPMI-1640, 5% FBS, 5x10^-5 M 2 ME, affinity-purified goat anti-mouse IgM 2.5 μg/ml Pharmingen, San Diego). B cells were then treated with APRIL-Flag protein plus different concentration of soluble BCMA-Fc protein for 72 hr and culture received 1 μCi of ^3^H thymidine during the last 18 hr. proliferation of B cell was quantitated by measuring the incorporation of radioactivity.

For analysis of Ig secretion from B cells, purified B cells 5x10^6/ml were cultured in 96-well flat bottom tissue culture plates in the presence of APRIL-Flag for six days. The culture supernatant were harvested and IgG, IgM and IgA levels were determined by an isotype specific sandwich ELISA technique. Ig concentration in test samples were determined by comparing triplicate test values with isotype control standard.

**Induction and detection of anti-keyhole limpet hemocyanin (KLH) and anti-Pneumovax antibodies.**
Mice (Balb/c females of 9-11 wk and 19-21 g, Charles River Laboratories, Wilmington, MA) were immunized on day 0 with 100 µg of KLH (Pierce, Rockford, IL) in CFA s.c. or with 115 µg of Pneumovax (Merck, West Point, PA) i.p. Starting on day 0, mice received 7 daily i.p. injections of 5 mg/Kg of either TACI-Fc or BCMA-Fc fusion proteins or non-fused Fc and were then bled on day 7. Anti-KLH and anti-Pneumovax IgG and IgM were measured in serum by ELISA. Briefly, for the measurement of anti-KLH antibodies, plates were coated with KLH in PBS, blocked, and added with dilutions of standard and test samples. Captured anti-KLH IgG or IgM were revealed using anti-IgG or anti-IgM biotinylated antibodies and neutravidin-conjugated HRP. For the measurement of anti-Pneumovax IgM, plates were coated with Pneumovax using poly-L-lysine, blocked, and added with dilutions of standard and test samples. Captured anti-Pneumovax IgM were revealed using an anti-IgM biotinylated antibody and neutravidin-conjugated HRP. Results were compared with the Student t test.

Results

G70/APRIL in vitro function

Human and mouse G70 also called APRIL was isolated and characterized (Figures 1, 2, and 3). FLAG-tagged soluble mouse G70 (smG70) was produced in E. coli purified and refolded (Figure 2). Soluble G70 (smG70) specifically stimulates B and T cell lymphoma cell proliferation in a dose-dependent manner (Figure 4). Furthermore, soluble G70:

1) specifically binds to cell-surface receptors expressed on human B and T lymphoma cells (Figure 5);
2) specifically stimulates proliferation of purified human peripheral blood B and T cells (Figure 6);
3) stimulates proliferation of purified murine spleen B and T cells in a dose-dependent manner (Figure 7);
4) acts synergistically with anti-CD28 antibody to stimulate proliferation of purified murine T cells (Figure 8);
5) has a strong costimulatory activity on purified murine T cells (Figure 9) in the presence of sub-optimal concentration of the T cell receptor activator: anti-CD3 antibody.

**G70/APRIL in vivo function**

A series of experiments were performed to elucidate soluble

G70/APRIL’s biological activity in normal mice in vivo. Each group consisted of 5 mice (BDF-1, 8 weeks of age, dosed at 1 mg/kg/day, 0.2 ml for 5 days). Spleen, thymus and mesenteric lymph nodes from three mice of each group was used for FACS analysis using a panel of T cell and B cell surface marker antibodies and all the mice were analyzed by standard necropsy and pathological analysis.

Spleen (Table 1A): murine soluble G70 caused an average about 60% decrease in the percentage of CD3+ T cells. In addition, there was an average 5-fold increase in T-helper cells activation and an average 22-fold increase in cytotoxic T cell activation as measured by IL-2 receptor expression. In addition the percentage of immature B cells increased about 2-fold while the percentage of mature B-cells increased 3- to 4-fold. The total percentage of lymphocytes (T+B) was unchanged compared to control.

Mesenteric Lymph Nodes (Table 1B): soluble G70 treated mice had an average of 25% decrease in the percentage of T cells. There was an average 3-fold increase in % activated T-helper cells and 36-fold increase in activated cytotoxic T-cells as measured by CD25/IL-2 receptor expression. In addition the percentage of immature B cells was increased on average 2-fold whereas mature B cells were up on average 4-fold.
In summary our preliminary observations indicate that G70/APRIL stimulates both T and B cells in the spleen and mesenteric lymph nodes. Pathological analysis revealed that soluble G70 treated mice have slightly enlarged spleens of normal morphology.

