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(54) Title: MULTIVARIABLE ANTIGENS COMPLEXED WITH TARGETING HUMANIZED MONOCLONAL ANTIBODY

Single Targeting mAb-Antigen Fusion Proteins



(57) Abstract: The present invention includes compositions and methods for designing, making and using modular recombinant antibodies or fragments thereof with one half of a cohesin-dockerin pair that permits the rapid assembly of multivalent antigen conjugates.

Multivariable Targeting mAb-Antigen Complexes

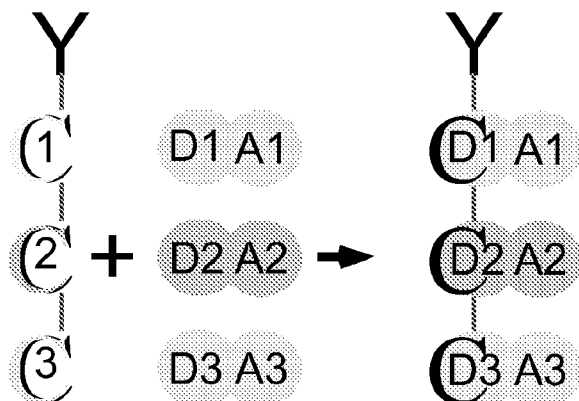


Figure 1

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MULTIVARIABLE ANTIGENS COMPLEXED WITH TARGETING HUMANIZED MONOCLONAL ANTIBODY

TECHNICAL FIELD OF THE INVENTION

The present invention relates in general to the field of novel vaccines, and more particularly, to the design, manufacture and use of multivariable antigens complexed with targeting humanized monoclonal antibodies.

5 BACKGROUND OF THE INVENTION

Without limiting the scope of the invention, its background is described in connection with vaccine development.

Protein engineering technology relating to monoclonal antibodies is highly advanced regarding humanization (i.e., rendering e.g., a rodent mAb sequence into a human mAb sequence while
10 preserving specific antigen combining sites of the the original mAb) and production (typically secreted from mammalian cell lines). In research and development are new applications of rAbs related to vaccination and are presently based on engineered rAb-antigen fusion proteins (typically with the antigen coding region placed in-frame with the C-terminal codon of the rAb heavy or H chain). A roadblock to this technology is the successful expression and production of
15 fully functional rAb-antigen. In many, perhaps most, cases the desired antigen confounds secretion of the engineered rAb-antigen. Also, the likelihood of poor or null expression is increased if the desired entity includes multiple antigen coding regions.

SUMMARY OF THE INVENTION

The invention provides methods for the assembly of rAb antigen complexes in a controlled
20 manner by simple mixing components and accomodates the ability to express and produce the rAb and antigen(s) in different expression – production systems that are best suited to the individual rAb and particular antigen. In addition, the invention demonstrates the novel application of the high affinity and high specificity cohesin-dockerin interaction to secreted mammalian expression systems, thus permitting the development of unique protein engineering
25 formats and production of new protein tools for research and clinical application.

More particularly, the present invention uses the cohesin-dockerin protein domains and their surrounding linker. For example, the invention permits the controlled assembly of recombinant monoclonal antibodies (rAbs) complexed to antigens, toxins, or cellular activating agents. The invention has wide potential application in vaccination and cancer therapy. Also claimed are

derivatives of this technology that permit the production of novel proteins with specific affinities for other proteins.

The invention is based on particular components of the well studied bacterial cellulose degrading protein complex called the cellulosome. Specifically, two protein domains (cohesin and dockerin) and natural protein linker sequences are utilized via the invention in novel contexts and applications.

The present invention is based on the discovery that particular cohesin and dockerin domains can be successfully and efficiently secreted from mammalian cells as fusion proteins while maintaining the specific and high affinity cohesin-dockerin protein-protein interaction. While the extensive cohesin-dockerin literature teaches the expectation that such fusion proteins should have this functionality, it does not describe production of such fusion proteins in mammalian secretion systems. The state of scientific knowledge does not allow the prediction of the discovery since the rules (other than features such as signal peptide) for successful secretion are not fully established. Furthermore, the cohesin linker regions are known to be glycosylated in their native bacteria, and the cohesin and dockerin domains contain predicted glycosylation sites. While this may actually favor secretion from mammalian cells, it is unclear if 'unnatural' glycosylation will perturb the cohesin-dockerin interaction.

While cohesin-dockerin interaction for various commercial applications has been published, the present invention is based on a previously unrealized potential for this interaction built around assembling specific protein complexes unrelated to the controlled assembly enzyme applications.

The invention includes the use of all cohesin-dockerin sequences from diverse cellulose degrading microbes, but describes the application of specific cohesin and dockerin and linker sequences from the microbe Clostridium thermocellum. For example, the sequence described herein encodes the H chain of a human IgG4 linked at the C-terminal codon to a Clostridium thermocellum dockerin sequence (called rAb.doc). Other embodiments of rAb.doc proteins are described similarly with examples that are engineered by simply transferring the dockerin coding region as a DNA fragment to vectors encoding the different H chain entities.

More particularly, the present invention includes a modular rAb carrier that includes an antigen-specific binding domain linked to one or more antigen carrier domains and one half of a cohesin-dockerin binding pair. The antigen-specific binding domain may be at least a portion of an antibody and the antibody is a fusion protein with and the binding pair in a fusion protein with

one half of a cohesin-dockerin binding pair. The rAb may also include a complementary half of the cohesin-dockerin binding pair bound to an antigen that forms a complex with the modular rAb carrier. The complementary half of the cohesin-dockerin binding pair may itself be a fusion protein with the antigen carried as part of the complex (modular rAb carrier (cohesin/dockerin) antigen complex). Examples of antigen specific domain include a full length antibody, an antibody variable region domain, an Fab fragment, a Fab' fragment, an F(ab)₂ fragment, and Fv fragment, and Fabc fragment and/or a Fab fragment with portions of the Fc domain. Non limiting examples of sources for the cohesin-dockerin binding pair include Clostridium thermocellum, Clostridium josui, Clostridium cellulolyticum and Bacteroides cellulosolvens and combinations thereof.

Non-limiting examples for targeting by the antigen-specific binding domain include: cell surface marker selected from MHC class I, MHC class II, CD1, CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD16, CD 19, CD20, CD29, CD31, CD40, CD43, CD44, CD45, CD54, CD56, CD57, CD58, CD83, CD86, CMRF-44, CMRF-56, DCIR, DC-ASPGR, CLEC-6, CD40, BDCA-2, MARCO, DEC-205, mannose receptor, Langerin, DECTIN-1, B7-1, B7-2, IFN- γ receptor and IL-2 receptor, ICAM-1, Fc γ receptor or other receptor relatively specifically expressed by antigen presenting cells.

The rAb of the present invention may also includes combinations of the domains that are defined as: an rAb.Doc; an rAb.Coh; an rAb.(Coh)_x; an rAb.(Doc)_x; an rAb.(Coh.Doc)_x; or an rAb.(Coh)_x(Doc)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. Examples of the modular rAb carrier in a complex include:

- an rAb.Doc:Coh.antigen;
 - an rAb.Coh:Doc.antigen;
 - an rAb.(Coh)_x:(Doc.antigen)_x;
 - an rAb.(Doc)_x:(Coh.antigen)_x;
 - an rAb.(Coh.Doc)_x:(Doc.antigen¹)(Coh.antigen²); or
 - an rAb.(Coh)_x(Doc)_x:(Doc.antigen¹)_x(Coh.antigen²)_x;
- wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

The present invention also include a vaccine of a modular rAb carrier that includes an antigen specific domain linked to one or more domains comprising one half of the cohesin-dockerin binding pair bound to a complementary half of the cohesin-dockerin binding pair bound to an

antigen. Non-limiting examples for targeting the rAb include immune cell surface protein selected from MHC class I, MHC class II, CD1, CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD16, CD 19, CD20, CD29, CD31, CD40, CD43, CD44, CD45, CD54, CD56, CD57, CD58, CD83, CD86, CMRF-44, CMRF-56, DCIR, DC-ASPGR, CLEC-6, CD40, BDCA-2, MARCO, DEC-205, mannose receptor, Langerin, DECTIN-1, B7-1, B7-2, IFN- γ receptor and IL-2 receptor, ICAM-1, Fc γ receptor or other receptor relatively specifically expressed by antigen presenting cells. Targets for vaccination with the rAb antigen carrier include, e.g., a bacterial, viral, fungal, protozoan or cancer protein and fragments thereof. The vaccine of claim 11, wherein the modular rAb carrier is further defined: an rAb.Doc:Coh.antigen; an rAb.Coh:Doc.antigen; an rAb.(Coh)_x:(Doc.antigen)_x; an rAb.(Doc)_x:(Coh.antigen)_x; an rAb.(Coh.Doc)_x:(Doc.antigen¹)(Coh.antigen²); or an rAb.(Coh)_x(Doc)_x:(Doc.antigen¹)_x(Coh.antigen²)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

The present invention also includes an isolated nucleic acid comprising a coding segment for a target-specific domain and one or more domains and one half of a cohesin-dockerin binding pair. For example, the target may be an antigen and the target specific domain may encode at least a portion of an antibody. The one or more domains can encode one or more cohesin domains, one or more dockerin domains or a combination of one or more cohesin and dockerin domains. The rAb is further defined as: an rAb.Doc; an rAb.Coh; an rAb.(Coh)_x; an rAb.(Doc)_x; an rAb.(Coh.Doc)_x; or an rAb.(Coh)_x(Doc)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

The present invention also includes a vector that includes a nucleic acid encoding an antigen specific domain and one or more domains that comprise one half of a cohesin-dockerin binding pair, a one half of a cohesin-dockerin binding pair with a protein molecule to be carried and combinations thereof. The one half of a cohesin-dockerin binding pair, a one half of a cohesin-dockerin binding pair with a protein molecule to be carried and combinations thereof are under the control of the same promoter, different promoters, transcribed in-line, transcribed in opposite directions.

The present invention also includes a host cell comprising a vector comprising a nucleic acid encoding an antigen specific domain and one or more domains and one half of a cohesin-dockerin binding pair.

A method of making a modular rAb carrier by combining an antigen specific domain linked to one or more domains of one half of a cohesin-dockerin binding pair. The rAb is further defined as: an rAb.Doc; an rAb.Coh; an rAb.(Coh)_x; an rAb.(Doc)_x; an rAb.(Coh.Doc)_x; or an

rAb.(Coh)_x(Doc)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Examples of the rAb is complexed with a complementary half of a cohesion:dokerin pair bound to an antigen and is selected from: an rAb.Doc:Coh.antigen; an rAb.Coh:Doc.antigen; an rAb.(Coh)_x:(Doc.antigen)_x; an rAb.(Doc)_x:(Coh.antigen)_x; an rAb.(Coh.Doc)_x:(Doc.antigen¹)(Coh.antigen²); or an rAb.(Coh)_x(Doc)_x:(Doc.antigen¹)_x(Coh.antigen²)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

The present invention may also be an immunotoxin that includes an rAb.Doc:Coh.toxin self-assembled conjugate, wherein the rAb is specific for a cell target. Examples of toxins include a radioactive isotope, metal, enzyme, botulin, tetanus, ricin, cholera, diphtheria, aflatoxins, perfringens toxin, mycotoxins, shigatoxin, staphylococcal enterotoxin B, T2, seguitoxin, saxitoxin, abrin, cyanoginosin, alphatoxin, tetrodotxin, aconotoxin, snake venom and spider venom. Cell targets for the immunotoxin include diseased or infected cells. Examples of diseased cells for targeting include cancer cell for, e.g., hematological cancers such as leukemias and lymphomas, neurological tumors such as astrocytomas or glioblastomas, melanoma, breast cancer, lung cancer, head and neck cancer, gastrointestinal tumors such as gastric or colon cancer, liver cancer, pancreatic cancer, genitourinary tumors such cervix, uterus, ovarian cancer, vaginal cancer, testicular cancer, prostate cancer or penile cancer, bone tumors, vascular tumors, or cancers of the lip, nasopharynx, pharynx and oral cavity, esophagus, rectum, gall bladder, biliary tree, larynx, lung and bronchus, bladder, kidney, brain and other parts of the nervous system, thyroid, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma and leukemia. The immunotoxin may target pathogens directly, e.g., bacteria, a protozoan, a helminth, a virally-infected cell or a fungus.

The present invention also includes a method for protein purification by separating a cohesin or dokerin fusion protein by interacting the fusion protein with a rAb that is conjugated to the complementary cohesin or dokerin bound to a substrate. The present invention may also use the cohesin as a fusion partner for toxins for conferring beneficial biochemical properties favoring ready purification of active cohesin.toxin fusion protein. The present invention may also use the anti-DC rAb.Doc to target DC for therapeutic applications where ablating DC. Therapeutic applications include, e.g., transplantation, autoimmune disease, infectious disease or cancer. The invention also includes an anti-DC-SIGN/L antibody provided in an amount that is sufficient to enhance the survival of dendritic cells, wherein the antibody matures and activates the dendritic cells for immunization. The antibody may target cells in vivo, e.g., dendritic cells as an adjuvant in vaccines.

Also invented is a bivalent and multivalent (rAb¹.Doc:Coh.rAb²) self-assembled conjugates as therapeutic, diagnostic, and industrial agents. Alternatively, the invention is a bivalent and multivalent (rAb.Doc:Coh.cytokine), (rAb.Coh:Doc.cytokine) or (cytokine¹.Coh:cytokine².Doc) self-assembled conjugates as therapeutic, cell proliferation or maturing agents. The modular rAbs carrier may be made by method that includes screening one or more multivalent rAb and/or rAb.cytokine and/or cytokine.cytokine combinations that are capable of specifically binding to a target cell and delivering the cytokine such that it exerts its effect on the target cell. Cytokines for use with the present invention include: interleukins, transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), epidermal growth factors (EGFs), connective tissue activated peptides (CTAPs), osteogenic factors, and biologically active analogs, fragments, and derivatives of such growth factors, B/T-cell differentiation factors, B/T-cell growth factors, mitogenic cytokines, chemotactic cytokines, colony stimulating factors, angiogenesis factors, IFN- α , IFN- β , IFN- γ , IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, etc., leptin, myostatin, macrophage stimulating protein, platelet-derived growth factor, TNF- α , TNF- β , NGF, CD40L, CD137L/4-1BBL, human lymphotoxin- β , G-CSF, M-CSF, GM-CSF, PDGF, IL-1 α , IL1- β , IP-10, PF4, GRO, 9E3, erythropoietin, endostatin, angiostatin, VEGF, transforming growth factor (TGF) supergene family include the beta transforming growth factors (for example TGF- β 1, TGF- β 2, TGF- β 3); bone morphogenetic proteins (for example, BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9); heparin-binding growth factors (fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)); Inhibins (for example, Inhibin A, Inhibin B); growth differentiating factors (for example, GDF-1); and Activins (for example, Activin A, Activin B, Activin AB).

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

FIGURE 1 compares the prior art (top portion) with an example of the multiple antigens targeted in a complex simultaneously with the same engineered humanized mAb (MATCHMAB)(bottom portion).

FIGURE 2 shows the use of the present invention to form Bi-specific mAbs.

FIGURE 3 shows Protein G affinity purified secreted rAb proteins analyzed by reducing SDS.PAGE and Coomassie Brilliant Blue staining. Lanes are from left to right.

FIGURES 4A and 4B show the measurement by anti-human IgFc ELISA of levels of secretion of various rAb.fusion proteins.

- 5 FIGURE 5 shows the measurement by anti-human IgFc ELISA (HRP activity) and LOX-1.alkaline phoshatase binding (AP activity) of secreted anti-LOX1_15C4 rAb.(blue symbols) and anti-LOX1_15C4.doc rAb (red symbols) proteins.

FIGURE 6 shows that when co-transfected with a mIgG kappa expression plasmid, rAB-pCMV(mIgG2bH-Dockerin) plasmid directs the efficient secretion of rAB- mIgG2b.Dockerin
10 fusion protein.

FIGURES 7A and 7B show that the secreted coh.alkaline phosphatase (coh.AP) but not AP binds efficiently and specifically to rAb.Doc immobilized on plastic..

FIGURES 8A and 8B shows various dilutions of a supernatant containing secreted G.AP bound to immobilized mIgG2a and mIgG2b, but not rAb.doc, while coh.AP bound rAb.doc
15 specifically..

FIGURE 9 shows the differential stability of complexes between a fixed amount of proG.AP or coh.AP or coh2.AP (0.1 ug) and immobilized mIgG2b or rAb.doc (0.25 ug) assembled by incubation for 1 hr in a micro-titre plate .

FIGURE 10 shows the differential stability in human serum of complexes between a fixed
20 amount of proG.AP or coh.AP (0.1 ug) and immobilized mIgG2b or rAb.doc (0.25 ug) were assembled by incubation for 1 hr in a micro-titre plate.

FIGURE 11 is a gel that shows the reduced vs. non-reduced SDS.PAGE analysis of rAb.doc:Coh2.AP complexes produced by sequential application of rAb.doc supernatant and coh.AP supernatant to the same protein G affinity column.

25 FIGURE 12 is a non-reduced SDS.PAGE analysis of rAb.doc:Coh.Flu HA5-1 complexes produced by sequential application of rAb.doc supernatant and coh.Flu HA5-1 supernatant to the same protein G affinity column.

FIGURE 13 shows that anti-DC_rAb.doc:coh.Flu M1 complex formed by mixing the individual purified components was effective in vitro in expanding Flu M1-specific T cells..

FIGURE 14 shows that Anti-DC_rAb.doc:coh.Flu M1 but not mIgG2b.doc:coh.Flu M1 complexes formed by mixing the individual purified components was effective in vitro in expanding Flu M1-specific T cells..

FIGURE 15 shows CD34+ human DC were sorted into CD1a+ and CD14+ subtypes and
5 cultured with and without 3 nM Anti-DC_rAb.Flu M1 PEP or Anti-DC_rAb.

FIGURE 16 shows E. coli harboring expression plasmids directing the synthesis of coh.pep proteins were grown and induced for specific protein production. Cells were harvested and broken by sonication.

FIGURE 17 shows that the DCIR.Doc rAb alone had no effect upon the survival of DCs, but
10 DC-SIGN/L.Doc rAb ehnaces their survival.

FIGURE 18shows that Coh.PE38 alone slightly increase the number of 7-AAD scored apoptotic cells (from 22.1-29.8%), but when linked to DCIR or DC-SIGN/L.Doc rAbs, Coh.PE38 greatly enhanced the number of 7-AAD scored apoptotic cells.

FIGURE 19 shows the expression of anti-DC-SIGN/L and Anti-DC-ASPGR rAb.Coh and
15 rAb.Doc were efficiently secreted.

FIGURE 20 shows the effect of IL-21 and Coh.IL-21 on the proliferation of human B cells.

DETAILED DESCRIPTION OF THE INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable
20 inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas
25 relevant to the present invention. Terms such as “a”, “an” and “the” are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

At present, protein engineering technology enables the ready and controlled addition of an
30 antigen (or different antigens to one of the chains) of a recombinant mAb (H or L, usually the C-

terminus of H is often used). If different antigens or different antigen sets need to be linked to the mAb, then the mAb needs to be re-engineered, expressed, and purified as a different entity.

The present invention provides for the complexing of multiple antigens or proteins (engineered, expressed, and purified independently from the primary mAb) in a controlled, multivariable fashion, to one single primary recombinant mAb. Presently, there are methods for engineering site-specific biotinylation sites that provide for the addition of different proteins (each engineered separately linked to streptavidin) to the one primary mAb. However, the present invention provides for addition to the primary mAb of multiple combinations, in fixed equimolar ratios and locations, of separately engineered proteins.

As used herein, the term “modular rAb carrier” is used to describe a recombinant antibody system that has been engineered to provide the controlled modular addition of diverse antigens, activating proteins, or other antibodies to a single recombinant monoclonal antibody (mAb). The rAb may be a monoclonal antibody made using standard hybridoma techniques, recombinant antibody display, humanized monoclonal antibodies and the like. The modular rAb carrier can be used to, e.g., target (via one primary recombinant antibody against an internalizing receptor, e.g., a human dendritic cell receptor) multiple antigens and/or antigens and an activating cytokine to dendritic cells (DC). The modular rAb carrier may also be used to join two different recombinant mAbs end-to-end in a controlled and defined manner.

The antigen binding portion of the “modular rAb carrier” may be one or more variable domains, one or more variable and the first constant domain, an Fab fragment, a Fab' fragment, an F(ab)₂ fragment, and Fv fragment, and Fabc fragment and/or a Fab fragment with portions of the Fc domain to which the cognate modular binding portions are added to the amino acid sequence and/or bound. The antibody for use in the modular rAb carrier can be of any isotype or class, subclass or from any source (animal and/or recombinant).

In one non-limiting example, the modular rAb carrier is engineered to have one or more modular cohesin-dockerin protein domains for making specific and defined protein complexes in the context of engineered recombinant mAbs. The mAb is a portion of a fusion protein that includes one or more modular cohesin-dockerin protein domains carboxy from the antigen binding domains of the mAb. The cohesin-dockerin protein domains may even be attached post-translationally, e.g., by using chemical cross-linkers and/or disulfide bonding.

The modular rAb carrier will be used to carry a separate molecule, e.g., a peptide, protein, lipid, carbohydrate, nucleic acid (oligonucleotide, aptamer, vector with or without base or backbone

modifications) or combinations thereof by binding that separate molecule to the complementary half of the cohesion:dockerin pair. For example, either the dockerin or cohesin made be made into a fusion protein or chemically bound to an antigen, a peptide, a protein, a toxin, a cytokine, an enzyme, a structural protein, an extracellular matrix protein, another antibody, a cell or fragments thereof. The modular rAb carrier may have one or more cohesin, dockerin or both cohesin and dockerin domains that allow the formation of a complex with one or more complementary cohesin/dockerin-molecules for delivery via the antigen recognition domain of the modular rAb carrier.

The term “antigen” as used herein refers to a molecule that can initiate a humoral and/or cellular immune response in a recipient of the antigen. Antigen may be used in two different contexts with the present invention: as a target for the antibody or other antigen recognition domain of the rAb or as the molecule that is carried to and/or into a cell or target by the rAb as part of a dockerin/cohesin-molecule complement to the modular rAb carrier. The antigen is usually an agent that causes a disease for which a vaccination would be advantageous treatment. When the antigen is presented on MHC, the peptide is often about 8 to about 25 amino acids. Antigens include any type of biologic molecule, including, for example, simple intermediary metabolites, sugars, lipids and hormones as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoal and other parasitic antigens, tumor antigens, antigens involved in autoimmune disease, allergy and graft rejection, and other miscellaneous antigens.

The modular rAb carrier is able to carry any number of active agents, e.g., antibiotics, anti-infective agents, antiviral agents, anti-tumoral agents, antipyretics, analgesics, anti-inflammatory agents, therapeutic agents for osteoporosis, enzymes, cytokines, anticoagulants, polysaccharides, collagen, cells, and combinations of two or more of the foregoing active agents. Examples of antibiotics for delivery using the present invention include, without limitation, tetracycline, aminoglycosides, penicillins, cephalosporins, sulfonamide drugs, chloramphenicol sodium succinate, erythromycin, vancomycin, lincomycin, clindamycin, nystatin, amphotericin B, amantidine, idoxuridine, p-amino salicylic acid, isoniazid, rifampin, antinomycin D, mithramycin, daunomycin, adriamycin, bleomycin, vinblastine, vincristine, procarbazine, imidazole carboxamide, and the like.

Examples of anti-tumor agents for delivery using the present invention include, without limitation, doxorubicin, Daunorubicin, taxol, methotrexate, and the like. Examples of

antipyretics and analgesics include aspirin, Motrin®, Ibuprofen®, naprosyn, acetaminophen, and the like.

5 Examples of anti-inflammatory agents for delivery using the present invention include, without limitation, include NSAIDS, aspirin, steroids, dexamethasone, hydrocortisone, prednisolone, Diclofenac Na, and the like.

Examples of therapeutic agents for treating osteoporosis and other factors acting on bone and skeleton include for delivery using the present invention include, without limitation, calcium, alendronate, bone GLa peptide, parathyroid hormone and its active fragments, histone H4-related bone formation and proliferation peptide and mutations, derivatives and analogs thereof.

10 Examples of enzymes and enzyme cofactors for delivery using the present invention include, without limitation, pancrease, L-asparaginase, hyaluronidase, chymotrypsin, trypsin, tPA, streptokinase, urokinase, pancreatin, collagenase, trypsinogen, chymotrypsinogen, plasminogen, streptokinase, adenyl cyclase, superoxide dismutase (SOD), and the like.

