

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 October 2002 (03.10.2002)

PCT

(10) International Publication Number
WO 02/076401 A2

- (51) International Patent Classification⁷: **A61K**
- (21) International Application Number: PCT/US02/09378
- (22) International Filing Date: 26 March 2002 (26.03.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/278,837 26 March 2001 (26.03.2001) US
- (71) Applicant (for all designated States except US):
DANA-FARBER CANCER INSTITUTE, INC.
[US/US]; 44 Binney Street, Boston, MA 02115 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **WILSON, S., Brian** [US/US]; 33 Homestead Street, Lexington, MA 02421 (US). **DRANOFF, Glenn** [US/US]; 28 Outlook Drive, Lexington, MA 02421 (US). **GILLESSEN, Silke** [CH/CH]; Rotachstr. 9, CH-9000 St. Gallen (CH).
- (74) Agent: **EISENSTEIN, Ronald, I.**; Nixon Peabody LLP, 101 Federal Street, Boston, MA 02110 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 02/076401 A2

(54) Title: METHOD OF ATTENUATING REACTIONS TO SKIN IRRITANTS

(57) Abstract: The present invention is directed to a method of inhibiting CD1d activation by administering a composition containing a moiety that blocks CD1d activation. Compositions of the invention are useful for the attenuation of CD1d-restricted immune responses, including treatment of skin disorders due to hyperactive immune responses (e.g., contact hypersensitivity), for systemic administration to attenuate ongoing immune responses, and to provide hypoallergenic cosmetic products including pharmaceutical, cosmetic, and skin care compositions. Preferably, these compositions are in a form intended for topical administration.

5

METHOD OF ATTENUATING REACTIONS
TO SKIN IRRITANTS

This invention was supported by National Institutes of Health Grants R01
A145051, K11 DK02345, and R01 CA74886 and the government of the United States
10 has certain rights thereto.

FIELD OF THE INVENTION

The present invention is directed to a method of attenuating and/or inhibiting a
variety of reactions such as rashes caused by irritants. Preferably, the attenuated
15 reaction is an immune response associated with CD1d activation.

BACKGROUND OF THE INVENTION

The skin represents the body's initial line of defense against the environment.
The body is continually exposed to a barrage of molecules, many of which can cause
20 an adverse reaction upon contact. These reactions can follow a spectrum from
causing a dramatic and detrimental effect on an individual to skin discoloration and
blotching.

The reactions include typical allergic dermatitis, including contact dermatitis and atopic dermatitis, as well as irritant dermatitis.

Contact dermatitis is an inflammation of the skin, which occurs when the skin comes in contact with substances that the skin is sensitive or allergic to. The reaction usually appears within 24-48 hours after exposure to the allergen. Common symptoms include redness, itching and swelling. Sometimes blistering and weeping of the skin also develop. The clinical symptoms of contact dermatitis can include acute eczema accompanied by erythema, edema, papula, vesicle, erosion, and itching. Repeated exposure to an irritant can lead to the development of eczema accompanying lichenification and infiltration. Allergic contact dermatitis can appear after initial or prolonged exposure to an irritant. Contact dermatitis includes irritant dermatitis, phototoxic dermatitis, allergic dermatitis, photoallergic dermatitis, contact urticaria, systemic contact-type dermatitis and the like.

A wide range of agents can cause allergic contact dermatitis including for example, metals (e.g. nickel, chromium, cobalt), fragrances, chemicals, cosmetics, textiles, pesticides, plastics, and pollen (see, for example, R. J. G. Rycroft et al. "Textbook of Contact Dermatitis").

Therapeutic agents such as drugs may also cause allergic contact dermatitis, particularly when administered transdermally. It is well known that many drugs, e.g., topical ointments, including some currently marketed in the United States (e.g. clonidine) sensitize the skin when used.

Skin sensitization may be produced not only by transdermally delivered drugs, but also by a non-sensitizing drug combined with skin sensitizing permeation enhancers, or a combination of a sensitizing drug and a sensitizing permeation

enhancer. Penetration of these sensitizing agents into the skin and the resulting adverse reaction of the skin may persist well beyond the time that the transdermal patch is removed from the skin. The reaction of the skin may be a source of discomfort and a clinical complication in patients suffering from such a reaction.

5 Atopic dermatitis is developed by exposure to various antigens, since an individual has an atopic disposition which is hypersensitivity against a certain substance. The clinical symptoms include marked itching, skin hypertrophy, infiltration, lichenification and the like.

Irritant dermatitis can occur when too much of a substance is used on the skin
10 or when the skin is sensitive to a certain substance. Susceptibility can include a genetic component. Skin-irritating agents are substances (e.g. soap) that cause an immediate and generally localized adverse response. The response is typically in the form of redness and/or inflammation and generally does not extend beyond the immediate area of contact. Symptoms that are commonly seen include redness,
15 scaling, and the skin looking irritated and sore.

Psoriasis is a skin condition associated with hyper-proliferation of skin cells and immunologic involvement. Psoriasis is a common, idiopathic chronic skin disease characterized by inflamed, scaling, skin lesions containing infiltrates of neutrophils, lymphocytes, and monocytes. Psoriasis manifests in many forms,
20 including cutaneous, mucosal, unguinal, and even psoriatic rheumatism. The most effective treatment in the control of localized psoriasis for most patients is the use of topical corticosteroids and topical coal-tar preparations. With certain patients who have generalized psoriasis, it has been necessary to use a variety of systemic chemotherapeutic agents, especially methotrexate.

Certain irritants may cause both allergic and non-allergic contact dermatitis. For example, latex. Latex refers to a type of plastic made from the milky sap of the rubber tree, and contains many proteins which can cause allergic reactions in sensitive individuals. Symptoms can range from watery eyes, hives, rash, swelling, wheezing and in severe cases, anaphylaxis. These responses can occur when latex items touch the skin; the mucous membranes (including the mouth, bladder, genitals, or rectum), and open wounds or bloodstream (especially during surgery). Anybody can develop latex sensitivity. People at increased risk for developing latex allergy include workers with ongoing latex contact (like health care workers), persons with many environmental allergies (hay fever), and those with spina bifida. Latex is found in a wide array of common products, including, for example: gloves, balloons, band-aids, tourniquets, bandages, catheters, rubber bands, IV, other tubing (ex. stethoscopes), art supplies, pacifiers, bottle nipples, diapers, condoms/diaphragms, elastic, chewing gum, carpeting, hand grips of bicycles and motorcycles, shoe soles, auto tires, swimming goggles and equipment.