\textbf{G70/APRIL is a ligand for BCMA and TACI}

G70/APRIL is related to the TNF ligand family member AGP3/BlyS. The TNFR receptor family member TACI (Fig.12) was recently shown to be a receptor for AGP3 ([A-570A patent application ser. no.]). Furthermore, TACI has a match to the orphan TNFR receptor family member BCMA (Figure 10) in a conserved extracellular cysteine rich domain (Figure 13). These observations together prompted us to investigate whether G70/APRIL is a ligand for BCMA and TACI and to test whether in addition to TACI AGP3/BlyS is also a ligand for BCMA.

Soluble mouse G70 specifically binds to 293 cells expressing exogenous BCMA (Figure 14). G70 also binds to 293 cells expressing TACI (Figure 15). Furthermore soluble G70 specifically blocks AGP3/BlyS binding to cell-surface receptors located on mouse B lymphoma cells (Figure 16). This suggest that G70 and AGP3 both binds to BCMA and TACI.

\textbf{smBCMA-Fc and shTACI-Fc prevent G70 and AGP3 ligand binding to cell-surface receptors}

Soluble BCMA (smBCMA-Fc; Fig. 10) and soluble TACI (shTACI-Fc) were produced in \textit{E. coli} purified to homogeneity and refolded.

Soluble TACI receptor specifically prevents G70 from binding to mouse B cells. (Figure 17). Furthermore, shBCMA-Fc and shTACI-Fc both prevent binding of AGP3 to B cells (Figure 18; and Figure 19 A). Soluble hBCMA-Fc also ameliorates G70 binding to A20 cells (Figure 19 B).

In summary: 1) both G70 and AGP3 binds the orphan TNFR receptor family members TACI and BCMA; 2) soluble BCMA and TACI
both effectively inhibits G70 and AGP3 from binding to B cells; 3) G70 and AGP3 competes for binding to cell-surface receptors.

Effects of TACI-Fc and BCMA-Fc treatment on the production of anti-KLH and anti-Pneumovax antibodies.

Treatment with either TACI-Fc or BCMA-Fc significantly inhibited the production of anti-KLH and anti-Pneumovax antibodies. Serum levels of both anti-KLH IgG and IgM were approximately 25% and 19% lower, respectively, in the TACI-Fc-treated mice than controls (Figure 20). Serum anti-KLH IgG and IgM were approximately 52% and 66% lower, respectively, in the BCMA-Fc-treated mice than controls (Figure 20). Serum levels of anti-Pneumovax IgM were also lower in the TACI-Fc- and BCMA-Fc-treated mice than controls (24% and 42%, respectively, Figure 20).
What is claimed is:

1. A method of inhibiting TACI activity, BCMA activity, or both in a mammal, which comprises administering a specific binding partner for APRIL, wherein the specific binding partner comprises
   a. the consensus region of TACI (SEQ ID NO: 16);
   b. the consensus region of BCMA (SEQ ID NO: 7);
   c. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13);
   but does not comprise the extracellular region of TACI (SEQ ID NO: 15) or the extracellular region of BCMA (SEQ ID NO: 6).

2. The method of Claim 1, further comprising administering a specific binding partner for AGP-3.

3. A method of treating B-cell lymphoproliferative disorders, which comprises administering a therapeutic agent comprising a specific binding partner selected from:
   a. the consensus region of TACI (SEQ ID NO: 16);
   b. the consensus region of BCMA (SEQ ID NO: 7); or
   c. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13)
   but not comprising the extracellular region of TACI (SEQ ID NO: 15) or the extracellular region of BCMA (SEQ ID NO: 6).

4. A method of treating T-cell lymphoproliferative disorders, which comprises administering a therapeutic agent comprising a specific binding partner selected from selected from:
   a. the consensus region of TACI (SEQ ID NO: 16);
   b. the consensus region of BCMA (SEQ ID NO: 7); or
   c. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13)
but not comprising the extracellular region of TACI (SEQ ID NO: 15)
or the extracellular region of BCMA (SEQ ID NO: 6).

