(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 October 2014 (09.10.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/164727 A1

(51) International Patent Classification:
C07K 16/14 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/US2014/023340

(22) International Filing Date:
11 March 2014 (11.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/777,842 12 March 2013 (12.03.2013) US

(71) Applicant: **WISCONSIN ALUMNI RESEARCH FOUNDATION** [US/US]; 614 Walnut Street, Madison, WI 53705 (US).

(72) Inventors: **KLEIN, Bruce, Steven**; 1709 Jefferson Street, Madison, WI 53711 (US). **BRANDHORST, Theodore, Tristan**; 305 Koster Street, Madison, WI 53713 (US). **SULLIVAN, Thomas**; 2305 Keyes Avenue, Madison, WI 53711 (US). **WUETHRICH, Marcel**; 458 Agnes Drive, Madison, WI 53711 (US).

(74) Agents: **BAKER, Jean, C.** et al.; Quarles & Brady LLP, 411 E. Wisconsin Ave., Milwaukee, WI 53202 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

[Continued on next page]

(54) Title: A METHOD OF TREATING FUNGAL INFECTION

(57) Abstract: A vaccine comprising Calnexin fragment and a method of using the vaccine to immunize a patient against fungi are disclosed. The Calnexin fragment may be either a full- length native version or a functionally equivalent version of full-length Calnexin.

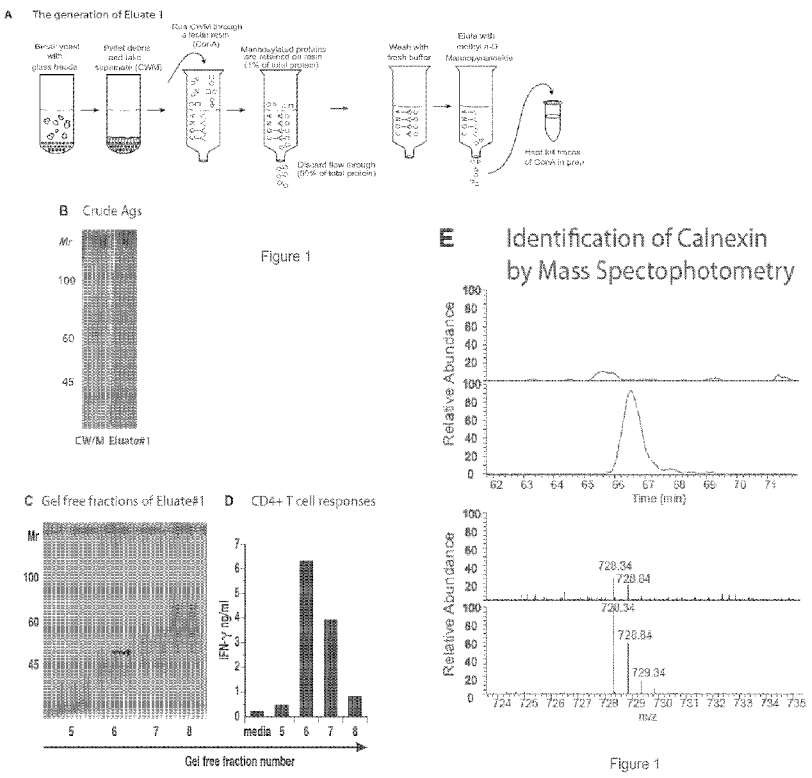


Figure 1

Figure 1

WO 2014/164727 A1

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

A METHOD OF TREATING FUNGAL INFECTION

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of US Provisional Patent Application Serial No. 61/777,842 filed on March 12, 2013, incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under AI035681 and AI040996 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] The incidence of fungal infections and mycoses has increased significantly in the past two decades, mainly due to the growing number of individuals who have reduced immunological function (immuno-compromised patients), such as cancer patients, patients who have undergone organ transplantation, patients with AIDS, patients undergoing hemodialysis, critically ill patients, patients after major surgery, patients with catheters, patients suffering from severe trauma or burns, patients having debilitative metabolic illnesses such as diabetes mellitus, persons whose blood is exposed to environmental microbes such as individuals having indwelling intravenous tubes, and even in some elderly individuals. Fungal infections are often also attributed to the frequent use of cytotoxic and/or antibacterial drugs, which alter the normal bacterial flora. Fungi include moulds, yeasts and higher fungi. All fungi are eukaryotic and have sterols but not peptidoglycan in their cell membrane. They are chemoheterotrophs (requiring organic nutrition) and most are aerobic. Many fungi are also saprophytes (living off dead organic matter) in soil and water and acquire their food by absorption. Characteristically fungi also produce sexual and asexual spores. There are over 100,000 species recognized, with 100 infectious members for humans.

[0004] Human fungal infections are uncommon in generally healthy persons, being confined to conditions such as *Candidiasis* (thrush) and dermatophyte skin infections such as athlete's foot. Nevertheless, yeast and other fungi infections are one of the human ailments which still present a formidable challenge to modern medicine. In an immuno-compromised host, a variety of normally mild or nonpathogenic fungi can cause potentially fatal infections. Furthermore, the relative ease with which human can now travel around the world provides the means for

unusual fungal infections to be imported from place to place. Therefore, wild and resistant strains of fungi are considered to be one of the most threatening and frequent cause of death mainly in hospitalized persons and immuno-compromised patients.

[0005] The identity of conserved antigens among pathogenic fungi is poorly understood. This is especially true for immunologically significant antigens that may serve as immunogens to vaccinate against infection. There are currently no commercial vaccines against fungi despite the growing problem of fungal infections. A vaccine against pathogenic fungi, especially one that protects against multiple fungal pathogens, would be of enormous clinical benefit, and of commercial interest.

[0006] An improved vaccine and a method of vaccination against fungi are needed in the art. Specifically, a vaccine antigenic to multiple fungi, e.g., multiple dimorphic fungi, and a method of using such vaccine are needed in the art.

[0007] There is currently no way to identify CD4 T cells in mammalian blood or tissue, and thus to determine an individuals profile of CD4 T cell based immune resistance or susceptibility. Therefore, needed in the art are compositions and methods for evaluating immunization status of a patient by identifying and evaluating CD4 T cells in the patient.

SUMMARY OF THE INVENTION

[0008] In one aspect, the present invention relates to a vaccine to immunize a patient against fungi, wherein the vaccine comprises a Calnexin fragment. The vaccine additionally comprises at least one of a stabilizer, a buffer, or an adjuvant. In one embodiment of the vaccine, the Calnexin fragment is either a full-length native version or a functionally equivalent version of full-length Calnexin. In one embodiment of the vaccine, the Calnexin fragment comprises or consists of at least the 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1). In another embodiment, the Calnexin fragment comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:2 - 9, 11, 13 -14, and 20 - 24. In yet another embodiment, the Calnexin fragment comprises or consists of a sequence selected from a group consisting of SEQ ID NOs:2, 6, 11, 12, 17, and 29. In one embodiment, the Calnexin fragment comprises a sequence selected from a group consisting of SEQ ID NOs:2 - 29. In another embodiment, the suitable calnexin fragment may comprise or consist of a sequence selected from a group consisting of the sequences presented in Figures 7A, 7B, 7C, 7D, 7E, and 7F. Specifically, the group may consist of those sequences highlighted in Figures 7A, 7B, 7C, 7D, 7E, and 7F.

[0009] In another aspect, the present invention relates to a method of protecting a patient from fungal infection comprising of the steps of obtaining the vaccine as disclosed, wherein the vaccine comprises a Calnexin fragment and providing a therapeutically effective amount of the vaccine to a subject, wherein the subject is protected from fungal infection. In one embodiment of the method, the fungi are either dimorphic fungi or non-dimorphic fungi, and the dimorphic fungi are selected from a group consisting of *Histoplasma, Coccidiodes, Paracoccidiodes, Penicillium, Blastomyces,* and *Sporothrix,* and the non-dimorphic fungi are selected from a group consisting of *Aspergillus, Pneumocystis, Magnaportha, Exophiala, Neuroaspora, Cryptococcus, Schizophyllum,* and *Candida.*

[0010] In one embodiment of the vaccine, the Calnexin fragment comprises or consists of at least the 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1). In another embodiment, the Calnexin fragment comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:2 - 9, 11, 13 -14, and 20 - 24. In yet another embodiment, the Calnexin fragment comprises or consists of a sequence selected from a group consisting of SEQ ID NOs:2, 6, 11, 12, 17, and 29. In one embodiment, the Calnexin fragment comprises a sequence selected from a group consisting of SEQ ID NOs:2 - 29. In another embodiment, the suitable calnexin fragment may comprise or consist of a sequence selected from a group consisting of the sequences presented in Figures 7A, 7B, 7C, 7D, 7E, and 7F. Specifically, the group may consist of those sequences highlighted in Figures 7A, 7B, 7C, 7D, 7E, and 7F.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The patent or application file contains at least one drawing in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0012] Figures 1A, 1B, 1C, 1D, and 1E are a set of graphs showing identity of shared fungal antigen (Ag). Figure 1A: Flow diagram that illustrates the generation of eluate #1 from the BAD1 vaccine strain #55. Figure 1B: Silver nitrate stain of PAGE of *B. dermatitidis* Ags CW/M and Eluate #1 (left to right). Figure 1C: Gel free separation of Eluate #1 into fractions by *Mr*. Figure 1D: Stimulation of 1807 TCR Tg cells *in vitro* by gel free fractions from panel C, as measured by IFN-γ response. The arrow in fraction 7 indicates the material that was subjected to MS/MS. Figure 1E: The identification of Calnexin by MS/MS. This figure shows data collected for one Calnexin-derived peptide, as an example. The top set of paired traces are a comparison of the HPLC separation of the non-stimulatory control fraction (upper) and the stimulatory fraction #7 (lower). The peak present in fraction #7 is not represented in the control.

MS analysis of this peak (bottom set of paired traces) identified it as the peptide: LQNSLNCGGAYMK [728.34Da; +2H], and this mass is significantly better represented in the stimulatory fraction #7 (lower) compared to the non-stimulatory control (upper).

[0013] Figures 2A, 2B, and 2C are a set of graphs showing experimental evidence proving that Calnexin is the shared antigen (Ag). Figure 2A: Induction of *E. coli* transformed with pET28c-Calnexin plasmid produces recombinant Calnexin (63kD). Figure 2B: Recombinant Calnexin stimulates 1807 T cells to produce IFN-γ *in vitro*. Figure 2C: Recombinant Calnexin activates (CD44) and induces proliferation (CFSE) of adoptively transferred 1807 cells *in vivo*.

[0014] Figures 3A, 3B, and 3C are a set of graphs showing identification of Calnexin's 1807 TCR epitope. Figure 3A: *In vitro* activation of 1807 T cells by Calnexin peptide 1. 10^5 BMDC were loaded with various concentrations of antigens or peptides shown and then co-cultured with 3 x 10^5 $CD4^+$ purified 1807 T cells. Three days later, T-cells were analyzed for activation by flow cytometry. Figure 3B: Naïve 1807 T cells were co-cultured as in Panel A, and cell culture supernatants analyzed for IFN-γ by ELISA. Figure 3C: *In vivo* activation of 1807 T cells by Calnexin peptide 1.

[0015] Figures 4A, 4B, and 4C are a set of graphs of experimental observations showing that Calnexin is present on the yeast surface. Figure 4A: Western-blot of the water-soluble extract. Figure 4B and Figure 4C: Surface staining of vaccine and challenge yeast.

[0016] Figures 5A and 5B are a set of graphs of experimental observations showing response to Calnexin. Figure 5A: Mice received adoptive transfer of 10^6 1807 T cells before vaccination, and were challenged with 2 x 10^4 *B. dermatitidis* yeast. 4d after infection, lungs were collected and 1807 T cells analyzed for cytokine products by FACS (Figure 5A) and lung CFU (Figure 5B).

[0017] Figure 6 is a set of graphs of Calnexin's protein sequence alignment among different strains, showing that Calnexin is highly conserved in dimorphic fungi. The deduced Calnexin protein sequences of *B. dermatitidis* strain 26199 (B.d. 26199), *H. capsulatum* strain G217B (H.c. G217B), *C. posadasii* strain C735 (C.p. C735) and *P. brasiliensis* strain PB01 (P.b. Pb01) were aligned using ClustalW software. Regions of identity (in at least three of the four species) are indicated in grey and boxed with a black border. Two different MHC class II peptide-binding prediction algorithms were use to analyze the Calnexin sequence of *B. dermatitidis* and the highest-ranking predictions are indicated on the sequence (Methods). The IEDB (red) boxes represent the regions where multiple overlapping peptides have been predicted. The six regions predicted to bind with an IC_{50} value less that 500nM are labeled -A through -E, based on lowest to highest value. The Marc Jenkins algorithm predicts nine amino-acid MHCII-binding

peptides. Ten predicted binding nanomers are shown, with two amino acids added to each end. These 13-mers were synthesized to test epitope-specific 1807 T-cell activation (see the Example and Figures 3A, 3B, and 3C). The peptides are labeled 1 through 10, based on the highest-to-lowest strength of the predicted binding.

[0018] Figures 7A, 7B, 7C, 7D, 7E, and 7F are diagrams showing an analysis of the predicted peptides that are suitable to work with the known epitope binding domain of several Human HLA DRB1 alleles. The diagram is produced by using the publicly available ProPred software (http://www.imtech.res.in/raghava/propred). In the output, the Blastomyces Calnexin sequence is shown on a separate line for each of 51 DRB1alleles, and peptides that are predicted to fit in the MHCII groove of that allele are indicated in blue, with red used to indicate a so-called anchor amino acid that would be at position one of the 9 amino acid core sequence. A peptide of interest is "promiscuous" if it is predicted to interact with many different human MHCII molecules. Since the human HLA locus is so polymorphic, a good vaccine for human's will have to have epitopes that are promiscuous, and can work with many different HLA MHC molecules in order to stimulate an immune response. The webarchive shows that Blastomyces Calnexin does, indeed, have several peptide sequences (blue) that are predicted to fit into the MHC groove for presentation to T-Cells. Of particular interest is that there is a predicted epitope for the sequence of Peptide1 (which was predicted for B6 mouse HLA interaction, and has been experimentally shown to do so with 1807 cells) at position 103 to 115. There are several other promiscuous epitopes throughout the Calnexin sequence as predicted by the ProPred software.

[0019] Figure 8 is a list showing the protein sequences of *Blastomyces* Calnexin of strains ATCC 18188 and ATCC 26199. The sequences are deduced from genomic sequences. (http://www.ncbi.nlm.nih.gov/protein/327357651; Protein database Accession number: EGE86508; Broad Institute predicted Gene name: BDDG_09453).

[0020] Figure 9 is a diagram showing the comparison analysis of Calnexin among dimorphic fungi, e.g., *Blastomyces, Histoplasma*, *Coccidioides* and *Paracoccidioides* and other, more distantly related fungi, e.g., *Aspergillus, Candida and Cryptococcus.*

[0021] Figure 10 is a diagram showing the formatted alignment and the comparison analysis of Calnexin among dimorphic fungi, e.g., *Blastomyces, Histoplasma, Coccidioides and Paracoccidioides* and other, more distantly related fungi, e.g., *Aspergillus, Candida and Cryptococcus.*

DETAILED DESCRIPTION OF THE INVENTION

[0022] As used herein, the term "patient" refers to a human or non-human mammalian patient in need of vaccination. The vaccines of the present invention may be intended for use by any species, including, for example, human, feline, canine, equine, porcine, bovine, ovine. Preferably, the vaccines of the present invention may be intended for use by human.

[0023] The term "fungi" or "funguses", as used herein, refers to a member of a large group of eukaryotic organisms that may include microorganisms, e.g., yeasts and molds. These organisms may be classified as a kingdom of fungi, which is separate from plants, animals, and bacteria. One major difference between fungi and the others is that fungal cells have cell walls that contain chitin, unlike the cell walls of plants, which contain cellulose.

[0024] These and other differences show that the fungi form a single group of related organisms, named the *Eumycota* (*true fungi* or *Eumycetes*), that share a common ancestor (a *monophyletic group*). This fungal group may be distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). Genetic studies have shown that fungi are more closely related to animals than to plants. In the present invention, the terms "fungi", "funguses", or "fungal" may refer to fungi which may cause infection in humans and animals.

[0025] In the embodiments of the present invention, fungi may include dimorphic fungi and non-dimorphic fungi.

[0026] The term "dimorphic fungi", as used herein, refers to fungi which may exist as mold/hyphal/filamentous form or as yeast. An example is *Penicillium marneffei*. At room temperature, it may grow as a mold. At body temperature, it may grow as a yeast. The exception to these conditions are *Candida* spp. *Candida* grows as a mold at body temperatures and as a yeast at room temperatures. Several species of dimorphic fungi may be potential pathogens, including *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Candida albicans*, *Ustilago maydis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum,* and *Sporothrix schenckii*.

[0027] The term "Calnexin", as used herein, refers to a 67kDa integral protein of the endoplasmic reticulum (ER) (Williams D. B., 2006; Myhill N., Lynes E. M., *et al.*, 2008).

[0028] Calnexin may appear variously as a 90kDa, 80kDa or 75kDa band on western blotting depending on the source of the antibody. Calnexin may consist of a large (50 kDa) N-terminal calcium-binding lumenal domain, a single transmembrane helix and a short (90

residues), acidic cytoplasmic tail. Calnexin may be one of the chaperone molecules, which may be characterized by their main function of assisting protein folding and quality control, ensuring that only properly folded and assembled proteins proceed further along the secretory pathway.

[0029] The function of Calnexin may include retaining unfolded or unassembled N-linked glycoproteins in the ER. Antibodies against Calnexin may be used as markers for the ER in immmunofluorescence experiments. Calnexin may bind only those N-glycoproteins that have GlcNAc2Man9Glc1 oligosaccharides. Oligosaccharides with three sequential glucose residues may be added to asparagine residues of the nascent proteins in the ER. The monoglucosylated oligosaccharides that are recognized by Calnexin result from the trimming of two glucose residues by the sequential action of two glucosidases, I and II. Glucosidase II may also remove the third and last glucose residue. ATP and calcium ions may be two of the cofactors involved in substrate binding for Calnexin.

[0030] Calnexin may also function as a chaperone for the folding of MHC class I alpha chain in the membrane of the ER. After folding is completed Calnexin is replaced by calreticulin, which assists in further assembly of MHC class I.

[0031] The term "Calnexin fragment", as used herein, refers to at least one portion or domain of the full-length version of wild-type Calnexin, or at least one portion or domain of the modified version or recombinant Calnexin. A Calnexin fragment may retain at least 90% activity of the wild-type version of Calnexin. A preferable fragment is at least 13 amino acids.

[0032] The term "functionally equivalent", as used herein, refers to a Calnexin fragment or a modified version of wild-type Calnexin that retains at least 90% activity of the wild-type version of Calnexin. In one embodiment, one may wish to use only selected domains of the native Calnexin protein.

[0033] The term "activity", as used herein, refers to antigenic reactivity of Calnexin fragments against fungi, as demonstrated below in the examples.

[0034] The term "therapeutically effective amount", as used herein, refers to an amount of an antigen or vaccine that would induce an immune response in a subject receiving the antigen or vaccine which is adequate to prevent signs or symptoms of disease, including adverse health effects or complications thereof, caused by infection with a pathogen, such as a virus or a bacterium. Humoral immunity or cell mediated immunity or both humoral and cell mediated immunity may be induced. The immunogenic response of an animal to a vaccine may be evaluated, e.g., indirectly through measurement of antibody titers, lymphocyte proliferation assays, or directly through monitoring signs and symptoms after challenge with wild-type strain. The protective immunity conferred by a vaccine may be evaluated by measuring, e.g., reduction

in clinical signs such as mortality, morbidity, temperature number, overall physical condition, and overall health and performance of the subject. The amount of a vaccine that is therapeutically effective may vary depending on the particular virus used, or the condition of the subject, and may be determined by a physician.

[0035] The term "protected", as used herein, refers to immunization of a patient against a disease. The immunization may be caused by administering a vaccine comprising an antigen. Specifically, in the present invention, the immunized patient is protected from fungal infection.

[0036] The term "vaccine", as used herein, refers to a composition that includes an antigen, as defined herein. Vaccine may also include a biological preparation that improves immunity to a particular disease. A vaccine may typically contain an agent that resembles a disease-causing microorganism, and the agent may often be made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent may stimulate the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters. Vaccines may be prophylactic, e.g., to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen, or therapeutic, e.g., to treat the disease. Administration of the vaccine to a subject results in an immune response, generally against one or more specific diseases. The amount of a vaccine that is therapeutically effective may vary depending on the particular virus used, or the condition of the patient, and may be determined by a physician. The vaccine may be introduced directly into the subject by the subcutaneous, oral, oronasal, or intranasal routes of administration.

[0037] The term "administration", as used herein, refers to the introduction of a substance, such as a vaccine, into a subject's body through or by way of a route that does not include the digestive tract. The administration, e.g., parenteral administration, may include subcutaneous administration, intramuscular administration, transcutaneous administration, intradermal administration, intraperitoneal administration, intraocular administration, intranasal administration and intravenous administration.