The more common reaction to latex products is not allergic, but rather, irritant contact dermatitis, which can cause dry, itchy, irritated areas on the skin, usually the hands. Skin reactions include a rash that usually begins 24 to 48 hours after contact. It may progress to oozing blisters or spread away from area touched by latex. Latex allergy (immediate hypersensitivity) is a more serious reaction. Certain proteins in latex cause an allergic reaction. The amount of exposure needed to cause symptoms is not known. Very low levels of exposure can trigger allergic reactions in some people, while having no affect to most people. Reactions usually begin within minutes of exposure to latex, but can occur hours later and have a variety of symptoms. Mild

reactions to latex usually cause skin redness, hives, or itching. More severe reactions can cause respiratory or breathing symptoms such as runny nose, sneezing, itchy eyes, scratchy throat, and asthma (trouble breathing, coughing, and wheezing).

Individuals can also develop allergic dermatitis and/or irritant dermatitis in response to insects and plants and shrubbery. For example, certain plants such as poison ivy excrete chemicals that upon contact can cause adverse reactions in humans. These reactions may particularly occur during gardening or nature walks.

Many animals can also suffer from a variety of skin irritations and inflammations generally known as dermatitis. For example, all animals can develop contact dermatitis caused by flea, mosquito, or other insect bites, allergies, external stimulation such as from prickly plants, and for other reasons. The condition has been notoriously difficult to treat. Veterinarians occasionally resort to injections of various medicines in an attempt to alleviate the symptoms and cure the dermatitis.

Presently known therapies for treating or preventing reactions associated with such irritants are inadequate. For example, steroidal agents and antihistamine agents have been used as therapeutic agents for contact dermatitis, and these and a part of the so-called anti-allergic agents have been used for atopic dermatitis. The most widely prescribed drugs to treat dermatologic disease are corticosteroids, also known as glucocorticosteroids or glucocorticoids. Approximately 50% of prescriptions written by dermatologists are for topical corticosteroids. However, these drugs can cause adverse reactions and/or are not fully effective. Systemic corticosteroids are often required in some severe dermatologic diseases but topical treatment is preferred in most responsive cases because it causes fewer systemic adverse effects.

Individual topical corticosteroid preparations vary in anti-inflammatory potency and clinical efficacy.

Though some steroids, particularly mid- to high-potency steroids, are efficacious in chronic dermatoses, long term use of steroids is associated with serious local side effects. These include skin atrophy (thinning, telangiectasia, striae) and a prompt rebound flare when the steroid is stopped. Treatment of large areas of skin and use of occlusive dressings can also increase the potential for adverse effects. This is especially the case in children.

Examples of anti-histamine agents include diphenhydramine hydrochloride, mequitazine, promethazine hydrochloride, and chlorpheniramine maleate anti-histamines have been used mainly to reduce itchiness. Anti-allergic agents include tranilast, ketotifen fumarate, oxatomide, and azelastine hydrochloride. In general, conventional so-called antiallergic agents are either ineffective or fail to show satisfactory therapeutic effects on contact dermatitis and atopic dermatitis.

Accordingly, there is a need for a treatment for skin reactions including allergic dermatitis (contact dermatitis and atopic dermatitis), as well as irritant dermatitis.

The pathophysiologic mechanisms involved in the above-described skin disorders and the evolution of such inflammatory processes are poorly understood. There are numerous skin conditions characterized by increased T cell activation and abnormal antigen presentation in the dermis and epidermis. Thus, there has been speculation that skin cells are important in the generation of a cutaneous inflammatory response (Kupper, "Immune and Inflammatory Processes in Cutaneous Tissues", J. Clin. Invest., 86, pp. 1783-89 (1990)).

CD1d-restricted NK T cells are among the immune system cells found in the skin. The *in vivo* functions of CD1d-restricted NK T cells are not fully known. They are involved in the IgG response to GPI-anchored proteins of various parasites, contribute to the IL-12-mediated rejection of tumors, and appear to regulate some autoimmune disorders and clearance of certain infections through the production of cytokines. Many of these functions have been observed by using lipid ligands that bind CD1d and activate NK T cells. For example, α -gal-cer.

In humans, the direct cellular targets for their immunomodulatory function(s) have remained enigmatic.

10

SUMMARY OF THE INVENTION

We have now discovered that one can attenuate and block CD1d activation. This can be done by administering compositions that attenuate CD1d-restricted NK T cell responses. Such compositions include compounds that act as antagonists by binding CD1d and inhibiting activation of CD1d-restricted NK T cells, compounds that block CD1d-specific receptors on NK T cells, and decoys, mimics and the like. Compositions include pharmaceutical and cosmetic compositions.

One preferred group of compositions include phospholipids that bind CD1d without activating NK T cells. Preferred phospholipids include 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine (DPPE) and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Poly(ethylene glycol) 2000] (DPPE-PEG).

20

Another preferred group of compositions includes glycolipid antagonists that bind CD1d without activating NK T cells. Preferred glycolipids include ceramide, α Man Cer, and β Gal Cer.

Another preferred group of compositions includes phosphatidyl inositol.

5 This method of attenuating CD1d activation can be used for treatment of conditions associated with activation of CD1d-restricted NK T cells. Preferred conditions include skin disorders due to hyperactive CD1d-restricted T cell responses and other disorders associated with ongoing CD1d-restricted immune responses.

Such CD1d associated skin disorders include contact dermatitis and further
10 eczematous dermatitises, atopic dermatitis, seborrheic dermatitis, psoriasis, Lichen planus, Pemphigus, bullous Pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinphilias, Lupus erythematosus, and Alopecia areata.

A preferred embodiment of the invention is directed to the treatment of contact
15 hypersensitivity.

Another embodiment of the invention provides hypoallergenic cosmetic products.

Another embodiment of the invention is directed to compositions that can be used prophylactically to prevent a reaction before one encounters the irritant. For
20 example, a composition to use before weeding.

One preferred embodiment of the invention provides preventing or treating skin sensitization produced by topical administration of therapeutic drugs.

Other embodiments of the invention provide systemic administration of compositions to attenuate ongoing CD1d-restricted immune responses. Systemic therapy can be used in any individual for which activation of NKT cells would be adverse. Systemic conditions can include individuals with autoimmune disease such as lupus. It can also be used in women at high risk for spontaneously aborting pregnancies.