5. A method of treating one or more solid tumors, which comprises
administering a therapeutic agent comprising a specific binding
partner selected from:
   a. the consensus region of TACI (SEQ ID NO: 16);
   b. the consensus region of BCMA (SEQ ID NO: 7); or
   c. the TACI/BCMA extracellular consensus sequence (SEQ ID
      NO: 13)

but not comprising the extracellular region of TACI (SEQ ID NO: 15)
or the extracellular region of BCMA (SEQ ID NO: 6).

6. The method of Claim 5, wherein the tumor is selected from lung,
gastrointestinal, pancreatic and prostate

7. The method of any of Claims 1, 3, 4, or 5, wherein the specific binding
partner is comprised within a molecule of the formula
\[(X^i)_a-F^i-(X^j)_b\]

wherein:
- \(F^i\) is a vehicle;
- \(X^i\) and \(X^j\) are each independently selected from -(L\(^1\))\(_c\)-P\(^i\), -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\), -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\)-P\(^k\), -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\)-P\(^k\)-P\(^f\), -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\)-P\(^k\)-P\(^f\)-P\(^g\), -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\)-P\(^k\)-P\(^f\)-P\(^g\)-P\(^h\), -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\)-P\(^k\)-P\(^f\)-P\(^g\)-P\(^h\)-P\(^i\), and -(L\(^1\))\(_c\)-P\(^i\)-(L\(^2\))\(_d\)-P\(^j\)-P\(^k\)-P\(^f\)-P\(^g\)-P\(^h\)-P\(^i\)-P\(^j\); the \(L^1, L^2, L^3,\) and \(L^4\) are each independently linkers; and
- \(a, b, c, d, e,\) and \(f\) are each independently 0 or 1, provided that at
  least one of \(a\) and \(b\) is 1.

8. The method of Claim 7, wherein the molecule comprises a structure of
   the formulae
   \[X^i-F^i\]
   or
   \[F^i-X^j.\]
9. The method of Claim 7, wherein the molecule comprises a structure of the formula

$$\text{F}^i - (L)^i_c - \text{P}^i$$.

10. The method of Claim 7, wherein the molecule comprises a structure of the formula

$$\text{F}^i - (L)^i_c - \text{P}^i - (L)^e_c - \text{P}^e$$

wherein one of $\text{P}^i$ and $\text{P}^e$ is the consensus region of TACI (SEQ ID NO: 16) and the other is the consensus region for BCMA (SEQ ID NO: 7).

11. The method of Claim 10, wherein the vehicle is an Fc domain.

12. The method of any of Claims 1, 3, 4, or 5, wherein the specific binding partner replaces a CDR region within an antibody molecule.

13. A composition of matter of the formula

$$(X)^i_e - \text{F}^i - (X)^e_b$$

wherein:

15. $\text{F}^i$ is a vehicle;

$X^i$ and $X^e$ are each independently selected from $-(L)^i_c - \text{P}^i$, $-(L)^e_c - \text{P}^e$, $-(L)^i_c - \text{P}^i - (L)^d_c - \text{P}^d$, $-(L)^e_c - \text{P}^e - (L)^d_c - \text{P}^d$, $-(L)^i_c - \text{P}^i - (L)^e_c - \text{P}^e - (L)^d_c - \text{P}^d$,

$P^i$, $P^d$, $P^e$, and $P^f$ are each independently

a. the consensus region of TACI (SEQ ID NO: 16);
b. the consensus region of BCMA (SEQ ID NO: 7); or
c. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13)

but not the extracellular region of TACI (SEQ ID NO: 15) or the extracellular region of BCMA (SEQ ID NO: 6...); and

a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a and b is 1.

14. The composition of matter of Claim 13 of the formulae

$$X^i - \text{F}^i$$
or
\[ F^1 \cdot X^2. \]

15. The composition of matter of Claim 14 of the formula
\[ F^i \cdot (L^j) \cdot P^i. \]

16. The composition of matter of Claim 14 of the formula
\[ F^i \cdot (L^j) \cdot P^i \cdot (L^j) \cdot P^j \]
wherein one of \( P^i \) and \( P^j \) is a specific binding partner for TACI and the other is a specific binding partner for BCMA.