[0038] The vaccine or the composition according to the invention may be administered to an individual according to methods known in the art. Such methods comprise application e.g. parenterally, such as through all routes of injection into or through the skin: e.g. intramuscular, intravenous, intraperitoneal, intradermal, mucosal, submucosal, or subcutaneous. Also, the vaccine may be applied by topical application as a drop, spray, gel or ointment to the mucosal epithelium of the eye, nose, mouth, anus, or vagina, or onto the epidermis of the outer skin at any part of the body. Other possible routes of application are by spray, aerosol, or powder

application through inhalation via the respiratory tract. In this last case the particle size that is used will determine how deep the particles will penetrate into the respiratory tract. Alternatively, application may be via the alimentary route, by combining with the food, feed or drinking water e.g. as a powder, a liquid, or tablet, or by administration directly into the mouth as a: liquid, a gel, a tablet, or a capsule, or to the anus as a suppository. The term "animal-based protein", as used herein, refers to proteins that are sourced from ruminant milk, and other sources, for example the muscle meat, of an animal, particularly a mammal. Suitable animal-based proteins may include, but are not limited to, digested protein extracts such as N-Z- Amine®, N-Z-Amine AS® and N-Z-Amine YT® (Sheffield Products Co., Norwich, N.Y.), which are casein enzymatic hydrolysates of bovine milk.

[0039] The term "vegetable-based protein", as used herein, refers to proteins from vegetables. A vegetable-based protein may include, without limitation, soy protein, wheat protein, corn gluten, rice protein and hemp protein, among others. Preferred vegetable based proteins in the present invention are soy proteins and corn gluten. Corn gluten is a mixture of various corn-derived proteins. The soy proteins can include 100% soy protein (available as VegeFuel® by Twinlab), textured soy protein, and soybean enzymatic digest. Textured soy protein is a soy protein that is made from defatted soy flour that is compressed and processed into granules or chunks. Soybean enzymatic digest describes soybean peptones that result from the partial hydrolysis of soybean proteins.

[0040] As used herein, the term "major histocompatibility complex" or "MHC" refers to a set of cell surface molecules encoded by a large gene family in all vertebrates. MHC molecules may mediate interactions of leukocytes, also called white blood cells (WBCs), which are immune cells, with other leukocytes or body cells. MHC determines compatibility of donors for organ transplant as well as one's susceptibility to an autoimmune disease via cross-reacting immunization. In humans, MHC is also called human leukocyte antigen (HLA).

[0041] Protein molecules—either of the host's own phenotype or of other biologic entities—are continually synthesized and degraded in a cell. Occurring on the cell surface, each MHC molecule displays a molecular fraction, called epitope, of a protein. The presented antigen can be either *self* or *nonself*.

[0042] The MHC gene family may be divided into three subgroups: class I, class II and class III. Diversity of antigen presentation, mediated by MHC classes I and II, may be attained in at least three ways: (1) an organism's MHC repertoire is polygenic (via multiple, interacting genes);

(2) MHC expression is codominant (from both sets of inherited alleles); (3) MHC gene variants are highly polymorphic (diversely varying from organism to organism within a species).

[0043] Of the three MHC classes identified, human attention commonly focuses on classes I and II. By interacting with CD4 molecules on surfaces of helper T cells, MHC class II mediates establishment of specific immunity (also called acquired immunity or adaptive immunity).

[0044] The present invention is generally applied to humans. In certain embodiments, non-human mammals, such as rats, may also be used for the purpose of demonstration. One may use the present invention for veterinary purpose. For example, one may wish to treat commercially important farm animals, such as cows, horses, pigs, rabbits, goats, and sheep. One may also wish to treat companion animals, such as cats and dogs.

Vaccines of the present invention

[0045] In one embodiment, the present invention relates to a vaccine against fungi comprising a Calnexin fragment. In one embodiment, the vaccine comprising a Calnexin fragment may be applicable to any fungi. In another embodiment, the vaccine comprising a Calnexin fragment may be applicable to any dimorphic fungi. In another embodiment, the vaccine comprising a Calnexin fragment may be applicable to a dimorphic fungus selected from a group consisting of *Histoplasma, Coccidiodes, Paracoccidiodes, Penicillium, Blastomyces,* and *Sporothrix.*

[0046] In another embodiment, the vaccine comprising a Calnexin fragment may be applicable to any non-dimorphic fungi. In another embodiment, the vaccine comprising a Calnexin fragment may be applicable to a non-dimorphic fungus selected from a group consisting of *Aspergillus, Pneumocystis, Magnaportha, Exophiala, Neuroaspora, Cryptococcus, Schizophyllum,* and *Candida.*

[0047] In one embodiment of the present invention, the Calnexin fragment is part of a full-length native version or a functionally equivalent version of full-length Calnexin. The Calnexin fragment may be produced and isolated from any fungi, e.g., those as discussed above and below. In one specific embodiment, the Calnexin fragment may be produced from any dimorphic fungi, e.g., those as discussed above. In yet another embodiment, the Calnexin fragment may be produce and isolated from any non-dimorphic fungi, e.g., those as discussed above. Further, the Calnexin fragment may also be produced from any other non-fungi sources. For example, the Calnexin fragment may be produced from bacteria and the as-produced Calnexin fragment may not be glycosylated. Thus, the as-produced Calnexin fragment may need to be glycosylated before it can be used as a vaccine.

[0048] In one specific embodiment, the Calnexin fragment of the present invention comprises or consists of the 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1). Table 1 shows a comparison of a Calnexin fragment of Calnexin peptide 1, the 13 amino acid sequence among fungi species and *Homo sapiens* (Calmegin). As shown in Table 1, to be a suitable vaccine, the Calnexin fragment, comprising the completely conserved 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1), may be produced from fungi s*pecies. The* Calnexin fragment, comprising the completely conserved 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1), may be produced from *Blastomyces dermatitidis* of strains 26199 (SEQ ID NO:2), 18808 (SEQ ID NO:3), Er-3 (SEQ ID NO:4), 14081 (SEQ ID NO:5); *Histoplasma capsulatum* of strains G186AR (SEQ ID NO:6), Nam1 (SEQ ID NO:7), H88 (SEQ ID NO:8), and H143 (SEQ ID NO:9), *Aspergillus sp.*1 of strains group.1, *A. flavus* (SEQ ID NO:17), and group.1, *A. oryzae* (SEQ ID NO:18), *A. terreus* (SEQ ID NO:19), and *Magnaporthe oryzae_70-15* (SEQ ID NO:26). In another preferred embodiment, the Calnexin fragment of the present invention comprises one or more of peptide 2, peptide 3, peptide 4, peptide 5, peptide 6, peptide 7, peptide 7, peptide 8, peptide 9, and peptide 10 as shown in Figure 6. In another embodiment, the Calnexin fragment of the present invention consists of peptide 2, peptide 3, peptide 4, peptide 5, peptide 6, peptide 7, peptide 7, peptide 8, peptide 9, and peptide 10 as shown in Figure 6.

Table 1. Calnexin peptide #1, 13 amino acid sequence

Genus species_strain														1807 reactive
Blastomyces dermatitidis (SEQ ID NOs:2-5)[a]	L	V	V	K	N	P	A	A	H	H	A	I	S	+
Histoplasma capsulatum (SEQ ID NOs:6-9)[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	+
*Paracoccidioides brasiliensis*_Pb18 (SEQ ID NO:10)	-	-	I	-	-	A	-	-	-	-	-	-	-	
*Paracoccidioides lutzii*_ Pb01 (SEQ ID NO:11)	-	-	I	-	-	A	-	-	-	-	-	-	-	+
*Coccidioides immitis.*_RS	-	-	-	-	-	A	-	-	-	-	-	-	-	

(SEQ ID NO:12)														
Coccidioides posadasii (SEQ ID NOs:13-14)[c]	-	-	-	-	-	A	-	-	-	-	-	-	-	+
Penicillium marneffei (SEQ ID NO:15)		-	L	-	-	-	-	-	-	-	-	-	-	
Penicillium chrysogenum (SEQ ID NO:16)		-	-	-	-	A	-	-	-	-	-	-	-	
*Aspergillus sp.*1. (SEQ ID NOs:17-19)[d]	-	-	-	-	-	-	-	-	-	-	-	-	-	
*Aspergillus sp.*2 (SEQ ID NOs:20-24)[e]	-	-	-	-	-	V	-	-	-	-	-	-	-	+
*Pneumocystis carinii*_Rat Form 1 (SEQ ID NO:25)	-	-	L	-	-	E	-	-	-	-	-	-	-	-
*Magnaporthe oryzae*_70-15 (SEQ ID NO:26)	-	-	-	-	-	-	-	-	-	-	-	-	-	
*Exophiala dermatitidis*_NIH/UT8656 (SEQ ID NO:27)	-	-	-	-	-	A	-	-	-	-	-	-	-	
*Neurospora crassa*_OR74A (SEQ ID NO:28)	-	-	-	-	-	A	-	-	-	-	-	-	-	
Cryptococcus neoformans (SEQ ID NO:29)	-	-	L	-	T	K	-	-	-	-	-	-	-	
*Schizophyllum commune*_H4-8 (SEQ ID NO:30)	-	-	A	-	T	K	-	-	-	-	-	-	-	
*Candida albicans*_5314	-	-	M	-	S	R	-	S	-	Y	-	-	-	-

(SEQ ID NO:31)														
Homo sapiens (Calmegin) (SEQ ID NO:32)	-	-	L	-	S	R	-	K	-	-	-	-	-	
Homo sapiens (Calnexin) (SEQ ID NO:33)	-	-	L	M	S	R	-	K	-	-	-	-	-	
Geomyces destructans (SEQ ID NO:34)[f]	-	-	-	-	-	A	-	-	-	-	-	-	-	

[a] *B. dermatitidis strains*: 26199, 18808, Er-3, 14081

[b] *H. capsulatum strains*: G186AR, Nam1, H88, H143

[c] *C. posadasii strains*: C35 Δ SOWgp, Silveira

[d] *Aspergillus species* group.1: *A. flavus, A, oryzae, A. terreus*

[e] *Aspergillus species* group 2: *A. nidulans, A. kawachii, A. niger, A. fumagatus* 293, *A. clavatus*

[f] *Geomyces destructans* now called *Pseudogymnoascus destructans*

[0049] In another embodiment of the present invention, a suitable Calnexin fragment, comprising 13 amino acid sequence of LVVKNPAAHHAIS (SEQ ID NO:1), may have at least one modified amino acid sequence among the 13 amino acid sequence. In one specific embodiment, the suitable Calnexin fragment may comprise LVVKNAAAHHAIS from *Coccidioides immitis.*_RS (SEQ ID NO:12). In another specific embodiment, the suitable Calnexin fragment may comprise LVVKNAAAHHAIS from *Coccidioides posadasii* of strains C35 Δ SOWgp (SEQ ID NO:13) and Silveira (SEQ ID NO:14). In another specific embodiment, the suitable Calnexin fragment may comprise LVLKNPAAHHAIS from *Penicillium marneffei* (SEQ ID NO:15). In another specific embodiment, the suitable Calnexin fragment may comprise LVVKNAAAHHAIS from *Penicillium chrysogenum* (SEQ ID NO:16). In yet another specific embodiment, the suitable Calnexin fragment may comprise LVVKNVAAHHAIS from *Aspergillus sp.2* of strains *group.2, A. nidulans* (SEQ ID NO:20), *group.2, A. kawachii* (SEQ ID NO:21*), group.2, A. niger* (SEQ ID NO:22*), group.2, A. fumagatus 293* (SEQ ID NO:23*),* or *group.2, A. clavatus* (SEQ ID NO:24). In yet another specific embodiment, the suitable Calnexin fragment may comprise LVVKNAAAHHAIS from *Exophiala dermatitidis_NIH/UT8656* (SEQ ID NO:27). In yet another specific embodiment, the suitable Calnexin fragment may comprise

LVVKNAAAHHAIS from *Neuroaspora crassa_OR74A* (SEQ ID NO:28). In another embodiment, the suitable Calnexin fragment may comprise LVVKNAAAHHAIS from *Geomyces destructans,* which are now called *Pseudogymnoascus destructans* (SEQ ID NO:34).

[0050] In another embodiment of the present invention, a suitable Calnexin fragment, comprising the 13 amino acid sequence of LVVKNPAAHHAIS (SEQ ID NO:1), may have at least two changed amino acid sequences among the 13 amino acid sequence. In one specific embodiment, the suitable Calnexin fragment may comprise LVIKNAAAHHAIS from *Paracoccidioides brasiliensis_Pb18* (SEQ ID NO:10). In another specific embodiment, the suitable Calnexin fragment may comprise LVIKNAAAHHAIS from *Paracoccidioides lutzii_ Pb01* (SEQ ID NO:11).

[0051] In another embodiment of the present invention, a suitable Calnexin fragment, comprising the 13 amino acid sequence of LVVKNPAAHHAIS (SEQ ID NO:1), may have at least three changed amino acid sequences among the 13 amino acid sequence. In one specific embodiment, the suitable Calnexin fragment may comprise LVLKTKAAHHAIS from *Cryptococcus neoformans* (SEQ ID NO:29). In another specific embodiment, the suitable Calnexin fragment may comprise LVAKTKAAHHAIS from *Schizophyllum commune_H4-8* (SEQ ID NO:30).

[0052] In another embodiment of the present invention, a suitable Calnexin fragment, comprising 13 amino acid sequence of LVVKNPAAHHAIS (SEQ ID NO:1), may have more than three changed amino acid sequences among the 13 amino acid sequence.

[0053] In one preferred embodiment, a suitable Calnexin fragment may comprise a sequence selected from the group consisting of SEQ ID NOs:2-11, 13-14, and 20-24.

[0054] In another preferred embodiment, a suitable Calnexin fragment may comprise a sequence selected from the group consisting of SEQ ID NOs:2, 6, 11, 12, 17, and 29.

[0055] In one embodiment, Applicants found or envisioned that the Calnexin fragment comprising LVLKNEAAHHAIS (SEQ ID NO:25) from *Pneumocystis carinii*_Rat Form 1, the Calnexin fragment comprising LVMKSRASHYAIS (SEQ ID NO:31) from *Candida albicans*_5314, and the Calnexin fragment comprising LVLKSRAKHHAIS (SEQ ID NO:32) from *Homo sapiens* (Calmegin) were not reactive with the 1807 cells. Thus, the Calnexin fragments from these species may not be suitable for a vaccine of the present invention.

[0056] In another embodiment, a suitable Calnexin fragment in the vaccine of the present invention may comprise a full-length native version of a Calnexin. In one specific embodiment, the full length native version of a Calnexin may comprise a sequence from *Blastomyces dermatitidis of* strains 26199 (SEQ ID NO:35) or 18188 (SEQ ID NO:36). In another

embodiment, a suitable Calnexin fragment in the vaccine of the present invention may comprise a functionally equivalent version of full-length wild-type Calnexin.

[0057] Applicants envision that many peptide sequences of Calnexin fragments would be suitable vaccines for human in the present invention. Figures 7A, 7B, 7C, 7D, 7E, and 7F show predicted peptide sequences of Calnexin fragments for 51 Human HLA DRB1 alleles, where the predicted peptide sequences of Calnexin fragments would fit in the known epitope binding domain of all the 51 Human HLA DRB1 alleles. In one embodiment, a suitable Calnexin fragment for human vaccination may comprise a sequence selected from a group consisting of each of the 51 amino acid sequences shown in Figures 7A, 7B, 7C, 7D, 7E, and 7F. In another embodiment, a suitable Calnexin fragment for human vaccination may comprise a sequence selected from a group consisting of each of the 51 amino acid sequences at least having the highlighted amino acid sequences as shown in Figures 7A, 7B, 7C, 7D, 7E, and 7F.

[0058] In one embodiment, a suitable calnexin fragment for human vaccination may comprise a sequence selected from a group consisting of at least one of the highlighted amino acid sequences as shown in Figures 7A, 7B, 7C, 7D, 7E, and 7F. In one embodiment, a suitable calnexin fragment for human vaccination may comprise a sequence selected from a group consisting of at least two of the highlighted amino acid sequences as shown in Figures 7A, 7B, 7C, 7D, 7E, and 7F. Applicants envision that the amino acid sequences highlighted in blue color can likely bind (based on motifs) to human HLA class II molecules and thus may be antigens for stimulating human CD4 T cells and eliciting calnexin antigen-dependent cellular immunity to fungi. In one embodiment, the suitable calnexin fragment may comprise or consist of a sequence selected from a group consisting of the sequences presented in Figures 7A, 7B, 7C, 7D, 7E, and 7F. Specifically, the group may consist of those sequences highlighted in Figures 7A, 7B, 7C, 7D, 7E, and 7F.

[0059] In another embodiment, the present invention relates to a method of vaccination for protecting a patient from fungal infections. The method of vaccination in the present invention may generally be applicable to any fungi comprising any dimorphic or non-dimorphic fungi. In a preferred embodiment, the method of vaccination may be used to protect a patient from the infections of dimorphic fungi. In one specific embodiment, the method of vaccination may be applicable to a dimorphic fungus selected from a group consisting of *Histoplasma, Coccidiodes, Paracoccidiodes, Penicillium, Blastomyces,* and *Sporothrix.* In another embodiment, the method of vaccination may be applicable to a non-dimorphic fungus selected from a group consisting of *Aspergillus, Pneumocystis, Magnaportha, Exophiala, Neuroaspora, Cryptococcus, Schizophyllum,* and *Candida.*

[0060] A Calnexin fragment suitable for a vaccine in the present invention may be in any form as discussed above. In one embodiment, a vaccine of a Calnexin fragment may be expressed in commercially available sources, e.g., *E. coli*. The vaccine of a Calnexin fragment may be then isolated and purified from the sources. The protein expression, isolation, and purifications are well know to a person having ordinary skill in the art. The Example demonstrated methods of expression, isolation, and purifications of a Calnexin fragment according to one embodiment of the present invention.

[0061] A vaccine comprising a Calnexin fragment may also comprise other suitable ingredients. In one embodiment, a vaccine may also comprise a carrier molecule as a stabilizer component. As the types of vaccines enclosed in the present invention may be rapidly degraded once injected into the body, the vaccine may be bound to a carrier molecule for stabilizing the vaccine during delivery and administration. A suitable carrier or stabilizer may comprise fusion proteins, polymers, liposome, micro or nanoparticles, or any other pharmaceutically acceptable carriers. A suitable carrier or stabilizer molecule may comprise a tertiary amine N-oxide, e.g., trimethylamine-N-oxide, a sugar, e.g., trehalose, a poly(ethylene glycol) (PEG), an animal-based protein, e.g., digested protein extracts such as N-Z- Amine®, N-Z-Amine AS® and N-Z-Amine YT® (Sheffield Products Co., Norwich, N.Y.), a vegetable-based protein, e.g., soy protein, wheat protein, corn gluten, rice protein and hemp protein, and any other suitable carrier molecules.

[0062] Suitable Carrier Or Vehicle

[0063] Suitable agents may include a suitable carrier or vehicle for delivery. As used herein, the term “carrier” refers to a pharmaceutically acceptable solid or liquid filler, diluent or encapsulating material. A water-containing liquid carrier can contain pharmaceutically acceptable additives such as acidifying agents, alkalizing agents, antimicrobial preservatives, antioxidants, buffering agents, chelating agents, complexing agents, solubilizing agents, humectants, solvents, suspending and/or viscosity-increasing agents, tonicity agents, wetting agents or other biocompatible materials. A tabulation of ingredients listed by the above categories, may be found in the *U.S. Pharmacopeia National Formulary,* 1857-1859, (1990).

[0064] Some examples of the materials which can serve as pharmaceutically acceptable carriers are sugars, such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as

cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols such as glycerin, sorbitol, mannitol and polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen free water; isotonic saline; Ringer's solution, ethyl alcohol and phosphate buffer solutions, as well as other non toxic compatible substances used in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions, according to the desires of the formulator.

[0065] Examples of pharmaceutically acceptable antioxidants include water soluble antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfite, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol and the like; and metal-chelating agents such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid and the like.

[0066] Stabilization Agent

[0067] In another configuration, the present formulation may also comprise other suitable agents that stabilize the formulations. For example, an approach for stabilizing solid protein formulations of the invention is to increase the physical stability of purified, e.g., lyophilized, protein. This will inhibit aggregation via hydrophobic interactions as well as via covalent pathways that may increase as proteins unfold. Stabilizing formulations in this context may often include polymer-based formulations, for example a biodegradable hydrogel formulation/delivery system. The critical role of water in protein structure, function, and stability is well known. Typically, proteins are relatively stable in the solid state with bulk water removed. However, solid therapeutic protein formulations may become hydrated upon storage at elevated humidities or during delivery from a sustained release composition or device. The stability of proteins generally drops with increasing hydration. Water may also play a significant role in solid protein aggregation, for example, by increasing protein flexibility resulting in enhanced accessibility of reactive groups, by providing a mobile phase for reactants, and by serving as a reactant in several deleterious processes such as beta-elimination and hydrolysis.

[0068] An effective method for stabilizing peptides and proteins against solid-state aggregation for delivery may be to control the water content in a solid formulation and maintain the water activity in the formulation at optimal levels. This level depends on the nature of the

protein, but in general, proteins maintained below their “monolayer” water coverage will exhibit superior solid-state stability.