In one embodiment of the invention, a locally administrable topical pharmaceutical or cosmetic composition is provided for the attenuation or treatment of skin conditions associated with CD1d-restricted immune responses. The locally administrable topical pharmaceutical composition includes a topical carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing that contact hypersensitivity is ablated in Cd1d knock out mice and in iNKT cell knock out mice. Mice at least seven weeks of age were sensitized epicutaneously on day 0 with 150 μ l of 3% oxazolone in 100% ethanol on the shaved abdomen. 6 days later the right ear was challenged with 1% oxazolone, 10 μ l on each side or carrier only. The hapten specific increase in ear thickness at 24 hours was determined using a micrometer.

Figure 2 is a graph showing that systemic treatment with α -ManCer inhibited oxazolone-induced contact hypersensitivity. Mice at least seven weeks of age were sensitized epicutaneously on day 0 with 70 μ l of 4% oxazolone in acetone/olive oil (4/1) with or without treatment with α -GalCer or α -ManCer. The hapten specific increase in ear thickness at 24 hours was determined using a micrometer. 5 days later

the right ear was challenged with 0.5% oxazolone, 10 μ l on each side or carrier only. The hapten specific increase in ear thickness at 24 hours was determined using a micrometer.

Figure 3 is a graph showing that topical DPPE-PEG (a CD1d-binding lipid) inhibits oxazolone-induced contact hypersensitivity. Mice at least seven weeks of age were sensitized epicutaneously on day 0 with 70 μ l of 4% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone, Sigma) in acetone/olive oil (4/1) with or without DPPE-PEG (100 mg/ml) and challenged five days later on the ear with 20 μ l of 0.5% oxazolone or carrier only. The hapten-specific increase in ear thickness at 24 hours was determined with a micrometer.

Figure 4A is a dose response curve showing that topical administration of DPPE-PEG inhibits oxazolone-induced contact hypersensitivity in a dose dependent fashion. Mice at least seven weeks of age were sensitized epicutaneously on day 0 with 70 μ l of 4% oxazolone in acetone/olive oil (4/1) with DPPE-PEG in a series of increasing dosage, ranging from 0 to 100 mg/ml. 5 days later the right ear was challenged with 0.5% oxazolone, 10 μ l on each side or carrier only. The hapten specific increase in ear thickness at 24 hours was determined using a micrometer.

Figure 4B is a graph showing topical administration of DPPE-PEG inhibits oxazolone-induced contact hypersensitivity both during recall and priming. Mice at least seven weeks of age were sensitized epicutaneously on day 0 with 70 μ l of 4% oxazolone in acetone/olive oil (4/1) with or without DPPE-PEG. 5 days later the right ear was challenged with 0.5% oxazolone, 10 μ l on each side or carrier only. One group that was primed with oxazolone only was challenged with a mixture of

oxazolone 0.05% and DPPE-PEG (100mg/ml). The hapten specific increase in ear thickness at 24 hours was determined using a micrometer.

Figure 5 is a graph showing that inhibition of CSH by DPPE-PEG was independent of MHC alleles. In bred strains of mice with unrelated MHC alleles were
5 challenged with oxazolone with or without DPPE-PEG at 100 mg/ml as described for Figure 4. The application of antagonist lipid inhibited CHS responses in SWR, C57Bl/10, DBA, and BALB/c mice.

DETAILED DESCRIPTION OF THE INVENTION

10 The present invention is directed to a method of inhibiting CD1d activation. The method comprises administering a composition containing a moiety that blocks CD1d activation.

Compositions of the invention are useful for the attenuation of CD1d-restricted immune responses, including treatment of skin disorders due to hyperactive
15 immune responses (e.g., contact hypersensitivity), for systemic administration to attenuate ongoing immune responses, and to provide hypoallergenic cosmetic products including pharmaceutical, cosmetic, and skin care compositions. Preferably, these compositions are in a form intended for topical administration.

We have discovered that certain antagonists bind CD1d and inhibit activation
20 of NK T cells. Consequently, by exposing CD1d-expressing cells to a CD1d-specific antagonist, one can attenuate immune responses by specific inhibition of CD1d-restricted events. Antagonists of the present invention include any molecules that inhibit activation of CD1d-restricted NK T cells. Thus, one can treat subjects

suffering from an activated CD1d associated disorder such as a skin disorder by administering an effective amount of CD1d specific antagonist.

Such compounds include antagonists that bind CD1d and inhibit NK T cell activation, antagonists that block CD1d-specific receptors such as on NK T cells, 5 decoys that prevent CD1d binding to the CD1d specific receptor, and the like.

Experiments in human models have revealed that CD1d-restricted CD161+ T cells specifically target myeloid dendritic (DC1) cells. DC1 dendritic cells are integral to the genesis of Th1 immune responses. Accordingly, their susceptibility to lysis by CD1d-restricted CD161+ T cells may be part of a negative feedback loop in 10 cell-mediated immune responses.

We tested dendritic cell function in CD1d null and wild type mice. The CD1d null mice had impaired priming responses in mixed lymphocyte reactions, to tumor vaccinations, and in skin contact hypersensitivity (see Figure 1). In addition, contact hypersensitivity could be blocked by the cutaneous addition of lipid ligands known to 15 bind CD1d but not activate NK T cells (see Figure 2).

Accordingly, one can readily determine whether a compound inhibits activation of CD1d by looking at activation of CD1d-restricted NK T cells *in vitro* using standard assays such as described herein. For example, the proliferation of NK T cells is indicative of their activation by binding to CD1d-expressing cells. 20 Proliferation can be measured, for example, by determining the incorporation of [³H] thymidine into V α 14 NK T cells (Kawano et al., *Science* 278:1626-29 (1997)). Other *in vitro* assays include the induction of cytokine production.

CD1d-specific antagonists include any antagonist that binds CD1d and inhibits activation of NK T cells. The binding of an antagonist to CD1d can be determined *in vitro* using standard assays. For example, surface plasmon resonance (Naidenko et al., *J. Exp. Med.* 190:1069-79 (1999)). Molecules known to bind CD1d include

5 antibodies, phospholipids and glycolipids, including highly glycosylated sphingolipids (gangliosides) (Kawano et al., *Science* 278: 1626-9 (1997); Naidenko et al., *J. Exp. Med.* 190:1069-79 (1999); Briken et al., *Sem. Immunol.* 12: 517-25 (2000); Kronenberg et al., *Proc. Natl. Acad. Sci. USA* 98: 2950-52 (2001)).