17. The composition of matter of Claim 16, wherein the vehicle is an Fc domain.
Human G70 cDNA (SEQ ID NO 1)
Length: 1465 bp

The proposed transmembrane domains are boxed

human G70 protein sequence (SEQ ID NO 2)

```
1 MPASSPFLLA PKGPPGNMGG PVREPALSVA LWLSWGAALG AVACAMALLT
51 QQTTELQSLRR EVSRLOQTGG GSQNGEGYPW QSLPEQSSDA LEAWEWSGERS
101 RKORAVLSTQ KQKQSQHSLHL VPINATSKDD SDVTEVMWPQ ALRRGRLQA
151 QGYGVRIQDA GYVLYLQSVL FQDTFTTMQQ VVSREGQQRQ ETLFRRCIRM
201 PSHPDRAYNS CYSAGVPGHLH QGDILSVTIP RARAKLNLSP HGTPLGFV
```
FIG. 2A

Sequence of mouse G70 (SEQ ID NOs: 3 and 4)

Mouse G70 (SEQ ID NO 3)

1  CATGCCCAGT  GCTTTGTGTG  TGTTACCCTGC  TCTAAGAACG  TGGCTGGGCA
51  GCGTCTCAC  GCCGTTGAGG  ACCACTTTA  CTGGCTGGCAT  TGCTACAAGA
101  ACTTGTGGGC  CAAGAAGTGT  GCTGGAATCA  AGAAACCCAT  CACTGGTTTT
151  GGTAAGAGCT  CCAAGTTGTG  GCCCTATGAA  GGACAATCTT  GGCAAGACTA
201  CTGGCTCTCC  TGCCAAAGAT  GTCCGGTGAA  TCTGGCCAAC  AAGCGCTTTG
251  TATTTCAATA  TGAGCAAGGT  TATGCTCCTG  ACTGTGCCAA  AAAGCGTCAA
301  CTTGAGCCTG  GCCCTGTCCT  TTCTAGTATA  TGGCACCATA  TTCTCTCTAG
351  GTAAGGGGGG  AAGGAAGTGC  AGAGTGTCAC  TAGCTGACC  CTGGGGACA
401  GGGGCTACTA  TGTTGATTTT  TTCTATTCT  CAAGTGCCTT
451  TTTATTTCTC  CTTGCGTAA  CCCGCTTTCT  CCTCTGTGTC  CTTTGCCTGT
501  ATTTCCAACC  TCCCTGTCTC  CTCTGTGCT  CCTACCTTCT  GAGAACACAG
551  CTGTTGGCG  GGTCCTCTAGC  TCAATCGAGC  GCTACTCTGC  GCGCACAGG
601  GGGGCTCAGT  CAGAGGACCA  GCCCTTTCCG  TTGCTCTTGG  GTTGAAGTGG
651  GGGGAGCTTC  TGGGGGGCT  AGCTGTGTC  GTGCGACTAC  TGATCCAAAC
701  GACAGAGCTG  CAAAGCCTAA  GGGCGGAGGT  GAAGGCCGTC  CAGCGGAGTG
751  GAGGGCGCTG  CCAAGACGCC  AGAGAGCAGG  CCCCTGTTGA  ATGCGACGG
801  CAGAAGCTGT  CGTCTCTCGA  ACTGCTGGAG  GATCTGCGGAG  AATCTCCAG
851  AAGGAAGGAA  GTACCTACCC  AAAGAGCAAA  GAGAAAGCAG  TCAGTCTCTC
901  ATCTTTGTCG  AGTTAAACAT  ACCTCACAAGG  ACTCTGACGT  GACAGAGTGT
951  ATGTCGGAAC  CAGTACTTATG  CGGTTCTGGG  CGCTGGAGG  CCCCGGGAGA
1001  CATTGTACGA  GTCTGGGACA  CTGGAATTATA  TTGGCTCTAT  AGTCAGGTC
1051  TGCTTTACAG  GTCTGACCTTC  ACAAATGGTC  AGCTGATATC  TCGGGAAGGA
1101  CAGAGAGAAG  GAAAGAATCT  ATGGCGATG  ATCAGAAGTA  TGGCTCTCTG
1151  TTCCTGACCTG  GCCAAGAAAT  GCTGCTAGCG  TGCAAGGCTC  TTCTATCTAT
1201  ATCAAGGGGA  TATTATCAGT  GCTAAAAATTC  CAGCGGCAAA  CGAACAACCT
1251  AGCCTTCTTC  GCAAGTGGAA  ATCTGCTGGG  TGTTGGAAAC  TATGATGTGT
1301  ATAAAGGAGG  TGGGAATTTT  CCCATTCAA  AACTGGTAG  ACAAAGGACA
1351  AGGAAAGGTC  AAAGAACGCT  TCTCATGGCT  TTGCTCTGAG  TGTTTGTCCT
1401  CTCCTTGCGT  TTCCCGCTCTC  CACTATGCG  GCTTTGACTC  CATGGATATT
1451  AAAAAAGTAG  AATATTTTGT  GTTTATCTCC  CAAAA