[0069] A variety of additives, diluents, bases and delivery vehicles may be provided within the invention that effectively control water content to enhance protein stability. These reagents and carrier materials effective as anti-aggregation agents in this sense may include, for example, polymers of various functionalities, such as polyethylene glycol, dextran, diethylaminoethyl dextran, and carboxymethyl cellulose, which significantly increase the stability and reduce the solid-phase aggregation of peptides and proteins admixed therewith or linked thereto. In some instances, the activity or physical stability of proteins may also be enhanced by various additives to aqueous solutions of the peptide or protein drugs. For example, additives, such as polyols (including sugars), amino acids, proteins such as collagen and gelatin, and various salts may be used.

[0070] Certain additives, in particular sugars and other polyols, may also impart significant physical stability to dry, e.g., lyophilized proteins. These additives may also be used within the invention to protect the proteins against aggregation not only during lyophilization but also during storage in the dry state. For example sucrose and Ficoll 70 (a polymer with sucrose units) exhibit significant protection against peptide or protein aggregation during solid-phase incubation under various conditions. These additives may also enhance the stability of solid proteins embedded within polymer matrices.

[0071] Yet additional additives, for example sucrose, stabilize proteins against solid-state aggregation in humid atmospheres at elevated temperatures, as may occur in certain sustained-release formulations of the invention. Proteins such as gelatin and collagen also serve as stabilizing or bulking agents to reduce denaturation and aggregation of unstable proteins in this context. These additives can be incorporated into polymeric melt processes and compositions within the invention. For example, polypeptide microparticles can be prepared by simply lyophilizing or spray drying a solution containing various stabilizing additives described above. Sustained release of unaggregated peptides and proteins can thereby be obtained over an extended period of time.

[0072] Various additional preparative components and methods, as well as specific formulation additives, are provided herein which yield formulations for mucosal delivery of aggregation-prone peptides and proteins, wherein the peptide or protein is stabilized in a substantially pure, unaggregated form using a solubilization agent. A range of components and additives are contemplated for use within these methods and formulations. Exemplary of these solubilization agents are cyclodextrins (CDs), which selectively bind hydrophobic side chains of

polypeptides. These CDs have been found to bind to hydrophobic patches of proteins in a manner that significantly inhibits aggregation. This inhibition is selective with respect to both the CD and the protein involved. Such selective inhibition of protein aggregation may provide additional advantages within the intranasal delivery methods and compositions of the invention.

[0073] Additional agents for use in this context include CD dimers, trimers and tetramers with varying geometries controlled by the linkers that specifically block aggregation of peptides and protein. Yet solubilization agents and methods for incorporation within the invention involve the use of peptides and peptide mimetics to selectively block protein-protein interactions. In one aspect, the specific binding of hydrophobic side chains reported for CD multimers may be extended to proteins via the use of peptides and peptide mimetics that similarly block protein aggregation. A wide range of suitable methods and anti-aggregation agents may be available for incorporation within the compositions and procedures of the invention.

[0074] Stabilizing Delivery Vehicle, Carrier, Support Or Complex-Forming Species

[0075] In another embodiment, the present formulation may also comprise other suitable agents such as a stabilizing delivery vehicle, carrier, support or complex-forming species. The coordinate administration methods and combinatorial formulations of the instant invention may optionally incorporate effective lipid or fatty acid based carriers, processing agents, or delivery vehicles, to provide improved formulations for delivery of Calnexin or functionally equivalent fragment proteins, analogs and mimetics, and other biologically active agents. For example, a variety of formulations and methods are provided for delivery which comprise one or more of these active agents, such as a peptide or protein, admixed or encapsulated by, or coordinately administered with, a liposome, mixed micellar carrier, or emulsion, to enhance chemical and physical stability and increase the half-life of the biologically active agents (e.g., by reducing susceptibility to proteolysis, chemical modification and/or denaturation) upon mucosal delivery.

[0076] Within certain aspects of the invention, specialized delivery systems for biologically active agents may comprise small lipid vesicles known as liposomes or micelles. These are typically made from natural, biodegradable, non-toxic, and non-immunogenic lipid molecules, and can efficiently entrap or bind drug molecules, including peptides and proteins, into, or onto, their membranes. The attractiveness of liposomes as a peptide and protein delivery system within the invention is increased by the fact that the encapsulated proteins can remain in their preferred aqueous environment within the vesicles, while the liposomal membrane protects them against proteolysis and other destabilizing factors. Even though not all liposome preparation methods known are feasible in the encapsulation of peptides and proteins due to

their unique physical and chemical properties, several methods allow the encapsulation of these macromolecules without substantial deactivation.

[0077] Additional delivery vehicles carrier, support or complex-forming species for use within the invention may include long and medium chain fatty acids, as well as surfactant mixed micelles with fatty acids. Most naturally occurring lipids in the form of esters have important implications with regard to their own transport across mucosal surfaces. Free fatty acids and their monoglycerides which have polar groups attached have been demonstrated in the form of mixed micelles to act on the intestinal barrier as penetration enhancers. This discovery of barrier modifying function of free fatty acids (carboxylic acids with a chain length varying from 12 to 20 carbon atoms) and their polar derivatives has stimulated extensive research on the application of these agents as mucosal absorption enhancers.

For use within the methods of the invention, long chain fatty acids, especially fusogenic lipids (unsaturated fatty acids and monoglycerides such as oleic acid, linoleic acid, linoleic acid, monoolein, etc.) provide useful carriers to enhance delivery of Calnexin or a functionally equivalent fragment, and other biologically active agents disclosed herein. Medium chain fatty acids (C6 to C12) and monoglycerides have also been shown to have enhancing activity in intestinal drug absorption and can be adapted for use within the mucosal delivery formulations and methods of the invention. In addition, sodium salts of medium and long chain fatty acids are effective delivery vehicles and absorption-enhancing agents for mucosal delivery of biologically active agents within the invention. Thus, fatty acids can be employed in soluble forms of sodium salts or by the addition of non-toxic surfactants, e.g., polyoxyethylated hydrogenated castor oil, sodium taurocholate, etc. Other fatty acid and mixed micellar preparations that are useful within the invention include, but are not limited to, Na caprylate (C8), Na caprate (C10), Na laurate (C12) or Na oleate (C18), optionally combined with bile salts, such as glycocholate and taurocholate.

[0078] The vaccine of the present invention may advantageously include a pharmaceutically acceptable excipient such as a suitable adjuvant. Suitable adjuvants include an aluminium salt such as aluminium hydroxide gel (alum) or aluminium phosphate (as described in WO93/24148), but may also be a salt of calcium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes. The suitable adjuvants may also comprise mannose-containing, carbohydrate based adjuvants such as fungal mannans.

[0079] The vaccine formulation may additionally include a biologically acceptable buffer to maintain a pH close to neutral (7.0-7.3). Such buffers preferably used are typically phosphates,

carboxylates, and bicarbonates. More preferred buffering agents are sodium phosphate, potassium phosphate, sodium citrate, calcium lactate, sodium succinate, sodium glutamate, sodium bicarbonate, and potassium bicarbonate. The buffer may comprise about 0.0001-5% (w/v) of the vaccine formulation, more preferably about 0.001-1% (w/v). The buffer(s) may be added as part of the stabilizer component during the preparation thereof, if desired. Other excipients, if desired, may be included as part of the final vaccine formulation.

[0080] The remainder of the vaccine formulation may be an acceptable diluent, to 100%, including water. The vaccine formulation may also be formulated as part of a water-in-oil, or oil-in-water emulsion.

[0081] Also provided as part of the invention is a method of preparation of the vaccine formulation herein described. Preparation of the vaccine formulation preferably takes place in two phases. The first phase typically involves the preparation of the stabilizer component. The first phase may typically involve the preparation of the stabilizer component. The stabilizer component may comprise any suitable components as discussed above. For example, a vegetable-based protein stock solution may be prepared by dissolving the vegetable-based protein in a diluent. The preferred diluent may be water, preferably distilled and/or purified so as to remove trace impurities (such as that sold as purified Super Q®). In a separate vessel an animal-based protein may be dissolved in a diluent, additionally with the sugar component and buffer additives. Preferably, an equal volume of the vegetable-based protein stock solution is added to the animal-based protein solution. It is desirable that after HCl / KOH adjustment to achieve a pH of approximately 7.2±0.1, the stabilizer component may be sterilized via autoclave. The stabilizer solution may be refrigerated for an extended period prior to introduction of the Calnexin fragment.

[0082] The second phase of preparation of the vaccine formulation may include introduction of the Calnexin fragment with the stabilizer component, thereby yielding the vaccine formulation. Preferably, the Calnexin fragment may be diluted with a buffer solution prior to its introduction to the stabilizer component.

[0083] Once this vaccine formulation solution has been achieved, the formulation may be separated into vials or other suitable containers. The vaccine formulation herein described may then be packaged in individual or multi-dose ampoules, or be subsequently lyophilized (freeze-dried) before packaging in individual or multi-dose ampoules. The vaccine formulation herein contemplated also includes the lyophilized version. The lyophilized vaccine formulation may be stored for extended periods of time without loss of viability at ambient temperatures. The lyophilized vaccine may be reconstituted by the end user, and administered to a patient.

[0084] The vaccine of the present invention may be either in a solid form or in a liquid form. Preferably, the vaccine of the present invention may be in a liquid form. The liquid form of the vaccine may have a concentration of 50-4,000 nanomolar (nM), preferably between 50-150 nM. In some embodiments, the concentration will be between 1-50,000 nM.

[0085] To vaccinate a patient, a therapeutically effective amount of vaccine comprising Calnexin fragments may be administered to a patient. The therapeutically effective amount of vaccine may typically be one or more doses, preferably in the range of about 0.01-10 mL, most preferably 0.1-1 mL, containing 20-200 micrograms, most preferably 1-50 micrograms of vaccine formulation/dose. The therapeutically effective amount may also depend on the vaccination species. For example, for smaller animals such as mice, a preferred dosage may be about 0.01-1mL of a 1-50 microgram solution of antigen. For a human patient, a preferred dosage may be about 0.1-1 mL of a 1-50 microgram solution of antigen. The therapeutically effective amount may also depend on other conditions including characteristics of the patient (age, body weight, gender, health condition, etc.), the species of fungi, and others.

[0086] A vaccine of the present invention may be administered by using any suitable means as disclosed above. Preferably, a vaccine of the present invention may be administered by intranasal delivery or intramuscular administration, e.g., needle injection.

[0087] After vaccination using a vaccine of the present invention, a patient may be immunized from at least one of fungi. In one specific embodiment, a patient after vaccination may be immunized from at least one of dimorphic fungi. In one preferred embodiment, a patient after vaccination may be immunized from multiple dimorphic fungi of *Histoplasma, Coccidiodes, Paracoccidiodes, Penicillium, Blastomyces, and Sporothrix.*

[0088] In one embodiment, the present invention relates to a therapeutic device for vaccination a patient against fungal infection. In one embodiment, the therapeutic device may comprise any suitable devices charged with a preparation of Calnexin or a functionally equivalent fragment. In another embodiment, the therapeutic device may comprise any suitable devices charged with a preparation of Calnexin or a functionally equivalent fragment and at least one additional active compound.

[0089] The instant invention may also include kits, packages and multicontainer units containing the above described pharmaceutical compositions, active ingredients, and/or means for administering the same for use in the prevention and treatment of diseases and other conditions in mammalian subjects. Briefly, these kits include a container or formulation that contains Calnexin or a functionally equivalent fragment, and/or other biologically active agents

in combination with mucosal delivery enhancing agents disclosed herein formulated in a pharmaceutical preparation for delivery.

Methods for determining the immunization status of a patient

[0090] In one aspect, the present application discloses diagnostic methods for determining immunization status of a patient. Applicants envision that the present methods would be used to access the status of receipt in a tissue transplantation procedure.

[0091] In one embodiment, the present application discloses proteins or peptides and methods of using such proteins or peptides to evaluate the immunization status of a patient. In one embodiment, proteins or peptides may be used to detect endogenous calnexin specific CD4 T cells. As discussed above, Applicants identified calnexin as a major shared antigen that is recognized by T cells that mediate protection against pathogenic fungi that are members of the broad fungal taxonomic group called Ascomycetes.

[0092] In one embodiment, the family of Ascomycetes may comprise *Blastomyces dermatitidis, Histoplasma capsulatum, Aspergillus fumigatus, Fonsecea pedrosoi,* and *Geomyces destructans* (the latter is the "white nose fungus", which is decimating bat populations in North America), to name a few.

[0093] In one preferred embodiment, the proteins or peptides may comprise peptide-MHCII tetramers (pMHC tetramers). Calnexin peptide #1 specific T cells recognize many of these fungi and confer protection against them. As used herein, calnexin peptide #1 specific T cells refers to the T cells that are directed against the calnexin peptide number 1 (that is, residues 103-115 of the calnexin protein; SEQ ID NOs:1-34). The examples of calnexin peptide #1 are shown in the Table 1.

[0094] Helper T cells play an essential role in protecting the host from infection and cancer. Each helper T cell expresses a unique receptor (TCR), which via the aid of the CD4 coreceptor is capable of binding to a specific foreign peptide embedded in a Major Histocompatibility Complex II (MHCII) molecule on the surface of another host cell – the so-called antigen-presenting cell. Recognition of the relevant peptide:MHCII ligand causes a helper T cell to produce various lymphokines that help B cells produce antibodies and enhance the microbicidal activities of phagocytes and cytotoxic lymphocytes. Therefore, The pMHC tetramers may be used to track the emergence and persistence of these T cells after exposure to the fungus in question.

[0095] In one embodiment, the fungus in question may include any fungi as discussed above and any others appreciated by one person having ordinary skill in the art.

[0096] The pMHCII tetramers may be produced from suitable methods. For example, the pMHCII tetramers may be synthesized by using the method described previously (http://www.jenkinslab.umn.edu/Jenkins_Lab/Protocols_files/New%20tetramer%20production%20052212.pdf). In one preferred embodiment, the pMHCII tetramers may comprise at least one fluorescent label. For example, the design of the tetramer may incorporate Fos-Jun leucine zipper motifs to force dimerize the coexpressed MHCII α and β chains (Teyton,et. al., *J. Exp. Med.* 183:2087), and the *E.coli* BirA signal sequence (Schatz, et. al., *Protein Science* 8:921) on the α chain to allow for site-specific biotinylation. The resulting biotinylated peptide:MHCII (pMHCII) heterodimers may be tetramerized with fluorochrome-labeled streptavidin.

[0097] In one embodiment, the present proteins or peptides such as the pMHC tetramers may be used to identify “endogenous” calnexin peptide #1 specific T cells that reside in the body of a patient before infection.

[0098] In one embodiment, the present proteins or peptides such as the pMHC tetramers may be used to quantify “endogenous” calnexin peptide #1 specific T cells that reside in the body of a patient before infection.

[0099] In one embodiment, the present proteins or peptides such as the pMHC tetramers may be used to monitor the response of calnexin peptide #1 specific T cells.

[00100] In one embodiment, the present proteins or peptides such as the pMHC tetramers may be used to monitor expansion and characteristics of the calnexin peptide #1 specific T cells after infection and vaccination.

[00101] In one embodiment, the present application discloses compositions to identify and track calnexin peptide specific T cells in a patient. In one embodiment, the compositions may comprise proteins or peptides. Specifically, the suitable proteins or peptides may comprise pMHC tetramers.

[00102] A composition comprising pMHC tetramers may also comprise other suitable ingredients. In one embodiment, the composition may also comprise a carrier molecule as a stabilizer component. As the types of proteins or peptides enclosed in the present invention may be rapidly degraded once injected into the body, the proteins or peptides may be bound to a carrier molecule for stabilizing the proteins or peptides during delivery and administration. A suitable carrier or stabilizer may comprise fusion proteins, polymers, liposome, micro or nanoparticles, or any other pharmaceutically acceptable carriers. A suitable carrier or stabilizer molecule may comprise a tertiary amine N-oxide, e.g., trimethylamine-N-oxide, a sugar, e.g.,

trehalose, a poly(ethylene glycol) (PEG), an animal-based protein, e.g., digested protein extracts such as N-Z- Amine®, N-Z-Amine AS® and N-Z-Amine YT® (Sheffield Products Co., Norwich, N.Y.), a vegetable-based protein, e.g., soy protein, wheat protein, corn gluten, rice protein and hemp protein, and any other suitable carrier molecules. The composition may also comprise any suitable carrier or vehicle, such as those as discussed above. The composition may also comprise other stabilization agents, such as those as discussed above.

[00103] In one embodiment, the composition may also comprise suitable stabilizing delivery vehicle, carrier, support or complex-forming species, such as those as discussed above. For example, the composition may additionally comprise at least one of a stabilizer, a buffer, or an adjuvant.

[00104] In one embodiment, the present application discloses methods for evaluating the immunization status of a patient.

[00105] In one specific embodiment, the present methods for evaluating the immunization status of a patient may be accomplished by detecting and evaluating "endogenous" calnexin peptide #1 specific T cells in a patient.

[00106] In one embodiment, a method for evaluating the immunization status of a patient against a fungus comprises the steps of 1) obtaining pMHC tetramers; 2) exposing a sample of a patient to a suitable amount of pMHC tetramers; 3) identifying helper T cells such as "endogenous" calnexin peptide #1 specific T cells in the patient's sample; 4) quantifying helper T cells such as "endogenous" calnexin peptide #1 specific T cells in the patient's sample; and 5) monitoring the response, expansion and characteristics of helper T cells such as calnexin peptide #1 specific T cells the after infection and vaccination, wherein the immunization status of a patient against the fungus is obtained by comparing the quantity, expansion and characteristics of the helper T cells before and after infection and vaccination.

[00107] In one specific embodiment, the suitable sample is a fresh blood sample from a patient.

[00108] In one embodiment, the peptide-MHCII tetramers comprise at least one fluorescent label. The fluorescent peptide-MHCII tetramers may bind to helper T cells such as "endogenous" calnexin peptide #1 specific T cells. One may identify the help T cells through a fluorescence detection technique.

[00109] In one embodiment, the method may be applied to evaluate the immunization status against any fungi such as dimorphic fungi or non-dimorphic fungi. In one embodiment, the method may be applied to evaluate the immunization status against a dimorphic fungus

selected from a group consisting of *Histoplasma, Coccidiodes, Paracoccidiodes, Penicillium, Blastomyces,* and *Sporothrix.*

[00110] In another embodiment, the method may be applied to evaluate the immunization status against a fungus selected from a group consisting of *Blastomyces dermatitidis, Histoplasma capsulatum, Aspergillus fumigatus, Fonsecea pedrosoi,* and *Geomyces destructans*.

[00111] In one aspect, the present application discloses a kit for evaluating the immunization status of a patient against a fungus. The kit may comprise (1) a container or formulation wherein the container or formulation comprises peptide-MHCII tetramers, (2) means for exposing peptide-MHCII tetramers to a sample of a patient, and (3) means for detecting helper T cells in the patient's sample, wherein the peptide-MHCII tetramers are binding to the helper T cells.

[00112] In one embodiment, the sample is a fresh blood sample of a patient.

[00113] In one embodiment, the peptide-MHCII tetramers may be either a powder or a solution. In one specific embodiment, the means for delivering peptide-MHCII tetramers is selected from a group consisting of subcutaneous administration, intramuscular administration, transcutaneous administration, intradermal administration, intraperitoneal administration, intraocular administration, intranasal administration and intravenous administration.

[00114] In another embodiment, the kit may used to evaluating the immunization status of a patient against a fungus selected from a group consisting of *Blastomyces dermatitidis, Histoplasma capsulatum, Aspergillus fumigatus, Fonsecea pedrosoi,* and *Geomyces destructans*.

[00115] In another embodiment, the kit may used to evaluating the immunization status of a patient against a fungus selected from a group consisting of *Blastomyces dermatitidis, Histoplasma capsulatum, Aspergillus fumigatus, Fonsecea pedrosoi,* and *Geomyces destructans*.

[00116] In one embodiment, the peptide-MHCII tetramers may comprise at least one fluorescent label. In one specific embodiment, the means of detection may be a fluorescence technique.

EXAMPLES

METHODS

[00117] Fungi.

[00118] Strains used were ATCC 26199 (Harvey, Schmid, *et al.*, 1978), a wild-type strain of *Blastomyces dermatitidis*, and the isogenic, attenuated mutant lacking BAD1, designated strain #55 (Brandhorst, Wuthrich, *et al.*, 1999), as well as *Histoplasma capsulatum* strain G217B, *Coccidiodes posadasii* (isolate C735) and *Candida albicans* strain #5314 (Wuthrich, Hung, *et al.*, 2011). *B. dermatitidis* was grown as yeast on Middlebrook 7H10 agar with oleic acid-albumin complex (Sigma) at 39°C. *H. capsulatum* was grown as yeast at 37°C and 5% CO_2 on brain-heart infusion agar (BHI) slants. *C. albicans* was grown on YPD plates. The saprobic phase of *C. posadasii* (isolate C735) was grown on GYE medium (1% glucose, 0.5% yeast extract, 1.5% agar) at 30°C for 3 to 4 weeks to generate a confluent layer of arthroconidia (spores) on the agar surface. Formalin killed spherules (FKS) of *C. posadasii* were generated as described (Levine, Cobb, *et al.*, 1960; Levine, Kong, *et al.*, 1965.).