15 Examples of cytokines for delivery using the present invention include, without limitation, interleukins, transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), epidermal growth factors (EGFs), connective tissue activated peptides (CTAPs), osteogenic factors, and biologically active analogs, fragments, and derivatives of such growth factors. Cytokines may be B/T-cell differentiation factors, B/T-cell growth factors, mitogenic cytokines, chemotactic cytokines, colony stimulating factors, 20 angiogenesis factors, IFN- α , IFN- β , IFN- γ , IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, etc., leptin, myostatin, macrophage stimulating protein, platelet-derived growth factor, TNF- α , TNF- β , NGF, CD40L, CD137L/4-1BBL, human lymphotoxin- β , G-CSF, M-CSF, GM-CSF, PDGF, IL-1 α , IL1- β , IP-10, PF4, GRO, 9E3, erythropoietin, endostatin, angiostatin, VEGF or any fragments or combinations thereof. Other 25 cytokines include members of the transforming growth factor (TGF) supergene family include the beta transforming growth factors (for example TGF- β 1, TGF- β 2, TGF- β 3); bone morphogenetic proteins (for example, BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9); heparin-binding growth factors (for example, fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like 30 growth factor (IGF)); Inhibins (for example, Inhibin A, Inhibin B); growth differentiating factors (for example, GDF-1); and Activins (for example, Activin A, Activin B, Activin AB).

Examples of growth factors for delivery using the present invention include, without limitation, growth factors that can be isolated from native or natural sources, such as from mammalian cells, or can be prepared synthetically, such as by recombinant DNA techniques or by various chemical processes. In addition, analogs, fragments, or derivatives of these factors can be used, provided that they exhibit at least some of the biological activity of the native molecule. For example, analogs can be prepared by expression of genes altered by site-specific mutagenesis or other genetic engineering techniques.

Examples of anticoagulants for delivery using the present invention include, without limitation, include warfarin, heparin, Hirudin, and the like. Examples of factors acting on the immune system include for delivery using the present invention include, without limitation, factors which control inflammation and malignant neoplasms and factors which attack infective microorganisms, such as chemotactic peptides and bradykinins.

Examples of viral antigens and/or viral antigenic targets include, but are not limited to, e.g., retroviral antigens such as retroviral antigens from the human immunodeficiency virus (HIV) antigens such as gene products of the gag, pol, and env genes, the Nef protein, reverse transcriptase, and other HIV components; hepatitis viral antigens such as the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, e.g., hepatitis A, B, and C, viral components such as hepatitis C viral RNA; influenza viral antigens such as hemagglutinin and neuraminidase and other influenza viral components; measles viral antigens such as the measles virus fusion protein and other measles virus components; rubella viral antigens such as proteins E1 and E2 and other rubella virus components; rotaviral antigens such as VP7sc and other rotaviral components; cytomegaloviral antigens such as envelope glycoprotein B and other cytomegaloviral antigen components; respiratory syncytial viral antigens such as the RSV fusion protein, the M2 protein and other respiratory syncytial viral antigen components; herpes simplex viral antigens such as immediate early proteins, glycoprotein D, and other herpes simplex viral antigen components; varicella zoster viral antigens such as gpI, gpII, and other varicella zoster viral antigen components; Japanese encephalitis viral antigens such as proteins E, M-E, M-E-NS1, NS1, NS1-NS2A, 80% E, and other Japanese encephalitis viral antigen components; rabies viral antigens such as rabies glycoprotein, rabies nucleoprotein and other rabies viral antigen components. See *Fundamental Virology*, Second Edition, eds. Fields, B. N. and Knipe, D. M. (Raven Press, New York, 1991) for additional examples of viral antigens.

Antigens and/or antigenic targets that may be delivered using the rAb-DC/DC-antigen vaccines of the present invention include genes encoding antigens such as viral antigens, bacterial antigens, fungal antigens or parasitic antigens. Viruses include picornavirus, coronavirus, togavirus, flavivirus, rhabdovirus, paramyxovirus, orthomyxovirus, bunyavirus, arenavirus, reovirus, retrovirus, papillomavirus, parvovirus, herpesvirus, poxvirus, hepadnavirus, and spongiform virus. Other viral targets include influenza, herpes simplex virus 1 and 2, measles, dengue, smallpox, polio or HIV. Pathogens include trypanosomes, tapeworms, roundworms, helminthes, malaria. Tumor markers, such as fetal antigen or prostate specific antigen, may be targeted in this manner. Other examples include: HIV env proteins and hepatitis B surface antigen. Administration of a vector according to the present invention for vaccination purposes would require that the vector-associated antigens be sufficiently non-immunogenic to enable long term expression of the transgene, for which a strong immune response would be desired. In some cases, vaccination of an individual may only be required infrequently, such as yearly or biennially, and provide long term immunologic protection against the infectious agent. Specific examples of organisms, allergens and nucleic and amino sequences for use in vectors and ultimately as antigens with the present invention may be found in U.S. Patent No. 6,541,011, relevant portions incorporated herein by reference, in particular, the tables that match organisms and specific sequences that may be used with the present invention.

Bacterial antigens for use with the rAb vaccine disclosed herein include, but are not limited to, e.g., bacterial antigens such as pertussis toxin, filamentous hemagglutinin, pertactin, FIM2, FIM3, adenylate cyclase and other pertussis bacterial antigen components; diptheria bacterial antigens such as diptheria toxin or toxoid and other diptheria bacterial antigen components; tetanus bacterial antigens such as tetanus toxin or toxoid and other tetanus bacterial antigen components; streptococcal bacterial antigens such as M proteins and other streptococcal bacterial antigen components; gram-negative bacilli bacterial antigens such as lipopolysaccharides and other gram-negative bacterial antigen components, Mycobacterium tuberculosis bacterial antigens such as mycolic acid, heat shock protein 65 (HSP65), the 30 kDa major secreted protein, antigen 85A and other mycobacterial antigen components; Helicobacter pylori bacterial antigen components; pneumococcal bacterial antigens such as pneumolysin, pneumococcal capsular polysaccharides and other pneumococcal bacterial antigen components; haemophilus influenza bacterial antigens such as capsular polysaccharides and other haemophilus influenza bacterial antigen components; anthrax bacterial antigens such as anthrax protective antigen and other anthrax bacterial antigen components; rickettsiae bacterial antigens

such as rompA and other rickettsiae bacterial antigen component. Also included with the bacterial antigens described herein are any other bacterial, mycobacterial, mycoplasmal, rickettsial, or chlamydial antigens. Partial or whole pathogens may also be: haemophilus influenza; Plasmodium falciparum; neisseria meningitidis; streptococcus pneumoniae; neisseria gonorrhoeae; salmonella serotype typhi; shigella; vibrio cholerae; Dengue Fever; Encephalitides; Japanese Encephalitis; lyme disease; Yersinia pestis; west nile virus; yellow fever; tularemia; hepatitis (viral; bacterial); RSV (respiratory syncytial virus); HPIV 1 and HPIV 3; adenovirus; small pox; allergies and cancers.

Fungal antigens for use with compositions and methods of the invention include, but are not limited to, e.g., candida fungal antigen components; histoplasma fungal antigens such as heat shock protein 60 (HSP60) and other histoplasma fungal antigen components; cryptococcal fungal antigens such as capsular polysaccharides and other cryptococcal fungal antigen components; coccidioides fungal antigens such as spherule antigens and other coccidioides fungal antigen components; and tinea fungal antigens such as trichophytin and other coccidioides fungal antigen components.

Examples of protozoal and other parasitic antigens include, but are not limited to, e.g., plasmodium falciparum antigens such as merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, blood-stage antigen pf 155/RESA and other plasmodial antigen components; toxoplasma antigens such as SAG-1, p30 and other toxoplasmal antigen components; schistosomae antigens such as glutathione-S-transferase, paramyosin, and other schistosomal antigen components; leishmania major and other leishmaniae antigens such as gp63, lipophosphoglycan and its associated protein and other leishmanial antigen components; and trypanosoma cruzi antigens such as the 75-77 kDa antigen, the 56 kDa antigen and other trypanosomal antigen components.

Target antigens on immune cell surfaces that can be targeted using the antigen recognition site of the antibody portion of the rAb of the present invention will generally be selected based on a number of factors, including: likelihood of internalization, level of immune cell specificity, type of immune cell targeted, level of immune cell maturity and/or activation and the like. Examples of cell surface markers for dendritic cells include, but are not limited to, MHC class I, MHC Class II, CD1, CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD16, CD 19, CD20, CD29, CD31, CD40, CD43, CD44, CD45, CD54, CD56, CD57, CD58, CD83, CD86, CMRF-44, CMRF-56, DCIR, DC-ASPGR, CLEC-6, CD40, BDCA-2, MARCO, DEC-205, mannose receptor, Langerin, DECTIN-1, B7-1, B7-2, IFN- γ receptor and IL-2 receptor, ICAM-1, Fc γ

receptor or other receptor relatively specifically expressed by antigen presenting cells. Examples of cell surface markers for antigen presenting cells include, but are not limited to, MHC class I, MHC Class II, CD1, CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD16, CD 19, CD20, CD29, CD31, CD40, CD43, CD44, CD45, CD54, CD56, CD57, CD58, CD83, CD86, CMRF-44, CMRF-56, DCIR, DC-ASPGR, CLEC-6, CD40, BDCA-2, MARCO, DEC-205, mannose receptor, Langerin, DECTIN-1, B7-1, B7-2, IFN- γ receptor and IL-2 receptor, ICAM-1, Fc γ receptor or other receptor relatively specifically expressed by antigen presenting cells. Examples of cell surface markers for T cells include, but are not limited to, CD3, CD4, CD8, CD 14, CD20, CD11b, CD16, CD45 and HLA-DR.

10 Target antigens on cell surfaces for delivery includes those characteristic of tumor antigens typically will be derived from the cell surface, cytoplasm, nucleus, organelles and the like of cells of tumor tissue. Examples of tumor targets for the antibody portion of the present invention include, without limitation, hematological cancers such as leukemias and lymphomas, neurological tumors such as astrocytomas or glioblastomas, melanoma, breast cancer, lung
15 cancer, head and neck cancer, gastrointestinal tumors such as gastric or colon cancer, liver cancer, pancreatic cancer, genitourinary tumors such cervix, uterus, ovarian cancer, vaginal cancer, testicular cancer, prostate cancer or penile cancer, bone tumors, vascular tumors, or cancers of the lip, nasopharynx, pharynx and oral cavity, esophagus, rectum, gall bladder, biliary tree, larynx, lung and bronchus, bladder, kidney, brain and other parts of the nervous system, thyroid, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma and leukemia.

Examples of antigens that may be delivered alone or in combination to immune cells for antigen presentation using the present invention include tumor proteins, e.g., mutated oncogenes; viral proteins associated with tumors; and tumor mucins and glycolipids. The antigens may be viral proteins associated with tumors would be those from the classes of viruses noted above. Certain
25 antigens may be characteristic of tumors (one subset being proteins not usually expressed by a tumor precursor cell), or may be a protein which is normally expressed in a tumor precursor cell, but having a mutation characteristic of a tumor. Other antigens include mutant variant(s) of the normal protein having an altered activity or subcellular distribution, e.g., mutations of genes giving rise to tumor antigens.

30 Specific non-limiting examples of tumor antigens include: CEA, prostate specific antigen (PSA), HER-2/neu, BAGE, GAGE, MAGE 1-4, 6 and 12, MUC (Mucin) (e.g., MUC-1, MUC-2, etc.), GM2 and GD2 gangliosides, ras, myc, tyrosinase, MART (melanoma antigen), Pmel 17(gp100), GnT-V intron V sequence (N-acetylglucoaminytransferase V intron V sequence), Prostate Ca

psm, PRAME (melanoma antigen), β -catenin, MUM-1-B (melanoma ubiquitous mutated gene product), GAGE (melanoma antigen) 1, BAGE (melanoma antigen) 2-10, c-ERB2 (Her2/neu), EBNA (Epstein-Barr Virus nuclear antigen) 1-6, gp75, human papilloma virus (HPV) E6 and E7, p53, lung resistance protein (LRP), Bcl-2, and Ki-67. In addition, the immunogenic
5 molecule can be an autoantigen involved in the initiation and/or propagation of an autoimmune disease, the pathology of which is largely due to the activity of antibodies specific for a molecule expressed by the relevant target organ, tissue, or cells, e.g., SLE or MG. In such diseases, it can be desirable to direct an ongoing antibody-mediated (i.e., a Th2-type) immune response to the relevant autoantigen towards a cellular (i.e., a Th1-type) immune response. Alternatively, it can
10 be desirable to prevent onset of or decrease the level of a Th2 response to the autoantigen in a subject not having, but who is suspected of being susceptible to, the relevant autoimmune disease by prophylactically inducing a Th1 response to the appropriate autoantigen. Autoantigens of interest include, without limitation: (a) with respect to SLE, the Smith protein, RNP ribonucleoprotein, and the SS-A and SS-B proteins; and (b) with respect to MG, the
15 acetylcholine receptor. Examples of other miscellaneous antigens involved in one or more types of autoimmune response include, e.g., endogenous hormones such as luteinizing hormone, follicular stimulating hormone, testosterone, growth hormone, prolactin, and other hormones.

Antigens involved in autoimmune diseases, allergy, and graft rejection can be used in the compositions and methods of the invention. For example, an antigen involved in any one or
20 more of the following autoimmune diseases or disorders can be used in the present invention: diabetes, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to
25 Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral
30 progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Crohn's disease, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis. Examples of

antigens involved in autoimmune disease include glutamic acid decarboxylase 65 (GAD 65), native DNA, myelin basic protein, myelin proteolipid protein, acetylcholine receptor components, thyroglobulin, and the thyroid stimulating hormone (TSH) receptor. Examples of antigens involved in allergy include pollen antigens such as Japanese cedar pollen antigens, ragweed pollen antigens, rye grass pollen antigens, animal derived antigens such as dust mite antigens and feline antigens, histocompatibility antigens, and penicillin and other therapeutic drugs. Examples of antigens involved in graft rejection include antigenic components of the graft to be transplanted into the graft recipient such as heart, lung, liver, pancreas, kidney, and neural graft components. The antigen may be an altered peptide ligand useful in treating an autoimmune disease.

As used herein, the term “epitope(s)” refer to a peptide or protein antigen that includes a primary, secondary or tertiary structure similar to an epitope located within any of a number of pathogen polypeptides encoded by the pathogen DNA or RNA. The level of similarity will generally be to such a degree that monoclonal or polyclonal antibodies directed against such polypeptides will also bind to, react with, or otherwise recognize, the peptide or protein antigen. Various immunoassay methods may be employed in conjunction with such antibodies, such as, for example, Western blotting, ELISA, RIA, and the like, all of which are known to those of skill in the art. The identification of pathogen epitopes, and/or their functional equivalents, suitable for use in vaccines is part of the present invention. Once isolated and identified, one may readily obtain functional equivalents. For example, one may employ the methods of Hopp, as taught in U.S. Pat. No. 4,554,101, incorporated herein by reference, which teaches the identification and preparation of epitopes from amino acid sequences on the basis of hydrophilicity. The methods described in several other papers, and software programs based thereon, can also be used to identify epitopic core sequences (see, for example, Jameson and Wolf, 1988; Wolf et al., 1988; U.S. Pat. No. 4,554,101). The amino acid sequence of these “epitopic core sequences” may then be readily incorporated into peptides, either through the application of peptide synthesis or recombinant technology.

As used herein, the term “promoter” describes a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The phrases “operatively positioned,” “operatively linked,” “under control,” and “under transcriptional control” mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence (i.e., ORF) to control

transcriptional initiation and/or expression of that sequence. A promoter may or may not be used in conjunction with an “enhancer,” which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence. A listing of promoters and/or enhancers that may be used with the present invention is described in, e.g., U.S. Patent
5 No. 6,410,241, relevant descriptions and tables incorporated herein by reference.

As used herein, the terms “cell,” “cell line,” and “cell culture” may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations, in vivo, ex vivo or in vitro. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, “host
10 cell” refers to a prokaryotic or eukaryotic cell, and it includes any transformable organism that is capable of expressing a heterologous gene encoded by a vector as delivered using the rAb protein vector of the present invention. A host cell can, and has been, used as a recipient for vectors. A host cell may be “transfected” or “transformed,” which refers to a process by which the exogenous nucleic acid expressing an antigen, as disclosed herein, is transferred or
15 introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

The preparation of vaccine compositions that includes the nucleic acids that encode antigens of the invention as the active ingredient, may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to infection can
20 also be prepared. The preparation may be emulsified, encapsulated in liposomes. The active immunogenic ingredients are often mixed with carriers which are pharmaceutically acceptable and compatible with the active ingredient.

The term “pharmaceutically acceptable carrier” refers to a carrier that does not cause an allergic reaction or other untoward effect in subjects to whom it is administered. Suitable
25 pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants that may be effective include but are not
30 limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, MTP-PE and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. Other examples of

adjuvants include DDA (dimethyldioctadecylammonium bromide), Freund's complete and incomplete adjuvants and QuilA. In addition, immune modulating substances such as lymphokines (e.g., IFN- γ , IL-2 and IL-12) or synthetic IFN- γ inducers such as poly I:C can be used in combination with adjuvants described herein.

5 Pharmaceutical products that may include a naked polynucleotide with a single or multiple copies of the specific nucleotide sequences that bind to specific DNA-binding sites of the apolipoproteins present on plasma lipoproteins as described in the current invention. The polynucleotide may encode a biologically active peptide, antisense RNA, or ribozyme and will be provided in a physiologically acceptable administrable form. Another pharmaceutical
10 product that may spring from the current invention may include a highly purified plasma lipoprotein fraction, isolated according to the methodology, described herein from either the patients blood or other source, and a polynucleotide containing single or multiple copies of the specific nucleotide sequences that bind to specific DNA-binding sites of the apolipoproteins present on plasma lipoproteins, prebound to the purified lipoprotein fraction in a physiologically
15 acceptable, administrable form.

Yet another pharmaceutical product may include a highly purified plasma lipoprotein fraction which contains recombinant apolipoprotein fragments containing single or multiple copies of specific DNA-binding motifs, prebound to a polynucleotide containing single or multiple copies of the specific nucleotide sequences, in a physiologically acceptable administrable form. Yet
20 another pharmaceutical product may include a highly purified plasma lipoprotein fraction which contains recombinant apolipoprotein fragments containing single or multiple copies of specific DNA-binding motifs, prebound to a polynucleotide containing single or multiple copies of the specific nucleotide sequences, in a physiologically acceptable administrable form.

The dosage to be administered depends to a great extent on the body weight and physical
25 condition of the subject being treated as well as the route of administration and frequency of treatment. A pharmaceutical composition that includes the naked polynucleotide prebound to a highly purified lipoprotein fraction may be administered in amounts ranging from 1 μ g to 1 mg polynucleotide and 1 μ g to 100 mg protein.

Administration of the therapeutic virus particle to a patient will follow general protocols for the
30 administration of chemotherapeutics, taking into account the toxicity, if any, of the vector. It is anticipated that the treatment cycles would be repeated as necessary. It also is contemplated that

various standard therapies, as well as surgical intervention, may be applied in combination with the described gene therapy.

Where clinical application of a gene therapy is contemplated, it will be necessary to prepare the complex as a pharmaceutical composition appropriate for the intended application. Generally this will entail preparing a pharmaceutical composition that is essentially free of pyrogens, as well as any other impurities that could be harmful to humans or animals. One also will generally desire to employ appropriate salts and buffers to render the complex stable and allow for complex uptake by target cells.

Aqueous compositions of the present invention may include an effective amount of the compound, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions can also be referred to as inocula. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions. The compositions of the present invention may include classic pharmaceutical preparations. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

Disease States. Depending on the particular disease to be treated, administration of therapeutic compositions according to the present invention will be via any common route so long as the target tissue is available via that route in order to maximize the delivery of antigen to a site for maximum (or in some cases minimum) immune response. Administration will generally be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Other areas for delivery include: oral, nasal, buccal, rectal, vaginal or topical. Topical administration would be particularly advantageous for treatment of skin cancers. Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients.

Vaccine or treatment compositions of the invention may be administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories, and in some cases, oral formulations or formulations suitable for distribution as aerosols. In the case of the oral formulations, the manipulation of T-cell subsets employing adjuvants, antigen packaging, or the

addition of individual cytokines to various formulation that result in improved oral vaccines with optimized immune responses. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25-70%.

10 The antigen encoding nucleic acids of the invention may be formulated into the vaccine or treatment compositions as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or with organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups
15 can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Vaccine or treatment compositions are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective.

20 The quantity to be administered depends on the subject to be treated, including, e.g., capacity of the subject's immune system to synthesize antibodies, and the degree of protection or treatment desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a range from about 0.1 mg to 1000 mg, such as in the range from about 1 mg to 300 mg, and preferably in the range from about 10 mg to 50 mg. Suitable regimens for
25 initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and may be peculiar to each subject. It will be apparent to those of skill in the art that the therapeutically effective amount of nucleic acid molecule or fusion polypeptides of this invention will depend,
30 inter alia, upon the administration schedule, the unit dose of antigen administered, whether the nucleic acid molecule or fusion polypeptide is administered in combination with other therapeutic agents, the immune status and health of the recipient, and the therapeutic activity of the particular nucleic acid molecule or fusion polypeptide.

The compositions can be given in a single dose schedule or in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include, e.g., 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the immune response, for example, at 1-4 months for a second dose, and if
5 needed, a subsequent dose(s) after several months. Periodic boosters at intervals of 1-5 years, usually 3 years, are desirable to maintain the desired levels of protective immunity. The course of the immunization can be followed by in vitro proliferation assays of peripheral blood lymphocytes (PBLs) co-cultured with ESAT6 or ST-CF, and by measuring the levels of IFN- γ released from the primed lymphocytes. The assays may be performed using conventional labels,
10 such as radionuclides, enzymes, fluorescent labels and the like. These techniques are known to one skilled in the art and can be found in U.S. Pat. Nos. 3,791,932, 4,174,384 and 3,949,064, relevant portions incorporated by reference.

The modular rAb carrier and/or conjugated rAb carrier-(cohesion/dockerin and/or dockerin-cohesin)-antigen complex (rAb-DC/DC-antigen vaccine) may be provided in one or more “unit
15 doses” depending on whether the nucleic acid vectors are used, the final purified proteins, or the final vaccine form is used. Unit dose is defined as containing a predetermined-quantity of the therapeutic composition calculated to produce the desired responses in association with its administration, i.e., the appropriate route and treatment regimen. The quantity to be administered, and the particular route and formulation, are within the skill of those in the clinical
20 arts. The subject to be treated may also be evaluated, in particular, the state of the subject’s immune system and the protection desired. A unit dose need not be administered as a single injection but may include continuous infusion over a set period of time. Unit dose of the present invention may conveniently may be described in terms of DNA/kg (or protein/Kg) body weight, with ranges between about 0.05, 0.10, 0.15, 0.20, 0.25, 0.5, 1, 10, 50, 100, 1,000 or more
25 mg/DNA or protein/kg body weight are administered. Likewise the amount of rAb-DC/DC-antigen vaccine delivered can vary from about 0.2 to about 8.0 mg/kg body weight. Thus, in particular embodiments, 0.4 mg, 0.5 mg, 0.8 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, 4.0 mg, 5.0 mg, 5.5 mg, 6.0 mg, 6.5 mg, 7.0 mg and 7.5 mg of the vaccine may be delivered to an individual in vivo. The dosage of rAb-DC/DC-antigen vaccine to be administered depends to a
30 great extent on the weight and physical condition of the subject being treated as well as the route of administration and the frequency of treatment. A pharmaceutical composition that includes a naked polynucleotide prebound to a liposomal or viral delivery vector may be administered in amounts ranging from 1 μ g to 1 mg polynucleotide to 1 μ g to 100 mg protein. Thus, particular

compositions may include between about 1 μ g, 5 μ g, 10 μ g, 20 μ g, 30 μ g, 40 μ g, 50 μ g, 60 μ g, 70 μ g, 80 μ g, 100 μ g, 150 μ g, 200 μ g, 250 μ g, 500 μ g, 600 μ g, 700 μ g, 800 μ g, 900 μ g or 1,000 μ g polynucleotide or protein that is bound independently to 1 μ g, 5 μ g, 10 μ g, 20 μ g, 30 μ g, 40 μ g, 50 μ g, 60 μ g, 70 μ g, 80 μ g, 100 μ g, 150 μ g, 200 μ g, 250 μ g, 500 μ g, 600 μ g, 700 μ g, 800 μ g, 900 μ g, 1 mg, 1.5 mg, 5 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg vector.