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FIG. 2B

Mouse G70 Length: 241 (SEQ ID NO 4)

1 MPASSPQHMG GSVREPALSV ALWLSWGAVL GAVTCAVALL IQQTELOSLR
51 REVSRLQRSG GPSQKQGERF WQSLWEPQSPD VLEAKDGAK SRRRAVLTQ
101 KHKKHSVLH LVPVNITSKD SDVTEVMWP QLRRGRGELA QGDIVRWWT
151 GIYLLYSQVL FHDVTFTMGQ VVSREGQGRR ETLFRCIRSM PSDDRAYS
201 CYSAGVFLHL QGDIIIVKLKIP RANAKLSLP HGTFLGFLKL *

G-70 FLAG des92 (smuG70) Strain #4081 (SEQ ID NO 19):

MDYKDDDKKHKKKSVLHLVPVNITSKDSDVTEVMWQPVLRGRGELAQGDIVRWDTGIY
LLYLSQVLFHDVTFTMGQVVSREGQGRRETLFRCIRSMPSDDRAYSNCYSAGVFLHQQDGII
TVKIIPRANAKLSLSPHGTFLGFLKL*
### FIG. 3

Alignm. of human and mouse G70

<table>
<thead>
<tr>
<th></th>
<th>mouse</th>
<th>human</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>MPASS------PGHMGGSVREPALSVALWLSWGAVLGAIVTCAVALQIQTELQSLRR</td>
<td>MPASS PG+MGG VREPALSVALWLSWA LGAV CA+ALL QQTTELQSLRR</td>
</tr>
<tr>
<td>60</td>
<td>MPASSPFLAPKGPGPNMGGFVREPALSVALWLSWAALGAVACAMALLQQTTELQSLRR</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>EVSRLQRSGGPSQKQGERPWQSLWEQSPDVLEAWKDGAKSRRRRRAVLTTKHKHHKHSVLH</td>
<td>EVSRLQ +GGPSQ PWQSL EQS D LEA+ G +SR+RAVLTTK KK+HSVHL</td>
</tr>
<tr>
<td>120</td>
<td>EVSRLQGTGGPSQNGEGYPWQSLPEQSSDASEAWSEMERSRKRAVLTTKQKKKQHHSVLH</td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>VPVNITSKD-SDVTEVWMQPVLRRGRGLEAQGDIVRVWDGTGILLYSQVLFHDTFTMGQ</td>
<td>VP+N TSKD SDVTEVWMQP LRRGRGL+AQG VR+ D G+YLLYSQVLF DTFTMGQ</td>
</tr>
<tr>
<td>180</td>
<td>VPINATSDKDSDVTEVWMQPALRRGRGQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQ</td>
<td></td>
</tr>
<tr>
<td>230</td>
<td>VVSREGQGRRETLFRCIRMSPSDPDRAYNCSYGAVFHHLQGDIITVPIRANAKLSP</td>
<td>VVSREGQ+ETLFRCIRMS PDRAYNCSYGAVFHHLQGDI+V IPRA AKL+LSP</td>
</tr>
<tr>
<td>240</td>
<td>VVSREGQGRQETLFRCIRMSHPDRAYNCSYGAVFHHLQGDILSVIIIPRANAKLNLSP</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>HGTFLGFVKL 240</td>
<td>HGTFLGFVKL</td>
</tr>
<tr>
<td>250</td>
<td>HGTFLGFVKL 250</td>
<td></td>
</tr>
</tbody>
</table>

*Note: The table represents the alignment of human and mouse G70 sequences.*
FIG. 4A

Effect of sG70/April on Raji cell proliferation

Effect of sG70/April on Jurkat cell proliferation

Effect of sG70/April on K562 cell proliferation
FIG. 4B

Effect of sG70/April on U937 cell proliferation

Effect of sG70/April on 293 T cell proliferation

Effect of sG70/April on 3T3 cell proliferation
FIG. 6

The effect of r-G70/April on human peripheral blood B cell, T cell and Granulocyte

![Graph showing the effect of r-G70/April on cell numbers.]