[00119] Mouse strains.

[00120] Inbred C57BL/6 mice were obtained from Jackson laboratory, Bar Harbor, ME. *Blastomyces*-specific TCR Tg 1807 mice bred to B6.PL (Thy1.1$^+$) mice to obtain Thy1.1$^+$ 1807 cells were described elsewhere (Wuthrich, Ersland, *et al.*, 2012). Mice were 7-8 weeks old at the time of these experiments. Mice were housed and cared for as per guidelines of the University of Wisconsin Animal Care Committee, who approved this work.

[00121] Generation of Eluate #1.

[00122] Cell wall membrane (CW/M) antigen (Ag) was extracted from BAD1 vaccine yeast (Brandhorst, Wuthrich, *et al.*, 1999) as previously described (Wuthrich, Filutowicz, *et al.*, 2000). Briefly, yeast were broken open with glass beads, debris pelleted, and the aqueous supernatant harvested. CW/M Ag was diluted to a protein concentration of 1.5mg/ml in binding buffer containing 20mM Tris, pH7.6, 0.3mM NaCl, 1mM $MnCl_2$, 1mM $MgCl_2$, 1mM $CaCl_2$ and centrifuged to remove insoluble complexes. To enrich the mannosylated proteins in the CW/M Ag preparation we used a Con A column (Figures 1A, 1B, 1C, 1D, and 1E). To prepare the column, we washed 0.75 ml Con A-Sepharose resin with 5 ml of binding buffer at least three times, each time the resin was pelleted by centrifugation at 1,000 x g for 3 min. After equilibration of the resin with an equal volume of binding buffer, the CW/M Ag extract was allowed to bind for 60 to 120 min under agitation at 4°C. The resin was then centrifuged at

1,000 x g for 3 min, and washed twice for 10 min with 15 ml of binding buffer containing 0.1% Tween 20. After a final wash with detergent free binding buffer, the bound fraction was eluted by incubating it for 10 min in 5 ml 20 mM Tris-HCL buffer pH 7.6 containing 500 mM α-D-methylmannopyranoside and 0.3 M NaCl. After pelleting the resin at 2,000 x g for 3 min, the supernatant was saved as eluate #1 and aliquoted for subsequent use. To inactivate Con A that might have leached from the resin, eluate #1 aliquots were heat treated for 15 min at 85˚C.

[00123] Enrichment of the shared Ag by Gel-free separation and identification by mass spec analysis.

[00124] Eluate #1 was applied to a Gel-free 8100 fractionation system (Protein Discovery, Knoxville, TN), and separated on a 10% Tris-Acetate cartridge. Fractions were collected that corresponded to separately eluted MW markers. These fractions were surveyed for protein content by PAGE analysis and silver stain. The fractions that activated 1807 T cells (quantified by production of INF-γ) were concentrated by FASP for MS analysis (below).

[00125] Filter aided sample preparation [FASP] method.

[00126] FASP sample preparation (Universal sample preparation method for proteome analysis (Wisniewski, Zougman, *et al.*, 2009) and mass spectrometric analysis was done at the Mass Spectrometry Facility at the Biotechnology Center, University of Wisconsin-Madison. In short, samples were bound to 10kDa MW cut-off Microcon filters (Millipore Corp., Bedford MA) and washed twice with 500µL of 25mM NH_4HCO_3 (pH8.5). Sample was denatured for 2 min in 100µL of 8M Urea / 50mM NH_4HCO_3 (pH8.5) then spun 6 min at 14,000xg. Disulfides were reduced at 37°C in 100µl of 6.4M Urea / 40mM NH_4HCO_3 (pH8.5) / 5mM DTT for 45 min then spun 2 min at 14,000xg. Cys alkylation was performed at room temperature in the dark for 15 min in 100µl of 6.4M Urea / 40mM NH_4HCO_3 (pH8.5) / 11mM IAA then spun 2 min at 14,000xg and washed once with 100µL of 8M Urea / 50mM NH_4HCO_3 (pH8.5) and once with 25mM NH_4HCO_3 (pH8.5). Digestion with 200ng trypsin (Promega Corporation, Madison WI) was performed in 50µl of 1M Urea / 20mM NH_4HCO_3 (pH8.5) / 5% ACN overnight at 37°C. Peptides were spun through the membrane and washed through with 50µl of 25mM NH_4HCO_3 (pH8.5), 5 min at 14,000xg. Eluted peptide solution was acidified with 2.5% TFA [Trifluoroacetic Acid] to 0.3% final and C18 solid phase extracted with OMIX SPE tips (Agilent Technologies, Santa Clara, CA). Peptides were eluted off the C18 column with 20ul of acetonitrile/H_2O/TFA (60%:40%:0.1%) into 1.5mL Protein LoBind tube (Eppendorf) dried in the SpeedVac to ~2µl, diluted to 18µl with 0.05% TFA and 8µl loaded for nanoLC-MS/MS analysis.

[00127] NanoLC-MS/MS.

[00128] Peptides were analyzed by nanoLC-MS/MS using the Agilent 1100 nanoflow system (Agilent Technologies) connected to a hybrid linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap XL, Thermo Fisher Scientific) equipped with a nanoelectrospray ion source. HPLC was performed using an in-house fabricated 15-cm C18 column packed with MAGIC C18AQ 3µm particles (MICHROM Bioresources Inc., Auburn, CA). Solvents were 0.1% formic acid in water (solvent A) and 0.1% formic acid, 95% acetonitrile in water (solvent B). The gradient consisted of 20 min loading and desalting at 1% solvent B, an increase to 40% B over 195 min, to 60% B over 20 min, and to 100% B over 5 min.

[00129] MS survey scans from m/z 300 to 2000 were collected in centroid mode at a resolving power of 100,000. Dynamic exclusion was employed to increase dynamic range and maximize peptide identifications, excluding precursors up to 0.55 m/z below and 1.05 m/z above previously selected precursors (40 sec expiration). Data was referenced against *B. dermatitidis* amino acid sequence database (19,126 protein entries) using in-house *Mascot* search engine 2.2.07 (Matrix Science, London, UK). Peptide mass tolerance was set at 20 ppm and fragment mass at 0.6 Da. Quantification was done with Scaffold software (version 3.6.3, Proteome Software Inc., Portland, OR). Protein identifications were reported above 95.0% probability within 0.9% False Discovery Rate and comprising at least 2 identified peptides. Probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii, Keller, *et al.*, 2003).

[00130] Generation and purification of recombinant Calnexin.

[00131] *Paracoccidiodes brasiliensis* Calnexin was amplified from the pGEM-Calnexin plasmid (dos Santos Feitosa, de Almeida Soares, et al., 2007), generously provided by Jose Daniel Lopes, using oligonucleotides designed to omit the stop codon and add *Nhe*I and *Sal*I restriction sites to the 5' and 3' ends, respectively. The resulting 1.7kb fragment was ligated into the pET28c vector digested with *Nhe*I and *Xho*I, in frame with a C-terminal 6xHis tag. The pET28c-Calnexin construct was transformed into BL21(DE3) *E. coli* for expression of recombinant Calnexin. Calnexin-expressing *E. coli* was grown at 37°C in LB medium supplemented with 50 ug/ml kanamycin to an OD600 of ~0.9, at which point isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.2 mM. Cells were induced for 24 hours at 15°C. Cells were harvested and resuspended in lysis buffer (50 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.1% Triton X-100, 5 mM DTT, and 0.1 mg/ml lysozyme supplemented with complete EDTA-free Protease Inhibitor Cocktail Tablet (Roche)), followed by sonication

and centrifugation. Calnexin was purified from the supernatant using a Ni-NTA column (Qiagen) and the wash and elution buffers were used according to manufacturer instructions for purification under native conditions. Calnexin eluate was then dialyzed into 1xPBS using 3,500 MWCO dialysis tubing (Pierce).

[00132] Generation of anti-Calnexin polyclonal antibody and staining of yeast.

[00133] Mice were vaccinated with 200 µg recombinant Calnexin (rCalnexin) thrice. For the first immunization, the protein was emulsified in CFA, the following two boosters were formulated in IFA (Wuthrich, Filutowicz, *et al.*, 2000). Two weeks after the last boost, mice were bled and the serum harvested. Oligospecific anti-Calnexin antibodies were purified from the serum using affinity-purification. Briefly, >200 µg purified recombinant Calnexin was run on an SDS-10% polyacrylamide gel at 20 mAmp for one hour, transferred to PVDF membrane (Millipore), and stained in Ponceau S. The band corresponding to Calnexin was excised from the membrane and probed overnight at 4°C with anti-Calnexin mouse serum diluted 1:2 in PBS. After washing once in PBS+0.1% Tween 20 and three times in PBS, the anti-Calnexin antibodies were eluted from the membrane in 100 mM glycine (pH 2.6). Following neutralization with 100 mM Tris-HCl (pH 8), the purified antibody was functionally verified by spectrophotometric analysis and Western blot.

[00134] For staining yeast, *B. dermatitidis* strain #55 was grown in liquid HMM for three days at 37°C, passed back to an OD600 of 0.8 and grown for an additional two days. Aliquots of 10^6 yeast were washed in PBS, resuspended in 90 µl PBS + 10 µl anti-Calnexin antibody, and incubated at 4°C for one hour. Cells were washed in PBS, and then incubated at room temperature for 40 minutes with rhodamine red-conjugated goat anti-mouse (Molecular Probes) diluted 1:100 in PBS containing 0.5% BSA and 2 mM EDTA. After washing in PBS, the yeast were fixed in 2% PFA, pelleted, and resuspended in PBS. Fluorescent microscopy was carried out on an Olympus BX60 using mirror cube U-MWIG, with images taken under a 40x objective using QCapture Pro software.

[00135] Comparison of Calnexin sequence among different fungi and prediction of its Class II epitopes.

[00136] To determine the degree of conservation of the Calnexin protein among the systemic dimorphic fungi, the deduced Calnexin protein sequences of *B. dermatitidis* strain 26199, *H. capsulatum* strain G217B, *C. posadasii* strain C735 and *P. brasiliensis* strain PB01 were aligned using ClustalW (Thompson, Higgins, *et al.*, 1994) in the MacVector software package (v. 12.5.1;

MacVector Inc., Carey, NC). To aid in determining possible epitopes within the Calnexin protein sequence, two different algorithms were used to predict binding peptides for the mouse C57/B6 MHC-class-II- allele, H2-IAb. In the first algorithm the Calnexin protein sequence of *B. dermatitidis* was analyzed using the Immune Epitope Database (IEDB) Analysis Resource (http://tools.immuneepitope.org/main/html/tcell_tools.html). The output of this software designates each peptide and its IC_{50} value. Several peptides, with nine amino-acid-core sequences that had IC_{50} values less than 500 nM (considered strong to moderate binding affinity) were predicted, and clustered into six regions of extended peptides within the *B. dermatitidis* Calnexin protein sequence (Figure 6). A second algorithm developed in the Laboratory of Marc Jenkins, University of Minnesota, which is based only on peptides that have been eluted from affinity purified H2-IAb molecules and sequenced by mass spec (Mark Jenkins, personal communication), generated ten strong-binding nanomers, with greater than 5 standard deviations above random peptides. The peptides were named Peptide 1 through Peptides 10, based on the strength of predicted binding to H2-IAb (Figure 6).

[00137] The ten predicted nanomers were synthesized as 13aa peptide - harboring an additional two flanking amino acids at each end - by GeneScript USA Inc. (Piscataway, NJ; http://www.genscript.com) and used to test epitope-specific 1807 T-cell activation.

[00138] Stimulation of 1807 T cells *in vitro*.

[00139] To test the antigenic properties of the Calnexin protein and peptides we loaded bone marrow derived dendritic cells (BMDC) with the respective antigens and cultured them with naïve 1807 T cells to assess T-cell activation and cytokine production. After three days of co-culture, the cell culture supernatants were harvested and analyzed for cytokines by ELISA and 1807 T cells stained for the activation markers CD44 and CD62L (Wuthrich, Ersland, *et al.*, 2012). In some experiments, the *Blastomyces* CW/M-reactive T-cell clone #5, whose TCR was cloned to generate 1807 transgenic mice (Wuthrich, Filutowicz, *et al.*, 2007), was used as a reporter T-cell to identify the presence of the antigen. Cell-culture supernatants were generated in 96-well plates in 0.2 ml containing 1 x 10^5 BMDC, 0.05 to 10 µg/ml of CW/M antigen (Wuthrich, Filutowicz, *et al.*, 2000), 0.05 to 50 µg/ml Calnexin and Drk1 (as a negative control)(Nemecek, Wuthrich, *et al.*, 2006) and 0.001 to 100 µM Calnexin peptides #1-10 (Figure 6). Supernatants were collected after 72 hours of co-culture. IFN-γ and IL-17A were measured by ELISA (R&D System, Minneapolis, MN) according to manufacturer specifications (detection limits were 0.05ng/ml).

[00140] Generation of a water-soluble extract from vaccine yeast.

[00141] Yeast surface proteins were extracted three times with three yeast-pellet volumes of water by agitating the yeast for one hour at 4˚C. The yeast were separated from the supernatant by centrifugation and filtration through a 0.2 µm filter. The water soluble-extract was concentrated by a Centricon column with a 30 kD cutoff.

[00142] Vaccination and infection.

[00143] Mice were vaccinated as described (Wuthrich, Filutowicz, *et al.*, 2000), twice, two weeks apart, subcutaneously (s.c.) with 20 to 200 µg recombinant Calnexin emulsified in complete Freund's adjuvant or with 10^8 heat killed *C. albicans* yeast and mineral oil. Mice were infected intratracheally (i.t.) with 2 x 10^3 or 2 x 10^4 wild-type yeast of *B. dermatitidis* strain 26199, 2 x 10^5 *H. capsulatum* G217B, 2 x 10^5 FKS or 60 spores of the virulent *C. posadasii* isolate C735 (Wuthrich, Filutowicz, *et al.*, 2000; Wisniewski, Zougman, *et al.*, 2009; Nesvizhskii, Keller, *et al.*, 2003; (dos Santos Feitosa, de Almeida Soares, *et al.*, 2007; Thompson, Higgins, *et al.*, 1994; Wuthrich, Filutowicz, *et al.*, 2007; Nemecek, Wuthrich, *et al.*, 2006; Wuthrich Gern, *et al.*, 2011). To assess the infiltration of primed CD4 T cells into the lungs, challenged mice were analyzed at day 4 post-infection. To analyze the extent of lung infection, homogenized lungs were plated and yeast colony forming units (CFU) enumerated on BHI agar (Difco, Detroit, MI), sheep-blood containing Mycosel plates, or GYE plates containing 50 µg/ml of chloramphenicol (Wuthrich, Gern, *et al.*, 2011).

[00144] Adoptive transfer of 1807 cells and experimental challenge.

[00145] To assess the T helper cytokine phenotype of Calnexin-specific $CD4^+$ T cells after vaccination with Calnexin and various adjuvants, we transferred 10^6 naïve 1807 Tg cells into C57BL/6 wild-type mice before vaccination. On the same day, recipients were vaccinated, boosted two weeks later and challenged two weeks after the boost.

[00146] Intracellular cytokine stain.

[00147] Lung cells were harvested at day 4 post-infection. Cells (0.5 x 10^6 cells/ml) were stimulated for 4 hours with anti-CD3 (clone 145-2C11; 0.1µg/mL) and anti-CD28 (clone 37.51; 1µg/mL) in the presence of Golgi-Stop (BD Biosciences). Stimulation with fungal ligands yielded comparable cytokine production by transgenic T-cells compared to CD3/CD28 stimulation (data not shown). After cells were washed and stained for surface CD4 and CD8 using anti-CD4 PerCp, anti-CD8 PeCy7, and anti-CD44-FITC mAbs (Pharmingen), they were

fixed and permeabilized in Cytofix/Cytoperm at 4° C overnight. Permeabilized cells were stained with anti-IL-17A PE and anti-IFN-γ –Alexa 700 (clone XMG1.2) conjugated mAbs (Pharmingen) in FACS buffer for 30 min at 4° C, washed, and analyzed by FACS. Cells were gated on CD4 and cytokine expression in each gate analyzed. The number of cytokine positive $CD4^+$ T cells per lung was calculated by multiplying the percent of cytokine-producing cells by the number of $CD4^+$ cells in the lung.

[00148] Cytokine protein measurements of *in vivo* primed T cells.

[00149] Cell-culture supernatants were generated in 24-well plates in 1 mL containing 5 x 10^6 splenocytes and lymph node cells and various concentrations of *Blastomyces* CW/M antigen (Wuthrich, Filutowicz, *et al.*, 2000), rCalnexin, Drk1, and Calnexin peptides. Supernatant was collected after 72 hours of co-culture. IFN-γ and IL-17A were measured by ELISA as above.

[00150] Statistical Analysis.

[00151] The number and percentage of activated, proliferating or cytokine producing T-cells and differences in number of CFU were analyzed using the Wilcoxon rank test for nonparametric data (Fisher and vanBelle, 1993) or the T-test when data were normally distributed. A *P* value of < 0.05 is considered statistically significant.

[00152] RESULTS

[00153] Steps used to identify Calnexin as the shared antigen (Ag).

[00154] 1807 TCR Tg cells recognize a protective antigen that is shared among systemic dimorphic fungi (Wuthrich, Hung, *et al.*, 2011; Wuthrich, Ersland, *et al.*, 2012). To identify the shared antigen, we prepared a cell wall membrane (CW/M) extract from *B. dermatitidis* vaccine yeast as previously described (Wuthrich, Filutowicz, *et al.*, 2000). After running CW/M through a Con A column that retains mannosylated proteins, we collected Eluate 1, which contained 1 % of the protein present in the starting material (Figure 1A). Traces of active Con A released from the column into Eluate #1 were heated to destroy its mitogenic activity (not shown). Eluate #1 (Figure 1B) was further fractioned in a gel free system to separate individual constituents by size (Figure 1C). Fractions 6 and 7 stimulated 1807 T cells to produce IFN-γ, whereas medium alone as a control, and fractions 5 and 8 did not (Figure 1D). To identify the T cell reactive Ag, we subjected fraction 7 to mass spec analysis. Proteins were identified by cross-referencing the mass of detected peptides against a database of the *B. dermatitidis* proteome. Proteins present in non-stimulatory fractions and proteins diverging from the mass parameters of the gel-free

fraction were discounted. This technique yielded a roster of five protein candidates potentially representing the shared antigen. Calnexin was one of these five proteins.

[00155] Proof positive that Calnexin is the Shared Antigen

[00156] To investigate whether Calnexin is the shared Ag that stimulates 1807 T cells, we cloned the gene into the plasmid pET28c and used IPTG to induce gene expression in transfected *E. coli*. 24 h later, the crude lysate from *E. coli* harbored an additional prominent band that migrated between 60 -70 kD, which corresponds with the predicted molecular weight of 63 kD for recombinant Calnexin (rCalnexin) (Figure 2A). We purified the recombinant protein over a Ni-NTA column (Figure 2A) and used the eluate to stimulate 1807 cells in an *in vitro* co-culture system with BMDC. In response to rCalnexin, 1807 T cells produced IFN-γ in a dose-dependent manner. The response to rCalnexin exceeded the response to CW/M extract, which also harbors Calnexin, but at a lower concentration (Figure 2B). In contrast, recombinant Drk1 - a hybrid histidine kinase of *B. dermatitidis* (Nemecek, Wuthrich, *et al.*, 2006) expressed and purified from *E. coli* as a control - did not induce IFN-γ production by 1807 T cells. Thus, rCalnexin (not LPS from *E. coli*) induced cytokine production by 1807 T cells specifically and in a dose-dependent manner.

[00157] To investigate whether rCalnexin induces activation and proliferation of 1807 cells *in vivo*, we adoptively transferred 1807 Tg T cells into naïve wild-type recipient mice prior to vaccination. Similar to live *B. dermatitidis* vaccine yeast, rCalnexin emulsified in complete Freund's adjuvant activated and stimulated proliferation of > 85% of the transferred 1807 cells (Figure 2C), whereas adjuvant alone did not. These results identify Calnexin as the shared Ag that is recognized by 1807 TCR Tg T cells, which confer resistance to multiple systemic dimorphic fungi (Wuthrich, Hung, *et al.*, 2011; Wuthrich, Ersland, *et al.*, 2012).

[00158] Identification of Calnexin's peptide epitope

[00159] To identify the 1807 T cell reactive peptide epitope, we first aligned the amino acid sequence of the fungal species that we have reported stimulate 1807 T cells *in vivo* (Wuthrich, Hung, *et al.*, 2011), including *B. dermatitidis*, *H. capsulatum*, *C. posadasii* and *P. brasiliensis*. We investigated regions of sequence conservation that might represent the shared epitope for the 1807 T-cell receptor. We found that Calnexin is highly conserved across the entire Calnexin sequence among this group of dimorphic fungi (Figure 6). Thus, the identification of highly conserved areas of the protein was not a sufficient measure to hone in on the 1807 epitope-containing sequence. To narrow the focus of possible peptides to test for 1807 reactivity, we

subjected *Blastomyces* Calnexin to two class II I-Ab restricted-epitope prediction algorithms (Figure 6). The IEBD algorithm predicted six regions of overlapping peptides with binding affinities values (IC_{50}) less that 500nM. In a second analysis, an algorithm developed in Marc Jenkins' laboratory (unpublished data) refined the above analysis, and predicted ten strong H2-IAb epitopes in *B.dermatitidis* Calnexin (Figure 6). We chemically synthesized peptides of thirteen amino acids in length, representing these ten predicted epitopes (named Peptide 1 though Peptide 10), and tested them to determine the cognate epitope for the 1807 T-cell receptor.