Phospholipids that bind CD1d without activating NK T cells include 1,2-

10 Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine (DPPE) (Naidenko et al., 1999). The phospholipid may be coupled to a conjugate, such as other lipids coupled to a carrier such as biotin or a poly(alkaline oxide), for example polyethylene glycol (PEG). Polymeric substances such as dextran, polyvinyl pyrrolidones, polysaccharides, starches, polyvinyl alcohols, polyacryl amides or other similar

15 polymers can be used. Polyethylene glycol (PEG) as the poly(alkylene oxide) is preferred. The poly(alkylene oxides) can include monomethoxy polyethylene glycol, polypropylene glycol, block copolymers of polyethylene glycol and polypropylene glycol and the like. The polymers can also be distally capped with C₁₋₄ alkyls instead of monomethoxy groups.

20 Other preferred phospholipids include phosphatidyl inositol.

Glycolipids that bind to CD1d share a common motif consisting of a hydrophobic portion composed of a branched or dual alkyl chain moiety with a covalently linked hydrophilic cap formed by a polar or charged group of the lipid of associated carbohydrates (Briken et al., 2000). The prototypical glycolipid antigen

presented by CD1d is β -galactosylceramide (Kawano et al., 1997). Thus, D1d antagonists can include glycolipids such as monoglycosylated ceramides and diglycosylated ceramides (Kawano et al., 1997).

Examples of monoglycosylated ceramides and diglycosylated ceramides which bind CD1d but do not activate NK T cells can include ceramides with inner sugar groups at the β -anomer position (such as Gal α 1-4Glc β 1-1'Cer), an axial configuration of the 2-hydroxyl group (such as α -ManCer), derivatives lacking the 3- and 4-hydroxyl groups on the phytosphingosine of α -GalCer (such as 3,4-deoxy α -GalCer), and ceramides with fatty acyl chain with less than C₂₆, and a sphingosine base less than C₁₈. Glycolipid antagonists can also be coupled to a conjugate such as biotin or a poly(alkylene oxide), for example PEG.

Other CD1d glycolipid antagonists include highly glycosylated sphingolipids, also known as gangliosides. Ganglioside antagonists include GM1 and GD1a (Naidenko et al., 1997). The antagonists can be coupled to a conjugate such as a biotin or poly (alkylene oxide).

Preferred examples of CD1d-specific lipid antagonists include but are not limited to: DPPE-PEG, phosphatidyl inositol, ceramide, α -ManCer, β -GalCer, Gal α 1-4Glc β 1-1'Cer, and 13,4-deoxy α -GalCer, GM1, and GD1a.

Other antagonists can include antibodies that specifically bind to CD1d and in doing so prevent CD1d from binding to a CD1d specific receptor. Single chain antibodies and humanized monoclonal antibodies are preferred. Alternatively, one can use molecules that block the CD1d-specific receptor. For example, a molecule. Alternatively, one can modify the lipids that activate CD1d binding by capping the

end that bind to CD1d (Kawano et al., *Science* 278: 1626-9 (1997); Naidenko et al., *J. Exp. Med.* 190:1069-79 (1999); Briken et al., *Sem. Immunol.* 12: 517-25 (2000); Kronenberg et al., *Proc. Natl. Acad. Sci. USA* 98: 2950-52 (2001)).

One can also use decoys that mimic CD1d receptors so that the CD1d
5 molecule does not bind to the receptor.

CD1d blocking molecules are presently preferred.

Compositions of the invention should be physiologically stable at
therapeutically effective concentrations. Physiological stable compounds are
compounds that do not break down or otherwise become ineffective upon introduction
10 to a patient prior to having a desired effect. Compounds are structurally resistant to
catabolism, and thus, physiologically stable, or coupled by electrostatic or covalent
bonds to specific reagents to increase physiological stability. Such reagents include
amino acids such as arginine, glycine, alanine, asparagine, glutamine, histidine or
lysine, nucleic acids including nucleosides or nucleotides, or substituents such as
15 carbohydrates, saccharides and polysaccharides, lipids, fatty acids, proteins, or protein
fragments. Useful coupling partners include, for example, glycol such as polyethylene
glycol, glucose, glycerol, glycerin and other related substances.

Preferably, the compositions are not substantially toxic, myelotoxic,
mutagenic or teratogenic at required dosages. Although side effects may occur,
20 preferably the benefits achieved from their use outweigh disadvantages attributable to
adverse side effects.

Compositions of the present invention can be used to prevent or treat any
condition associated with activation of CD1d-restricted NK T cells, in which

attenuation of CD1d activation, such as attenuation of the CD1d-restricted NK T cell response, would be desirable. Preferred embodiments of the invention include topical administration to treat skin disorders due to hyperactive immune responses (e.g., contact hypersensitivity) and systemic administration. Prophylactic use to attenuate
5 immune responses. For example, to avoid reactions to certain plants such as poison ivy, poison oak, etc. when you are going to be in an area where exposure to such a substance is likely, for example, if you are going to be in the woods, gardening, etc.

Systemic administration is preferred in any individual for which activation of NKT cells would be adverse.

10 Certain women have a problem with spontaneous abortion which appears to be associated with high levels of CD1d. These individuals could use the present compositions before trying to conceive, preferably using systemic administration.

Certain individuals with autoimmune diseases have complications associated in part with CD1d activation. These compositions can be used with such individuals.

15 One problem with allergic or irritant caused itching is that the scratching can in fact result in further irritation that causes rashes and irritation long after the initial stimulus is gone. This is a particular problem with non-human animals. These compositions can be used to attenuate, treat or prevent such conditions. Another preferred embodiment includes the composition in hypoallergenic cosmetic products.

20 In one embodiment of the invention, a locally administrable topical pharmaceutical composition is provided for the prevention or treatment of skin conditions associated with CD1d-restricted T cell responses. Skin conditions include, but are not limited to, contact dermatitis and further eczematous dermatitises, atypical

dermatitis, seborrheic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinphilias, Lupus erythematosus, and Alopecia areata.