The effect of IL-2 and G70/April on human peripheral T cell proliferation

![Graph showing the effect of IL-2 and G70/April on cell proliferation.]
FIG. 7

Effect of sG70/April on murine B cell proliferation

Effect of sG70/April on murine T cell proliferation
FIG. 8

Effect of G70/April on murine T cell proliferation costimulated through CD28 antibody

- G70/April +CD28 ab
- sG70/April

CPM vs. G70/April (ng/ml)
FIG. 9

Co-stimulatory activity of G70/April on mouse T cells

![Graph showing 3H-TdR incorporation (cpm x 10^-3) for different treatments.]

- Anti-CD3: 
  - - - - + + + +

- G70 ng/ml: 
  - 0 10 100 1000 0 10 100 1000
Human (SEQ ID NO: 5):

1 MAGQCSQNEY FDSLHACIP CQLRSSNTP PLTCQRYCNA SVTNSVKGTN
51 AILWTCLGLS LIISAVFVL MFLRKKISSE PLKDEFKNTG SGGLGMANID
101 LEKSRGTDEI ILPRGYETV YEECTCDEIK SKPVDSDHIC FPLPAMEEGA
151 TILVTKTND YCKSLPAALS ATEIEKSISA R

Human (SEQ ID NO: 5):

MAGQCSQ NEYFDSLHHA CIPCQLRSS NTPPLTCQRY CNASVTNSVK
GTNA IILWTCL GLSLIISLAV FVLMIFFLLRKI SSEPLKDEFK NTGSSGLGMA
NIDLEKSRTG DEIIILPRGDE YTVEECTCDE CIKSKPVDS DHCFLPAME
EGATILVTIT TNDYCKSLPA ALSATEIEKS ISAR

hBCMA's extracellular domain (SEQ ID NO: 6):

MAGQCSQ NEYFDSLHHA CIPCQLRSS NTPPLTCQRY CNASVTNSVK
GTNA

hBCMA's cysteine-rich consensus region (SEQ ID NO: 7):

CSQ NEYFDSLHHA CIPCQLRSS NTPPLTCQRY C

hBCMA's transmembrane region (SEQ ID NO: 8):

ILWTCL GLSLIISLAV FVLMF

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FIG. 10B

huBCMA-Fc (SEQ ID NO: 9):

MAQQCSQNEYFDSLHACIPCQLRCSNTTPPLTCQRYCNASVTNSVKGNTAGGGG
GGDKHTHTCPPCPAPELLGGPSVFLPFPKPKDTPDMISRTPEVTCKVTVVSHEDEPVK
FNWYVDGVEVHNAKTTPREEQSYNSTYRVVSVLTVHQQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRLDELTKNQVSLTCLVKGFYPSDIAVHESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGK*

muBCMA-Fc (SEQ ID NO: 10):

MAQQCFHEYFDSLHACKPCHLRCENPPATCQPYCDPSVTSSVKGSYTGGGGG
DKTHTCPPCPAPELLGGPSVFLPFPKPKDTPDMISRTPEVTCKVTVVSHEDEPVKFN
WYVDGVEVHNAKTTPREEQSYNSTYRVVSVLTVHQQDWLNGKEYKCKVSNKALP
PAPIEKTISKAKGQPREPQVYTLPPSRLDELTKNQVSLTCLVKGFYPSDIAVHESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
LSLSPGK*
FIG. 11
Alignment of human BCMA amino acid sequence and murine BCMA amino acid sequence

murine BCMA amino acid sequence Length: 185 (SEQ ID NO: 11):

1  MAQQCFHSEY FDSLHACKP CHLRCSNPPA TCQPYCDPSV TSSVKGTYYTV
51 LWIFLGLTLV LSLALFTISF LLRKMNPEAL KDEPQSPGQL DGSAQLDKAD
101 TELTRIRAGD DRIFPRSLEY TVEECTCDEC VKSKPKGDSD HFPPLPAMEEE
151 GATILVTTKT GDYGKSSVPT ALQSVMGMEK PTHTR

alignment of human BCMA amino acid sequence and murine BCMA amino acid sequence.