[00160] To test whether the synthetic peptides activate naïve 1807 T cells *in vitro,* we loaded BMDC with individual peptides and co-cultured them with 1807 cells. Peptide #1 strongly activated naïve 1807 T cells as measured by their reduced expression of CD62L (Figure 3A) and increased expression of CD44 (data not shown). In contrast, an irrelevant control OT2 peptide, and all other synthetic Calnexin peptides did not activate 1807 cells. Peptide 1 also stimulated the production of IFN-γ by 1807 cells in a dose dependent manner (Figure 3B). As little as 1 to 10 nM of peptide 1 stimulated as much IFN-γ as 10 µg/ml of CW/M Ag, which has been shown to induce substantial amounts of the cytokine (data not shown). Neither Calnexin Peptide 5, nor the other synthesized Calnexin peptides, induced the production of IFN-γ by 1807 cells.

[00161] Evidence that Calnexin is displayed on the yeast surface

[00162] Among fungal pathogens, most of the virulence factors and antigenic proteins are secreted or associated with the cell wall or surface. Despite the fact that Calnexin is a molecular chaperone and folding sensor that regulates the transport of proteins from the ER to the Golgi apparatus, (Ellgaard and Helenius, 2003) vaccination with *B. dermatitidis* yeast efficiently stimulates 1807 T cell responses *in vivo*. Thus, we wondered how presumably intracellular Calnexin is accessed by antigen-presenting cells and displayed to T cells. To address this conundrum, we sought to investigate whether Calnexin is instead present on the yeast surface. During our search for the shared Ag, we found that a water-soluble extract of surface proteins from the vaccine yeast activated 1807 T cells (data not shown). Western-blot analysis of the water-soluble extract detected a doublet that migrated on SDS-PAGE at the same position as rCalnexin produced by *E. coli* (Figure 4A). To investigate whether vaccine yeast harbor Calnexin on their surface, we stained yeast *in vitro* at 37˚C and yeast harvested from the site of vaccination (subcutaneous tissue) with polyclonal anti-Calnexin antibodies. Both *in vitro* and *in vivo* grown vaccine yeast stained positively with the anti-Calnexin serum

(Figures 4B and 4C). The virulent parental strain 26199 that is used for the pulmonary challenge of mice also harbored Calnexin on the yeast surface when harvested and stained at day 4 post-infection (Figure 4C). These results indicate that Calnexin is present on the surface of vaccine and challenge yeast.

[00163] Functional relevance of Calnexin and peptide T cell responses.

[00164] To determine whether vaccination with Calnexin induces protective immunity against lethal *B. dermatitidis* infection, we immunized mice with soluble recombinant protein plus either complete Freund's adjuvant (CFA) or heat killed *C. albicans* yeast (contains fungal PAMPs) to polarize naïve T cells into Th1 cells or Th17, respectively (LeibundGut-Landmann, Gross, *et al.*, 2007). To evaluate whether these vaccine formulations efficiently stimulate the generation and recruitment of Th17 and Th1 cells to the lung upon recall, we adoptively transferred naïve 1807 T cells into mice prior to vaccination and determined the number of cytokine producing 1807 T cells at day 4 post-infection. Mice vaccinated with Calnexin recruited Th17 and Th1 cells into the lung in a dose and Ag-specific manner. The antigen formulation prepared with heat killed *C. albicans* yeast expanded more 1807 T cells than that prepared with CFA (Figure 5A). Most strikingly, mice that were vaccinated with rCalnexin and *C. albicans* yeast as the adjuvant completely cleared lung infection by day 4 post-infection, whereas mice vaccinated with either *Candida* adjuvant alone or Calnexin and CFA together did not (Figure 5B). These data indicate that recombinant Calnexin protein has the capacity to protect vaccinated mice against lethal pulmonary infection when Ag-specific T cells have been primed in sufficient numbers.

[00165] Peptide Prediction Of Calnexin Fragments To Human.

[00166] Applicants performed an analysis of the predicted peptides that could work with the known epitope binding domain of several Human HLA DRB1 alleles, using the publicly available ProPred software (http://www.imtech.res.in/raghava/propred/). The results were shown in Figures 7A, 7B, 7C, 7D, 7E, and 7F . In the output, the Blasto Calnexin sequence was shown on a separate line for each of 51 DRB1 alleles, and peptides that are predicted to fit in the MHCII groove of that allele were indicated in blue, with red used to indicate a so-called anchor amino acid that would be at position one of the 9 amino acid core sequence. A peptide of interest is "promiscuous" if it is predicted to interact with many different human MHCII molecules. Since the human HLA locus is so polymorphic, a good vaccine for humans will have to have epitopes that are promiscuous, and can work with many different HLA MHC molecules in order to stimulate an immune response. The results in Figures 7A, 7B, 7C, 7D, 7E, and 7F

show that Blasto Calnexin does, indeed, have several peptide sequences (blue) that are predicted to fit into the MHC groove for presentation to T-Cells. Of particular interest is that there is a predicted epitope for the sequence of Peptide1 (which was predicted for B6 mouse HLA interaction, and has been experimentally shown to do so with 1807 cells) at position 103 to 115. There were several other promiscuous epitopes throughout the Calnexin sequence as predicted by the ProPred software.

[00167] Peptide MHCII Tetramers To Detect Endogenous Calnexin Specific Cd4 T Cells

[00168] Applicants have taken advantage of the discovery of calnexin as a major shared antigen that is recognized by T cells that mediate protection against pathogenic fungi that are members of the broad fungal taxonomic group called Ascomycetes. Having already discovered that calnexin peptide #1 specific T cells recognize many of these fungi and confer protection against them, Applicants created an immunogical tool - peptide-MHCII tetramers (pMHC tetramers) - to track the emergence and persistence of these T cells after exposure to the fungus in question. The synthesis of pMHCII tetramers has been previously described. The present application discloses methods of creating reagents to identify and track calnexin peptide specific T cells.

[00169] Applicants have now used the tetramers to find and quantify "endogenous" calnexin peptide #1 specific T cells that reside in the body before infection, and then to monitor their response, expansion and characteristics after infection and vaccination. Applicants initiated this work by studying mice before and after infection with *Blastomyces dermatitidis* or after vaccination with calnexin recombinant protein or attenuated *B. dermatitidis.* Applicants envision that the process of the experiments may be extended to other fungi that are members of the family of ascomycetes. Other fungi may include *Histoplasma capsulatum, Aspergillus fumigatus, Fonsecea pedrosoi,* and *Geomyces destructans* (the latter is the "white nose fungus", which is decimating bat populations in North America), to name a few. Applicants results suggest that infection with these fungi activates and expands endogenous calnexin peptide #1 specific T cells.

[00170] The tetramers that we are developing pave the way toward a clinical application. Individuals with cancer or other disorders who are to receive bone marrow or stem cell transplants may be at risk for opportunistic fungal infection with *Asperillus species*. These infections may carry high morbidity and mortality rates that reach 80-90%. It would be clinically advantageous to use the tetramer to screen and discern whether a bone marrow or stem cell *donor* has evidence of strong immunity against *Aspergillus* as a way of planning the clinical

management of the *recipient*. For example, the tetramers in the present application may be used to, 1) gauge the risk of *Aspergillus* infection in the transplanted recipient (who will receive the immune or non-immune cells); 2) to plan anti-fungal prophylaxis strategies for the at-risk recipient, or 3) plan vaccination of the *donor* (pre- transplant) to induce calnexin or peptide #1 antigen-specific T cells.

REFERENCES

1. Harvey, R.P., Schmid, E.S., Carrington, C.C., and Stevens, D.A. 1978. Mouse model of pulmonary blastomycosis: utility, simplicity, and quantitative parameters. *American Review of Respiratory Disease* 117:695-703.

2. Brandhorst, T.T., Wüthrich, M., Warner, T., and Klein, B. 1999. Targeted gene disruption reveals an adhesin indispensable for pathogenicity of Blastomyces dermatitidis. *J Exp Med* 189:1207-1216.

3. Wüthrich, M., Hung, C.Y., Gern, B.H., Pick-Jacobs, J.C., Galles, K.J., Filutowicz, H.I., Cole, G.T., and Klein, B.S. 2011. A TCR Transgenic Mouse Reactive with Multiple Systemic Dimorphic Fungi. *J Immunol* 187:1421-1431.

4. Levine, H.B., Cobb, J.M., and Smith, C.E. 1960. Immunity to coccidioi-domycosis induced in mice by purified spherule, arthrospore, and mycelial vaccines. *Trans N Y Acad Sci* 22:436-449.

5. Levine, H.B., Kong, Y.C., and Smith, C. 1965. Immunization of Mice to Coccidioides Immitis: Dose, Regimen and Spherulation Stage of Killed Spherule Vaccines. *J Immunol* 94:132-142.

6. Wüthrich, M., Ersland, K., Sullivan, T., Galles, K., and Klein, B.S. 2012. Fungi subvert vaccine T cell priming at the respiratory mucosa by preventing chemokine-induced influx of inflammatory monocytes. *Immunity* 36:680-692.

7. Wüthrich, M., Filutowicz, H.I., and Klein, B.S. 2000. Mutation of the WI-1 gene yields an attenuated Blastomyces dermatitidis strain that induces host resistance. *J Clin Invest* 106:1381-1389.

8. Wisniewski, J.R., Zougman, A., Nagaraj, N., and Mann, M. 2009. Universal sample preparation method for proteome analysis. *Nat Methods* 6:359-362.

9. Nesvizhskii, A.I., Keller, A., Kolker, E., and Aebersold, R. 2003. A statistical model for identifying proteins by tandem mass spectrometry. *Anal Chem* 75:4646-4658.

10. dos Santos Feitosa, L., de Almeida Soares, C.M., Dos Santos, M.R., Bailao, A.M., Xander, P., Mortara, R.A., and Lopes, J.D. 2007. Cloning, characterization and expression of a calnexin homologue from the pathogenic fungus Paracoccidioides brasiliensis. *Yeast* 24:79-87.

11. Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673-4680.

12. Wüthrich, M., Filutowicz, H.I., Allen, H.L., Deepe, G.S., and Klein, B.S. 2007. V{beta}1+ J{beta}1.1+/V{alpha}2+ J{alpha}49+ CD4+ T Cells Mediate Resistance against Infection with Blastomyces dermatitidis. *Infect Immun* 75:193-200.

13. Nemecek, J.C., Wüthrich, M., and Klein, B.S. 2006. Global control of dimorphism and virulence in fungi. *Science* 312:583-588.

14. Wüthrich, M., Gern, B., Hung, C.Y., Ersland, K., Rocco, N., Pick-Jacobs, J., Galles, K., Filutowicz, H., Warner, T., Evans, M., et al. 2011. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. *J Clin Invest* 121:554-568.

15. Wüthrich, M., Gern, B., Hung, C.Y., Ersland, K., Rocco, N., Pick-Jacobs, J., Galles, K., Filutowicz, H., Warner, T., Evans, M., et al. 2011. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. *J Clin Invest*.

16. Fisher, L.D., and van Belle, G. 1993. Biostatistics: A Methodology for the Health Sciences. *John Wiley & Sons, New York.*:611-613.

17. Ellgaard, L., and Helenius, A. 2003. Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4:181-191.

18. LeibundGut-Landmann, S., Gross, O., Robinson, M.J., Osorio, F., Slack, E.C., Tsoni, S.V., Schweighoffer, E., Tybulewicz, V., Brown, G.D., Ruland, J., et al. 2007. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 8:630-638.

19. Myhill Nathan, Lynes Emily M., Nanji Jalal A., Blagoveshchenskaya Anastassia D., Fei Hao, Simmen Katia Carmine, Cooper Timothy J., Thomas Gary, Simmen Thomas, The Subcellular Distribution of Calnexin Is Mediated by PACS-2. *Molecular Biology of the Cell* 2008, 19, 2777–2788.

20. Williams David B. Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. *Journal of Cell Science*, 2006, 119, 615-623

CLAIMS

We claim:

1. A vaccine to immunize a patient against fungi, wherein the vaccine comprises a Calnexin fragment.

2. The vaccine of claim 1, additionally comprising at least one of a stabilizer, a buffer, or an adjuvant.

3. The vaccine of claim 1, wherein the Calnexin fragment is either a full-length native version or a functionally equivalent version of full-length Calnexin.

4. The vaccine of claim 1, wherein the Calnexin fragment comprises at least the 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1).

5. The vaccine of claim 1, wherein the fragment consists of SEQ ID NO:1.

6. The vaccine of claim 1, wherein the Calnexin fragment comprises a sequence selected from the group consisting of SEQ ID NOs:2 - 9, 11, 13 -14, and 20 - 24.

7. The vaccine of claim 1, wherein the Calnexin fragment consists of a sequence selected from the group consisting of SEQ ID NOs:2 - 9, 11, 13 -14 and 20 - 24.

8. The vaccine of claim 1, wherein the Calnexin fragment comprises a sequence selected from a group consisting of SEQ ID NOs:2 - 29.

9. The vaccine of claim 1, wherein the Calnexin fragment comprises a sequence selected from a group consisting of SEQ ID NOs:2, 6, 11, 12, 17, and 29.

10. The vaccine of claim 1, wherein the Calnexin fragment consists of a sequence selected from a group consisting of SEQ ID NOs:2, 6, 11, 12, 17, and 29.

11. The vaccine of claim 1, wherein the Calnexin fragment comprises a sequence selected from a group consisting of the sequences presented in Figures 7A, 7B, 7C, 7D, 7E, and 7F.

12. A method of protecting a patient from fungal infection comprising of the steps of:
    a. obtaining the vaccine of claim 1, wherein the vaccine comprises a Calnexin fragment and
    b. providing a therapeutically effective amount of the vaccine to a subject, wherein the subject is protected from fungal infection.

13. The method of claim 12, wherein the Calnexin fragment is either a full length native version or a functionally equivalent version of full-length Calnexin.

14. The method of claim 12, wherein the fungi are either dimorphic fungi or non-dimorphic fungi.

15. The method of claim 14, wherein the dimorphic fungi are selected from a group consisting of *Histoplasma, Coccidiodes, Paracoccidiodes, Penicillium, Blastomyces,* and *Sporothrix.*

16. The method of claim 14, wherein the non-dimorphic fungi are selected from a group consisting of *Aspergillus, Pneumocystis, Magnaportha, Exophiala, Neuroaspora, Cryptococcus, Schizophyllum,* and *Candida.*

17. The method of claim 12, wherein the Calnexin fragment either comprises or consists of the 13 amino acid sequence LVVKNPAAHHAIS (SEQ ID NO:1).

18. The method of claim 12, wherein the Calnexin fragment either comprises or consists of a sequence selected from a group consisting of SEQ ID NOs:2-30.

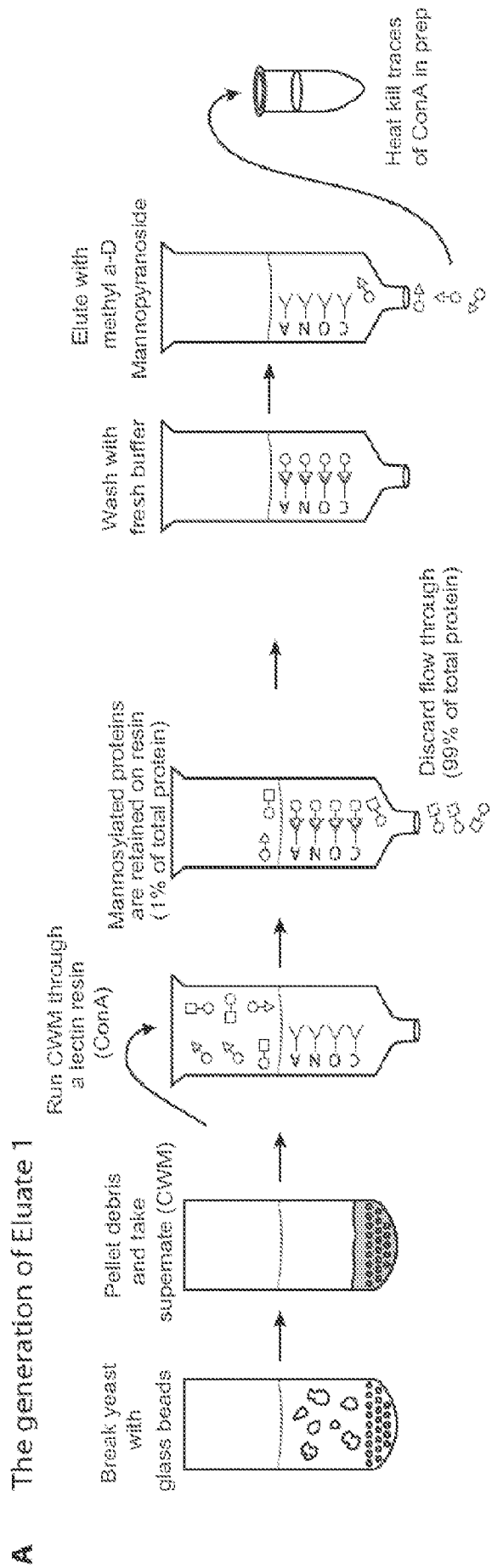
19. The method of claim 12, wherein the Calnexin fragment comprises or consists of a sequence selected from a group consisting of SEQ ID NOs:2, 6, 11, 12, 17, and 29.

20. The method of claim 12, wherein the Calnexin fragment comprises or consists of a sequence selected from a group consisting of SEQ ID NOs:2 - 9, 11, 13 -14, and 20 - 24.

21. The method of claim 12, wherein the Calnexin fragment comprises or consists of a sequence selected from a group consisting of the sequences presented in Figures 7A, 7B, 7C, 7D, 7E, and 7F.