A preferred skin condition is contact dermatitis. Contact dermatitis can be
5 caused by a variety of irritants. The most widely known natural allergens which are capable of sensitizing and causing contact dermatitis in many people are antigenic plants of the genus Rhus, such as poison ivy, poison oak, and poison sumac. Other widely known skin irritants are commercial products such as insecticides containing Pyrethrum or Rotenone, dye intermediates such as aniline, nitro compounds,
10 anthracene, and derivatives thereof, dyes such as paraphenylenediamine and aniline black, photo developers such as hydroquinone and para-amido-phenol, antioxidants such as hexamethylene tetramine, and synthetic and natural resins such as wood rosin and phenol formaldehyde, and detergents and constituents of rubber and latex gloves

Other agents that cause contact dermatitis include, for example, metals (e.g.
15 nickel, chromium, cobalt, including exposure during body piercing), latex, fragrances, chemicals, cosmetics, textiles, pesticides, plastics, pollen, and the like. Therapeutic agents such as drugs may also cause allergic contact dermatitis, particularly when administered topically or transdermally. Agents that cause non-allergic contact dermatitis include skin-irritating agents such as water, skin cleansers, industrial
20 cleaning agents, alkalis, acids, oils, organic solvents, oxidizing agents, reducing agents, plant matter, animal matter, combinations thereof, and the like.

Another preferred skin condition is psoriasis. Psoriasis is a skin condition associated with hyper-proliferation of skin cells and immunologic involvement. Psoriasis is a common, idiopathic chronic skin disease characterized by inflamed,

scaling, skin lesions containing infiltrates of neutrophils, lymphocytes, and monocytes. The compositions and methods of the present invention can be used to treat any form of psoriasis, including cutaneous, mucosal, unguinal, and even psoriatic rheumatism. Compositions and methods of the present invention can be used to treat
5 localized and generalized psoriasis.

One preferred embodiment of the invention provides prophylactic treatment to minimize skin sensitization produced by topical administration of therapeutic drugs. In another embodiment, the composition can be administered with the therapeutic drug or cosmetic. In still another embodiment, the composition is used for treatment
10 after the reaction has occurred.

Another embodiment of the invention provides systemic administration of compositions to attenuate ongoing CD1d-restricted immune responses. This embodiment is preferable for any individual for whom activation of NKT cells would be adverse. Systemic conditions can include certain high risk spontaneously aborting
15 pregnancies. It can also be used with autoimmune diseases such as lupus.

In another embodiment of the invention, a locally administrable topical cosmetic composition is provided, for example to provide hypoallergenic products.

The compositions of the present invention include those suitable for topical and systemic administration including oral, rectal, intravaginal, nasal, ophthalmic or
20 parenteral administration, all of which may be used as routes of administration using the materials of the present invention. A preferred route of administration is topical. The topical composition may be in the form of a pharmaceutical but it does not have to be. For example, it can be a cosmetic.

In one embodiment of the invention, the locally administrable topical composition is provided for the prevention or treatment of skin conditions associated with CD1d-restricted immune responses. The locally administrable topical composition includes a topical carrier.

5 The topical carrier, as noted above, is one which is generally suited to topical drug administration and includes any such materials known in the art. The topical carrier is selected so as to provide the composition in the desired form, e.g., as a liquid, lotion, cream, paste, gel, powder, or ointment, and may be comprised of a material of either naturally occurring or synthetic origin. It is essential that the
10 selected carrier not adversely affect the active agent or other components of the topical formulation. Examples of suitable topical carriers for use herein include water, alcohols and other nontoxic organic solvents, glycerin, mineral oil, silicone, petroleum jelly, lanolin, fatty acids, vegetable oils, parabens, waxes, and the like. The composition of the invention may also be administered in the form of a shampoo, in
15 which case conventional components of such a formulation are included as well, e.g., surfactants, conditioners, viscosity modifying agents, humectants, and the like.

Particularly preferred formulations herein are colorless, odorless ointments, lotions, creams and gels.

Ointments are semisolid preparations which are typically based on petrolatum
20 or other petroleum derivatives. The specific ointment base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery, and, preferably, will provide for other desired characteristics as well, e.g., emolliency. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing. As explained in Remington: The Science and

Practice of Pharmacy, 19th Ed. (Easton, Pa.: Mack Publishing Co., 1995), at pages 1399-1404, ointment bases may be grouped in four classes: oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Oleaginous ointment bases include, for example, vegetable oils, fats obtained from animals, and semisolid hydrocarbons obtained from petroleum. Emulsifiable ointment bases, also known as absorbent ointment bases, contain little or no water and include, for example, hydroxystearin sulfate, anhydrous lanolin and hydrophilic petrolatum. Emulsion ointment bases are either water-in-oil (W/O) emulsions or oil-in-water (OW) emulsions, and include, for example, cetyl alcohol, glyceryl monostearate, lanolin and stearic acid. Preferred water-soluble ointment bases are prepared from polyethylene glycols of varying molecular weight (Remington: The Science and Practice of Pharmacy).

Lotions are preparations to be applied to the skin surface without friction, and are typically liquid or semiliquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and preferably, for the present purpose, comprise a liquid oily emulsion of the oil-in-water type. Lotions are preferred formulations herein for treating large body areas, because of the ease of applying a more fluid composition. It is generally necessary that the insoluble matter in a lotion be finely divided. Lotions will typically contain suspending agents to produce better dispersions as well as compounds useful for localizing and holding the active agent in contact with the skin, e.g., methylcellulose or sodium carboxymethyl-cellulose, or the like. A particularly preferred lotion formulation for use in conjunction with the present invention contains propylene glycol mixed with a hydrophilic petrolatum such as that which may be

obtained under the trademark Aquaphor.RTM. from Beiersdorf, Inc. (Norwalk, Conn.).

Creams containing the selected agent are, as known in the art, viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-
5 washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase, also sometimes called the "internal" phase, is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation, as explained in
10 Remington, supra, is generally a nonionic, anionic, cationic or amphoteric surfactant.

Gels formulations are preferred for application to the scalp. As will be appreciated by those working in the field of topical drug formulation, gels are semisolid, suspension-type systems. Single-phase gels contain organic macromolecules distributed substantially uniformly throughout the carrier liquid,
15 which is typically aqueous, but also, preferably, contain an alcohol and, optionally, an oil.

Shampoos may be formulated with the standard shampoo components, i.e., cleansing agents, thickening agents, and preservatives with the cleansing agent representing the primary ingredient, typically an anionic surfactant or a mixture of an
20 anionic and an amphoteric surfactant.

Various additives, known to those skilled in the art, may be included in the topical formulations of the invention. For example, solvents may be used to solubilize certain drug substances. Other optional additives include skin permeation enhancers, opacifiers, anti-oxidants, gelling agents, thickening agents, stabilizers, and the like.

Other agents may also be added, such as antimicrobial agents, antifungal agents, antibiotics and anti-inflammatory agents such as steroids.