Query:  4  MAQQCSQNEYFDSLHACIPCQLRCSSHNPPLTQRYCNASVTNSVKGTNAILWTCLGLS 63
         MA QC  +EYFDSLHAC PC LRCS+ PP TCQ YC+ SVT+SVKGT +LW LGL+
Sbjct:  1  MAQQCFHSEYFDSLHACKPCHLRCSN--PPATCQPYCDPSVTSSVKGTYTVLWIFLGLT 58

Query:  64 LIISLAVFVLMFLLRKSIESEPLKDKEFKNTG----SGLLGMANIDLEKRSRTGDIEILPRGL 119
         L++SLA+F  +FLLRK++ E LKDE ++ G S L A+ +L + R GD+ I PR L
Sbjct:  59 LVLSALFTISFLLRKNMPEALKDEPQSPGQLDGSAQLDKADTELTRIRAGDRIIFPRSL 118

Query:  120 EYTVVECTCDECISKPKVDSDHPPLPAMEEGATIlVTKTNDYCKS-LPAAL-SATEI 177
         EYTVVECTCDEC+KSKPK DSDH FPLPAMEEGATILVTKT D Y KS ++P AL S +
Sbjct:  119 EYTVVECTCDECVSKPKGDSDHFPPLPAMEEGATILVTKTGDYGKSSVPTALQSVMG 178

Query:  178 EKSSAR 184
         EK R
Sbjct:  179 EKPTHTR 185
FIG. 12A

Human TACI

huTACI (SEQ ID NO: 14):
1 MSGLGRSRRG GRSRVDQEER FPQGLWTGVAMRSCPEEQYW DPLLGTCSMC
51 KTICNHQSQR TCAAFCRSLS CRKEQGKFYD HLLRDCISCA SICGQHPKQC
101 AYFCENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL
151 PGLKLSADQV ALVYSTLGLC LCAVLCCFLV AVACFLKRG DPCSCQPRSR
201 PRQSPAKSSQ DHAMEAGSPV STSEPVTETC SFCFPECRAP TQESAVTPGT
251 PDPTCAGRWG CHTRTTVLQP CPHPDSGLG IVCVPAQEGG PGA

huTACI's extracellular domain (SEQ ID NO: 15):
1 MSGLGRSRRG GRSRVDQEER FPQGLWTGVAMRSCPEEQYW DPLLGTCSMC
51 KTICNHQSQR TCAAFCRSLS CRKEQGKFYD HLLRDCISCA SICGQHPKQC
101 AYFCENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL
151 PGLKLSADQV ALVYST
FIG. 12B

huTACI’s cysteine-rich consensus region (SEQ ID NO: 16):
CPEEQYWDPLLGTMSCKTICNHQSQRTCAAFC and
CRKEQGKFDHLLRKCISCASICGQHPKQCAYFC

transmembrane region (SEQ ID NO: 17):
LGLCLCAVLCFLVAVACFL

hTACI-Fc (SEQ ID NO: 18):

1  MSGLGRSRRG GRSRVDQEBR FPQGLMTGVA MRSCEPEEQYW DPLLGTCMSC
51  KTICNHQSQR TCAAFCRSLS CRKEQGKFDY HLLRDCISCA SICGQHPKQC
101  AYFCENKLRS PVNLPPRLRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL
151  PGLKLSDAQV ALVYSSGGGG DKTHTCPPCP APELLGGPSV FLFPPKPDKT
201  LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
251  RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQREPQVYVT
301  LPSSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPVLDS
351  DGSFFLYSKL TVDKSRWQQQ NVFSCSVMHE ALHNYHTQKS LSLSPGK*
FIG. 13

Alignment of cysteine rich extracellular regions of human TACI and human BCMA.

34 CPEEQYWDPLLGTCSCKTNHQSS.RTCAAFCRSLSCRKEQGKFYDHL 82
8 CSQNEYFDSLHACIPQLCSSNTPPLTCQRYCNASVTNSVKGT..NAI 55

83 LRDCISCASI 92
56 LWTCLGLSLI 65
FIG. 14A

293 cells transfected with vector only

FIG. 14B

293 cells transfected with antisense BCMA
FIG. 14C

293 cells transfected with BCMA sense
FIG. 18

A20 AGP3 binding
A20 AGP3
binding + 10X
humanBCMA
A20 AGP3
binding + 10X TACI