The present invention was tested in an in vitro cellular system that measures immune stimulation of human Flu-specific T cells by dendritic cells to which Flu antigen has been targeted. The results shown herein demonstrate the specific expansion of such antigen specific cells at doses of the antigen which are by themselves ineffective in this system.

The present invention may also be used to make a modular rAb carrier that is, e.g., a recombinant humanized mAb (directed to a specific human dendritic cell receptor) complexed with protective antigens from Ricin, Anthrax toxin, and Staphylococcus B enterotoxin. The potential market for this entity is vaccination of all military personnel and stored vaccine held in reserve to administer to large population centers in response to any biothreat related to these agents. The invention has broad application to the design of vaccines in general, both for human and animal use. Industries of interest is pharmaceutical and biotechnology

One commercial application of the invention is a recombinant humanized mAb (directed to the specific human dendritic cell receptor DCIR) fused through the Ab heavy chain to antigens known or suspected to encode protective antigens. These include as examples for vaccination against various agents - hemagglutinins from Influenza H5N1; HIV gag from attenuated toxins from Ricin, Anthrax toxin, and Staphylococcus B enterotoxin; 'strings' of antigenic peptides from melanoma antigens, etc. The potential market for this entity is preventative or therapeutic vaccination of at risk or infected people. The invention has broad application for vaccination against many diseases and cancers, both for human and animal use. Industries of interest are pharmaceutical and biotechnology. In addition, this invention has implications beyond anti-DCIR application since it describes a method to identify particularly favorable sequences to enhance secretion of recombinant antibodies.

The application of anti-DCIR combining regions for making engineered recombinant monoclonal antibodies fused to antigens as potent therapeutic or preventative vaccination agents. Use of different V-region sequences against the same combining specificity to find those most

compatible with efficient expression of a H chain C-terminal linked antigen or other protein sequence.

EXAMPLE 1. Multiple Antigens Targeted in a Complex simultaneously with the same engineered Humanized mAb (MATCHMAB).

5 One type of therapeutic (in this case, vaccination) entity envisioned is a humanized DC-targeting mAb-antigen fusion protein, where the antibody variable region specificity is directed against an internalizing human dendritic cell receptor. The present state-of-the art is to engineer the fusion of the desired antigen to the C-terminus of the mAb H chain. This paradigm obviously allows different antigens (A1, A2, A3) to be engineered to the same proven targeting mAb backbone (Y
10 in the figure below), thus extending the utility of the one mAb to immunizing against different pathogenic agents. This concept can be further extended by engineering, e.g., the A1, A2, A3 coding regions end-to-end fused to the IgGFc C-terminal coding region.

The present invention disclosed a new paradigm for linking the antigen to the targeting mAb that extends the concept for the first time to multiple antigens targeted in a complex simultaneously
15 with the same engineered humanized mAb (MATCHMAB).

Figure 1 compares the prior art (top portion) with an example of the multiple antigens targeted in a complex simultaneously with the same engineered humanized mAb (MATCHMAB)(bottom portion). Y represents the humanized anti-DC targeting mAb; A1, A2, A3 are independent protective antigens, or any other desired protein domains; C1, C2, C2 are specific high affinity capture domains for, respectively, docking domains D1, D2, D3; and DnAn are the
20 corresponding docking-antigen fusion proteins. Note that the various domains are not drawn to scale. The mAb itself is ~150 kDa, C is ~17 Da, D is ~8 kDa and A varies, but is usually >20 kDa).

The MATCHMAB is based on using cellulosome-assembly cohesin-dockerin sequences to form
25 modular non-covalent targeting mAb-antigen complexes. The relatively small and specific cohesin-dockerin protein-protein interaction domains can allow simple customized formulation of targeting mAb-antigen complexes. Thus, a single manufactured humanized mAb (in the above notation: Y.C1.C2.C3.Cn) can be use as the basis of delivering multiple antigens in various, yet strictly defined, combinations.

30 Example of sequence encoding C1.C2.C3.Cn is taken from the public sequence >gi|50656899|gb|AAT79550.1| of cellulosomal anchoring scaffoldin B precursor (*Bacteroides cellulosolvens*). Below with blue showing the leader secretion sequence and yellow and grey

technology can also be used to enhance ('mature') the affinity of a C-D interaction, should this be desired. Also, engineering cysteine residues at opposing faces of the C-D interaction (based on modeling from the published C-D structures) could be used to make a covalent bond between C-D to strengthen the interaction. Furthermore, the dimeric nature of the mAb (and therefore the
5 linked C-domains) can be used to advantage for affinity enhancement purposes. In this embodiment, e.g., the D-antigen fusion protein is engineered either with a second identical dockerin domain (D-antigen-D, or D-D-antigen), or with a homodimerization domain. This configuration, provided the linkers between domains were not constraining, will result in the preferred simultaneous binding of both D domains to the same mAb, with greatly enhanced
10 stability compared to the single interaction.

Based on the crystal structure of the cohesin-dockerin complex (e.g., see PNAS 2003,13809-13814, Cellulosome assembly revealed by the crystal structure of the cohesin-dockerin complex. Ana L. Carvalho *, Fernando M. V. Dias, José A. M. Prates, Tibor Nagy, Harry J. Gilbert, Gideon J. Davies, Luís M. A. Ferreira, Maria J. Romão and Carlos M. G. A. Fonte), it is
15 apparent that one embodiment is an antigen-dockerin fusion proteins (i.e., antigen fused to the N-terminus of a dockerin). However, both from the structure and from the nature of cohesin domain organization within scaffoldins, it is apparent that cohesions can be fused end-to-end, even without spacer sequences. Furthermore, it is apparent that well-described techniques are available to engineer miniaturized versions of the cohesin and dockerin domains (see for
20 example, Proc. Natl. Acad. Sci. USA Vol. 94, pp. 10080-10085, September 1997. Structural mimicry of a native protein by a minimized binding domain. Melissa A. Starovasnik, Andrew C. Braisted, And James A. Wells).

It is recognized herein that the linker sequences have a propensity for O-linked glycosylation resulting from ST richness. Also, both the C and D domains can have potential N-linked sites.
25 These features can be advantageous in enhancing the solubility of the mammalian cell-expressed engineered mAb through decoration with carbohydrates. Of course, the consequences of glycosylation of the C domains needs to be check by function (binding to the cognate D), and if needed rectified by site directed mutagenesis. An attractive feature of this invention is that D-A can be expressed in whatever system is known to be best. For example, the tumor antigen
30 MART1 is a membrane protein and is best prepared in high yield via E. coli inclusion bodies. Schema using antigens directly fused to the mAb are restricted to antigens that are compatible with mammalian-cell expression.

Another embodiment of the invention is the use of the D-C interaction to make bi-specific mAbs joined tail-to-tail. Figure 2 shows the use of the present invention to form Bi-specific mAbs. mAb1 (black) is expressed with C-terminal C1 and mAb2 (magenta) is expressed with C-terminal D1. Mixing equimolar mAb1 and mAb2 will result in a bi-specific 1:1 complex. Note that, since each mAb molecule contains two molar equivalents of C or D (the mAb is itself a dimeric structure), the bi-specific mAb will be greatly stabilized by two concurrent C-D interactions. Especially at lower (mAb), this will be the most stable configuration.

EXAMPLE 2. Combination of Antibody and Cohesion/Dockerin domains and antigens.

Example 2 shows that particular cohesin and dockerin domains can be successfully and efficiently secreted from mammalian cells as fusion proteins while maintaining the specific and high affinity cohesin-dockerin protein-protein interaction. While the extensive cohesin-dockerin literature teaches the expectation that such fusion proteins should have this functionality, it does not describe production of such fusion proteins in mammalian secretion systems. The state of scientific knowledge does not allow the prediction of the discovery since the rules (other than features such as signal peptide) for successful secretion are not fully established. Furthermore, the cohesin linker regions are known to be glycosylated in their native bacteria, and the cohesin and dockerin domains contain predicted glycosylation sites. While this may actually favor secretion from mammalian cells, it is unclear if 'unnatural' glycosylation will perturb the cohesin-dockerin interaction.

While cohesin-dockerin interaction for various commercial applications has been published, the present invention is based on a previously unrealized utility for this interaction built around assembling specific protein complexes unrelated to the envisioned controlled assembly enzyme applications.

The invention includes the use of all cohesin-dockerin sequences from diverse cellulose degrading microbes, but describes the application of specific cohesin and dockerin and linker sequences from the microbe *Clostridium thermocellum*. For example, the sequence described in Table 1 encodes the H chain of a human IgG4 linked at the C-terminal codon to a *Clostridium thermocellum* dockerin sequence (called rAb.doc). Other embodiments of rAb.doc proteins are described similarly in Table 2 and these are engineered by simply transferring the dockerin coding region as a DNA fragment to vectors encoding the different H chain entities.

TABLE 1 shows the nucleic acid and amino acid sequences for rAB-pIRES2(hIgG4H-Dockerin) or C52. DNA (entire coding region) and amino acid sequence (the predicted secreted product) of

human IgG4H.doc fusion protein is shown below. The dockerin domain (taken from Clostridium thermocellum celD is highlighted in yellow and the H chain and dockerin joining sequence is underlined. The highly predicted N-linked glycosylation site within the dockerin domain is highlighted in red.

5 TABLE 1. rAB-pIRES2(hIgG4H-Dockerin) or C52.

ATGGACCTCCTGTGCAAGAACATGAAGCACCTGTGGTTCTTCCTCCTGCTGGTGGCGGCTCCAGATGGGTCTGTCCCAGGCTGCA
 GCTGCAGGAGTCGGGCCAGGCCTGCTGAAGCCTTCGGTGACCTGTCCCTCACCTGCACTGTCTCGGGTGACTCCGTCGCCAGTA
 GTTCTTATTACTGGGGCTGGGTCCGTCAGCCCCAGGGAAGGACTCGAGTGGATAGGGACTATCAATTTTAGTGGAATATGTAT
 10 TATAGTCCGTCCTCAGGAGTCGAGTGACCATGTCGGCAGACATGTCGGAGAACTCCTTCTATCTGAAATTGGACTCTGTGACCGC
 AGCAGACACGGCCGTCTATTATTGTGCGCAGGACACCTCGTTATGGGATTGGGGCCCACTGGGGACAGGGAAAAGTGGTCTCG
 TCTCTCCAGCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGC
 TGCCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCGC
 TGCTTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTGGGGCACGAAGACCTACACCTGCA
 15 ACGTAGATCACAAAGCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAAATATGGTCCCCCATGCCACCCTGCCAGCACCT
 GAGTTCGAAGGGGGACCATCAGTCTTCTGTTCCCCCAAAACCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTG
 CGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAA
 AGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAG
 TACAAGTGAAGGTCTCCAACAAAGGCCCTCCCGTCTCCATCGAGAAAACCATCTCAAAGCCAAAGGGCAGCCCCGAGAGCCACA
 20 GGTGTACACCCTGCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCTGGTCAAAGGCTTCTACCCAGCG
 ACATCGCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACAACTACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTC
 TTCCTTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCA
 CAACCACTACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAAGCTAGCAATCTCCTCAAAATGAAGTACTGTACGGAGATGTGA
 ATGATGACGGAAAAGTAACTCCACTGACTTGACTTTGTAAAAAGATATGTTCTTAAAGCCGTCTCAACTCTCCCTTCTCCAAA
 25 GCTGAAAAGAAGCAGATGTAAATCGTGACGGAAGAGTTAATTCAGTGATGTACAATACTTCAAGATATTTGATAAGGGTAAT
 CGAGAAATTACCAATATAA (SEQ ID NO.:2)

RLQLQESGPGLLKPSVTLTCTVSGDSVASSSYWGWVRQPPGKLEWIGTINFSGNMYYSPSLRSRVTMSADMSSENSFYKLDLDS
 VTAADTAVYCAAGHLVMGFGAHWGQKLVSVSPASTKGPSVFFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
 30 FPAVLQSSGLYSLSSVTVFSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPEPFEFEGGSPVFLFPKPKDLMISRTPE
 VTCVVVDVQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPR
 EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVME
 ALHNHYTQKLSLSLGLKASNSPQNEVLYGDVINDDGKVINSTDITLLKRYVLKAVSTLPSKAEKRNADVNRDGRV DVTILSRYL
 RVIEKLPI (SEQ ID NO.:3)

35 TABLE 2 shows the nucleic acid and amino acid sequences for rAB-pIRES2(mAnti-DCIR2C9H-LV-hIgG4H-C-Dockerin) or C82. DNA (entire coding region) and amino acid sequence (the predicted secreted product) is shown below. The dockerin domain is highlighted in yellow and the H chain and dockerin joining sequence is underlined. The IgG variable region is highlighted in blue. The highly predicted N-linked glycosylation site within the dockerin domain
 40 is highlighted in red.

TABLE 2. rAB-pIRES2(mAnti-DCIR2C9H-LV-hIgG4H-C-Dockerin) or C82.

ATGAAATGCAGCTGGGTCACTTCTTCTCCTGATGGCAGTGGTTACAGGGGTCAATTGAGAGGTTGAGTGCAGCAGTCTGGGGCTGA
 GCTTGTGAGGCCAGGGCCCTTAGTCAAGTTGTCCTGCAAAGCTTCTGGCTTCAACATTAATGACTACTATATCCACTGGGTGAAGC
 45 AGCGGCTGAACAGGGCCTGGAGCGGATTGGATGGATTGATCCTGACAATGGTAATACTATATATGACCCGAAGTTCAGGGCAAG
 GCCAGTATAACAGCAGACACATCCCCAACACAGCCTACCTGCAGCTCAGCAGCCTGACATCTGAGGACACTGCCGTCTATTACTG
 TGCTAGAACCCGATCTCCTATGGTTACGACGGGGTTGTTACTGGGCCAAGGGACTGTGGTCACTGTCTCTGCAGCCAAAACGA
 AGGGCCATCCGTCTTCCCTTGGCGCCCTGCTCCAGGAGCACTCCGAGAGCACAGCCGCTGGGCTGCCTGGTCAAGGACTAC
 TTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCGCTGTCTACAGTCTCAGG
 50 ACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAAGCCCA
 GCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAAATATGGTCCCCCATGCCACCCTGCCAGCACCTGAGTTCGAAGGGGGACCA
 TCAGTCTTCTGTTCCCCCAAACCAAGGACACTCTCATGATCTCCCGACCCCTGAGGTACAGTGCCTGGTGGTGGACGTGAG
 CCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACRAAGCCGCGGGAGGAGCAGT

TCACAGCACGTACCGTGTGGTCAGCGTCTCCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTCTCC
 AACAAAGGCCTCCCGTCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCC
 ATCCCAGGAGGAGATGACCAAGAACCAGGTGACGCTGACCTGGCTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGG
 5 AGAGCAATGGGCAGCCGAGAACAACCTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTACAGCAGGCTA
 ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAA
 GAGCCTCTCCCTGTCTCTGGGTAAAGCTAGCAATCTCCTCAAATGAAGTACTGTACGGAGATGTGAATGATGACGGAAAAGTAA
 ACTCCACTGACTTGTGTTAAAAAGATATGTTCTTAAAGCCGCTCAACTCTCCCTTCTCCAAAGCTGAAAAGAACGCAGAT
 GTAAATCGTGACGGAAGAGTTAATTCCAGTGATGTACAATACTTTCAAGATATTTGATAAGGGTAATCGAGAAATTACCAATATA
 A (SEQ ID NO.:4)

EVQLQDSGAEIVRPPGALVPLSCKAGGPHINDYYTHVKORPEQGLERIGWIDFDNHTIYDPEFOGKASTADTSNTAYLQLSSD
 EGEDTAVVYFCARTFSEHVTGFEVYGGGTVVVSAAKTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSVGH
 TFPVAVLQSSGLYSLSVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPAPEFEGGSPVFLFPPKPKDTLMISRTF
 10 EVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQP
 REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
 15 EALHNHYTQKSLSLSLGKASNSPQNEVLYGDVNDGKVNSTDLTLLKRYVLKAVSTLPSKAEKNADVNRDGRV DVTILSRYL
 IRVIEKLPI. (SEQ ID NO.:5)

TABLE 3 shows the nucleic acid and amino acid sequences for rAB-(mAnti-
 20 ASGPR_49C11_7H-SLAML-V-hIgG4H-C-Dockerin) or C153. DNA (entire coding region) and
 amino acid sequence (the predicted secreted product) is shown below. The dockerin domain is
 highlighted in yellow and the H chain and dockerin joining sequence is underlined. The IgG
 variable region is highlighted in blue. The highly predicted N-linked glycosylation site within
 the dockerin domain is highlighted in red.

25 TABLE 3. rAB-(mAnti-ASGPR_49C11_7H-SLAML-V-hIgG4H-C-Dockerin) or C153.

ATGGACCCCAAAGGCTCCCTTTCTGGAGAATACTTCTGTTTCTCTCCCTGGCTTTTGTAGTTGTCGTACGGAGATGTGCAGCTTCA
 GGAGTCAGGACCTGACCTGGTGAAACCTTCTCAGTCACTTCACTCACCTGCACTGTCACTGGCTACTCCATCACCAGTGGTTATA
 GCTGGCACTGGATCCGGCAGTTTCCAGGAAACAAACTGGAATGGATGGGTACATACTCTTCACTGGTAGCTAACTACAACCA
 30 TCTCTGAAAAGTGAATCTCTATCACTCGAGACACATCCAAGAACCAGTTCTTCTGCACTTGAATCTGTGACTACTGAGGACAC
 AGCCACATATTTCTGTGCAAGATCTAATATGGTTCCTTTGCTTCTCTGGGGCCAAAGGACTCTGGTCACTGTCTCTGCAGCCAAAA
 CAAAGGGCCCATCCGCTTCCCCCTGGCGCCTGCTCCAGGACACCTCCGAGAGCACAGCCGCCCTGGCTGGCTGGTCAAGGAC
 TACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCCCTGACCAGCGGCTGCACACCTTCCCGGCTGCTTACAGTCCCTC
 AGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAGC
 35 CCAGCAACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCTGCCAGCACCTGAGTTCGAAGGGGGA
 CCATCAGTCTTCTGTTCCCCCAAAACCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAGTGCCTGGTGGTGGACGT
 GAGCCAGGAAGACCCCGAGGTCCAGTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
 AGTTCAACAGCAGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTC
 40 TCCAACAAGGCCTCCCGTCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCGAGAGCCACAGGTGTACACCCTGCC
 CCCATCCAGGAGGAGATGACCAAGAACCAGGTGACGCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGT
 GGGAGAGCAATGGGCAGCCGAGAAACAACCTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTACAGCAGG
 CTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACA
 GAAGAGCCTCTCCCTGTCTCTGGGTAAAGCTAGCAATCTCCTCAAATGAAGTACTGTACGGAGATGTGAATGATGACGGAAAAG
 TAACTCCACTGACTTGTGTTAAAAAGATATGTTCTTAAAGCCGCTCAACTCTCCCTTCTTCCAAAGCTGAAAAGAACGCA
 45 GATGTAATCGTGACGGAAGGTTAATTCAGTGATGTACAATACTTTCAAGATATTTGATAAGGGTAATCGAGAAATTACCAAT
 ATAA (SEQ ID NO.:6)

DVQLQDSGAEIVRPPGALVPLSCKAGGPHINDYYTHVKORPEQGLERIGWIDFDNHTIYDPEFOGKASTADTSNTAYLQLSSD
 EGEDTAVVYFCARTFSEHVTGFEVYGGGTVVVSAAKTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSVGH
 50 TFPVAVLQSSGLYSLSVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPAPEFEGGSPVFLFPPKPKDTLMISRTPEVTC
 VVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQ
 VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH
 NHYTQKSLSLSLGKASNSPQNEVLYGDVNDGKVNSTDLTLLKRYVLKAVSTLPSKAEKNADVNRDGRV DVTILSRYLIRVI
 EKLPI (SEQ ID NO.:7)

55 TABLE 4 shows the nucleic acid and amino acid sequences for rAB-pIRES2(mAnti-DC-
 SIGNAL16E7H-LV-hIgG4H-C-Dockerin) or C92. DNA (entire coding region) and amino acid

sequence (the predicted secreted product) is shown below. The dockerin domain is highlighted in yellow and the H chain and dockerin joining sequence is underlined. The IgG variable region is highlighted in blue. The highly predicted N-linked glycosylation site within the dockerin domain is highlighted in red.

5 TABLE 4. rAB-pIRES2(mAnti-DC-SIGNL16E7H-LV-hIgG4H-C-Dockerin) or C92

10 ATGGAAGGCACTGGATCTTTCTCTTCTGTTTTTCAGTAACTGCAGGTGTCCACTCCCAGGTCCAGCTTCAGCAGTCTGGGGCTGA
 GCTGGCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACCTTTACTACCTACTGGATGCACTGGGTA
 AGAGCCCTGGACAGGGTCTGGAATGGATTGGATACATTAATCCTATCACTGGTTATACTGAGTACAATCAGAAGTTCAAGGACAAG
 GCCACCTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTTCAGCTTATTACTG
 TGCAAGAGAGGGTTTTAAGTGTATGGACTATTGGGGTCAGGGAACCTCAGTCACCGTCACCTCAGCCAAAACACGGGCCCATCCG
 TCTTCCCCCTGGCGCCCTGTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
 GTGACGGTGTGCTGGAAGTCAAGCGCCCTGACCAGCGCGTGCACACCTTCCGGCTGTCTACAGTCTCAGGACTCTACTCCCT
 CAGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCAGCAACACCAAGG
 TGGACAAGAGAGTTGAGTCCAAATATGGTCCCCATGCCACCCTGCCAGCACCTGAGTTCGAAGGGGACCATCAGTCTTCTG
 15 TTCCCCCAAACCAAGGACACTTCTATGATCTCCCGGACCCCTGAGGTACGTCGCTGGTGGTGGACGTGAGCCAGGAAGACC
 CGAGGTCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTCAACAGCACGT
 ACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGGCCTC
 CCGTCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCATCCCAGGAGGA
 GATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
 20 AGCCGGAGAACAACACTAAGACCACGCTCCCGTGTGGACTCCGACGGTCTCTTCTTCTTACAGCAGGCTAACCGTGGACAAAG
 AGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCT
 GTCTCTGGGTAAGCTAGCAATTCTCTCAAATGAAGTACTGTACGGAGATGTGAATGATGACGGAAAAGTAACTCCACTGACT
 TGACTTTGTTAAAAGATATGTTCTTAAAGCCGTCTCAACTCTCCCTTCTTCCAAAGCTGAAAAGAACGCAGATGTAATCGTGAC
 25 GGAAGAGTTAATCCAGTGATGCACAATACTTTCAAGATATTTGATAAGGGTAATCGAGAAATTACCAATATAA (SEQ ID
 NO. : 8)

30 QVQLQSGAELAPPGASVFMSCFASGYTFTYMHYKQEPQGLEIGYINFTGYTEINCKFDKATLADKSSQAYINQSSZ
 TSEDSAVLYCAREGLSAMDYKGGTQSVTVYSAKTTGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSQVHTFPA
 VLQSSGLYSLSSVTVTPSSSLGTKTYTCNVDRKPSNTKVDKRVESESKYGPCCPFCPEPEFEGGSPVFLFPPKPKDTLMISRTPEVTC
 VVVDVSEQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQ
 VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNIVFSCSVMHEALH
 NHYTQKSLSLGKASNSPQNEVLYGDVNDGKVNSTDLTLTKRYVLKAVSTLPSKAEKINADVNIRDGRVSDVTTLSRYLIRVI
 40 EKLPI (SEQ ID NO. : 9)

Mammalian expression plasmids encoding such rAb.doc IgG H chain proteins are created using standard molecular biology techniques and can be based on commercially available expression plasmid vectors such as pIRES2-DsRed2 (BD Biosciences). To produce secreted rAb.doc, mammalian cells are co-transfected with this expression plasmid and an expression plasmid encoding a complimentary IgG L chain (exemplified in Table 3). Standard protocols (such as the FreeStyle™ 293 Expression System, Invitrogen) are used as for mammalian cells, transfection reagents, and culture media. Transfected cells are cultured for 3-7 days and the culture supernatant is harvested by centrifugation, clarified by filtration, and the rAB.doc protein purified by Protein G affinity chromatography using protocols from the column manufacturer (GE Pharmacia).