22. The method of claim 12, wherein the Calnexin fragment of step (a) is expressed and isolated from *E. Coli.*

Figure 1


A The generation of Eluate 1
Break yeast with glass beads
Pellet debris and take supernate (CWM)
Run CWM through a lectin resin (ConA)
Mannosylated proteins are retained on resin (1% of total protein)
Discard flow through (99% of total protein)
Wash with fresh buffer
Elute with methyl a-D Mannopyranoside
Heat kill traces of ConA in prep

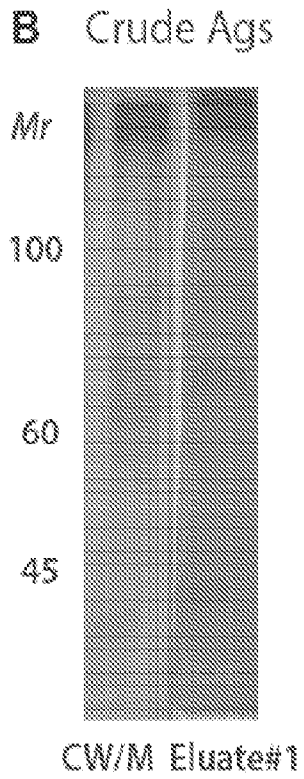
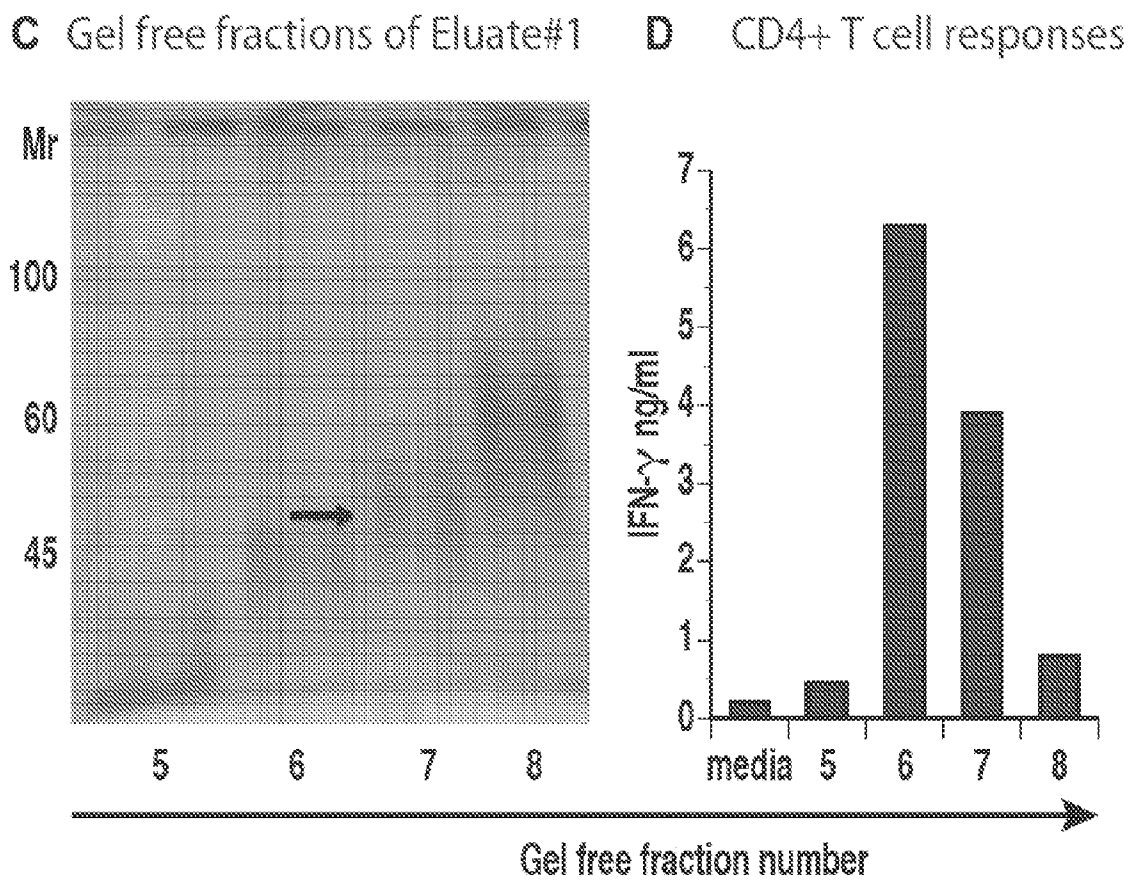

B Crude Ags
Mr
100
60
45
CW/M Eluate#1

Figure 1


C Gel free fractions of Eluate#1
D CD4+ T cell responses
Mr
100
60
45
5
6
7
8
IFN-γ ng/ml
0
1
2
3
4
5
6
7
media
5
6
7
8
Gel free fraction number

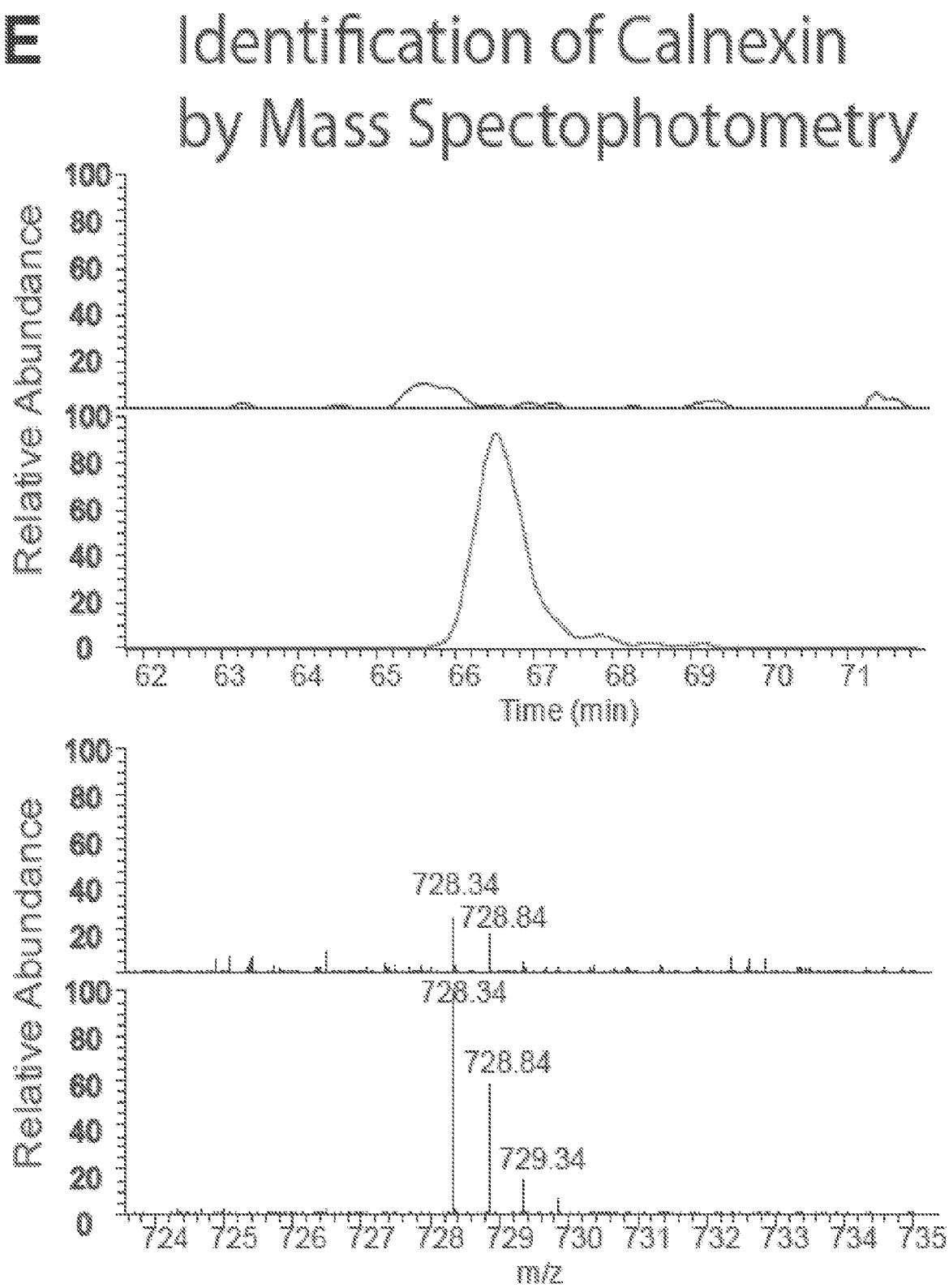

E
Identification of Calnexin by Mass Spectophotometry
Relative Abundance
100
80
60
40
20
0
62
63
64
65
66
67
68
69
70
71
Time (min)
728.34
728.84
729.34
724
725
726
727
728
729
730
731
732
733
734
735
m/z

Figure 1

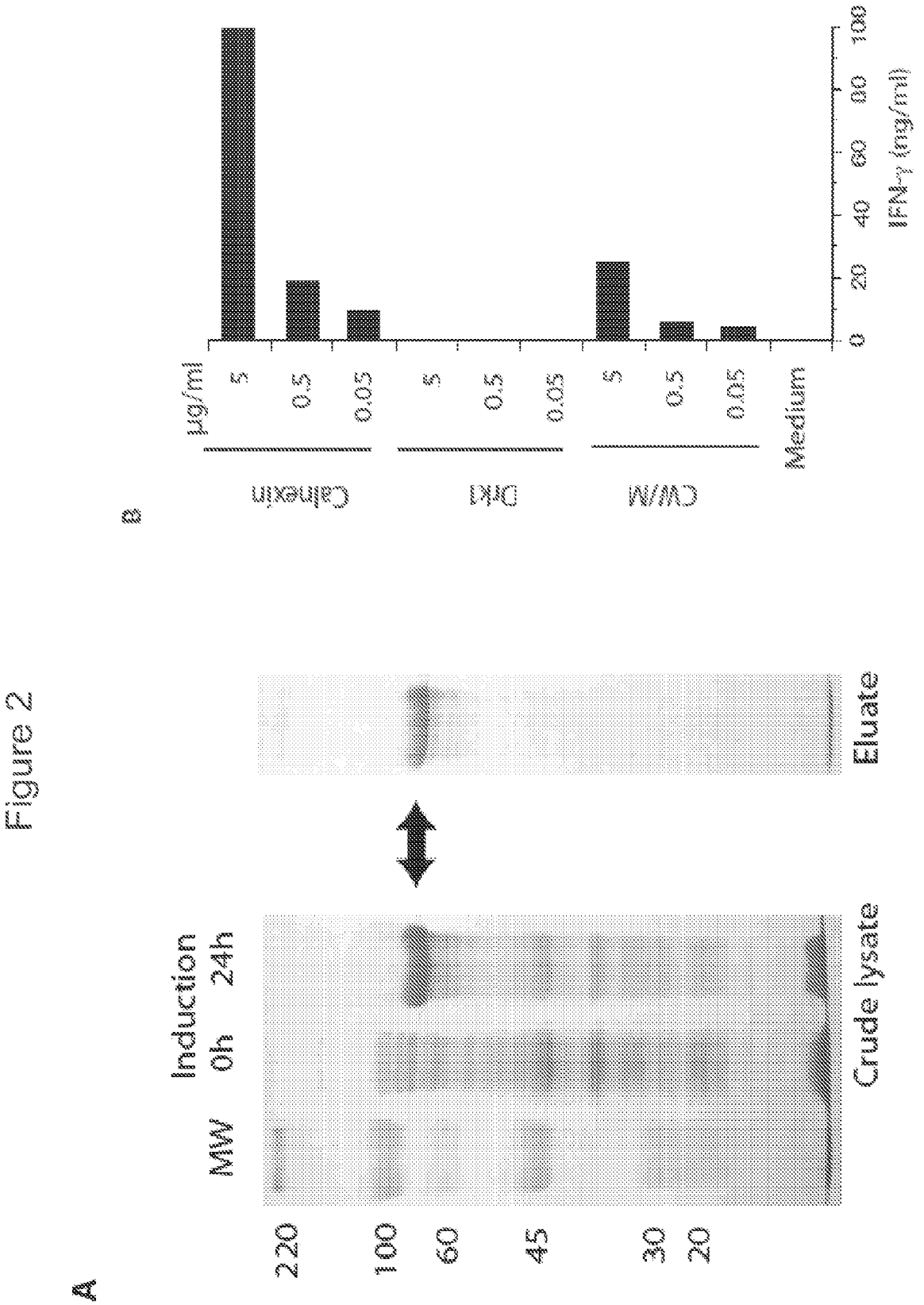

A
220
100
60
45
30
20
MW
Induction
0h
24h
Crude lysate
Eluate
B
CW/M
Dkl
Calnexin
µg/ml
5
0.5
0.05
Medium
IFN-γ (ng/ml)
0
20
40
60
80
100

Figure 2

Figure 2

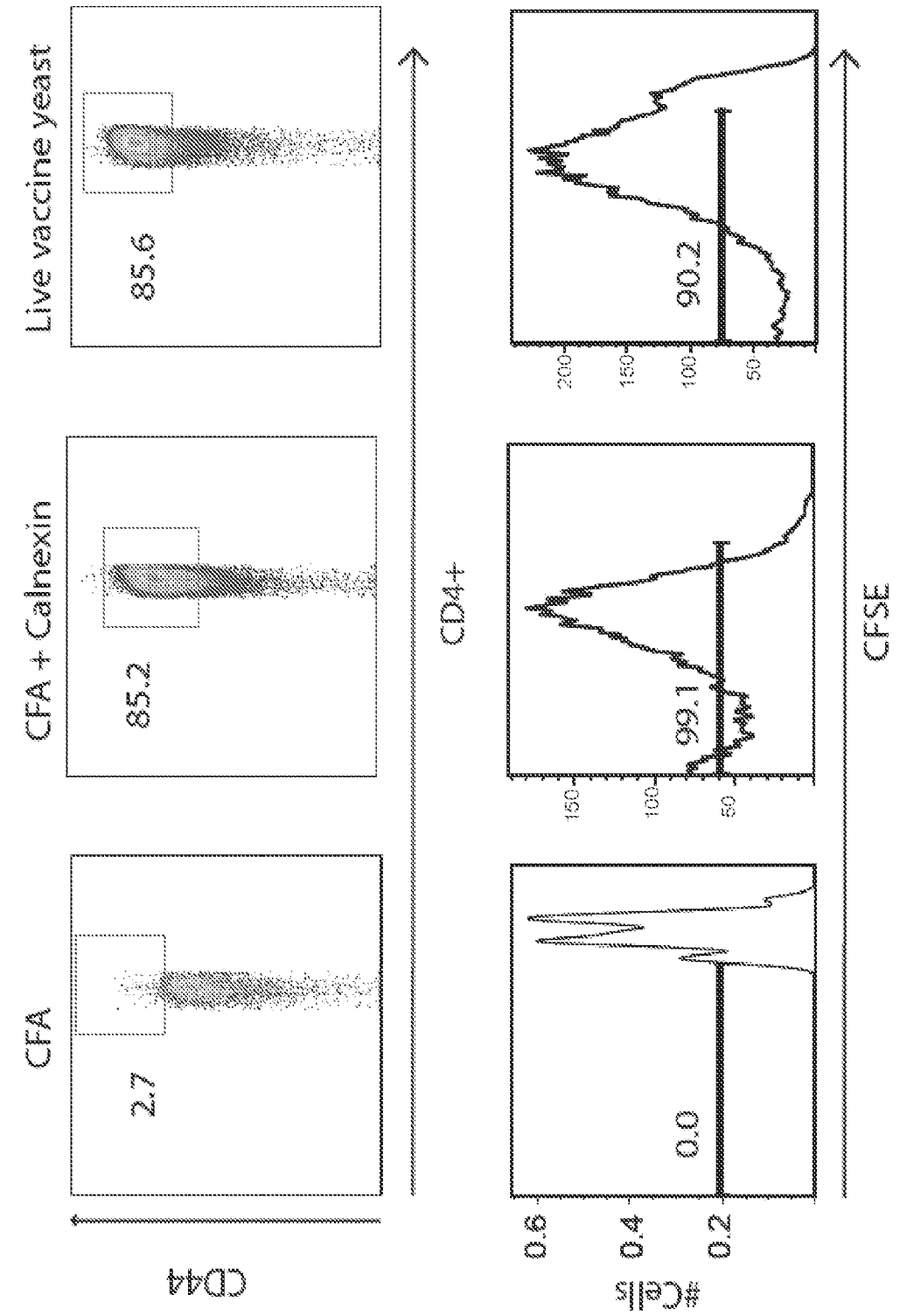
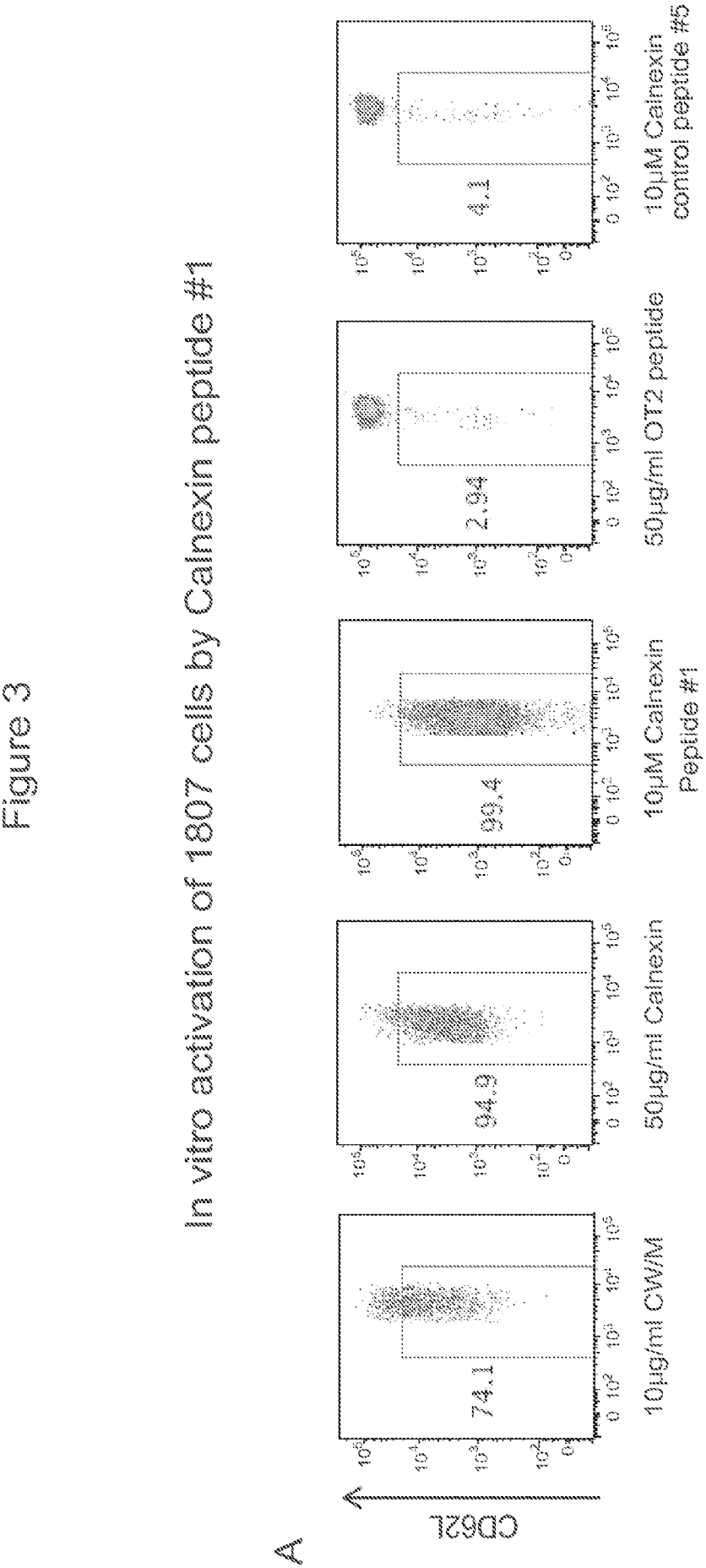

C
CD44
CD4+
CFSE
#Cells
CFA
2.7
CFA + Calnexin
85.2
Live vaccine yeast
85.6
0.0
99.1
90.2
0.6
0.4
0.2
150
100
50
200
150
100
50

Figure 3


In vitro activation of 1807 cells by Calnexin peptide #1
A
CD62L
CD4
10μg/ml CW/M
74.1
50μg/ml Calnexin
94.9
10μM Calnexin
Peptide #1
99.4
50μg/ml OT2 peptide
2.94
10μM Calnexin
control peptide #5
4.1