In the preferred topical formulations of the invention, the active agent is present in an amount which is generally less than 10% by weight of the total composition, preferably less than about 1% by weight, and most preferably less than
5 about 0.1% by weight.

The topical compositions of the invention may also be delivered to the skin using a time-release mechanism. For example, "transdermal"-type patches, wherein the CD1d activation-blocking composition is contained within a laminated structure
10 that serves as a drug delivery device to be affixed to the skin. In such a structure, the drug composition is contained in a layer, or "reservoir," underlying an upper backing layer. The laminated structure may contain a single reservoir, or it may contain multiple reservoirs. In one embodiment, the reservoir comprises a polymeric matrix of a pharmaceutically acceptable contact adhesive material that serves to affix the
15 system to the skin during drug delivery. Examples of suitable skin contact adhesive materials include, but are not limited to, polyethylenes, polysiloxanes, polyisobutylenes, polyacrylates, polyurethanes, and the like. The particular polymeric adhesive selected will depend on the particular drug, vehicle, etc., i.e., the adhesive must be compatible with all components of the drug-containing composition. In an
20 alternative embodiment, the drug-containing reservoir and skin contact adhesive are present as separate and distinct layers, with the adhesive underlying the reservoir which, in this case, may be either a polymeric matrix as described above, or it may be a liquid or hydrogel reservoir, or may take some other form.

The backing layer in these laminates, which serves as the upper surface of the device, functions as the primary structural element of the laminated structure and provides the device with much of its flexibility. The material selected for the backing material should be selected so that it is substantially impermeable to the CD1d
5 activation-blocking composition and to any other components of the composition, thus preventing loss of any components through the upper surface of the device. The backing layer may be either occlusive or nonocclusive, depending on whether it is desired that the skin become hydrated during drug delivery. The backing is preferably made of a sheet or film of a preferably flexible elastomeric material. Examples of
10 polymers that are suitable for the backing layer include polyethylene, polypropylene, polyesters, and the like.

During storage and prior to use, the laminated structure preferably includes a release liner. Immediately prior to use, this layer is removed from the device to expose the basal surface thereof, either the drug reservoir or a separate contact
15 adhesive layer, so that the system may be affixed to the skin. The release liner should be made from a drug/vehicle impermeable material.

Such devices may be fabricated using conventional techniques, known in the art, for example by casting a fluid admixture of adhesive, drug and vehicle onto the backing layer, followed by lamination of the release liner. Similarly, the adhesive
20 mixture may be cast onto the release liner, followed by lamination of the backing layer. Alternatively, the drug reservoir may be prepared in the absence of drug or excipient, and then loaded by "soaking" in a drug/vehicle mixture.

As with the topical formulations of the invention, CD1d activation blocking composition contained within the reservoirs of these laminated system may contain a

number of components. In some cases, the blocking composition may be delivered "neat," i.e., in the absence of additional liquid. In most cases, however, the composition will be dissolved, dispersed or suspended in a suitable pharmaceutically acceptable vehicle, typically a solvent or gel. Other components which may be present
5 include preservatives, stabilizers, surfactants, and the like.

Preferably, the topical formulations and the laminated delivery systems also contain a skin permeation enhancer. A skin permeation enhancer can be co-administered. Suitable enhancers are well known in the art and include, for example, dimethylsulfoxide (DMSO), dimethyl formamide (DMF), N,N-dimethylacetamide
10 (DMA), decylmethylsulfoxide (C₁₀ MSO), C₂-C₆ alkanediols, and the 1-substituted azacycloheptan-2-ones, particularly 1-n-dodecylcyclazacycloheptan-2-one (available under the trademark Azone.RTM. from Whitby Research Incorporated, Richmond, Va.), alcohols, and the like.

The ointments, pastes, creams and gels also may contain excipients, such as
15 animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays also can contain excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary
20 propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The topical compositions according to this invention may also include one or more preservatives or bacteriostatic agents, e.g., methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chlorides, and the like. The topical

compositions also can contain other active ingredients such as antimicrobial agents, particularly antibiotics, anesthetics, analgesics, and antipruritic agents.

Antimicrobial agents that can be used in the topical administration of the present invention are those compatible with skin and soluble in solvents. In addition, antimicrobial agents are active against a broad spectrum of microorganisms, including but are not limited to, gram positive and gram negative bacteria, yeast, and mold. Examples of the antimicrobial agents include, but are not limited to, triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol which is also known as Irgasan.TM. DP 300 manufactured by Ciba-Geigy Corporation), hexetidine (5-amino-1,3-bis(2-ethylhexyl)-5-methyl-hexahydropyrimidine), chlorhexidine salts (salts of N,N"-Bis(4-chlorophenyl)-3,12-diimino-2,4,11,14-tetraazatetradecanediimidi amide), 2-bromo-2-nitropropane-1,3-diol, hexyresorcinol, benzalkonium chloride, cetylpyridinium chloride, alkylbenzyltrimethylammonium chlorides, iodine, phenol derivatives, povidone-iodine (polyvinylpyrrolidinone-iodine), parabens, hydantoin (2,4-imidazolidinedione), hydantoin derivatives (derivatives of 2,4-imidazolidinedione), phenoxyethanol, cis isomer of 1-(3-chloroallyl)-3,5,6-triaza-1-azoniaadamantane chloride (quarternium-15 which is also known as Dowicil 200 manufactured by Dow Chemical Company), diazolidinyl urea, benzethonium chloride, methylbenzethonium chloride, and mixtures thereof. Examples of hydantoin derivatives include, but are not limited to, dimethylol-5,5-dimethylhydantoin (glydant). Preferably, examples of the antimicrobial agents include triclosan, cis isomer of 1-(3-chloroallyl)-3,5,6-triaza-1-azoniaadamantane chloride (quarternium-15), hydantoin, hydantoin derivatives such as dimethylol-5,5-dimethylhydantoin (glydant), and mixtures thereof.

The percentage of the antimicrobial agents in the composition is about 0.01 wt. % to about 10 wt. %, and preferably, about 0.01 wt. % to about 2 wt. %.

In one embodiment such as where a latex condom or diaphragm is to be used, the CD1d activation block compositions can be included in ointments, foams, and
5 creams that can be used during sex. For example, they can be administered preferably prior to or just after sexual contact such as intercourse. One preferred composition would be a vaginal foam, including a spermicide, containing one of the compounds.