Figure 3 shows analysis of typical secreted rAb.doc products by reducing SDS.PAGE with staining by Coomassie Brilliant Blue. This analysis shows that the rAb.doc is efficiently produced as a secreted H + L chain dimer. Heterogeneity in the H chain likely reflects N-linked

glycosylation at a highly predicted (Potential 0.6426, NetNGlyc 1.0 Server - Technical University of Denmark) site within the dockerin sequence.

TABLE 5 shows the nucleic acid and amino acid sequences for rAB-pIRES2(mAnti-DC-SIGNL16E7K-LV-hIgGK-C) or C73. DNA (entire coding region) and amino acid sequence (the predicted secreted product) of IgG Kappa protein fusing the V region from the mAnti-DC-SIGNL16E7 hybridoma (highlighted in blue) to a human C region (highlighted in yellow).

TABLE 5. rAB-pIRES2(mAnti-DC-SIGNL16E7K-LV-hIgGK-C) or C73.

10 ATGCATCGCACCAGCATGGGCATCAAGATGGAGTCACAGATTCAGGCATTTGTATTTCGTGTTTCTCTGGTTGTCTGGTGTGGCGG
 AGACATTGTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTCAGGATG
 TGACTTCTGCTGTAGCCTGGTATCAACAAAAACCAGGGCAATCTCCTAAACTACTGATTTACTGGGCATCCACCCGGCACACTGGA
 15 GTCCCTGATCGCTTTCACAGGCAGTGGATCTGGGACAGATTATACTCTCACCATCAGCAGTGGGCAGGCTGAAGACCTGGCACTTA
 TTACTGTCACCAATATTATAGCGCTCCTCGGACGTTTCGGTGGAGGCACCAAGCTCGAGATCAAACGAACTGTGGCTGCACCATCTG
 TCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAG
 GCCAAAGTACAGTGAAGGTGGATAACGCCTCCAATCGGTAAGTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCAC
 20 CTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACAAAGTCTATGCCTGCGAAGTCACCCATCAGGGCC
 TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO. :10)

20 DIVMTQSHRFEMSTCVGDRVGVTCAGQDVTGAVANVQOKFGQSEKLLIYVASTFHTGVPDRFTGSGSGTDYTLTISSGQAEDLALY
 YCHQYYSAFPTTEGGSTLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSST
 YLSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO. :11)

FIGURE 3 shows Protein G affinity purified secreted rAb proteins analyzed by reducing SDS.PAGE and Coomassie Brilliant Blue staining. Lanes are from left to right.

The invention embodies the unanticipated presence and use of this glycosylation site that likely confers onto mammalian cell-secreted dockerin fusion proteins desirable solubility and pharmacokinetic properties well known to be associated with glycosylation. Figure 2 shows that rAb.antigen fusion proteins employing identical IgG H and L sequences can differ dramatically in efficiency of secretion. In both sited examples, rAb.doc entities are well expressed compared to rAb fused to Influenza HA5 sequences which typically express very poorly. The invention also embodies the unanticipated capacity of the dockerin domain to not significantly hinder the secretion of the associated rAb entity. Furthermore, the invention embodies the property of the dockerin domain to not hinder the functionality of the rAb specific antigen combining regions. This property is exemplified in Figure 5 which shows concordance between IgFc reactivity and LOX-1 reactivity between anti-LOX1_15C4 rAb proteins and anti-LOX1_15C4.doc.

FIGURES 4A and 4B show the measurement by anti-human IgFc ELISA of levels of secretion of various rAb.fusion proteins. 2.5 ug each of the H and L chain expression plasmids were transfected into 293F cells and two-fold dilutions of supernatant samples were tested after three days of culture. Y axis values are arbitrary HRP activity.

FIGURE 5 shows the measurement by anti-human IgFc ELISA (HRP activity) and LOX-1.alkaline phoshatase binding (AP activity) of secreted anti-LOX1_15C4 rAb.(blue symbols)and anti-LOX1_15C4.doc rAb (red symbols) proteins. Different ratios totalling 5 ug of the H and L chain expression plasmids were transfected into 293F cells and supernatant samples were tested after three days of culture.

The invention embodies the property of the dockerin domain to be efficiently and functionally expressed in the context of fusion proteins other than hIgG4 and its close derivatives. For example, Table 6 shows the sequence of a rAb.doc entity based on a mouse IgG2b H chain fusion protein.

TABLE 6 shows the nucleic acid and amino acid sequences for rAB-pCMV(mIgG2bH-Dockerin) or C19. DNA (entire coding region) and amino acid sequence (the predicted secreted product) is shown. The dockerin domain is highlighted in yellow and the H chain and dockerin joining sequence is underlined. The highly predicted N-linked glycosylation site within the dockerin domain is highlighted in red.

TABLE 6. rAB-pCMV(mIgG2bH-Dockerin) or C19.

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTACAGGTCCAAGTGCAGCAGCCTGGGGCTGA
 GCTGGTGAGGCCTGGGACTTCAGTGAAGTTGTCCTGCAAGGCTTCTGGTTACATCTTTACCAGCTACTGGATGCACTGGGTAAAGC
 AGAGGCTGGACAAGGCTTGAGTGGATCGGACTGATTGATCCTTCTGATAGTTATAGTAAGTACAATCAAAGTTCAAGGGCAAG
 GCCACATTGACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTG
 TGCAAGAGGGGAGCTCAGTGAAGTTCTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACAACACCCCATCAGTCTATC
 CACTGGCCCTGGGTGTGGAGATAACAACCTGGTTCTCTGTGACTCTGGGATGCCTGGTCAAGGGCTACTTCCCTGAGTCAGTGA
 GTGACTTGGAACTCTGGATCCCTGTCCAGCAGTGTGCACACCTTCCCAGCTCTCCTGCAGTCTGGACTCTACACTATGAGCAGCTC
 AGTGACTGTCCCCTCCAGCACCTGGCCAAGTCAGACCGTCACCTGCAGCGTTGCTCACCCAGCCAGCAGCACCACGGTGGACAAAA
 AACTTGAGCCCAGCGGGCCATTTCAACAATCAACCCCTGCCTCCATGCAAGGAGTGTCAAAATGCCAGCTCCTAACCTCGAG
 GGTGGACCATCCGTCTTCATCTTCCCTCCAAATATCAAGGATGTACTCATGATCTCCCTGACACCCAAGGTACGTGTGTGGTGGT
 GGATGTGAGCAGGATGACCCAGACGTCGGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACAAAACCCATA
 GAGAGGATTACAACAGTACTATCCGGGTGGTTCAGTGCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGC
 AAGGTCAAACAACAAAGACTCCCATCACCATCGAGAGAACCATCTCAAAAATTAAGGGCTAGTCAGAGCTCCACAAGTATACAT
 CTTGCCGCCACCAGCAGAGCAGTTGTCCAGGAAAGATGTCAGTCTCACTTGCTGGTCTGGGCTTCAACCCCTGGAGACATCAGTG
 TGGAGTGGACCAGCAATGGGCATACAGAGGAGAACTACAAGGACACCGCACCAGTCTGGACTCTGACGGTTCTTACTTCATATAC
 AGCAAGCTCGATATAAAAACAAGCAAGTGGGAGAAAACAGATTCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTA
 CCTGAAGAAGACCATCTCCCGGTCTCCGGTAAAGCTAGCAATTCTCCTCAAAAATGAAGTACTGTACGGAGATGTGAATGATGACG
 GAAAAGTAAACTCCACTGACTTGACTTTGTTAAAAAGATATGTTCTTAAAGCCGTCTCAACTCTGCCTTCTTCAAAGCTGAAAAG
 AACGCAGATGTAATCGTGACGGAAGAGTTAATCCAGTGATGTCACAATACTTTCAAGATATTTGATAAGGGTAATCGAGAAATT
 ACCAATATA (SEQ ID NO.:12)

QVQLQPGAELVLRPGTSVKLSCKASGYIFTSYWMHWKQRPQGLEWIGLIDPDSYSKYNQKFKGKATLTVDTSSTAYMQLSSL
 TSEDSAVYYCARGELSDFWGQGTTLTVSSAKTTPPSVYPLAPGCGDITGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALL
 QSGLYTMSSTVTPSSTWPSQTVTCSVAHPASSTTVDKKLEPSGPISTINPCPPCKECKCPAPNLEGGPSVFIFFPNIKDVLMS
 LTPKVTVCVVVDVSEDDPDVRI SWFVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMGKEFKCKVNNKDLPSPIERTISKIK
 GLVRAPQVYILPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSGYSYFIYSKLDIKTSKWEKTDSEFCN
 VRHEGLKNYLLKKTISRSPGKASNSPQNEVLYGDVNDGKVNSTDLTLLKRYVLKAVSTLPSKAEKNADVNRDGRV DVTILS
 RYLIRVIEKLPI. (SEQ ID NO.:13)

Figure 6 shows that when co-transfected with a mIgG kappa expression plasmid, rAB-pCMV(mIgG2bH-Dockerin) plasmid directs the efficient secretion of rAB- mIgG2b.Dockerin fusion protein. In FIGURE 6, a Protein G affinity purified rAb proteins secreted from

transfected 293 F cells analyzed by reducing SDS.PAGE and Coomassie Brilliant Blue staining. Lanes 11 and 12 show mIgG2b.doc products.

The use of the rAb.doc invention detailed above is the assembly of rAb-antigen or toxin or activator or enzyme complexes via the specificity and tenacity of the dockerin-cohesin interaction. Table 5 shows one embodiment of the invention in the form of a cohesin.alkaline phosphatase fusion protein (coh.AP). Also described are additional embodiments such as an alkaline phosphatase fusion protein containing two cohesion domains (coh.coh.AP) and other proteins are examples of the generality of the invention such as the single cohesin domain fused to other sequences such as the mature sequence of human prostate specific antigen (coh.hPSA) and to the HA1 domain of influenza A HA5 (coh.Flu HA5-1).

TABLE 7 shows the nucleic acid and amino acid sequences for Mam-pCDM8(Cohesin-SLAML-AP-6xHis) or C16. DNA (entire coding region) and amino acid sequence (the predicted secreted product) is shown below. The cohesin domain is highlighted in yellow and the cohesin and alkaline phosphatase joining sequence is underlined. The highly predicted (G-score > 0.5, NetOGlyc 3.1 Server - Technical University of Denmark) O-linked glycosylation sites within the cohesin domain and the linker distal to the cohesin domain are highlighted in red. Residues highlighted grey are a C-terminal His tag to facilitate purification via metal affinity chromatography.

TABLE 7. Mam-pCDM8(Cohesin-SLAML-AP-6xHis) or C16.

20 ATGGATCCCAAAGGATCCCTTTCTCTGGAGAATACTTCTGTTTCTCTCCCTGGCTTTTGGAGTTGAGCTACGGACTCGACGATCTGGA
 TGCAGTAAGGATTAAGTGGACACAGTAAATGCAAAACCGGGAGACACAGTAAGAATACCTGTAAGATTCAGCGGTATACCATCCA
 AGGGAATAGCAAACCTGTGACTTTGTATACAGCTATGACCCGAATGTACTTGAGATAATAGAGATAGAACCAGGGAGACATAATAGTT
 GACCCGAATCCTGACAAGAGCTTTGATACTGCAGTATATCCTGACAGAAAGATAATAGTATTCCTGTTTGCAGAAGACAGCGGAAC
 25 AGGAGCGTATGCAATAACTAAAGACGGAGTATTTGCTACGATAGTAGCGAAAGTAAAAGAAGGAGCACCTAACGGACTCAGTGTA
 TCAAATTTGTAGAAGTAGCGGATTTGCGAACAATGACCTTGTAAGAACAGAACACAGTTCCTTTGACGGTGGAGTAAATGTTGGA
 GATACAACAGAACCCTGCAACACCTACAACACCTGTAACAACACCGACAACAACAGATGATCTGGATGCACTCGAGATCATCCCAGT
 TGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCAAGAAGCTGCAGCCTGCACAGACAGCCG
 CCAAGAACCTCATCATCTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGAC
 AACTGGGGCCTGAGTTACCCTGGCCATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATAACAATGTAGACAAACATGTGCC
 30 AGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCCGCTTTA
 ACCAGTGAACACAGACACGCGGCAACGAGGTTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACC
 ACCACACGAGTGCAGCACGCTCGCCAGCCGGCACCTACGCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCCTGC
 CTCGGCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATTGACGTGATCCTAGGTGGAGGCCGAA
 AGTACATGTTTCGCATGGGAACCCAGACCCTGAGTACCCAGATGACTACGCCAAGGTGGGACCAGGCTGGACGGGAAGAATCTG
 35 GTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTACGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGT
 GACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCTGATGGAGA
 TGACAGGGCTGCCCTGGCCTGCTGAGCAGGAACCCCGGGCTTCTTCTTTCGTGGAGGGTGGTTCGCATCGACCATGGTTCAT
 CATGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTTCGACGACGCCATTGAGAGGGGCGGGCCAGCTCACCAGCGAGGA
 GGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCTTCGGAGGCTACCCCTGCGAGGGAGCTCCATCTTCGGGC
 40 TGGCCCTTGCAAGGCCCGGGACAGGAAGCCTACACGGTCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCC
 CGGCCGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCTGGACGAAGAGACCCACGCAGG
 CGAGGACGTGGCGGTGTTTCGCGCGCGGCCCGAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTTCATGG
 CCTTCGCCGCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCGCGGCACCACCACCATCACCATCACCATTGA
 45 (SEQ ID NO.:14)

LDDLDVARIKVDTVNAKPGDTPVRIIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEIPGELIVDPNPKSFDTAVYVDRKMIVFLFAE
 EDSGTGAYAITEDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGD~~EPAP~~~~VP~~~~P~~~~DDLDAL~~
 EIIIPVEEENPDFWNREAAEALGAAKKLQPAQTAANKLIIIFLGDGMGVSTVTAARILKGQKKDKLGPPELPLAMDRFPYVALSKTYNV
 DKHVPD SGATATAYLCGVKGNFQTI GLSAAARFNQCNTTRGNEVISVMNRKAKGKSVGVVTTTRVQHASPAGTYAHTVNRNWYS
 ADVPASARQEGCQDIATQLISNMMDIVILGGGRKYMFRMGTDPPEYPPDDYSQGGTRLDGKNLVQEWLAKRQGARYVWNRTELMQAS
 LDPSVTHLMGLFEPGDMKYEIHRDSTLDP SLMEMTEAALRLLSRNPRGFFLVEEGRIDHGHESRAYRALTETIMFDDAIERAGQ
 LTSEEDTLSLVTADHSHVFSFGGYPLRGSIFGLAPGKARDRKAYTVLLYGNPGYVLKDGARPDVTESESGSPEYRQQSAVPLDE
 ETHAGEDVAVFARGPQAHLVHGVQEQTFAHVMAFAACLEPYTACDLAPPAGTT~~HHHHHH~~ (SEQ ID NO.:15)

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 10
 15
 TABLE 8 shows the nucleic acid and amino acid sequences for Mam-pCDM8(Cohesin-Cohesin-SLAML-AP-6xHis) or C17. DNA (entire coding region) and amino acid sequence (the predicted secreted product) is shown below. The cohesin domain is highlighted in yellow and the cohesin and alkaline phosphatase joining sequence is underlined. The highly predicted O-linked glycosylation sites within the linker distal to the cohesin domains are highlighted in red as is a single highly predicted N-linked glycosylation site (NPT). Residues highlighted grey are a C-terminal His tag to facilitate purification via metal affinity chromatography.

TABLE 8. Mam-pCDM8(Cohesin-Cohesin-SLAML-AP-6xHis) or C17.

20
 25
 30
 35
 40
 45
 ATGGATCCCAAAGGATCCCTTCTCTGGAGAATACTTCTGTTTCTCTCCCTGGCTTTTGTAGTTGAGCTACGGACTCGACGATCTGGA
 TGCAGTAAGGATTAAGTGGACACAGTAAATGCAAAACCGGGAGACACAGTAAAGAATACCTGTAAGATTACAGCGGTATACCATCCA
 AGGGAATAGCAAACCTGTGACTTTGTATACAGCTATGACCCGAATGTACTTGAGATAATAGAGATAAAACCGGGAGAATTGATAGTT
 GACCCGAATCCTGACAAGAGCTTTGATACTGCAGTATATCCTGACAGAAAGATAATAGTATTCCTGTTTGCAGAAGACAGCGGAAC
 AGGAGCGTATGCAATAACTAAAGACGGAGTATTTGCTACGATAGTAGCGAAAGTAAAATCCGGAGCACCTAACGGACTCAGTGTA
 TCAAATTTGTAGAAGTAGGCGGATTTGCGAATAATGACCTTGTAGAACAGAAGACACAGTTCTTTGACGGTGGAGTAAATGTTGGA
 GATACAACAAGAACCTGCAACACCTACAACACCTGTAACAACACCCGACAACAACAGATGATCTGGATGCAGTAAGGATTAAGATGGA
 CACAGTAAATGCAAAACCGGGAGACACAGTAAATATACCTGTAAGATTCAAGTGTGATACCAATCCAAAGGAATAGCAAACCTGTGACT
 TTGTATACAGCTATGACCCGAATGTACTTGAGATAATAGAGATAAAACCGGGAGAATTGATAGTTGACCCGAATCCTACCAAGAGC
 TTTGATACTGCAGTATATCCTGACAGAAAGATGATAGTATTCCTGTTTGCAGAAAGACAGCGGAACAGGAGCGTATGCAATAACTAA
 AGACGGAGTATTTGCTACGATAGTAGCGAAAGTAAAAGAGGAGCACCTAACGGACTCAGTGAATCAAATTTGTAGAAGTAGCGG
 GATTTGCGAACAATGACCTTGTAGAACAGAAGACACAGTTCTTTGACGGTGGAGTAAATGTTGGAGATACAACAGAACCTGCAACA
 CCTACAACACCTGTAACAACACCCGACAACAACAGATGATCTGGATGCATCCAGTTCGAGGAGGAGAAACCCGGACTT
 CTGGAACCGCGAGGCGCGGCTGGGTGCGCCAAAGAGCTGCAGCTGCACAGACAGCCGCAAGAACCTCATCATCTTCC
 TGGGCGATGGGATGGGGGTGTCTACGGTGTACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGCGCTGAGTTACCC
 CTGGCCATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCAC
 GGCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCCGCCGCTTAAACCAGTGAACACGACACGCG
 GCAACGAGGTATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACAGGAGTGCAGCACGCC
 TCGCCAGCCGGCACCTACGCCACACGGTGAACCGCAACTGGTACTCGGACCGCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTG
 CCAGGACATCGTACCGAGCTCATCTCCAACATGGACATTGACGTGATCCTAGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAA
 CCCCAGACCCTGAGTACCAGATGACTACAGCCAAAGTGGACAGGCTGACGCGGAAGAATCTGGTGCAGGAATGGCTGGCGGAAG
 CGCCAGGGTGGCCGTACGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCTGGACCCGTCTGTGACCCATCTCATGGGTCTCTT
 TGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGCAGAGGCTGCCCTGCGCC
 TGCTGAGCAGGAACCCCGCGGCTTCTTCTCTTCTCGTGGAGGGTGGTGCATCGACCATGGTTCATCATGAAAGCAGGGCTTACCGG
 GCACTGACTGAGACGATCATGTTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACAGCGAGGAGGACACGCTGAGCCTCGTCAC
 TGCCGACCACTCCCACGCTTCTCTCTTCCGAGGCTACCCCTGCGAGGGAGCTCCATCTTCGGGTGGCCCTGGCAAGGCCCGGG
 ACAGGAAGGCCCTACCGTCTCTCTATACGGAACCGTCCAGGCTATGTGCTCAAGGACGGCCCGCCGGATGTTACCGGAGC
 GAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGGCCCTGACGGAAGAGACCCACGCGAGGACGAGGAGGCGGTGTTGCG
 GCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTATGGCCTTCGCCGCTGCCTGGAGC
 CCTACACCGCTGCGACCTGGCGCCCCCGCCGGCACCACCACCATCACCATCACCATTGA (SEQ ID NO.:16)

50
 55
 LDDLDVARIKVDTVNAKPGDTPVRIIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIKPGELIVDPNPKSFDTAVYVDRKIIIVFLFAE
 DSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGD~~EPAP~~~~VP~~~~P~~~~DDLDVAVR~~
 IKVDTVNAKPGDTPVRIIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIKPGELIVDPNPKSFDTAVYVDRKIIIVFLFAEDSGTGAY
 AITKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGD~~EPAP~~~~VP~~~~P~~~~DDLDAL~~
 NPDFWNREAAEALGAAKKLQPAQTAANKLIIIFLGDGMGVSTVTAARILKGQKKDKLGPPELPLAMDRFPYVALSKTYNVDKHVPDSG
 ATATAYLCGVKGNFQTI GLSAAARFNQCNTTRGNEVISVMNRKAKGKSVGVVTTTRVQHASPAGTYAHTVNRNWYSADAVPASAR
 QEGCQDIATQLISNMMDIVILGGGRKYMFRMGTDPPEYPPDDYSQGGTRLDGKNLVQEWLAKRQGARYVWNRTELMQASLDPSVTHL
 MGLFEPGDMKYEIHRDSTLDP SLMEMTEAALRLLSRNPRGFFLVEEGRIDHGHESRAYRALTETIMFDDAIERAGQLTSEEDT
 SLVTADHSHVFSFGGYPLRGSIFGLAPGKARDRKAYTVLLYGNPGYVLKDGARPDVTESESGSPEYRQQSAVPLDEETHAGEDV
 AVFARGPQAHLVHGVQEQTFAHVMAFAACLEPYTACDLAPPAGTT~~HHHHHH~~ (SEQ ID NO.:17)

TABLE 9 shows the nucleic acid and amino acid sequences for Mam-pCDM8(SLAML-Cohesin-hPSA) or C149. DNA (entire coding region) and amino acid sequence (the predicted secreted product) is shown below. The cohesin domain is highlighted in yellow and the cohesin and hPSA joining sequence is underlined. The highly predicted O-linked glycosylation sites within the linker distal to the cohesin domains and a single highly predicted N-linked glycosylation site within the cohesin domain are highlighted in red.

TABLE 9. Mam-pCDM8(SLAML-Cohesin-hPSA)or C149.

10 ATGGATCCCAAAGGATCCCTTTCTGGAGAATACTTCTGTTTCTCTCCCTGGCTTTTGTAGTTGAGCTACGGACTCGACGATCTGGA
 TGCAGTAAGGATTAAGTGGACACAGTAAATGCAAACCGGGAGACACAGTAAGAATACCTGTAAGATTACAGCGGTATACCATCCA
 AGGGAATAGCAAAGTGTGACTTTGTATACAGCTATGACCCGAATGTACTTGAGATAATAGAGATAGAACCAGGGAGACATAATAGTT
 GACCCGAATCCTGACAAGAGCTTTGATACTGCAGTATATCCTGACAGAAAGATAATAGTATTCCTGTTTGCAGAAGACAGCGGAAC
 AGGAGCGTATGCAATAACTAAAGACGGAGTATTTGCTACGATAGTAGCGAAAGTAAAAGAAGGAGCACCTAACGGACTCAGTGTA
 TCAAATTTGTAGAAGTAGGCGGATTTGCGAACAATGACCTTGTAGAACAGAAAGACACAGTTCTTTGACGGTGGAGTAAATGTTGGA
 15 GATACAACAGAACCTGCAACACCTACAACACCTGTAACAACACCGACAACAACAGATGATCTGGATGCACTCGAGGCGCCCTCAT
 CCTGTCTCGGATTGTGGGAGGCTGGGAGTGCAGAAAGCATTTCCAACCTGGCAGGTGCTTGTGGCCTCTCGTGGCAGGGCAGTCT
 GCGGCGGTGTTCTGGTGCACCCCAAGTGGGTCTCACAGCTGCCACTGCATCAGGAACAAAAGCGTGATCTTGTGGGTGGGCAC
 AGCCTGTTTCATCCTGAAGACACAGGCCAGGTATTTTCAAGTCCAGCTCAGCCACAGCTTCCACACCCGCTCTACGATATGAGCCTCCTGAA
 GAATCGATTCTCAGGCCAGGTGATGACTCCAGCCACGACCTCATGCTGCTCCGCCTGTCAGAGCCTGCCAGCTCACGGATGCTG
 TGAAGGTCATGGACCTGCCACCCAGGAGCCAGCACTGGGGACCACCTGCTACGCCTCAGGCTGGGGCAGCATTGAACCAGAGGAG
 20 TTCTTGACCCCAAAGAACTTCAAGTGTGTGGACCTCCATGTTATTTCCAATGACGTGTGCGCGCAAGTTCACCTCAGAAGGTGAC
 CAAGTTCATGCTGTGTGCTGGACGCTGGACAGGGGGCAAAGCACCTGCTCGGGTGATTCTGGGGGCCACTTGTCTGTAATGGTG
 TGCTTCAAGGTATCACGTCATGGGGCAGTGAACCATGTGCCCTGCCGAAAGGCCTTCCCTGTACACCAAGGTGGTGCATTACCGG
 AAGTGGATCAAGGACACCATCGTGGCCAACCCCTGA (SEQ ID NO.:18)

25 LDDLDVARIKVDTVNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIEIEIEPGELIVDENPKSFDTAVYPPDRKMIVFLFA
 EDSGTGAYAITEDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTFQFFDGGVNVGD^{REDACTED}EPA^{REDACTED}P^{REDACTED}PV^{REDACTED}P^{REDACTED}DDLAL
 EAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSIVILLGRHSLFHPEDTGQVFQVSHSFPHPLYD
 30 MSLLNKFLRPGDDSSHDMLLRLLRSEPAELTDVAVKVMDLPTQEPALGTTCYASGWSIEPEEFLTPKKLQCVDLHVISNDVCAQVH
 PQKVTKFMCLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP (SEQ ID NO.:
 19)

TABLE 10 shows the nucleic acid and amino acid sequences for Mam-pCDM8(SLAML-Cohesin-FluHA5-1-6xHis)or C24. DNA (entire coding region) and amino acid sequence (the predicted secreted product) is shown below. The cohesin domain is highlighted in yellow and the cohesin and Flu HA5-1 joining sequence is underlined. The highly predicted O-linked glycosylation sites within the linker distal to the cohesin domains and a single highly predicted N-linked glycosylation site within the cohesin domain are highlighted in red. Residues highlighted grey are a C-terminal His tag to facilitate purification via metal affinity chromatography.