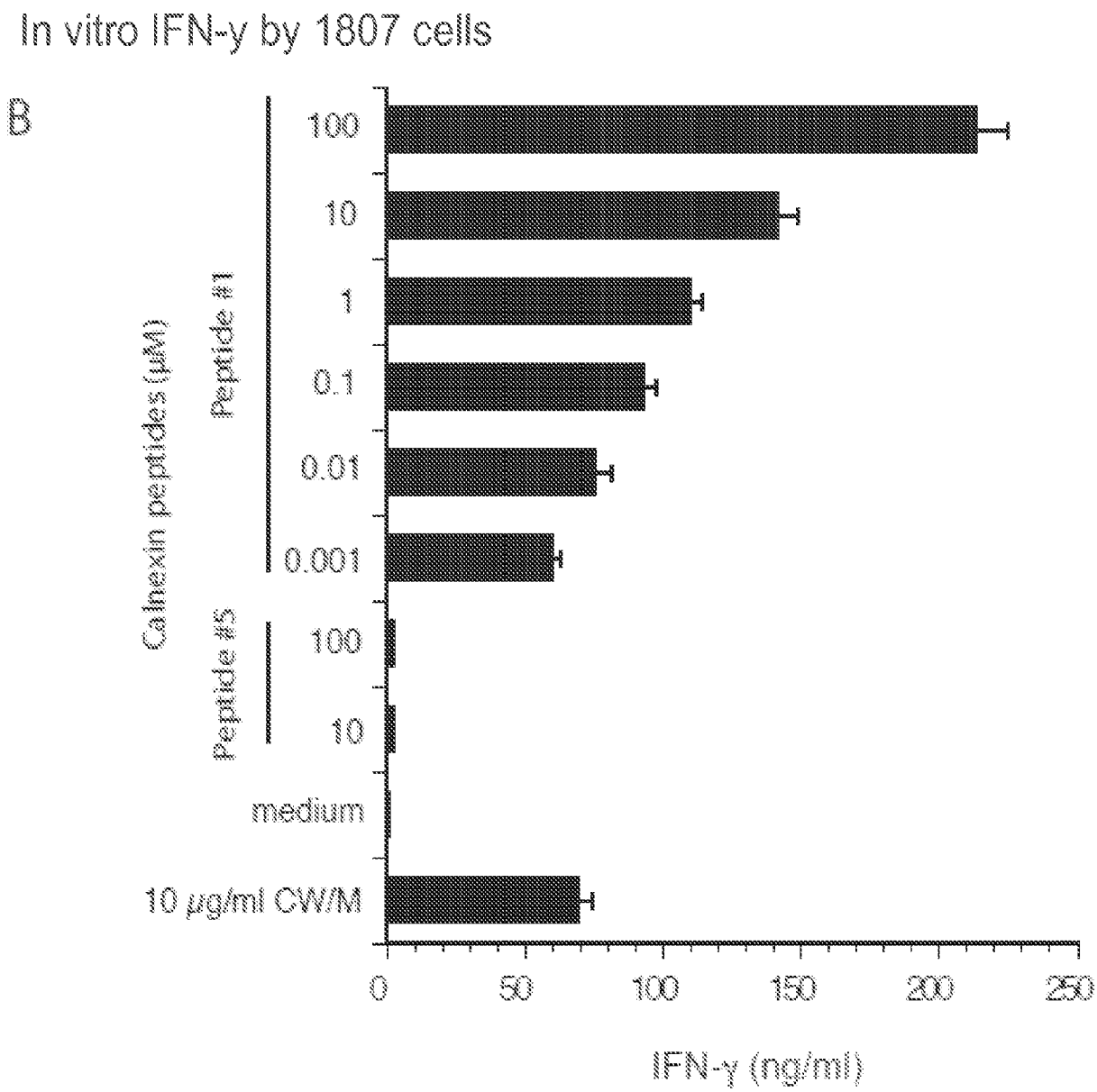
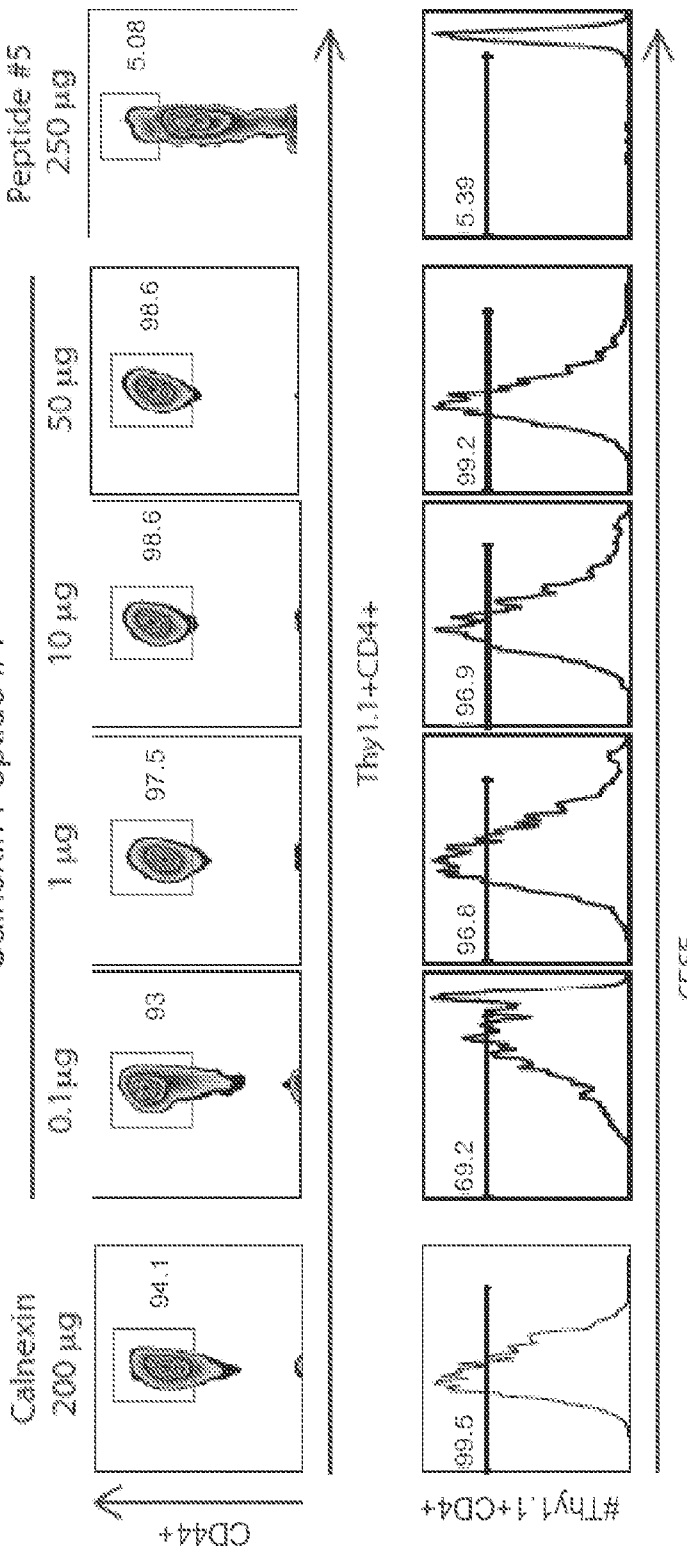

In vitro IFN-γ by 1807 cells
B
Calnexin peptides (μM)
Peptide #1
100
10
1
0.1
0.01
0.001
Peptide #5
100
10
medium
10 μg/ml CW/M
0
50
100
150
200
250
IFN-γ (ng/ml)

Figure 3

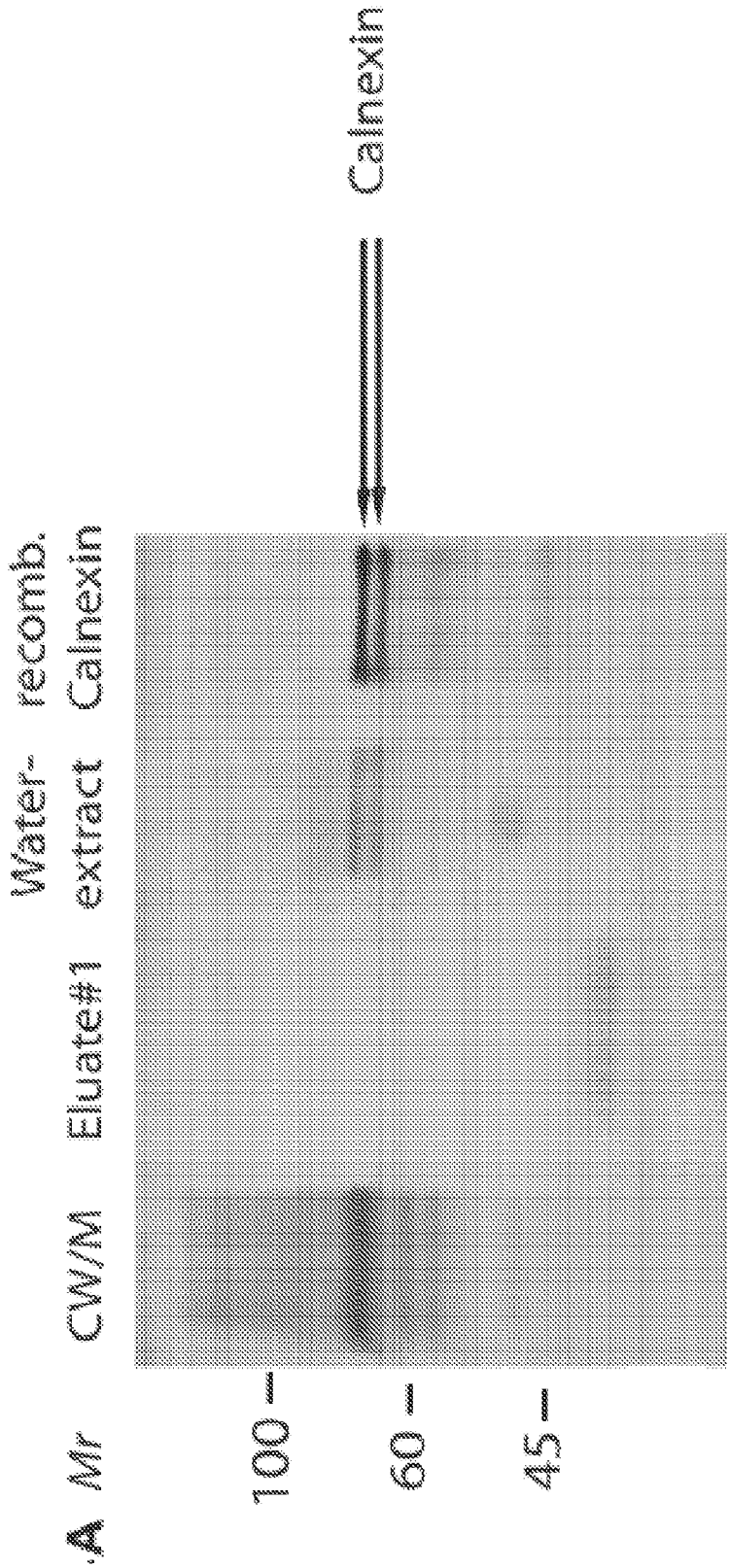
Figure 3

In vivo activation of 1807 cells by Calnexin peptide #1



C

Figure 4


A
Mr
CW/M
Eluate#1
Water-extract
recomb. Calnexin
100
60
45
Calnexin

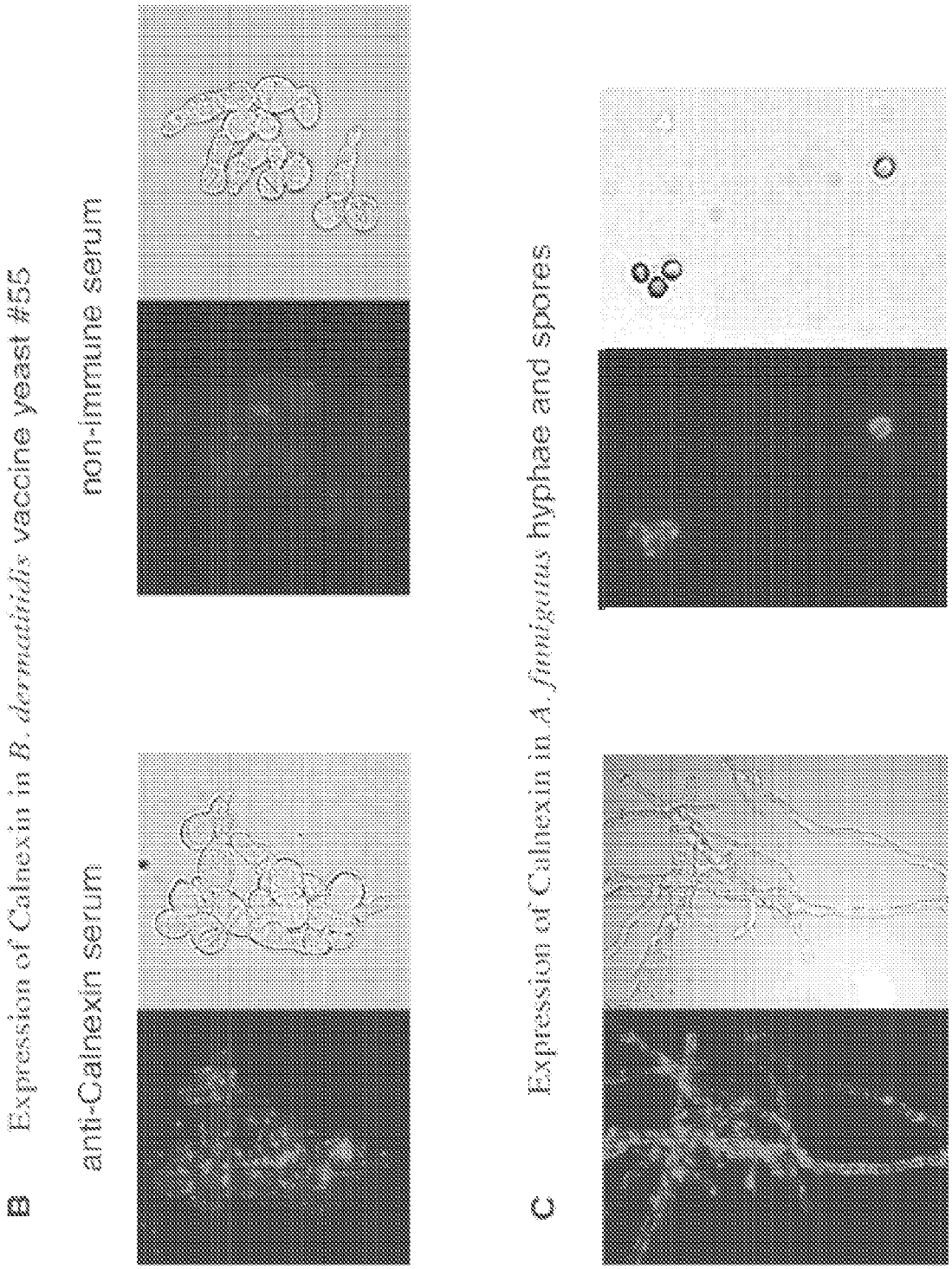

B
Expression of Calnexin in B. dermatitidis vaccine yeast #55
anti-Calnexin serum
non-immune serum
C
Expression of Calnexin in A. fumigatus hyphae and spores

Figure 4

A

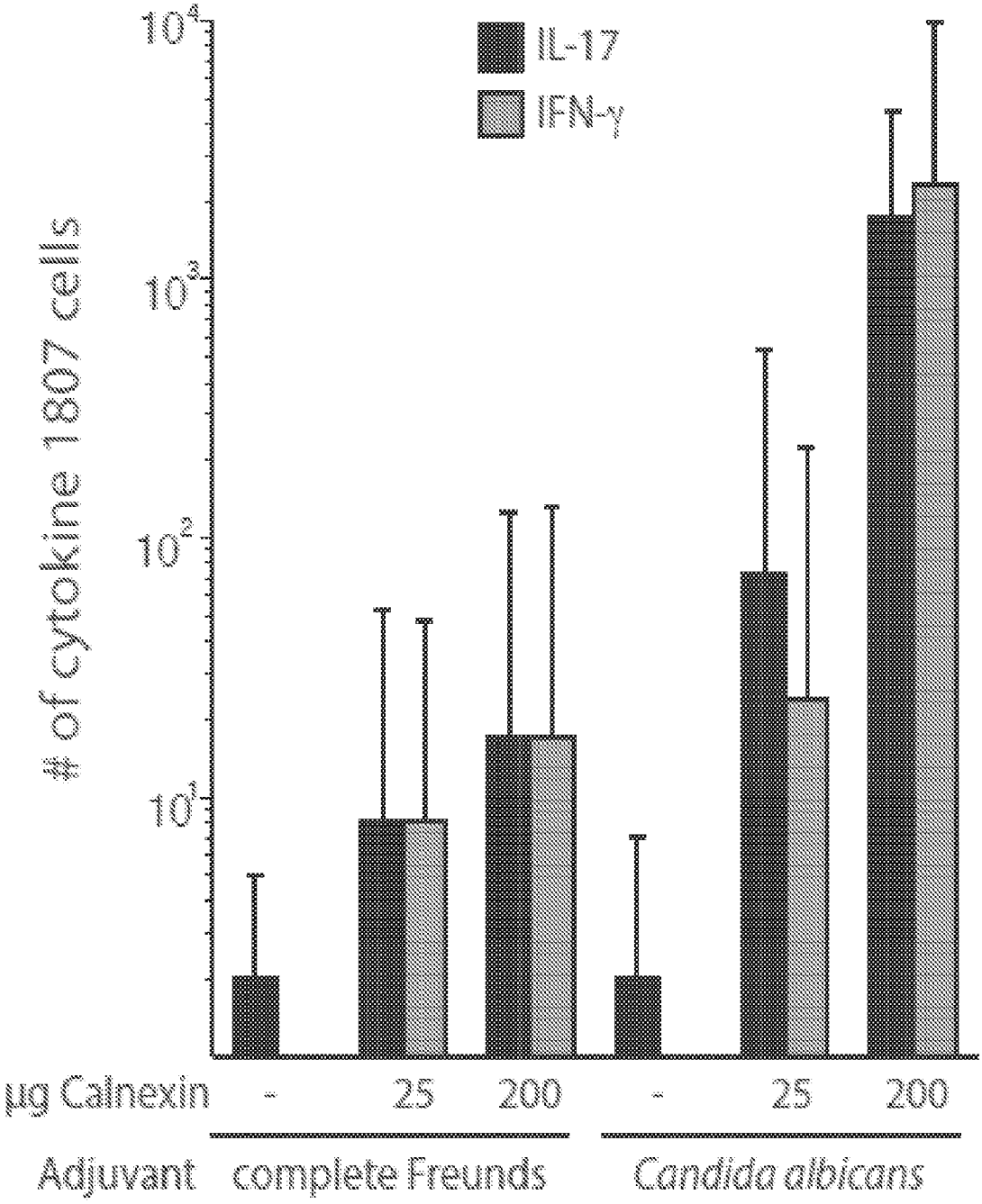

IL-17
IFN-γ
# of cytokine 1807 cells
10^4
10^3
10^2
10^1
µg Calnexin
-
25
200
-
25
200
Adjuvant
complete Freunds
Candida albicans

Figure 5

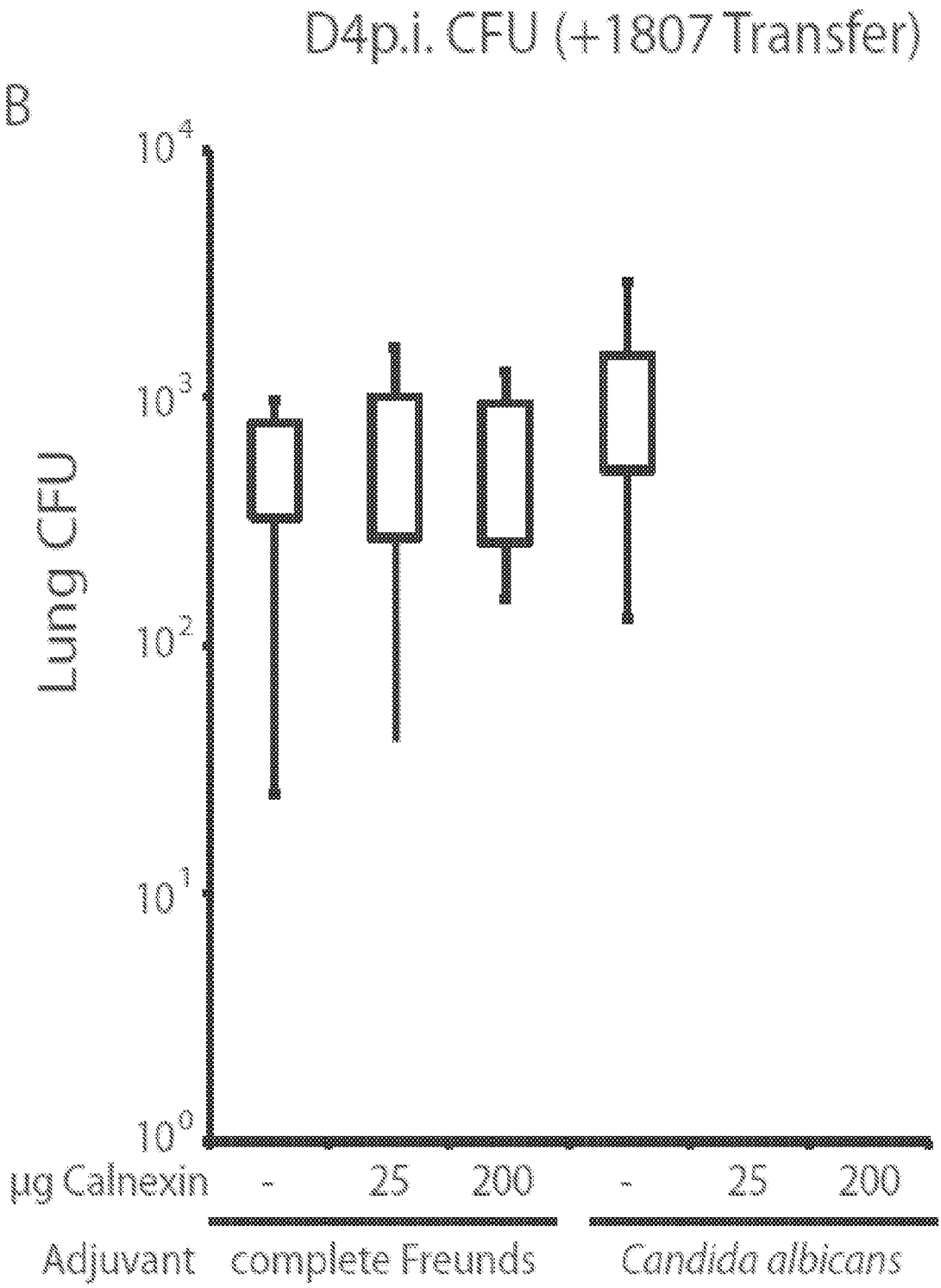

D4p.i. CFU (+1807 Transfer)
B
Lung CFU
10^4
10^3
10^2
10^1
10^0
µg Calnexin
-
25
200
-
25
200
Adjuvant
complete Freunds
Candida albicans