The topical compositions and drug delivery systems of the invention can be used in the prevention or treatment of the skin conditions identified above. When used
10 in a preventive (prophylactic) method, susceptible skin can be treated prior to exposure or just after exposure but any visible lesions on areas known to be susceptible to such lesions are observed. In treating skin conditions, it will be recognized by those skilled in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the condition being
15 treated, the form, route and site of administration, and the particular individual undergoing treatment, and that such optimums can be determined by conventional techniques. It will also be appreciated by one skilled in the art that the optimal dosing regimen, i.e., the number of doses can be ascertained using conventional course of treatment determination tests. Generally, a dosing regimen will involve administration
20 of the selected topical formulation at least once daily, and preferably one to four times daily, until the symptoms have subsided.

Systemic administration of a composition may be by oral, parenteral, sublingual, rectal such as suppository or enteral administration, or by pulmonary absorption. Parenteral administration may be by intravenous injection, subcutaneous

injection, intramuscular injection, intra-arterial injection, intrathecal injection, intra
peritoneal injection or direct injection or other administration to one or more specific
sites. When long term administration by injection is necessary, venous access devices
such as medi-ports, in-dwelling catheters, or automatic pumping mechanisms are also
5 preferred wherein direct and immediate access is provided to the arteries in and
around the heart and other major organs and organ systems.

Compositions may also be administered to the nasal passages as a spray.
Arteries of the nasal area provide a rapid and efficient access to the bloodstream and
immediate access to the pulmonary system. Access to the gastrointestinal tract, which
10 can also rapidly introduce substances to the blood stream, can be gained using oral
enema, or injectable forms of administration. Compositions may be administered as a
bolus injection or spray, or administered sequentially over time (episodically) such as
every two, four, six or eight hours, every day (QD) or every other day (QOD), or over
longer periods of time such as weeks to months. Compositions may also be
15 administered in a timed-release fashion such as by using slow-release resins and other
timed or delayed release materials and devices.

Where systemic administration is desired, orally active compositions are
preferred as oral administration is a convenient and economical mode of drug
delivery. Oral compositions may be poorly absorbed through the gastrointestinal
20 lining. Compounds which are poorly absorbed tend to be highly polar. Preferably,
such compositions are designed to reduce or eliminate their polarity. This can be
accomplished by known means such as formulating the oral composition with a
complimentary reagent which neutralizes its polarity, or by modifying the compound
with a neutralizing chemical group. Preferably, the molecular structure is similarly

modified to withstand very low pH conditions and resist the enzymes of the gastric mucosa such as by neutralizing an ionic group, by covalently bonding an ionic interaction, or by stabilizing or removing a disulfide bond or other relatively labile bond.

5 Treatments to the patient may be therapeutic or prophylactic. Therapeutic treatment involves administration of one or more compositions of the invention to a patient suffering from one or more symptoms of the disorder. Relief and even partial relief from one or more symptoms can correspond to an increased life span or simply an increased quality of life. Further, treatments that alleviate a pathological symptom
10 can allow for other treatments to be administered.

The term “compatible”, as used herein, means that the components of the compositions are capable of being commingled with the CD1d blocking agents of the present invention, and with each other, in a manner such that does not substantially impair the desired efficacy.

15 Doses of the pharmaceutical compositions of the invention will vary depending on the subject and upon the particular route of administration used. Dosages can range from 0.1 to 100,000 $\mu\text{g}/\text{kg}$ per day, more preferably 1 to 10,000 $\mu\text{g}/\text{kg}$. By way of an example only, an overall dose range of from about, for example, 1 microgram to about 300 micrograms might be used for human use. This dose can
20 be delivered at periodic intervals based upon the composition.

EXAMPLES

Animals

8-12 week old female C57Bl/6 mice were purchased from Taconic Farms, Inc. (Germantown, NY). The CD1d-null allele (generated in a 129 x C57Bl/6 founder
5 (25) (26) was backcrossed 7 generations into the C57Bl/6 strain. (15) Homozygous CD1d deficient mice were obtained from littermate pairings and used to generate heterozygote CD1d deficient and wild type controls. Breeding pairs of mice deficient for CD1d on the BALB/c background were purchased from Jackson Labs (Bar Harbor, ME). The C57Bl/6 and BALB/c J 281 null mice were established by
10 specific deletion of the J 281 gene segment(27) (28). All mouse experiments were approved and conducted under IACUC guidelines.

Reagents

Contact hapten, 4-ethoxymethylene-2-phenyl-2-phenyl-2-oxazolin-5-one (oxazolone) was purchased from SIGMA, GalCer [(2S, 3S, 4R)-1-O-(D-
15 galactopyranosyl)-2-(N-hexacosanoylamino)-1,3,4-octadecanetriol was kindly provided by Pharmaceutical Research Laboratories, Kirin Brewery, Gunma, Japan, and CD1d-binding antagonists Polyethylene glycol (PEG)₂₀₀₀ dipalmitoyl-L-phosphatidylethanolamine (DPPE-PEG) and (PEG)₂₀₀₀ ceramide were purchased from Northern Lipids, Inc.

20 Contact hypersensitivity

Mice at least seven weeks of age were sensitised epicutaneously on day 0 with 70 µl of 4% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone, Sigma, St. Louis, MO) in acetone/olive oil (4/1) and challenged five days later on the right ear

with 20 μ l of 0.5% oxazolone or carrier only on the left ear. The hapten-specific increase in ear thickness at 24 hours was determined with a micrometer. For experiments using DPPE-PEG, 4% oxazolone in acetone/olive oil (4/1) solutions with or without DPPE-PEG (in dosages ranging from 5mg/ml to 100 mg/ml) were applied.

- 5 Finally, a mixture of 0.5% oxazolone and 50mg/ml DPPE-PEG was used for the inhibition of oxazolone recall.

Analysis of invariant V α 14J α 281 TCR frequency. Total RNA was isolated from spleens of individual mice using TRIZOL (Gibco BRL, Grand Island, New York) according to the manufacturer's recommendations. First-strand cDNA
10 synthesis was performed using oligo(dT) as a primer for reverse transcription of 2 μ g of total RNA in a 50 μ l reaction mixture using MMLV-RT (Life Technologies, GIBCO-BRL, Gaithersburg, MA). Quantitative analysis of V α 14J α 281 T cell frequency was done using multiplex RT-PCR by comparing the intensity of the TCR α -chain CDR3 band with the invariant INKT cell specific band, as previously
15 described (29).

Treatment protocols

Mice received a series of 5 intraperitoneal injections of either 2 μ g of α ManCer (2 μ g i.p. diluted in a solution of phosphate buffered saline and 0.5% Tween-20 every other day, starting 3 days prior to skin sensitization and continuing
20 through to re-challenge.