TABLE 10. Mam-pCDM8(SLAML-Cohesin-FluHA5-1-6xHis)or C24.

40 ATGGATCCCAAAGGATCCCTTTCTGGAGAATACTTCTGTTTCTCTCCCTGGCTTTTGTAGTTGAGCTACGGACTCGACGATCTGGA
 TGCAGTAAGGATTAAGTGGACACAGTAAATGCAAACCGGGAGACACAGTAAGAATACCTGTAAGATTACAGCGGTATACCATCCA
 AGGGAATAGCAAAGTGTGACTTTGTATACAGCTATGACCCGAATGTACTTGAGATAATAGAGATAGAACCAGGGAGACATAATAGTT
 GACCCGAATCCTGACAAGAGCTTTGATACTGCAGTATATCCTGACAGAAAGATAATAGTATTCCTGTTTGCAGAAGACAGCGGAAC
 45 AGGAGCGTATGCAATAACTAAAGACGGAGTATTTGCTACGATAGTAGCGAAAGTAAAAGAAGGAGCACCTAACGGACTCAGTGTA
 TCAAATTTGTAGAAGTAGGCGGATTTGCGAACAATGACCTTGTAGAACAGAAAGACACAGTTCTTTGACGGTGGAGTAAATGTTGGA
 GATACAACAGAACCTGCAACACCTACAACACCTGTAACAACACCGACAACAACAGATGATCTGGATGCACTCGAGGATCAGATTTG
 CATTGGTTACCATGCAACAACCTCGACAGAGCAGGTTGACACAATAATGAAAAAGACGTTACTGTTACACATGCCAAAGACATAC
 TGGAAAAGAAAACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTGTAGAGATTGTAGCGTAGCTGGATGGCTC

CTCGGAAACCAATGTGTGACGAATTCATCAATGTGCCGGAATGGTCTTACATAGTGGAGAAGGCCAATCCAGTCAATGACCTCTG
 TTACCCAGGGGATTTCAATGACTATGAAAAATTGAAACACCTATTGAGCAGAATAAACCATTTTGTAGAAAAATTCAGATCATCCCCA
 AAAGTTCTTGGTCCAGTCATGAAGCCTCATTAGGGGTGAGCTCAGCATGTCCATACCAGGGAAAGTCCCTCTTTTTCAGAAATGTG
 5 GTATGGCTTATCAAAAAGAACAGTACATACCCAACAATAAAGAGGAGCTACAATAATACCAACCAAGAAGATCTTTTGGTACTGTG
 GGGGATTCACCATCCTAATGATGCGGCAGAGCAGACAAAGCTCTATCAAAACCAACCACCTATATTTCCGTTGGGACATCAACAC
 TAAACCAGAGATTGGTACCAAGAATAGCTACTAGATCCAAAGTAAACGGGCAAAGTGGGAAGGATGGAGTCTTCTGGACAATTTTA
 AAGCCGAATGATGCAATCAACTTCGAGAGTAATGGAAATTTTCATTGCTCCAGAATATGCATACAAAATTGTCAAGAAAGGGGACTC
 AACAAATTATGAAAAGTGAATTGGAATATGGTAACTGCAACACCAAGTGTCAAACCTCCAATGGGGGCGATAAACTCTAGCATGCCAT
 10 TCCACAATATACACCCTCTACCATTGGGGAATGCCCAAATATGTGAAATCAAACAGATTAGTCTTGCGCACCATCACCATCAC
 CATTGA (SEQ ID NO.:20)

LDDLDAVRIKVDVTVNAKPGDVRIPVRFSGIPSKGIANCDFVYSYDPNVLEIEIEIEPGEIIVDFNPKSFDTAVYPDRKMIVFLFA
 EDSGTGAYAITEDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTOFFDGGVINVDGEPAPPVPPDDLDAL
 15 EDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKKNHNGKLCDLGDGKPLILRDCSVAGWLLGNPMCDEFINVPESYIIVEKANP
 VNDLCYPGDFNDYEKLLKHLLSRINHFEDIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNVVWLIKKNSTYPTIKRSYNNNTNQED
 LLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSKVNQSGRMEFFWTILKPNDAINFESNGNFIAPAYAYKIV
 KKG DSTIMKSELEYGNCTKQTPMGAINSSMPFHNIHPLTIGECPKYVKS NRLVLAHHHHHH. (SEQ ID NO.:21)

Similar to the above mentioned rAb.doc constructs, the invention embodies the efficient
 secretion from mammalian cells of functional cohesin fusion proteins (called herein
 20 coh.fusions). It was not obvious that cohesin domains could be so successfully secreted while
 retaining dockerin-binding function. Figure 5 demonstrates that supernatant containing secreted
 coh.alkaline phosphatase (coh.AP) binds specifically to a rAb.doc protein immobilized on a
 plastic surface.

FIGURES 7A and 7B show that the Expression plasmids encoding secreted alkaline phosphatase
 25 (AP) or coh.AP directed secretion of functional proteins from transfected 293F cells. After 3
 days of culture supernatants were harvested and tested for their ability to bind 0.25 ug of either
 rAb.doc (top panel) or rAb (lower panel) bound to a 96 well micro-titre plate. After 1 hr of
 incubation the plates were washed and developed with a chromogenic AP substrate.

The invention embodies the application of assembly of specific protein complexes based on the
 30 cohesin.dockerin interaction. Specific antibody.antigen complexes can also be assembled using
 the established interaction of protein A or protein G IgFc binding domains. The invention
 embodies unique properties of the cohesin.dockerin interaction that result in greatly superior
 complex formation compared to the e.g., protein G interaction with IgG. In figures 6 and 7 the
 interaction of a cohesin.AP (called Coh.AP) protein is shown to be specific for a rAb.Doc
 35 protein.

FIGURES 8A and 8B shows various dilutions of a supernatant containing secreted G.AP were
 incubated for 1 hr in micro-titre wells containing 0.25 ug of immobilized mIgG2a, mIgG2b, or a
 mIgG2b-based rAb.doc. After washing the bound AP activity was developed using chromogenic
 AP substrate. The proG.AP did not bind to the rAb.doc since it was an isotype variant of
 40 mIgG2b that did not interact with the particular protein G domain used in the proG.AP construct.

Figure 8B shows an identical study, but employing dilutions of a supernatant containing secreted Coh.AP. Coh.AP binds only to rAb.doc, again demonstrating the specificity of the coh.doc interaction.

Figure 9 demonstrates the vastly superior stability of preassembled complexes based on coh.doc interaction compared to proG.IgGFc interaction. FIGURE 9 shows the formation of complexes
5 between a fixed amount of proG.AP or coh.AP or coh2.AP (0.1 ug) and immobilized mIgG2b or rAb.doc (0.25 ug) were assembled by incubation for 1 hr in a micro-titre plate. At various times a 20-fold excess of soluble mIgG2b or rAb.doc were added and incubation continued for various times. Plates were then washed and bound AP activity accessed by addition of chromogenic AP
10 substrate.

This example shows the use of such coh.doc complexes in settings containing serum (e.g., tissue culture media and in vivo administration). Figure 10 demonstrates the vast superiority of coh.doc complexes compared to proG.IgGFc complexes in such a setting. Under the conditions used, ~15
5 ug/ml Ig was sufficient to completely displace bound proG.AP, while the coh.AP remained stably bound to rAb.doc even in the presence of pure serum (15 mg/ml Ig)
15

FIGURE 10 shows the formation of complexes between a fixed amount of proG.AP or coh.AP (0.1 ug) and immobilized mIgG2b or rAb.doc (0.25 ug) were assembled by incubation for 1 hr in a micro-titre plate. Various dilutions of human serum were added and incubation continued for 4
10 hrs. Plates were then washed and bound AP activity accessed by addition of chromogenic AP substrate.
20

The invention also embodies a particular utility of the coh.doc interaction that permits a production process that ensures complete complex formation and that can be concomitant with a purification process for the coh.fusion protein entity. This invention is exemplified in Figures 11 and 12, which illustrate this process via sequential capture of rAb.doc from culture supernatant
25 by protein G affinity chromatography, followed by capture of coh.antigen from culture supernatant by the proteinG:rAb.doc column. Elution with low pH then releases pure rAb.doc:coh.antigen. If there is an excess of coh.antigen over rAb.doc, then full and complete complex should result. A related embodiment of this invention would be application to the protein G captured rAb.doc of excess pure or partially purified coh.fusion protein.

FIGURE 11 shows a gel of reduced vs. non-reduced SDS.PAGE analysis of rAb.doc:Coh2.AP complexes produced by sequential application of rAb.doc supernatant and coh.AP supernatant to the same protein G affinity column. Lanes 2 and 4 show that Coh2.AP co-purifies with rAb.doc.
30

FIGURE 12 is a non-reduced SDS.PAGE analysis of rAb.doc:Coh.Flu HA5-1 complexes produced by sequential application of rAb.doc supernatant and coh.Flu HA5-1 supernatant to the same protein G affinity column. Lanes 1 to 4 left to right show that Coh.Flu HA5-1 co-purifies with rAb.doc.

5 A well described feature of cohesin domains is their compatibility with the standard E. coli bacterial expression system. The invention embodies the novel use of expression of dockerin fusion proteins in mammalian secretion systems, and it also encompasses the formation of coh.doc complexes where the different components (i.e., coh and doc) are expressed in different systems. This is a great advantage since it affords the possibility of using the most favorable
10 expression system for each component. For example, coh.Flu M1 expression constructs failed to efficiently direct the synthesis of secreted product from transfected mammalian cells. However, coh.Flu M1 was very efficiently expressed as a soluble protein in E. coli. Table 6 shows the sequence of the coh.Flu M1 used in this example.

TABLE 11 shows the nucleic and amino acid sequence for E coli-pET28(Cohesin-FluM1-
15 6xHis) or C32 is shown below. In the amino acid sequence the cohesin domain is highlighted in yellow and the point of fusion between cohesion and influenza A M1 protein is underlined. Residues highlighted grey are a C-terminal His tag to facilitate purification via metal affinity chromatography.

TABLE 11. E coli-pET28(Cohesin-FluM1-6xHis) or C32.

20 ATGGATCTGGATGCAGTAAGGATTAAGTGGACACAGTAAATGCAAAACCGGGAGACACAGTAAATATACCTGTAAGATTCAGTGG
TATACCATCCAAGGGAATAGCAAACCTGTGACTTTGTATACAGCTATGACCCGAATGTACTTGAGATAATAGAGATAAAACCGGGAG
AATTGATAGTTGACCCGAATCCTACCAAGAGCTTTGATACTGCAGTATATCCTGACAGAAAGATGATAGTATTCCTGTTTGGCGAA
25 GACAGCGGAACAGGAGCGTATGCAATAACTAAAGACGGAGTATTTGCTACGATAGTAGCGAAAGTAAAAGAAGGAGCACCTAACGG
GCTCAGTGTAAATCAAATTTGTAGAAGTAGGCGGATTTGCGAACAAATGACCTTGTAAGAACAGAACACAGTTCCTTGACGGTGGAG
TAAATGTTGGAGATACAACAGAACCTGCAACACCTACAACACCTGTAACAAACACCGACAACAACAGATGATCTGGATGCAGCTAGC
CTTCTAACCGAGGTGCAAAACGTACGTTCTCTATCATCCCGTCAGGCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATGT
CTTTGCAGGGAAGAACACCGATCTTGAGGTTCTCATGGAATGGCTAAAGACAAGACCAATCCTGTCACCTCTGACTAAGGGGATTT
TAGGATTTGTGTTACGCTCACCGTGCCAGTGAGCGGGGACTGCAGCGTAGACGCTTTGTCCAAAATGCTCTTAATGGGAACGGA
30 GATCCAAATAACATGGCAAAGCAGTTAACTGTATAGGAAGCTTAAGAGGGAGATAACATTCATGGGGCCAAAAGAAATAGCACT
CAGTTATCTGCTGGTGCACTTGCCAGTTGTATGGCCCTCATATAACAAGGATGGGGGCTGTGACCACTGAAGTGGCATTGGCC
TGGTATGCGCAACCTGTGAACAGATTGCTGACTCCCAGCATCGGTCTCATAGGCAAATGGTGACAACAACCAATCCACTAATCAGA
CATGAGAACAGAAATGGTTCTAGCCAGCACTACAGCTAAGGCTATGGAGCAAATGGCTGGATCGAGTGAGCAAGCAGCAGAGGCCAT
GGATATTGCTAGTCAGGCCAGGCAAATGGTGCAGGCGATGAGAACCATTGGGACTCATCCTAGCTCCAGTGTGGTCTAAAAGATG
ATCTTCTGAAAATTTGCAGGCTTACCAGAAACGGATGGGGGTGCAGATGCAGCGATTCAAGCTCGAGCACCACCACCACCACCAC
35 TGA (SEQ ID NO. :22)

MDLDVARIKVDTVNAKPGDVTNIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIKPGELIVDPNPTKSFDTAVYPDRKMIVFLFAE
DSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPTVTTPTTTDDLLDAAS
40 LLTEVETYVLSIIPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGILGFVFTLTVPSEGLQRRRFVQNALNNGN
DPNNMDKAVKLYRKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTINPLIR
HENRMVLAQTAKAMEQAGSSEQAAEAMDIASQARQMVQAMRTIIGTHPSSAGLKDLDLENLQAYQKRMGVQMQRFKLE
(SEQ ID NO. :23)

The invention embodies the use of the dockerin.cohesin interaction to assemble ordered and specific complexes for various therapeutic or vaccination purposes. An example is the use of

rAb.doc with binding specificity to an internalizing human Dendritic Cell (DC) receptor complexed with coh.Flu M1 protein. Figure 11 demonstrates this utility by an in vitro study. DC cultured with anti-DC_rAb.doc:coh.Flu M1, then co-cultured with autologous T cells, directed the expansion of T cells with specific memory of Flu M1. Equivalent doses of coh.Flu M1 alone had no such effect. The study shows at least a 50-fold enhancement of Flu M1-specific T cell expansion via the anti-DC_rAb.doc:coh.Flu M1 compared to coh.Flu M1 alone.

FIGURE 13 shows that functional anti-DC_rAb.doc:coh.Flu M1 complex was formed by mixing the individual purified components. Various amounts of the complex, or coh.Flu M1 alone, were incubated in culture medium with 5E4 human DC (from a HLA201 donor) and 10E5 autologous T cells. After 24 hr, the DC were activated with CD40L and incubation was continued for an additional 9 days. Cells were harvested and stained with a PE-labeled Flu M1 peptide GILGFVFTL (SEQ ID NO.:24) HLA-A2 tetramer and analyzed for the frequency of antigen-specific CD8+ cells.

Figure 14 shows a similar example incorporating the additional control of coh.Flu M1 complexed to an isotype-matched mAb.doc with no binding to the human DC. Figure 12 shows that Anti-DC_rAb directly linked via an H chain fusion to a peptide fragment spanning the Flu M1 GILGFVFTL epitope is also effective in eliciting DC targeted antigen delivery resulting in expansion of Flu M1-specific T cells. However the Anti-DC_rAb.Flu M1 PEP entity was secreted very poorly from mammalian cells, likely precluding production of such a vaccine. This problem illustrates the embodiment of the invention that allows production issues to be solved by employing expression systems appropriate for the (in this case) vaccine antigen.

FIGURE 14 shows that Anti-DC_rAb.doc:coh.Flu M1 or mIgG2b.doc:coh.Flu M1 complexes were formed by mixing the individual purified components. Various amounts of the complexes, or coh.Flu M1 alone, were incubated in culture medium with 5E4 human DC (from a HLA201 donor) and 10E5 autologous T cells. After 24 hr, the DC were activated with CD40L and incubation was continued for an additional 9 days. Cells were harvested and stained with a PE-labeled Flu M1 peptide GILGFVFTL (SEQ ID NO.:24) HLA-A2 tetramer and analyzed for the frequency of antigen-specific CD8+ cells. Concentrations for mIgG2.doc complexes were the same as those for Anti-DC_rAb complexes.

FIGURE 15 shows CD34+ human DC were sorted into CD1a+ and CD14+ subtypes and cultured with and without 3 nM Anti-DC_rAb.Flu M1 PEP or Anti-DC_rAb. Autologous T cells were added after 1 day and culture continued for a further 8 days. Analysis was as described

above. The CD1a+ cells were very efficient in expanding Flu M1-specific CD8+ cells only with Anti-DC_rAb.Flu M1 PEP treatment.

While one type of embodiment of the invention is a vaccine composed of an Anti-DC-rAb.doc:coh.antigen complex, it is envisioned that in some cases a preferred DC-targeting vaccine will be Anti-DC-rAb.antigen where antigen is likely a string of protective antigens. Identification of such antigens in efficacious combinations compatible with efficient expression in production systems is extremely problematic. One embodiment of the invention affords a method to streamline testing of antigen epitope combinations for the development of such vaccines. Specifically, the invention teaches a method to screen likely antigen epitopes alone and in combinations for efficacy as a prelude to addressing production of the desired Anti-DC-rAb.antigen. For example, TABLE 13 shows the sequences of exemplative cohesin.peptide constructs which can be readily expressed via E. coli systems. Using techniques similar to those described in Figure 11, diverse collections of coh.pep proteins can be readily tested for efficacy as complexes with a single anti-DC_rAb.doc entity. The most efficacious coh.pep compounds can then be engineered directly as anti-DC_rAb.peptide fusion proteins. Figure 16 shows examples of purified coh.PEP proteins expressed in E. coli.

TABLE 12 shows the amino acid sequence of the melanoma-associated antigen gp100. Well known HLA-A201-restricted dominant peptides are shaded and detailed below the sequence. Peptide sequences labeled M are variants with enhanced affinity for HLA-A201. C180 is an E. coli expression construct that encodes the sequence shown below in which the cohesin domain is shaded blue and the gp100 peptide is shaded grey. Underlined residues bounding the peptide are native to gp100. C-terminal His tags are to facilitate purification via metal affinity chromatography.

Shown below is the gp100 sequence and the associated peptides referred to above.

MDLVLKRCLLHLAVIGALLAVGATKVPNRNQDWLGVSRQLRTKAWNRQLYPEWTEAQRLD^CWRGGQVSLKVSNDGPTLIGANASFSI
 ALNFPGSQKVLDPDGQVIWVNNTIINGSQVWGGQPVPYPOETDDACIFPDGGPCPSGSWSQKR^{SFVYVWKTWQYVQV}LGGPVSGLSI
 GTGRAMLGTHTMEVTVYHR^{RGRS}SYVPLAHSSSAFTI^{ITDQVPFSVSVS}QLRALDGGNKHFLRNQPLTFALQLHDPGYLEADLSY
 TWDFGDSSTLISRALVVHT^{YLEEGCVTAQ}VVLQAAIPLTSCGSSPVPGTDDGHRPTAEAPNTTAGQVPTTEVVGTTPGQAPTAE
 PSGTTSVQVPTTEVI^{STAPVQMPTAESTGMTPEKVPVSEVMGTTLAEMSTPEATGMTPAEVS}IVVLSGTTAAQVTTTEWVETTARE
 LPIPEPEGPDASSIMSTESITGSLGPLLDGTATLRLVKRQVPLDCVLYRYGSFVTLTDIVQGI^{ESAEILQAVPSGEGDAFELTVS}
 QGGLPK^{EACMEISSPGCQPPAQR}LCQPVLPSPAC^{QLVLH}QILKGGSGTY^{CLNVSLADTNSLAVVSTQLIMPGQEAGLQGVPLIVGI}
 LLVLM^{AVV}LASLIYRRRLMKQDFSV^QLP^{HSSSHWLR}LPRIFCSCPIGENSPLLSGQQV (SEQ ID NO.:25)

The HLA-A0201 restricted peptide sequences are:

GP100 WT: 154-162: KTWGQYVQV (SEQ ID NO.:26)

GP100 M: 209-217 (2M): IMDQVPFSV (SEQ ID NO.:27); 209–217 WT: ITDQVPFSV (SEQ ID NO.:28)

GP100 M: 280-288 (9V): YLEPGPVTV (SEQ ID NO.:29) 280-288 WT: YLEPGPVTA (SEQ ID NO.:30)

C180 is E. coli-pET28(Cohesin-hgp100-PeptideA-6xHis):

5 MDLDAVRIKVDTVNAKPGDTVNI PVRFSGI PSKGI ANCDFVYSYDPNVLEI IEIKPGELI VDPNPTKSFD TAVYPDRKMI VFLFAE DSGTGAYAI TKDGVFATI VAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLDAA SAFTIMDQVFFSVSVSAS GAAALEHHHHHH. (SEQ ID NO.:31)

10 TABLE 13 shows the amino acid sequence of the melanoma antigen MART-1. Well known HLA-A201-restricted dominant peptides are shaded and detailed below the sequence. M peptides show peptide sequence variants with enhanced affinity for HLA-A201. C181 is an E. coli expression construct that encodes the sequence shown below in which the cohesin domain is shaded yellow and the MART-1 peptide is shaded grey. Underlined residues bounding the peptide are native to MART-1. C172 and C174 are two constructs directing the expression of anti-DC_rAb.MART-1 peptide and a matching control rAb.MART-1 peptide H chain. Only the sequences appended to the C-terminal residue are shown. C-terminal His tags are to facilitate 15 purification via metal affinity chromatography.

MART-1 is:

MPREDAHF IYGYPKKGHGHSYTTAE EAAGIGILT VILGVLLLI GCWYCRRRNGYRALMDKSLHVGTQCALTRRC PQEGFDHRDSKV SLQEKNCPEVVPNAPPAYEKLSAEQSPPPYSP (SEQ ID NO.:32)

The HLA-A0201 restricted peptides sequences are:

20 MARTI WT: 9 mer: AAGIGILTV (SEQ ID NO.:33)
MARTI WT: 10 mer: EAAGIGILTV (SEQ ID NO.:34)
MARTI M: 10 mer: ELAGIGILTV (SEQ ID NO.:35)

C181 is E. coli - pET28(Cohesin-hMART-1-PeptideB-6xHis)

25 MDLDAVRIKVDTVNAKPGDTVNI PVRFSGI PSKGI ANCDFVYSYDPNVLEI IEIKPGELI VDPNPTKSFD TAVYPDRKMI VFLFAE DSGTGAYAI TKDGVFATI VAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLDAA TAEELAGIGILTV ILGAS GAAALEHHHHHH. (SEQ ID NO.:36)

C186 is E. coli-pET28(Cohesin-Flex-hMART-1-PeptideA-6xHis)

30 MDLDAVRIKVDTVNAKPGDTVNI PVRFSGI PSKGI ANCDFVYSYDPNVLEI IEIKPGELI VDPNPTKSFD TAVYPDRKMI VFLFAE DSGTGAYAI TKDGVFATI VAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLDAA S DTTEARHPHPVTTPTTTD GTTAEELAGIGILTV ILGG TNNSTPTKGEFCRYPSHWRPLEHHHHHH (SEQ ID NO.:37)

C172 is rAB-pIRES2(mAnti-ASGPR_49C11_7H-LV-hIgG4H-hMART-1-PeptideA)

C174 is rAB-pIRES2(hIgG4H-hMART-1-PeptideA)

...ASDTTEARHPHPVTTPTTTD GTTAEELAGIGILTV ILGG TNNSTPTKGEFCRYPSHWRPRL (SEQ ID NO.:38)

35 FIGURE 16 shows E. coli harboring expression plasmids directing the synthesis of coh.pep proteins were grown and induced for specific protein production. Cells were harvested and broken by sonication. The supernatant fractions were applied purified by metal affinity

chromatography. Analysis was by reducing SDS.PAGE gel stained by Coomassie Brilliant Blue. The figure shows typical product coh.pep proteins labeled from left to right.