Figure 5

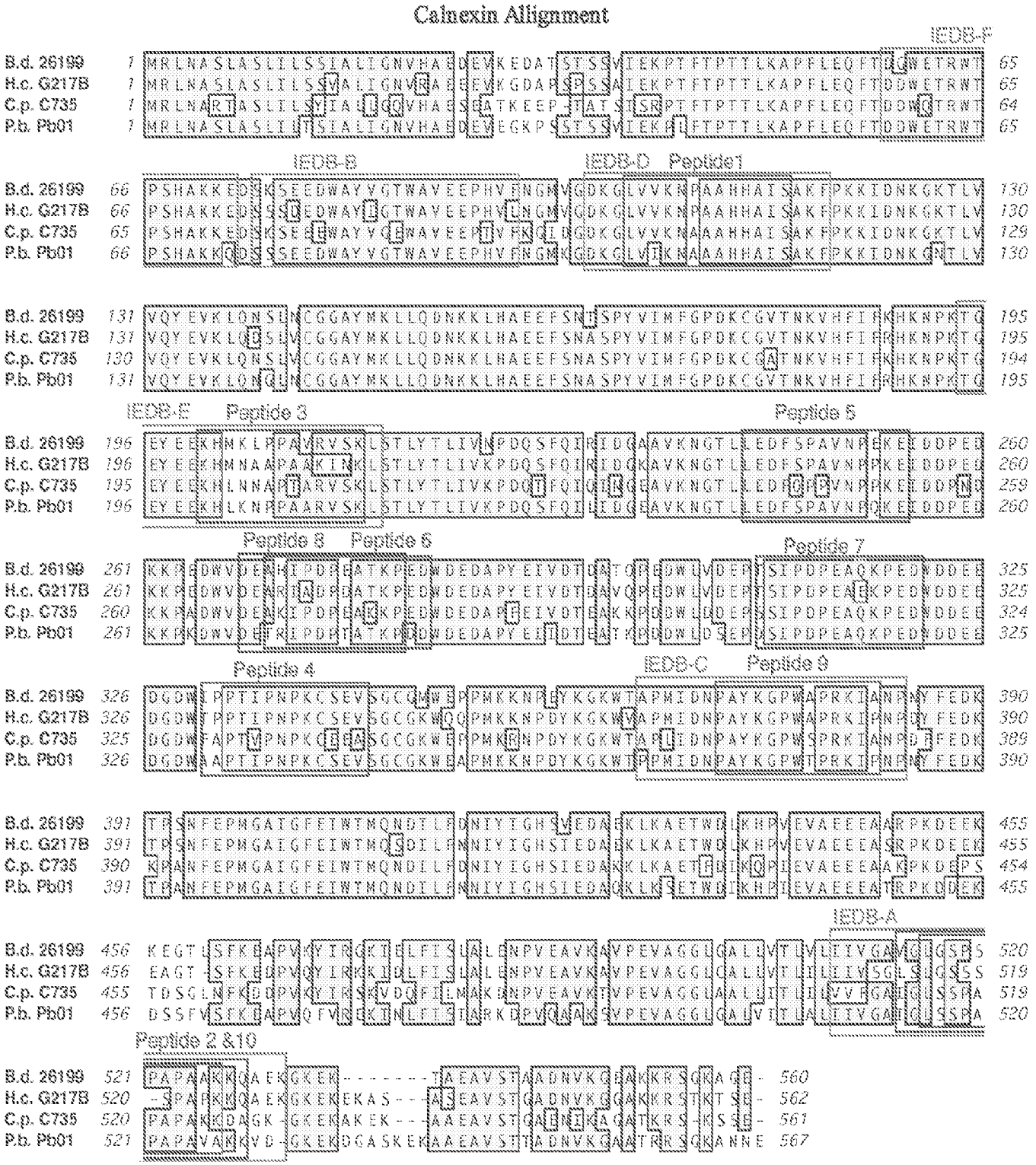

Calnexin Alignment
IEDB-F
IEDB-B
IEDB-D
Peptide1
IEDB-E
Peptide 3
Peptide 5
Peptide 8
Peptide 6
Peptide 7
Peptide 4
IEDB-C
Peptide 9
IEDB-A
Peptide 2 &10
B.d. 26199
H.c. G217B
C.p. C735
P.b. Pb01

Figure 6


10 20 30 40 50 60 70 80
DRB1_0101: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_0102: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_0301: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0305: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0306: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0307: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0308: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0309: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0311: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0401: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0402: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0404: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0405: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEE
DRB1_0408: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0410: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0421: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0423: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0426: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_0701: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0703: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0801: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0802: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_0804: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEE
DRB1_0806: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEE
DRB1_0813: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_0817: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEE
DRB1_1101: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDV
DRB1_1102: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1104: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1106: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1107: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSE
DRB1_1114: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDV
DRB1_1120: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1121: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDV
DRB1_1128: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1301: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1302: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1304: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1305: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1307: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEE
DRB1_1311: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDV
DRB1_1321: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1322: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1323: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1327: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEE
DRB1_1328: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1501: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1502: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB1_1506: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEED
DRB5_0101: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDW
DRB5_0105: MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTLKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDV

FIGURE 7

FIGURE 7 (continued)

FIGURE 7 (continued)

FIGURE 7 (continued)

FIGURE 7 (continued)

FIGURE 7 (continued)

FIGURE 7 (continued)

FIGURE 7 (continued)

> B.d. 26199 calnexin (deduced from genomic sequence)
MRLNASLASLILSSIALIGNVHAEDEVKEDATSTSSVIEKPTFTPTTILKAPFLEQFTDGWETRWTPSHAKKEDSKSEEDWAYVGTWAVEE
PHVFNGMVGDKGLVVKNPAAHHAISAKFPKKIDNKGKTLVVQYEVKLQNSLNCGGAYMKLLQDNKKLHAEEFSNTSPYVIMFGPDKC
GVTNKVHFIFKHKNPKTGEYEEKHMKLPPAVRVSKLSTLYTLIVNPDQSFQIRIDGAAVKNGTLLEDFSPAVNPFKEIDDPEDKKPEDWV
DEAHIPDPEATKPEDWDEDAPYEIVDTDATQPEDWLVIDEPTSIPDPEAQKPEDWIDDEEDGDWIPPTIPNPKCSEVSGCGMWEPPMKKNP
EYKGKWTAPMIDNPAYKGPWAPRKIANPNYFEDKTPSNFEPMGAIGFEIWTMQNDILFDNIYICHSVEDAEKLKAETWDLKHPVEVAEE
EAARPKDEEKKEGTLSFKEAPVKYIRGKIELFISLALENPVEAVKAVPEVAGGLGALLVTLVLIIVGAVGLGSPSPAPAAKKQAEKGKEKT
AEAVSTAADNVKGEAKKRSGKAGE

Links to Calnexin Protein sequence in GenBank:

-Note that these links are for a the Calnexin sequence for the strain 1818S, but the protein sequence is identical to that in strain 26199

http://www.ncbi.nlm.nih.gov/protein/327337631
Protein database Accession number: EGE86308
Broad Institute predicted Gene name: BDDG_09453

FIGURE 8

ClustalW (v1.83) multiple sequence alignment

7 Sequences Aligned Processing time: 0.7 seconds
Gaps Inserted = 85 Conserved Identities = 152
Score = 51436

Pairwise Alignment Mode: Fast
Pairwise Alignment Parameters:
ktup = 1 Gap Penalty = 3 Top Diagonals = 5 Window Size = 5
Similarity Matrix: gonnet

Multiple Alignment Parameters:
Open Gap Penalty = 10.0 Extend Gap Penalty = 0.2
Delay Divergent = 40% Gap Distance = 8
Similarity Matrix: gonnet

Identity Scores (%)

	B.d. 26199	P.b. Pb01	C.i. RS	H.c. G186AR	A flavus	C.a.5314	C. neoform.
B.d. 26199	100.0	82.9	78.9	87.1	73.9	32.5	49.0
P.b. Pb01	90.3	100.0	77.5	80.5	72.6	33.1	49.7
C.i. RS	87.6	85.9	100.0	77.5	72.3	33.8	50.0
H.c. G186AR	92.0	88.4	87.1	100.0	72.6	33.6	48.9
A flavus	85.5	84.4	85.5	83.6	100.0	34.6	51.7
C.a.5314	46.0	47.8	47.3	46.8	46.6	100.0	33.5
C. neoform.	63.1	63.6	64.0	62.0	64.4	46.4	100.0

Similarity Scores (%)

FIGURE 9

WO 2014/164727
PCT/US2014/023340

simple calnexin pro Alignments
Friday, January 25, 2013 2:25 PM 24/26

Formatted Alignments

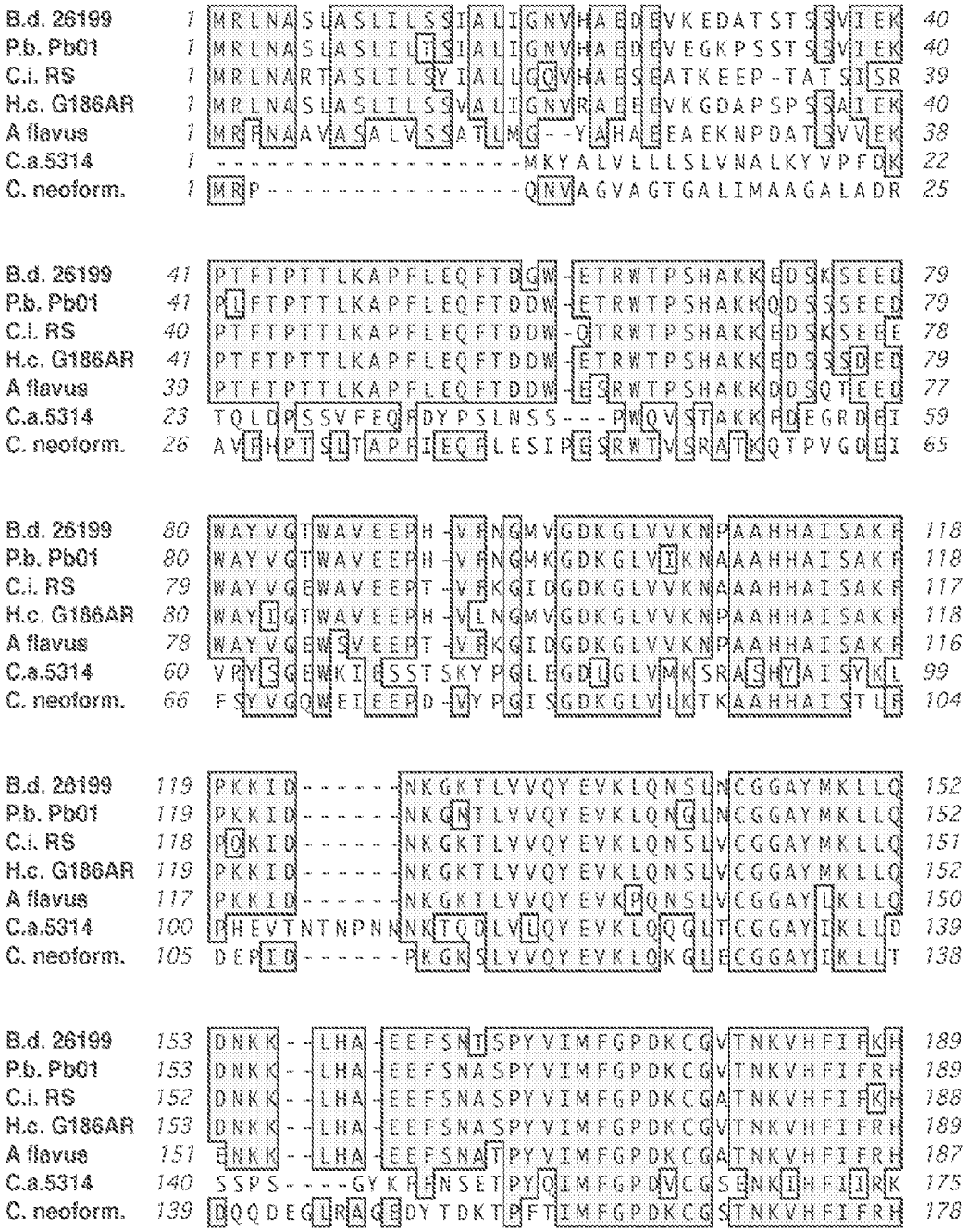


FIGURE 10

simple calnexin pro Alignments
Friday, January 25, 2013 2:25 PM

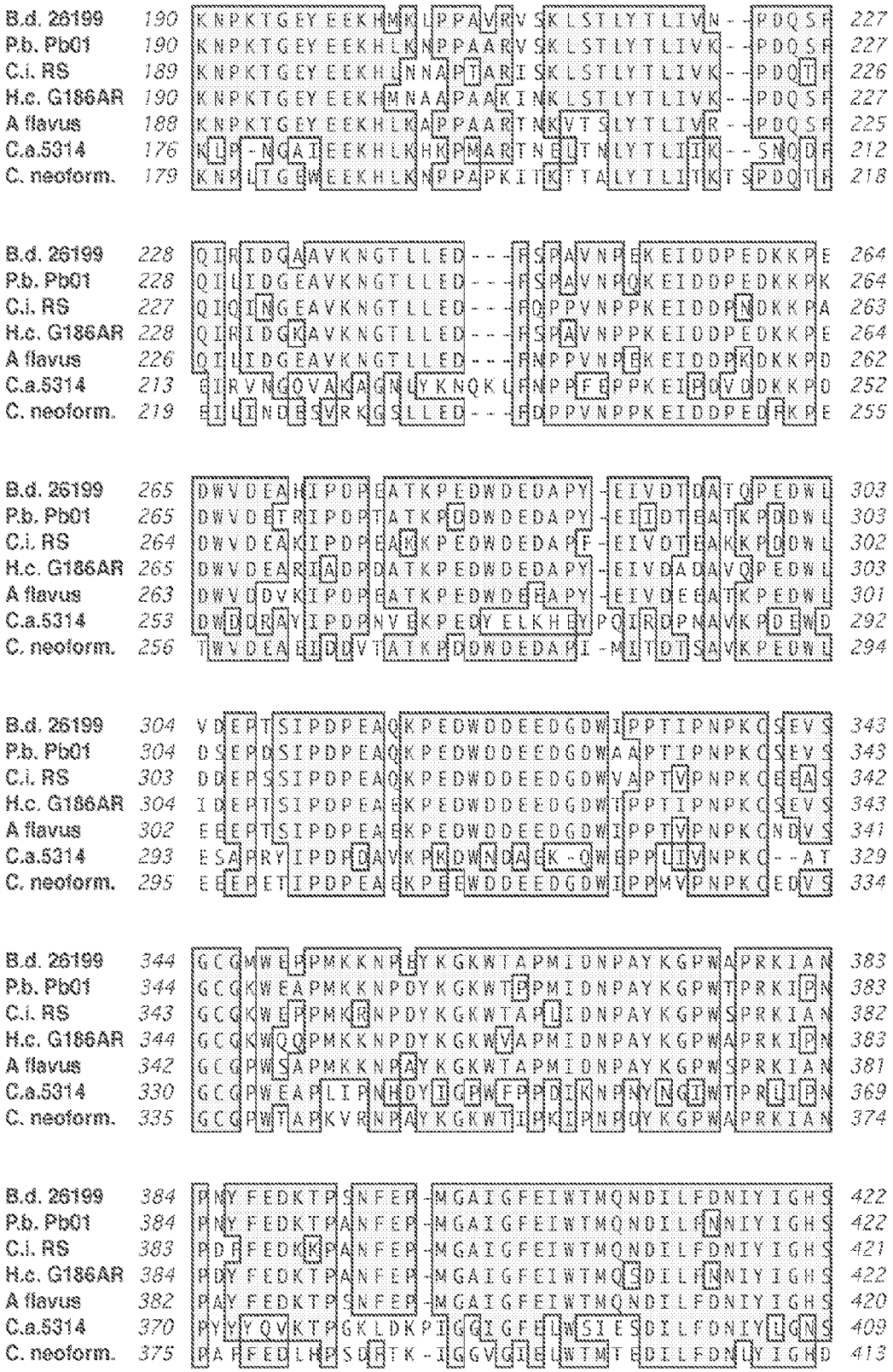


FIGURE 10 - continued

simple calnexin pro Alignments 26/26
Friday, January 25, 2013 2:25 PM

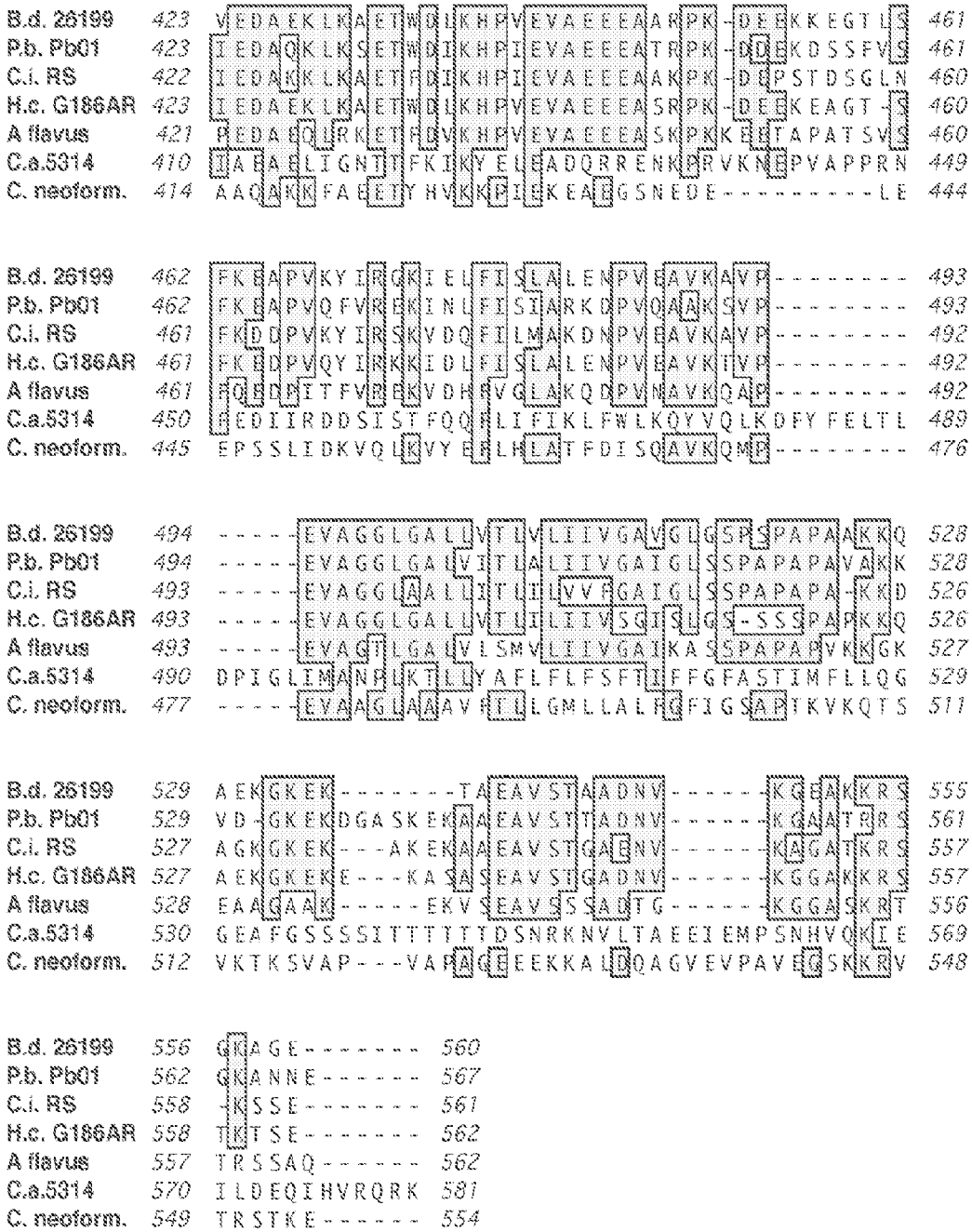


FIGURE 10 - continued

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/023340

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/14
ADD. A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/049329 A1 (SUNBIO BIOTECH PHARMACEUTICALS [CN]; BEIJING SUNBIO BIOTECH CO LTD [CN]) 2 May 2008 (2008-05-02) paragraph [0068]; claims 1,2	1-4,6,8, 9,11
A	LUCIANO DOS SANTOS FEITOSA ET AL: "Cloning, characterization and expression of a calnexin homologue from the pathogenic fungusParacoccidioides brasiliensis", YEAST, vol. 24, no. 2, 1 February 2007 (2007-02-01), pages 79-87, XP055133360, ISSN: 0749-503X, DOI: 10.1002/yea.1438 the whole document	1-22

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2014	02/09/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kalsner, Inge

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/023340

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BEI WANG ET AL: "Lentiviral calnexin-modified dendritic cells promote expansion of high-avidity effector T cells with central memory phenotype", IMMUNOLOGY, vol. 128, no. 1, 1 September 2009 (2009-09-01), pages 43-57, XP055133376, ISSN: 0019-2805, DOI: 10.1111/j.1365-2567.2009.03067.x the whole document -----	1-22
A	MARCEL WÜTHRICH ET AL: "Fungi Subvert Vaccine T Cell Priming at the Respiratory Mucosa by Preventing Chemokine-Induced Influx of Inflammatory Monocytes", IMMUNITY, vol. 36, no. 4, 1 April 2012 (2012-04-01), pages 680-692, XP055133659, ISSN: 1074-7613, DOI: 10.1016/j.immuni.2012.02.015 the whole document -----	1-22
A	M. WUTHRICH ET AL: "A TCR Transgenic Mouse Reactive with Multiple Systemic Dimorphic Fungi", THE JOURNAL OF IMMUNOLOGY, vol. 187, no. 3, 24 June 2011 (2011-06-24) , pages 1421-1431, XP055134017, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1100921 abstract -----	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/023340

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008049329 A1	02-05-2008	AU 2007308576 A1	02-05-2008
		CN 101528255 A	09-09-2009
		EP 2089053 A1	19-08-2009
		SG 175660 A1	28-11-2011
		US 2010158930 A1	24-06-2010
		WO 2008046251 A1	24-04-2008
		WO 2008049329 A1	02-05-2008