This Example shows the successful use of cohesin and dockerin fusion proteins secreted from mammalian cells. If both fusion partners are rAbs with different specificities (i.e., rAb1.doc and rAb2.coh), then simple mixing results in rAb1.doc:rAb2.coh which is a bi-specific antibody. Bispecific antibodies have many potential therapeutic and technical applications. The invention provides a simple and predictable means to assemble such entities through the doc:coh interaction. Alternately, if rAb1.doc:rAb1.coh were assembled such entities represent controlled cross-linked mAbs with potentially unique biological properties.

Cohesin.dockerin modules exist in diverse cellulose degrading species. While they have sequence similarities, they can have specificities that do not cross between species. This affords an opportunity to build novel scaffolds composed of cohesins with different specificities and use this scaffold to assemble high order complexes in a spatially and numerically controlled manner. Others have described the core technology for using this notion for biotechnology applications (see Fierobe, H.-P., Mechaly, A., Tardif, C., Belaich, A., Lamed, R., Shoham, Y., Belaich, J.-P., and Bayer, E. A. (2001) Design and production of active cellulosome chimeras: Selective incorporation of dockerin-containing enzymes into defined functional complexes. *J. Biol. Chem.* 276, 21257-21261.). The invention embodies the specific use of this technology for applications related to manufacture of rAb.(doc:coh.fusion)_n complexes where n represents >1 pairings of doc:coh interactions with unique specificities. Thus, the invention envisions the assembly (by simple mixing of components) of spatially ordered complexes between rAb.doc1.doc2.doc3.etc. and coh1.fusionA, coh2.fusionB, coh3.fusion3, etc. The coh.fusion proteins could represent different antigens, or combinations of antigens and activating agents like cytokines.

By extension multiple coh:doc specificities could also be used to make bivalent rAbs with higher order antigen specificities. Cellulose degrading bacteria and similar organisms also use cellulose binding domains (CBD) to organize the degradation machinery. The structure of a CBD from *Clostridium thermocellum* shows that the N and C-termini are in close proximity and are not an integral part of the CBD functional structure. In fact CBD typically occurs linked to other domains such as coh.CBD.coh in cipA. The invention encompasses the use of entities such as coh.CBD.coh to assemble spatially and numerically ordered complexes mimicking antibodies and multi subunit receptors. For example, a IgG kappa chain v region fused to doc1 and a IgG H chain V region linked to doc2 can assemble with coh1.CBD.coh2 to yield VL.doc1:coh1.CBD.coh2:VH.doc2 to yield an entity with affinity and binding specificity

analogous to the original mAb. Such entities should be e.g., very useful screening tools for refining mAb specificities through mutagenesis procedures, particularly since the VL and VH component could be mutated independently and combined by mixing in various combinations. As described above, this technology can be readily extended to multiple controlled coh:V.doc combinations potentially yielding binding entities with extremely high specificities and affinities. An extension of this would be using e.g., coh1.coh2.CBD.coh3 as a template for assembly of cytoR1.doc + cytoR2.doc + cytoR3.doc (where cytoR represents the ectodomain of one subunit of a complex cytokine receptor). Such entities will have utility for blocking cytokine interactions for therapy and in biotechnology for measuring cytokines in complex supernatants.

10 EXAMPLE 3. Using Cohesin-Dockerin Technology for Immunotoxin Therapy.

Currently 1.2 million Americans develop cancer each year and about 500,000 die from the disease, because most cancers cannot be cured once they have metastasized. To develop a new treatment for metastatic cancer, genetic engineering has been used to modify a powerful bacterial toxin, Pseudomonas exotoxin A (PE), so that instead of killing normal cells it selectively kills cancer cells. PE is a three domain protein composed of 613 amino acids. Anti-cancer agents are produced by deleting its binding domain (aa 1-252) and replacing it with the Fv fragment of an antibody or with a growth factor that binds to antigens present on cancer cells. These agents are termed recombinant immunotoxins (RITs). RITs have been made that target Ley present on colon, breast, lung and other epithelial cancers (B3(Fv)-PE38), that target the EGF receptor overexpressed on glioblastomas (TGF-alpha-PE38), that target mutant EGF receptors present on glioblastomas (MR-1(Fv)-PE38KDEL), and that target the IL-2 receptor present on many T and B cell leukemias and lymphomas LMB-2 or anti-Tac(Fv)-PE38 and that target CD22 on B cell malignancies and that target BL22 or RFB4(dsFv)-PE38 ovarian cancers and mesotheliomas (SS1P). These agents are produced in E. coli because large amounts can be readily purified from this source and because the toxin itself would kill mammalian cells expressing it. When administered to mice with the appropriate human cancer xenograft, all these RITs produce complete tumor regressions. Most of these agents are now in clinical trials in humans and several have produced complete and partial remissions in humans with cancer.

An ideal immunotoxin should be very active so that only small amounts need to be given to cause tumor regressions, stable so it remains functional during the 5-10 hours required to reach the interior of a tumor, and non immunogenic so it can be given repeatedly. Initially, recombinant immunotoxins contained amino acids 253-613 of PE (domains II and III). It has been determined that amino acids 364-395 can be deleted without loss of activity. Increased

stability can be addressed by linking the toxin to a whole antibody, which are well known to have long half-lives and the technology in the invention provides this solution.

While the rAb.Doc:Coh.toxin technology can be applied to known cancer antigens, it can also be tested to kill intra-tumoral DC that are suspected to foster escape of the tumor from immune surveillance. In this latter case, anti-DC toxin therapy could be doubly advantageous since build up of immunity against the administered toxin itself should be suppressed (that is because DC themselves are key to the initiation of this immune response via uptake and processing of the antigen. In this therapy, the DCs that uptake the antigen die and cannot mount the anti-toxin response).

5 Frankel (Clinical Cancer Research, 8, 942-944, 2002) describes issues hindering the wider application of immunotoxins. These include production problems which often require refolding of E. coli inclusion body expressed material where misfolding contaminants are problematic. Also, affinity of the immunotoxin for its target is often difficult to obtain in sufficient strength. The technology basis of this invention addresses both these issues – firstly, we found that
15 cohesin.PE38 fusion protein is expressed in E. coli as a soluble protein that can be purified in a fully functional state (with both cohesin and toxin activities in tact) by simple biochemical means without complex refolding. Secondly, high affinity monoclonal antibodies against target antigens can be routinely obtained by one practiced in the art. What is difficult is engineering the antibody variable regions in a form that is fused with toxin and fully functional for target
20 binding. The usual means (e.g., sFv forms) of engineering invariably lead to significant loss of affinity against the target compared to the initial monoclonal antibody. The rAb.Doc:Coh.toxin technology circumvents this issue affording a means to preserve both the high affinity binding sites of the initial mAb (note that humanization of mouse mAb V regions while maintaining high and specific binding activity is routine to one practiced in the art), as well as the beneficial
25 properties of long half-life and non-antigenicity of a full recombinant hIgG context.

Furthermore since the cohesin.toxin is produced independently, one formulation of the toxin can be conjugated to any number of separately produced targeting rAb.Doc proteins by simple mixing of the component prior to injection of the patient. This greatly simplifies manufacturing as well as research development time. The technology described in the invention can be readily
30 applied to any toxin and any rAb specificity.

Details of the rAb.Doc:Coh.toxin technology. pRB 391 (from Dr. Pastan) Pastan, Chief of the Laboratory of Molecular Biology, Division of Basic Sciences. NCI, NIH) was used as a template

for PCR with primers PE38-N3
 (cacggtcaccgtctccaaagcttccggagctagcGAGGGCGGCAGCCTGGCCGCGCT (SEQ ID NO.:39))
 and PE38-C3
 (GGCCGGCTCCTGCGAAGGGAGCCGGCCGGTTCGCGGCCGCTTACTTCAGGTCCTCGC
 5 GCGGCGGTTTGCCG (SEQ ID NO.:40)).

Cloning was into the previously established construct C21 or E. coli-pET28(Cohesin-6xHis) to generate a fusion protein encoding Cohesin-PE38 corresponding to the amino acid sequence shown below (grey residues are cohesin; yellow residues are PE38, separated by a linker sequence native to the cohesin domain).

10 M~~DLDAVRIKFDVTVNAKPGDTVHIFVRFSGIPSKGIANCDFVYSYDPNVLEIIEIKPGELTVDPNFKGFDTAVYFDKMIYFLFAE~~
~~DSGTGAYATTKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFAIMDLVEQKTQFFDGGVIVGDTTEPATPTTPVTTPTTTTDDLLDAAS~~
~~EGGSLAALTAHQACHLPLETFFTRHRQPRGWEQLEQCGYPVORLVALYLAARLSWNQVDQVIRNALASPGSGGDLGEAIREQPEQAR~~
~~LALTLAAEESERFVROGTGNDEAGAANGPADSGDALLERNYPTGAEFLGDGGDVSFSTRGTQNWTVRLLQAHROLEERGYVFGY~~
 15 H~~GTFFLEAAQSIVFGGVRARSQDLDAIWRGFYIAGDPALAYGYAQDQEPDARGRIRNGALLRVYVPRSSLPGFYRTSLTLAAPEAAG~~
~~EVERLIGHPLPLRLDAITGPEEEGGRLETILGWPLAERTVVI~~PSAIP~~TDPRIVGGDLDPSSIPDKEQAI~~SALPDYASQPGKPPRED~~~~
~~LK~~ (SEQ ID NO.:41)

Expression and purification of recombinant Coh.PE38 protein – E. coli cells from each 1 L fermentation were resuspended in 25 ml ice-cold 50 mM Tris, 1 mM EDTA pH 8.0 with 0.1 ml of protease inhibitor Cocktail II (Calbiochem). The cells were sonicated on ice 2x 5 min at setting 18 (Fisher Sonic Dismembrator 60) with a 5 min rest period and then spun at 17,000 r.p.m. (Sorvall SA-600) for 20 min at 4°C. The supernatant was passed through 1 ml ANX Sepharose column equilibrated in 50 mM Tris, 1 mM EDTA pH 8.0 and and eluted with a 0-1 M NaCl gradient in Buffer B. Fractions containing Cohesin.PE38 were identified by SDS.PAGE and pooled fractions were further purified by purification via anti-cohesin mAb affinity chromatography with elution by 0.1 M glycine pH 2.7.

Selective killing of human DC by rAb.Doc targeted Coh.PE38 – Human DC were prepared from blood monocytes by culture for 6 days in with GM-CSF and IL-4. The DCs were then cultured with either Coh.PE38 alone, anti-DC-SIGN/L 16E7 rAb.Doc alone, anti-DCIR 24A5.Doc alone, or the rAb.Docs together with Coh.PE38 (1.25 ug/ml of agents were added). After 48 hr the cells were stained with a reagent (7-AAD) that detects apoptotic cells and analyzed by FACS scoring forward versus side scatter and 7-AAD fluorescence.

Figure 17 shows that the DCIR.Doc rAb alone had no effect upon the survival of DCs. However, DC-SIGN/L alone has a survival enhancing effect upon the DC (evidenced both by the scatter analysis and the 7-AAD staining. Figure 18 shows that Coh.PE38 alone slightly increase the number of 7-AAD scored apoptotic cells (from 22.1-29.8%). However, targeting the Coh.PE38 toxin via DCIR.Doc increased the 7-AAD positive population to 55.3%. The scatter analysis

even more dramatically revealed an almost complete loss of the population characteristic of viable DC. Targeting the Coh.PE38 toxin via DC-SIGN/L.Doc increased the 7-AAD positive population to 53.7%.with a similar loss of the viable DC scatter population. However, this latter result should be viewed in the context of the survival effect of the DC-SIGN/L.Doc rAb, meaning that the killing can be viewed as from 3.1-53.1% 7-AAD positive.

Using Cohesin-Dockerin Technology to make Multivalent Antibodies. A Cohesin domain was engineered in-frame with the C-terminus of a rAb H chain using PCR based on C17 (Mam-pCDM8(Cohesin-Cohesin-SLAMF1-AP-6xHis))as template. The resulting secreted H chain sequence is shown below (the cohesin domain is highlighted in grey and the C-terminal H chain residue is in bold):

```

15 QIQLVQSGPELKKPGETVNIISCKASGYSTFTNYGMNHWKQAPGKGLKRWGWINTYTIGESTYADDFKGRFAFSLETSASTAYLQISNL
KNEEMATYPCARGDERYYYFDYWGQGTTLTGSSAKTKGSPVPLAPCSRSTSESTAALGCLVKDYFPEFVTVSWNSGALTGGVHTF
PAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNVDRKPSNTRVDERVESKYGPCCPCPAPEFEGGSPVFLFPPKPKDTLMISRTPEV
TCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYPRVVSFLVTLHQDWLNGKEYKCKVSNKGLPSSIEKTIISKAKGQPRE
20 PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKGRWQEGNVPSCGMHEA
LHNHYTQKSLSLGLKASTTEFATPTTPTPTPTTTDLDKVEIKTQVNAKPGDNTIYFPGQSPGGLIAPFVYFPPYDEL
EETLPPGELTIDPDTISIDKFFDPRMIVTEFREDDSGTGRVAITFDGVAITFANVYKESAPENGLYVYFVYGGSPALHDLVQDT
KPKGKPKVDT. (SEQ ID NO.:42)

```

This expression construct was co-transfected with the appropriate rAb L chain into 293F cells and expression of secreted rAb was appraised by anti-hIgGFc ELISA at day 3. Figure 19 shows the expression of anti-DC-SIGN/L and Anti-DC-ASPGR rAb.Coh were efficiently secreted.

Thus both Cohesin and Dockerin domains are readily expressed as rAb fusion proteins. This property is essential for the use of (rAb1.Coh:rAb2.Doc) complexes as bivalent antibodies (i.e., having two different combining specificities in one protein). Bivalent antibodies have many desirable features suited to industrial, analytic, and therapeutic applications. They are, however, difficult to develop and molecular tools used to engineer them typically adulterate desirable features of high affinity and specificity inherent to the parent monoclonal antibodies. The (rAb1.Coh:rAb2.Doc) technology circumvents this obstacle and is, moreover, extensible to higher (than 2) valency of combining power by incorporating multiple Cohesin or Dockerin strings with pair wise specificities as described elsewhere in this application. Furthermore, this technology can be extended to using, e.g., a cytokine to provide the additional valency (i.e., rAb1.Doc:Coh.cytokine).

For example, a fusion protein between a Cohesin domain and IL-21 was engineered as an expression construct and the Coh.IL-21 protein was efficiently secreted from transiently transfected 293F cells and easily purified by sequential Q Sepharose and anti-Cohesin affinity chromatography. The sequence of the secreted product is shown below with the cohesin domain

shown in grey and the IL-21 domain in yellow. This product was fully functional as determined by it's efficacy in sustaining proliferation of human B cells.

Mam-pCDM8(SLAML-Cohesin-hIL-21)

5 LDLIDANRLEKYDTQNAEPDITVRIKPVSDGLESKGIANGDEVTGSDPHYLELIEIEPQDIIVLDFEEDKSYDPAAYEDRKLIVLEA
 ELGCTGAYALTRKGVFATIVAPVKEGAPNGLSYIKTVVGGCFARHDLVPAKTPQFFGGVHVGDTTEPATPTTPVTPTTTDDLAL
 EADQGGQDPRMIRMPQLIDIVDQLKHYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGMNERIINVSTKKLFRKPPSTNA
 GPRQKHPLTCFSCDSYEKKPFKEFLERFKSLLOKMIHQHLSRTHGSEDS (SEQ ID NO.:43)

Thus rAb.Doc:Coh.IL-21 can deliver concomitant proliferation and activation signals to a B cell (i.e., if the rAb itself has activation properties). This notion can be extended to any rAb with biological properties directed to a particular cell type and any cytokine with activity directed to the same cell type. Figure 20 shows the effect of IL-21 and Coh.IL-21 on the proliferation of human B cells.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or
5 open-ended and do not exclude additional, unrecited elements or method steps.

The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this
10 example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

All of the compositions and/or methods disclosed and claimed herein can be made and executed
15 without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and
20 modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

What is claimed is:

1. A modular rAb carrier comprising an antigen-specific binding domain linked to one or more antigen carrier domains comprising one half of a cohesin-dockerin binding pair.
2. The rAb of claim 1, wherein the antigen-specific binding domain comprises at least a
5 portion of an antibody.
3. The rAb of claim 1, wherein the antigen-specific binding domain comprises at least a portion of an antibody in a fusion protein with the one half of the cohesin-dockerin binding pair.
4. The rAb of claim 1, further comprising a complementary half of the cohesin-dockerin binding pair bound to an antigen that forms a complex with the modular rAb carrier.
- 10 5. The rAb of claim 1, further comprising a complementary half of the cohesin-dockerin binding pair that is a fusion protein with an antigen.
6. The rAb of claim 1, wherein the antigen specific domain comprises a full length antibody, an antibody variable region domain, an Fab fragment, a Fab' fragment, an F(ab)₂ fragment, and Fv fragment, and Fabc fragment and/or a Fab fragment with portions of the Fc
15 domain.
7. The rAb of claim 1, wherein the cohesin-dockerin are selected from Clostridium thermocellum, Clostridium josui, Clostridium cellulolyticum and Bacteroides cellulosolvens and combinations thereof.
8. The rAb of claim 1, wherein the antigen-specific binding domain binds a cell surface
20 marker selected from MHC class I, MHC class II, CD1, CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD16, CD 19, CD20, CD29, CD31, CD40, CD43, CD44, CD45, CD54, CD56, CD57, CD58, CD83, CD86, CMRF-44, CMRF-56, DCIR, DC-ASPGR, CLEC-6, CD40, BDCA-2, MARCO, DEC-205, mannose receptor, Langerin, DECTIN-1, B7-1, B7-2, IFN- γ receptor and IL-2 receptor, ICAM-1, Fc γ receptor or other receptor relatively specifically expressed by
25 antigen presenting cells.
9. The rAb of claim 1, wherein the rAb is further defined as: an rAb.Doc; an rAb.Coh; an rAb.(Coh)_x; an rAb.(Doc)_x; an rAb.(Coh.Doc)_x; or an rAb.(Coh)_x(Doc)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.
10. The rAb of claim 1, wherein the rAb is further defined as being part of a complex:
30 an rAb.Doc:Coh.antigen;

an rAb.Coh:Doc.antigen;

an rAb.(Coh)_x:(Doc.antigen)_x;

an rAb.(Doc)_x:(Coh.antigen)_x;

an rAb.(Coh.Doc)_x:(Doc.antigen¹)(Coh.antigen²); or

5 an rAb.(Coh)_x(Doc)_x:(Doc.antigen¹)_x(Coh.antigen²)_x;

wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

11. A vaccine comprising a modular rAb carrier comprising an antigen specific domain linked to one or more domains comprising one half of the cohesin-dockerin binding pair bound to a complementary half of the cohesin-dockerin binding pair bound to an antigen.

10 12. The vaccine of claim 11, wherein the antigen specific domain is specific for an immune cell surface protein selected from MHC class I, MHC class II, CD1, CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD16, CD 19, CD20, CD29, CD31, CD40,CD43, CD44, CD45, CD54, CD56, CD57, CD58, CD83, CD86, CMRF-44, CMRF-56, DCIR, DC-ASPGR, CLEC-6, CD40, BDCA-2, MARCO, DEC-205, mannose receptor, Langerin, DECTIN-1, B7-1, B7-2, IFN- γ receptor and IL-2 receptor, ICAM-1, Fc γ receptor or other receptor relatively specifically expressed by antigen presenting cells.

13. The vaccine of claim 11, wherein the antigen comprises a bacterial, viral, fungal, protozoan or cancer protein.

14. The vaccine of claim 11, wherein the modular rAb carrier is further defined:

20 an rAb.Doc:Coh.antigen;

an rAb.Coh:Doc.antigen;

an rAb.(Coh)_x:(Doc.antigen)_x;

an rAb.(Doc)_x:(Coh.antigen)_x;

an rAb.(Coh.Doc)_x:(Doc.antigen¹)(Coh.antigen²); or

25 an rAb.(Coh)_x(Doc)_x:(Doc.antigen¹)_x(Coh.antigen²)_x;

wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

15. An isolated nucleic acid comprising a coding segment for an target specific domain and one or more domains and one half of a cohesin-dockerin binding pair.

16. The nucleic acid of claim 15, wherein the target is an antigen and the specific domain encodes at least a portion of an antibody.
17. The nucleic acid of claim 15, wherein the one or more domains encodes one or more cohesin domains, one or more dockerin domains or a combination of one or more cohesin and
5 dockerin domains.
18. The nucleic acid of claim 15, wherein the target specific domain comprises an rAb is further defined as: an rAb.Doc; an rAb.Coh; an rAb.(Coh)_x; an rAb.(Doc)_x; an rAb.(Coh.Doc)_x; or an rAb.(Coh)_x(Doc)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.
19. A vector comprising a nucleic acid encoding an antigen specific domain and one or more
10 domains that comprise one half of a cohesin-dockerin binding pair, a one half of a cohesin-dockerin binding pair with a protein molecule to be carried and combinations thereof.
20. The vector of claim 19, wherein the one half of a cohesin-dockerin binding pair, a one half of a cohesin-dockerin binding pair with a protein molecule to be carried and combinations thereof are under the control of the same promoter, different promoters, transcribed in-line,
15 transcribed in opposite directions.
21. A host cell comprising a vector comprising a nucleic acid encoding an antigen specific domain and one or more domains and one half of a cohesin-dockerin binding pair.
22. A method of making a modular rAb carrier comprising:
combining an antigen specific domain linked to one or more domains comprising one half of a
20 cohesin-dockerin binding pair.
23. The method of claim 22, wherein the rAb is further defined as: an rAb.Doc; an rAb.Coh; an rAb.(Coh)_x; an rAb.(Doc)_x; an rAb.(Coh.Doc)_x; or an rAb.(Coh)_x(Doc)_x; wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.
24. The method of claim 22, wherein the rAb is complexed with a complementary half of a
25 cohesion:dokerin pair bound to an antigen and is selected from:
an rAb.Doc:Coh.antigen;
an rAb.Coh:Doc.antigen;
an rAb.(Coh)_x:(Doc.antigen)_x;
an rAb.(Doc)_x:(Coh.antigen)_x;

an rAb.(Coh.Doc)_x:(Doc.antigen¹)(Coh.antigen²); or

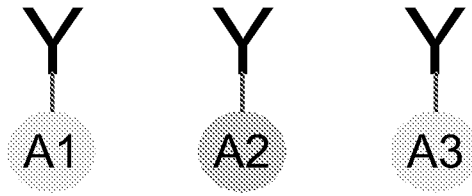
an rAb.(Coh)_x(Doc)_x:(Doc.antigen¹)_x(Coh.antigen²)_x;

wherein x is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

25. An immunotoxin comprising an rAb.Doc:Coh.toxin self-assembled conjugate, wherein
5 the rAb is specific for a cell target.
26. The immunotoxin of claim 25, wherein the toxin is selected from wherein the toxin is
selected from the group consisting of a radioactive isotope, metal, enzyme, botulin, tetanus,
ricin, cholera, diphtheria, aflatoxins, perfringens toxin, mycotoxins, shigatoxin, staphylococcal
enterotoxin B, T2, seguitoxin, saxitoxin, abrin, cyanoginosin, alphatoxin, tetrodotoxin,
10 aconotoxin, snake venom and spider venom.
27. The immunotoxin of claim 25, wherein the cell target comprises a cancer cell selected
from hematological cancers, leukemias, lymphomas, neurological tumors, astrocytomas or
glioblastomas, melanoma, breast cancer, lung cancer, head and neck cancer, gastrointestinal
tumors such as gastric or colon cancer, liver cancer, pancreatic cancer, genitourinary tumors
15 such cervix, uterus, ovarian cancer, vaginal cancer, testicular cancer, prostate cancer or penile
cancer, bone tumors, vascular tumors, or cancers of the lip, nasopharynx, pharynx and oral
cavity, esophagus, rectum, gall bladder, biliary tree, larynx, lung and bronchus, bladder, kidney,
brain and other parts of the nervous system, thyroid, Hodgkin's disease, non-Hodgkin's
lymphoma, multiple myeloma and leukemia.
- 20 28. The immunotoxin of claim 25, wherein the cell target comprises a pathogen selected
from a bacteria, a protozoan, a helminth, a virally-infected cell or a fungus.
29. A method for protein purification, comprising:
separating a cohesin or dockerin fusion protein by interacting the fusion protein with a rAb that
is conjugated to the complementary cohesin or dockerin bound to a substrate.
- 25 30. The method of claim 29, further comprising the step of administering the protein in a
therapeutic application comprising transplantation, autoimmune disease, infectious disease or
cancer.
31. The use of the cohesin as a fusion partner for toxins for conferring beneficial biochemical
properties favoring ready purification of active cohesin.toxin fusion protein.
- 30 32. The use of anti-DC rAb.Doc to target DC for therapeutic applications where ablating DC.

33. An anti-DC-SIGN/L antibody provided in an amount that is sufficient to enhance the survival of dendritic cells, wherein the antibody matures and activates the dendritic cells for immunization.
34. The antibody of claim 33, wherein the antibody is targeted in vivo to dendritic cells as an adjuvant in vaccines.
35. A bivalent and multivalent (rAb¹.Doc:Coh.rAb²) self-assembled conjugates as therapeutic, diagnostic, and industrial agents.
36. A bivalent and multivalent (rAb.Doc:Coh.cytokine), (rAb.Coh:Doc.cytokine) or (cytokine¹.Coh:cytokine².Doc) self-assembled conjugates as therapeutic, cell proliferation or maturing agents.
37. A method for making modular rAb comprising:
screening one or more multivalent rAb and/or rAb.cytokine and/or cytokine.cytokine combinations that are capable of specifically binding to a target cell and delivering the cytokine such that it exerts its effect on the target cell.
38. The method of claim 37, wherein the cytokine comprises interleukins, transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), epidermal growth factors (EGFs), connective tissue activated peptides (CTAPs), osteogenic factors, and biologically active analogs, fragments, and derivatives of such growth factors, B/T-cell differentiation factors, B/T-cell growth factors, mitogenic cytokines, chemotactic cytokines, colony stimulating factors, angiogenesis factors, IFN- α , IFN- β , IFN- γ , IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, etc., leptin, myostatin, macrophage stimulating protein, platelet-derived growth factor, TNF- α , TNF- β , NGF, CD40L, CD137L/4-1BBL, human lymphotoxin- β , G-CSF, M-CSF, GM-CSF, PDGF, IL-1 α , IL1- β , IP-10, PF4, GRO, 9E3, erythropoietin, endostatin, angiostatin, VEGF, transforming growth factor (TGF) supergene family include the beta transforming growth factors (for example TGF- β 1, TGF- β 2, TGF- β 3); bone morphogenetic proteins (for example, BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9); heparin-binding growth factors (fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)); Inhibins (for example, Inhibin A, Inhibin B); growth differentiating factors (for example, GDF-1); and Activins (for example, Activin A, Activin B, Activin AB).

Single Targeting mAb-Antigen Fusion Proteins



Multivariable Targeting mAb-Antigen Complexes

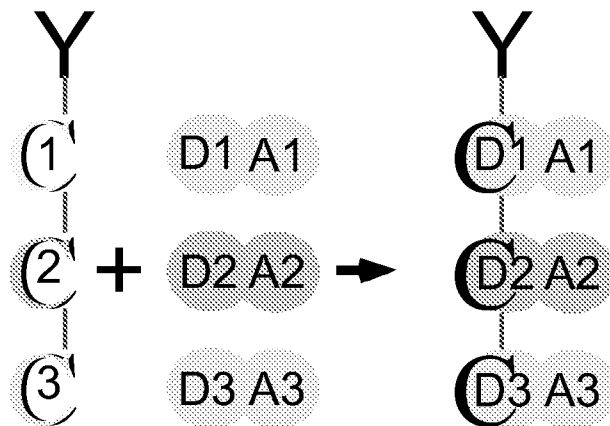


Figure 1

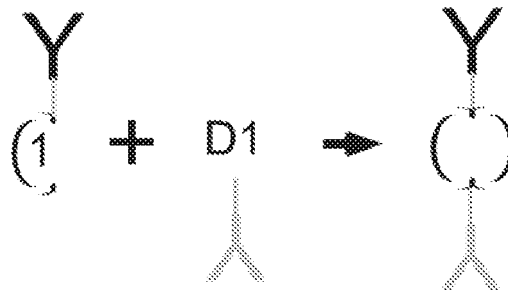
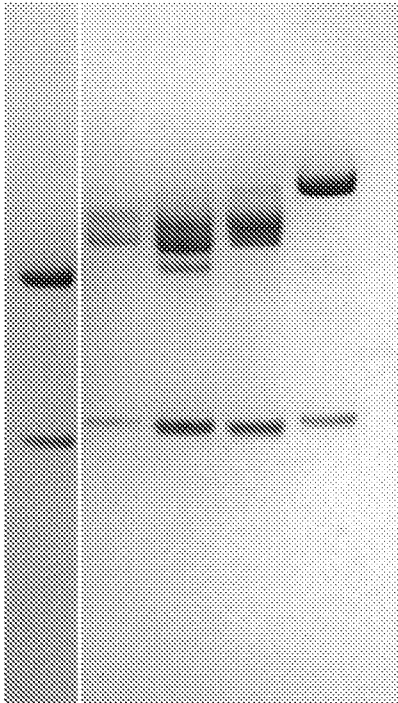


Figure 2



Lane 1: mIgG2b ~1 ug
 Lane 2: anti-DCIR_2C9.doc 15 uL
 Lane 3: anti-ASGPR_49C11.doc 15 uL
 Lane 4: anti-DCSIGN/L_16E7.doc 15 uL
 Lane 5: anti-LOX1_15C4.PSA 15 uL

Figure 3

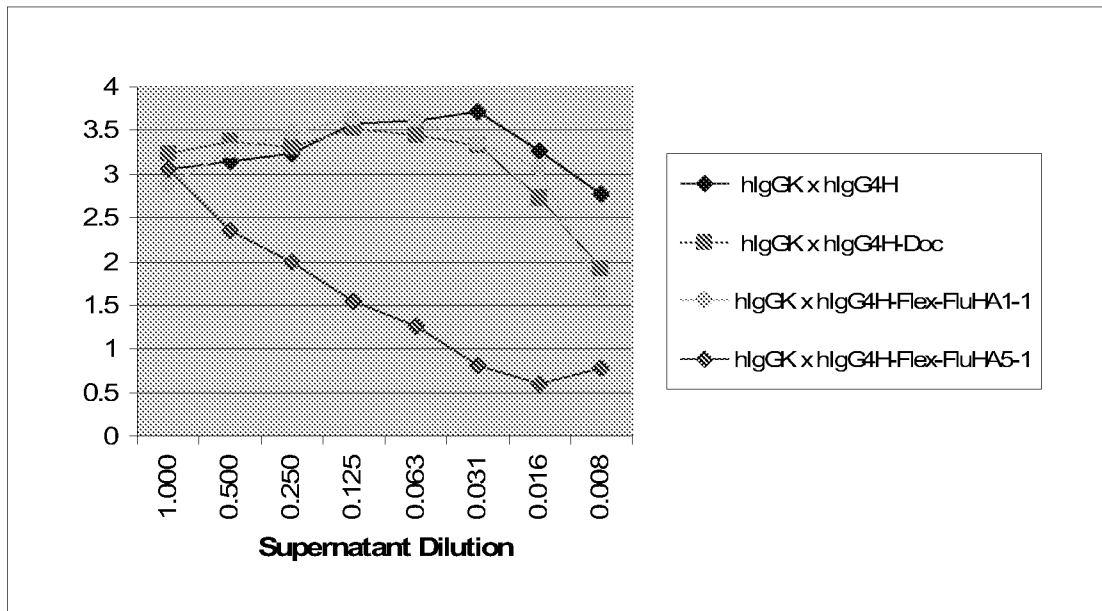


Figure 4A

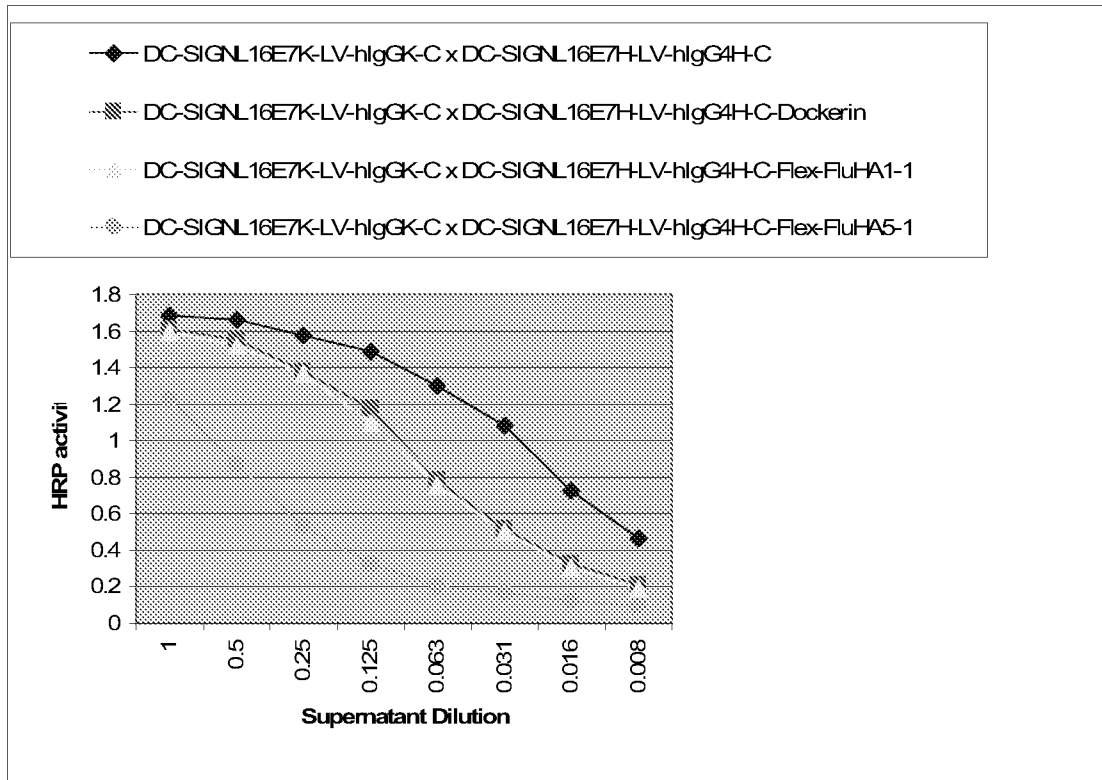


Figure 4B

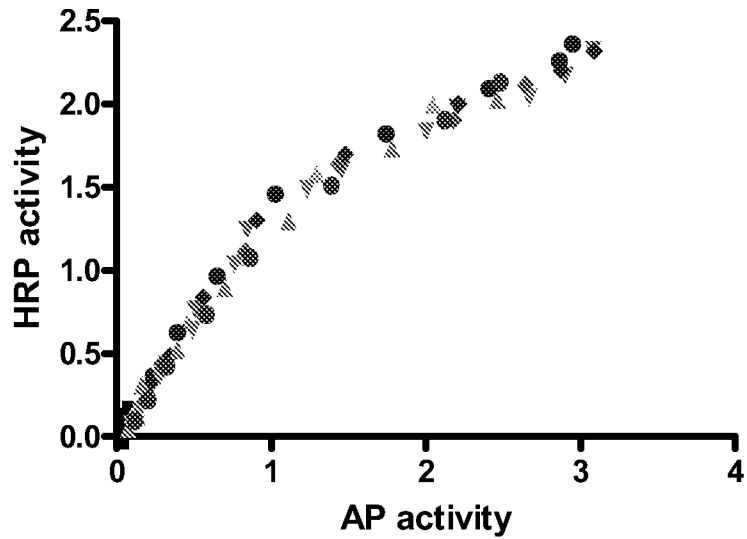


Figure 5

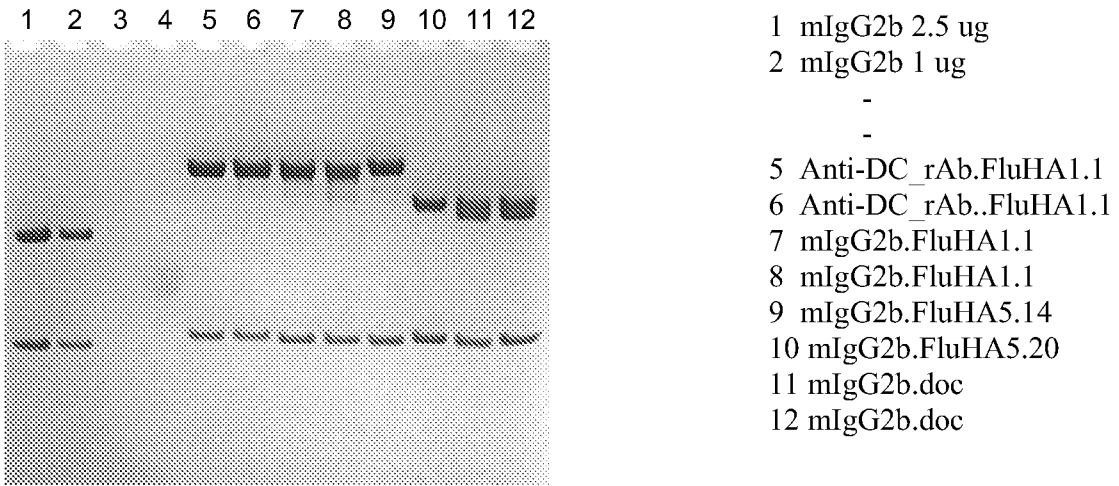


Figure 6

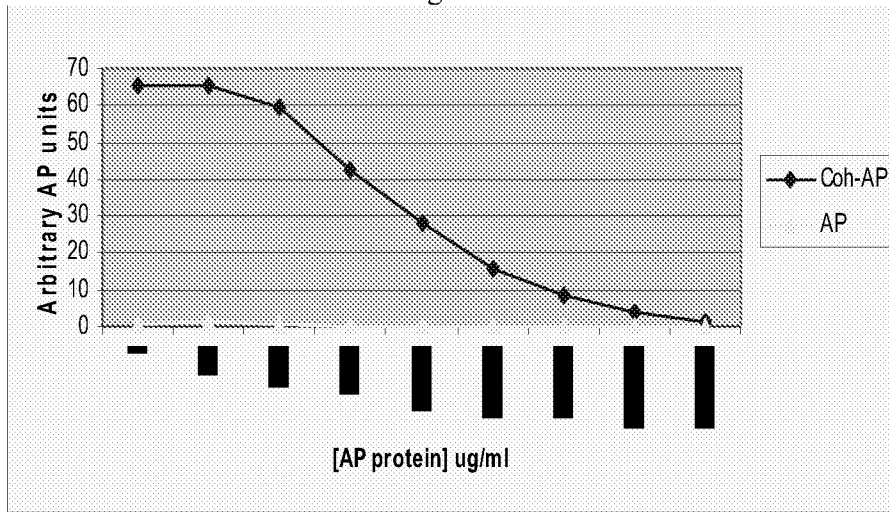


Figure 7A

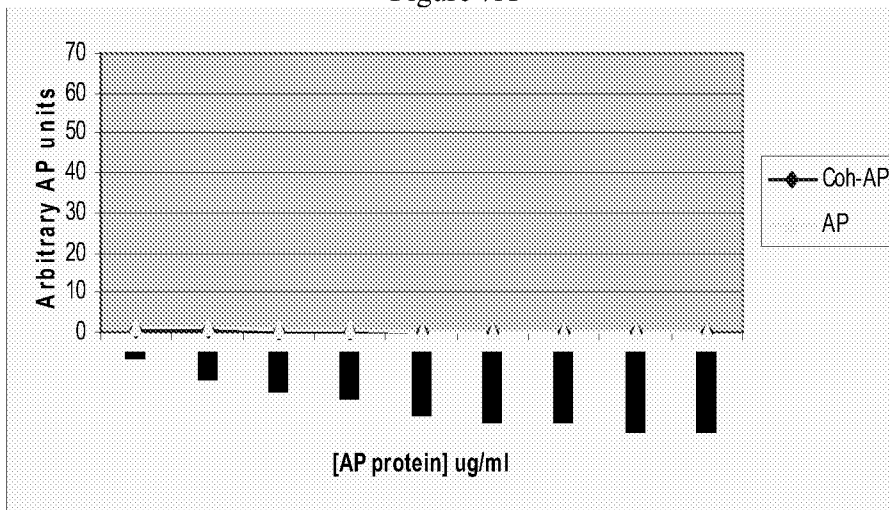


Figure 7B

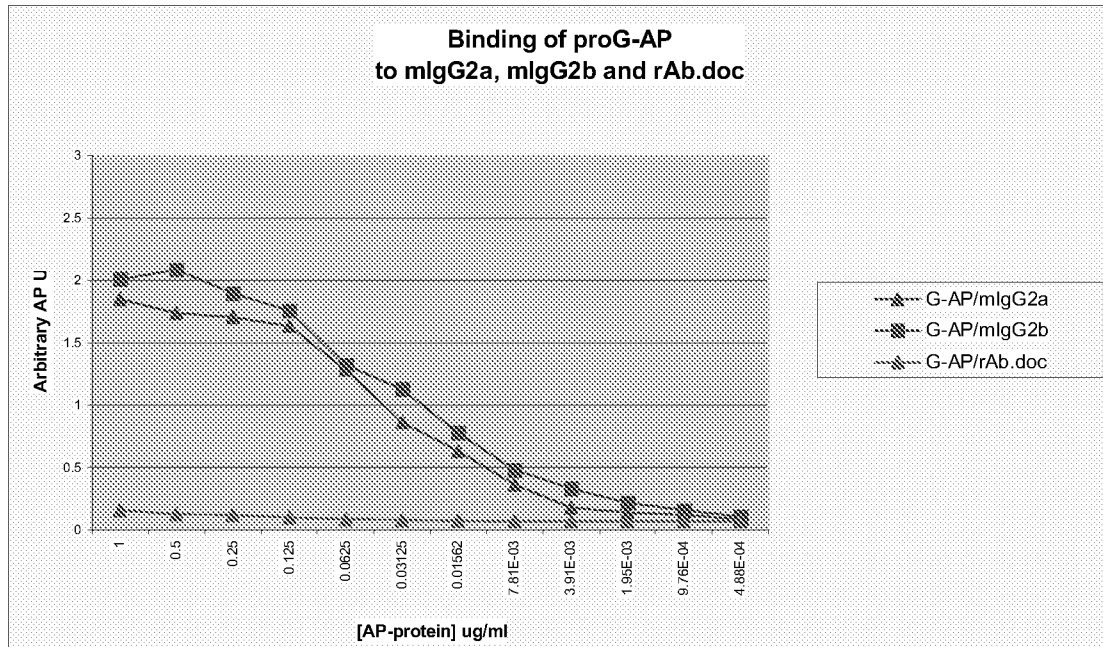


Figure 8A

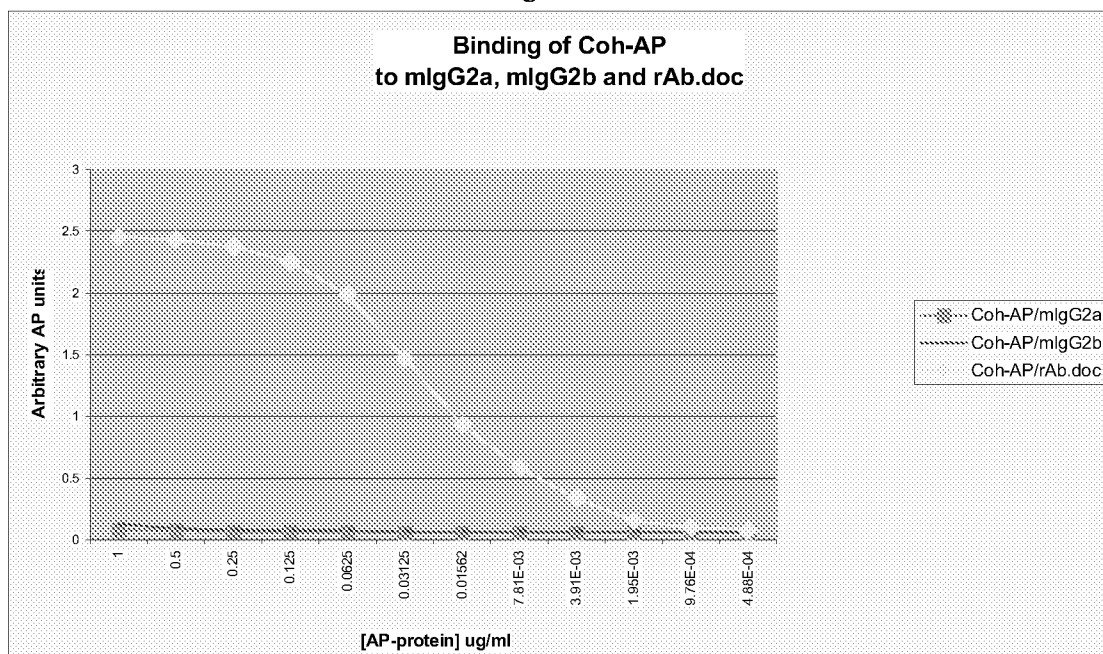


Figure 8B

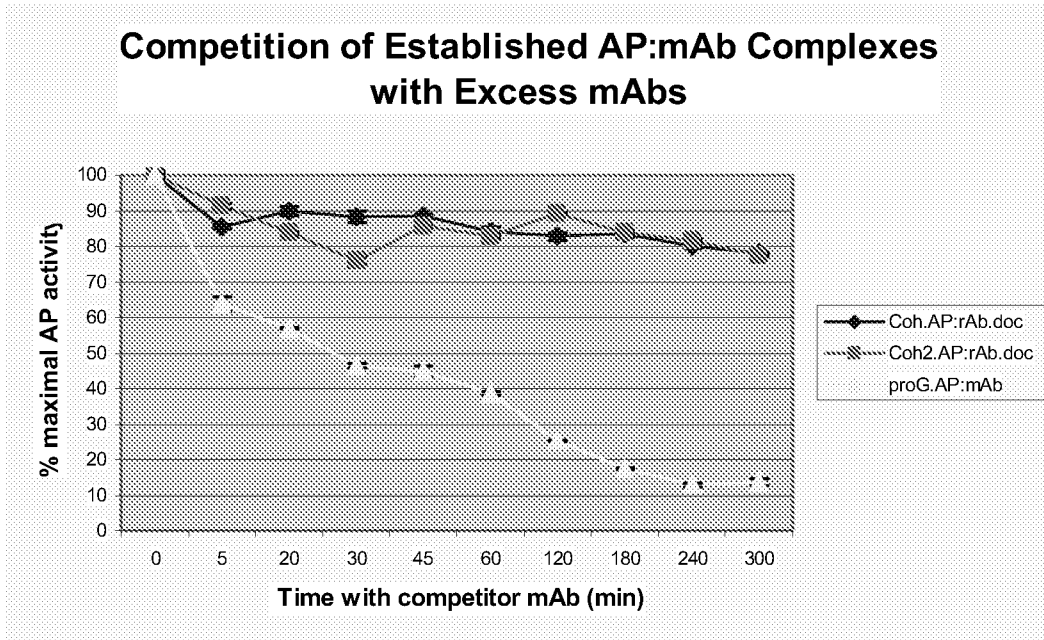


Figure 9

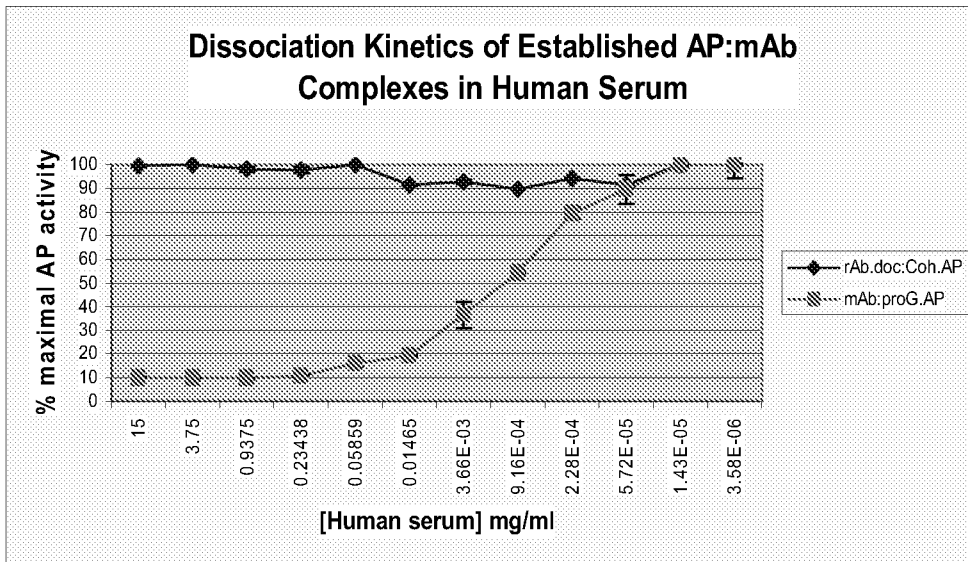


Figure 10

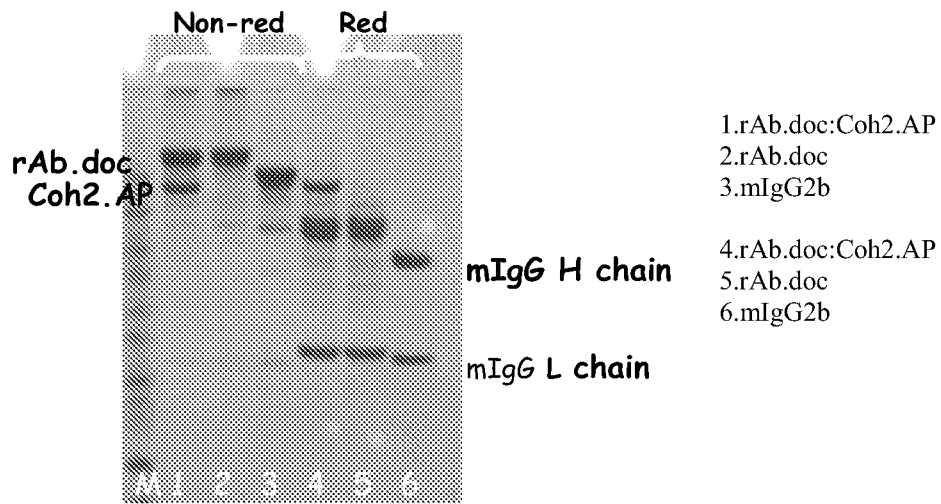


Figure 11

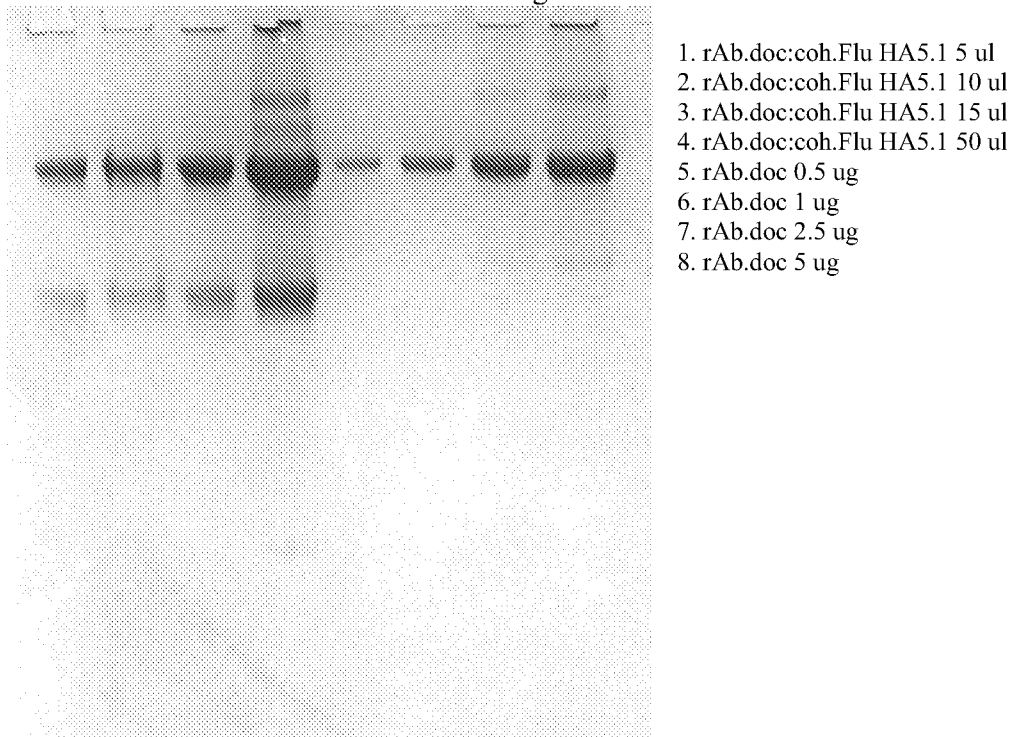


Figure 12

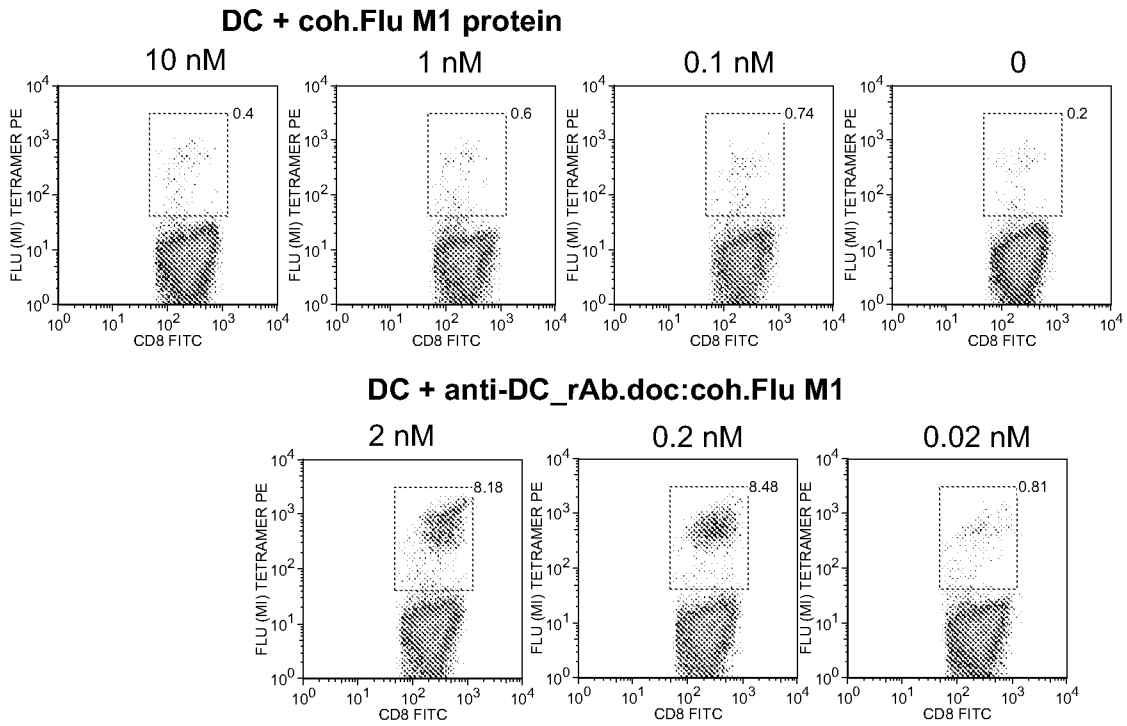


Figure 13

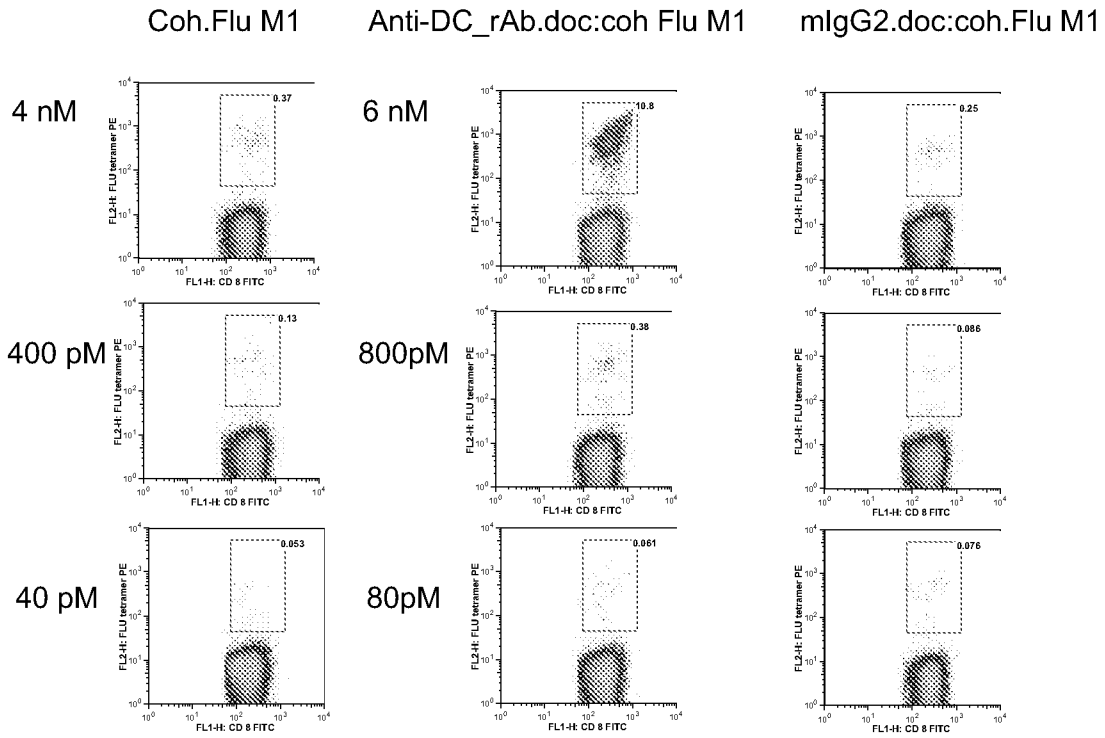
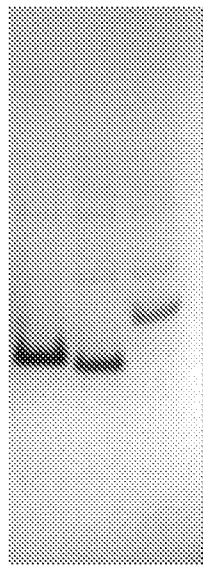
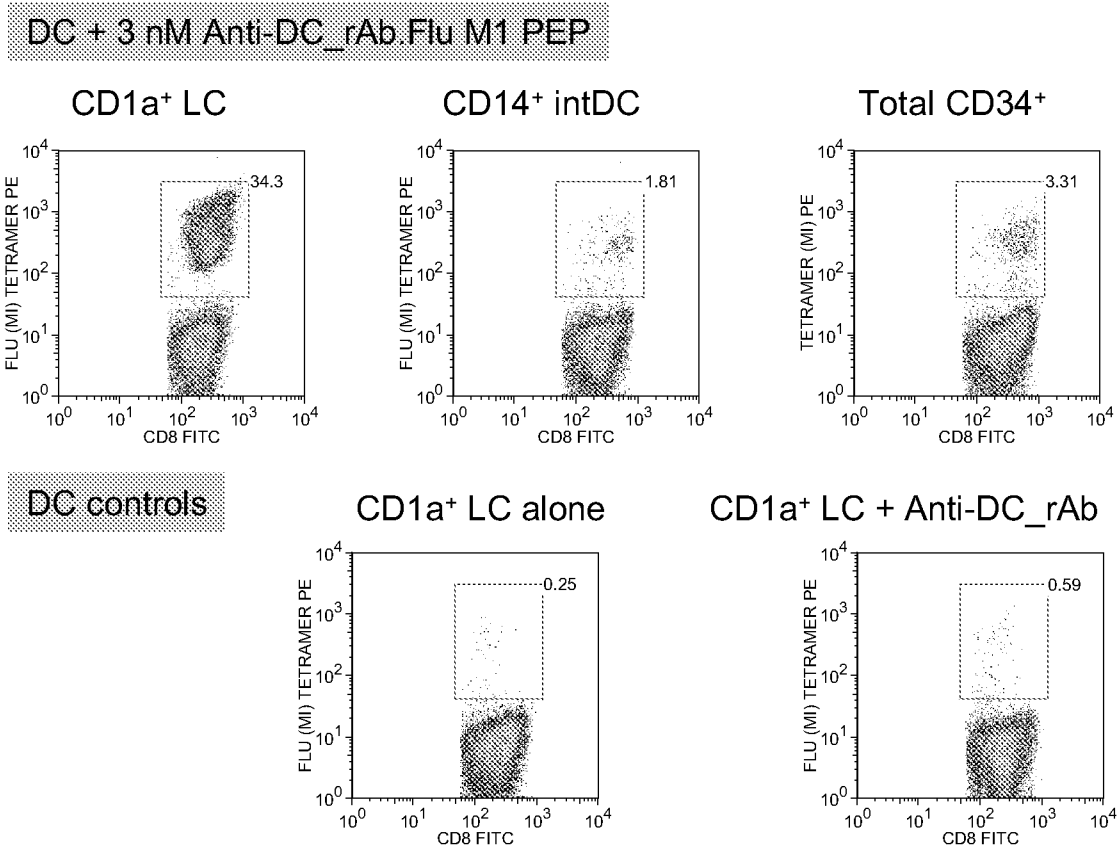


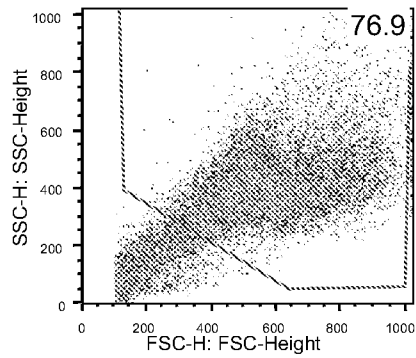
Figure 14



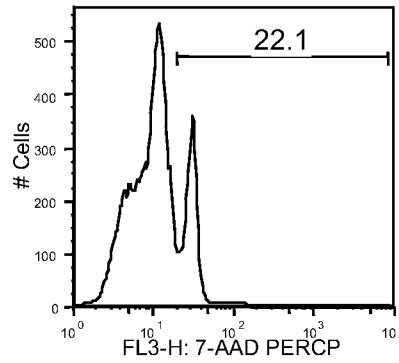
- 1: Cohesin-hgp100-PeptideA
- 2: Cohesin-hMART-1-PeptideB
- 3: Cohesin-Flex-hMART-1-PeptideA

Figure 16

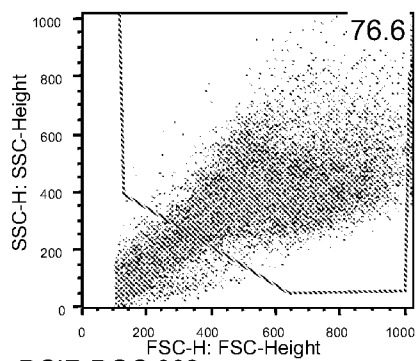
6 days GM IL-4 DCs ND 161 + DCIR.DOC or DC
SIGN/L.DOC + Coh.PE38
2.5 ug/ml, 48HRS incubation



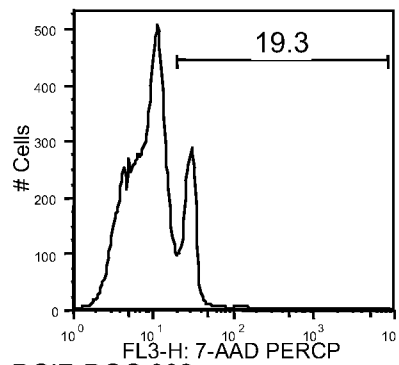
6d IL4 DCs alone.001



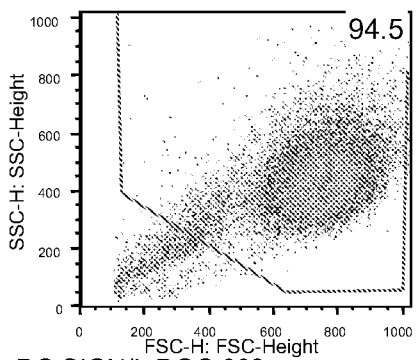
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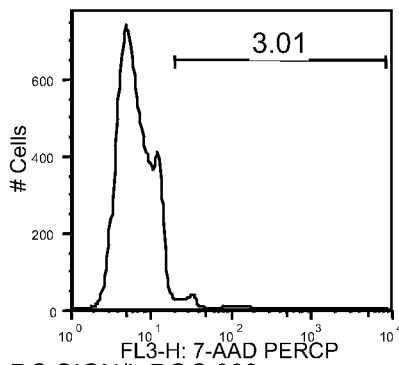
DCIR.DOC.002



DCIR.DOC.002

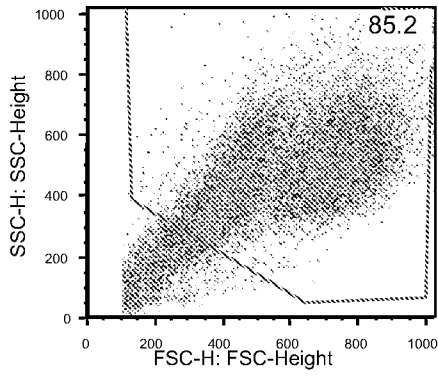


DC SIGN/L.DOC.003

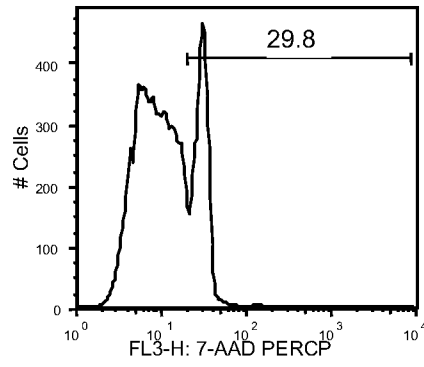


DC SIGN/L.DOC.003

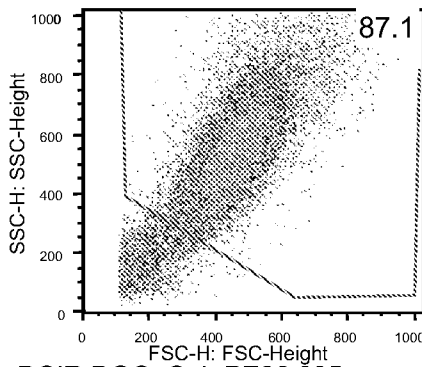
Figure 17



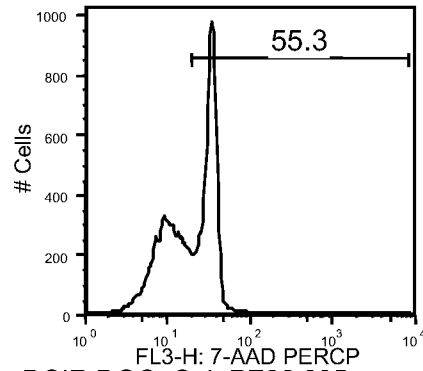
Coh.PE38.004



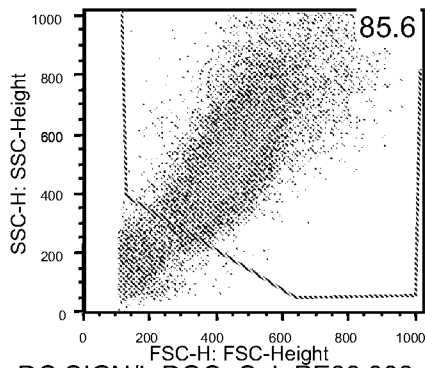
Coh.PE38.004



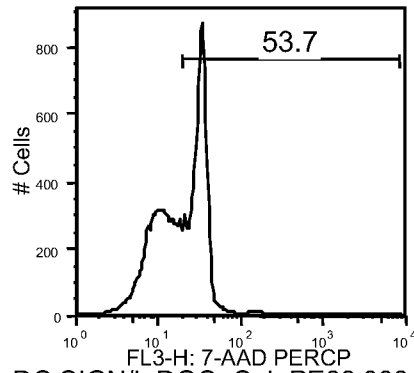
DCIR.DOC+Coh.PE38.005



DCIR.DOC+Coh.PE38.005



DC SIGN/L.DOC+Coh.PE38.006



DC SIGN/L.DOC+Coh.PE38.006

Figure 18

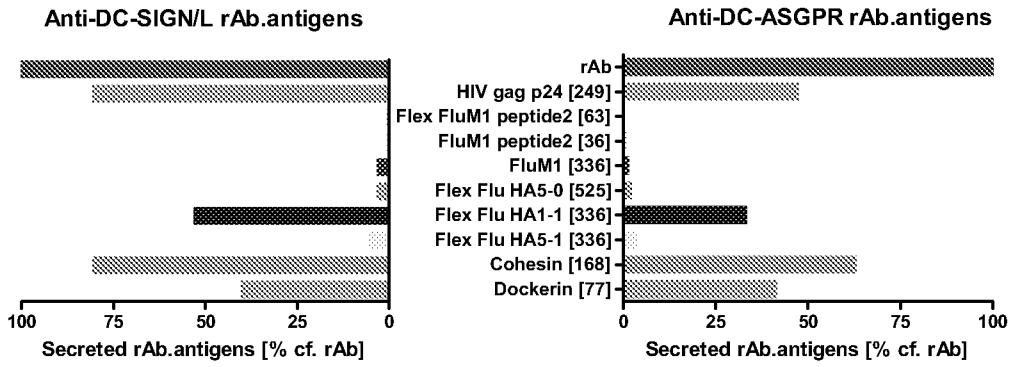


Figure 19

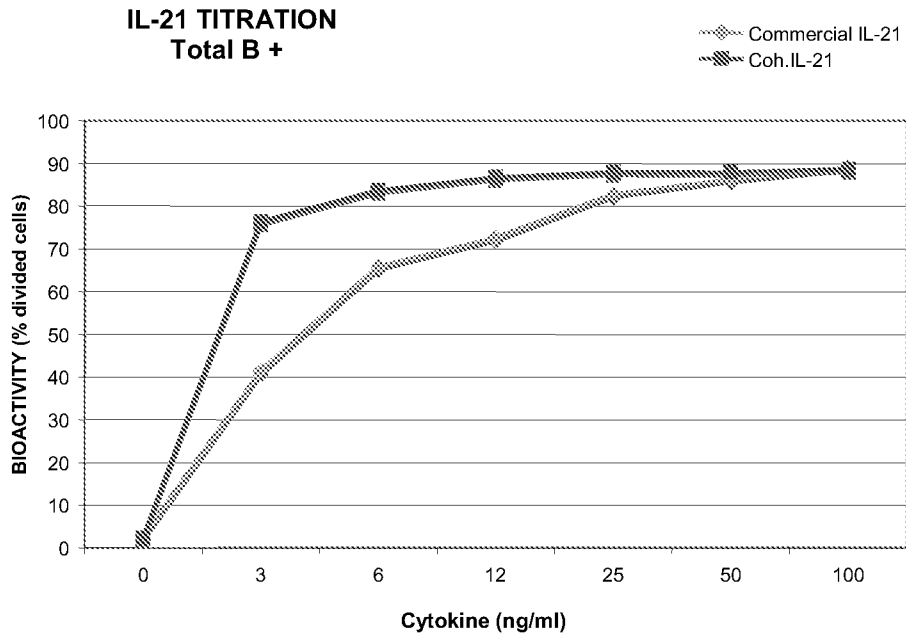


Figure 20