Statistical analysis

Significant differences between groups were evaluated with a Mann-Whitney test, or where appropriate, a two tailed Student's t test. Mean differences were considered significant when $p < 0.05$.

Contact hypersensitivity is ablated both in CD1d knock out and in iNKT cell-
5 deficient J α 281 knock out mice. Mice were sensitized epicutaneously on day 0 with oxazolone and challenged six days later on the ear with oxazolone or carrier only, as described above. The hapten-specific increase in ear thickness at 24 hours was determined with a micrometer. (See Figure 1).

To examine the functional importance of CD1d restricted iNKT cells in CHS
10 we measured the ability of iNKT cell-deficient mice to generate contact hypersensitivity to oxazolone. This reaction is a form of delayed-type hypersensitivity in which hapten-protein conjugates are presented by cutaneous dendritic cells, followed by their migration to regional lymph nodes, to hapten-specific CD4 and CD8 positive T lymphocytes (1)(2)(30)(3). Upon secondary
15 hapten challenge, sensitized T cells initiate a local inflammatory response. In this system, activation of iNKT cells by CD1d on skin DC is thought to be an important initiation step followed by the elaboration of cytokines such as IL-4 (10) (31). Interestingly, contact hypersensitivity to oxazolone was significantly impaired in iNKT cell-deficient mice on both the C57Bl/6 and Balb/c backgrounds (Figure 1),
20 revealing a requirement for CD1d-restricted T cells for maximal hapten-specific immunity.

Systemical administration of an inactive CD1d binding lipid, α -ManCer inhibits contact hypersensitivity to oxazolone. Since CD1d- or J α 281-null mice had impaired CHS responses, we examined whether activation or inhibition of iNKT cells

with the prototypic CD1d-dependent glycolipid agonist α -GalCer or antagonist α -ManCer, a glycolipid that is known to bind CD1d but fails to activate iNKT cells, would modulate CSH responses. Mice were treated with the α -GalCer or α -ManCer by i.p. injections administered prior to and during the sensitization phase with

5 oxazolone. Pretreatment of mice with α -ManCer inhibited the CSH response by 50% when compared to the control group (Figure 2). Conversely, administration of α -GalCer did not alter the CSH response to oxazolone. Thus, CHS responses were not augmented by iNKT cell activation, but could be significantly inhibited by systemic treatment with CD1d antagonists previously demonstrated not to activate iNKT cells

10 in vivo and in vitro.

Topical DPPE-PEG (a CD1d-binding lipid) inhibits oxazolone-induced contact hypersensitivity (Figure 3). Mice were sensitized epicutaneously on day 0 with oxazolone with or without DPPE-PEG (100 mg/ml) and challenged five days later on the ear with oxazolone or carrier only, as described above. The hapten-

15 specific increase in ear thickness at 24 hours was determined with a micrometer.

Topical administration of iNKT antagonist lipids inhibited the generation of CHS. Increasing doses of DPPE-PEG inhibit oxazolone-induced hypersensitivity in a dose-dependent manner, as shown in Figure 4A. We evaluated whether administration of CD1d antagonists could be used topically to block the generation of

20 CSH responses. Since a synthetically modified lipid, DPPE-PEG, was recently demonstrated to be a significantly more soluble and effective competitor of α -GalCer presentation than α -ManCer and was available in pharmacologic quantities, (22) this reagent was chosen for use as a topical inhibitor of CSH. In control experiments systemic administration of DPPE-PEG or PEG-ceramide inhibited CHS as effectively

as α -ManCer (data not shown). For these experiments, wild type C57Bl/6 mice were sensitized with oxazolone mice in the presence of increasing doses of DPPE-PEG. Half-maximal inhibition of CSH was achieved at 25 mg/ml of DPPE-PEG (Figure 4A) and CSH could be inhibited in a dose response fashion.

5 Figure 4B shows that topical DPPE-PEG inhibits contact hypersensitivity during recall and priming. Inclusion of DPPE-PEG can inhibit recall to oxazolone even after priming with the agent. To determine whether the inhibitory effect of DPPE-PEG was dependent on when the inhibitor was used, oxazolone-induced CSH responses were tested when DPPE-PEG was present at both phases, or only during
10 priming or recall. Contact hypersensitivity responses were maximally inhibited by the inclusion of DPPE-PEG during both the challenge and recall portions of the experiment (Figure 4B). Notably, the CSH responses were also inhibited, but to a lesser extent, when the reagent was used only during the challenge or recall phases of the response. Importantly, the effect was specific for DPPE-PEG since neither
15 palmitic acid, nor PEG alone were effective inhibitors of CSH (Figure 2 and data not shown).

Inhibition of CSH by DPPE-PEG is strain independent (Figure 5). The CD1d antigen presentation system can respond rapidly to cues in the environment and this function has been conserved since the evolutionary divergence of rodents and humans
20 (32) (24). To test whether the effect of DPPE-PEG was MHC or strain dependent, oxazolone-induced CSH was determined for 4 additional MHC-unrelated congenic mouse strains in the presence or absence of the inhibitor. The addition of DPPE-PEG was an effective inhibitor in all strains tested (Figure 5). There was, however, significant strain variability in both the magnitude of the responses to oxazolone and

the ability of DPPE-PEG to inhibit CSH. Therefore, DPPE-PEG was an effective inhibitor of oxazolone-induced CSH in a MHC-independent fashion and in all strains tested.

CSH reactions were inhibited in mice deficient in iNKT cells directly
5 confirming an important role for iNKT cells in CSH. Furthermore, CHS was specifically inhibited in those mice that were treated with CD1d-binding antagonists. Thus, blockade of the CD1d system could be used to treat ACD in an antigen- and MHC-independent fashion.

REFERENCES

1. Eisen, H.N., L. Orris, and S. Belman. 1952. Elicitation of delayed allergic skin reactions with haptens: the dependence of elicitation on hapten combination with protein. *Journal of Experimental Medicine* 95:473.
- 5 2. Silberberg, I., R.L. Baer, and S.A. Rosenthal. 1976. The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. *J Invest Dermatol* 66, no. 4:210.
3. Gocinski, B.L., and R.E. Tigelaar. 1990. Roles of CD4+ and CD8+ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. *J*
10 *Immunol* 144, no. 11:4121.
4. Dieli, F., M. Taniguchi, G.L. Asherson, G. Sireci, N. Caccamo, E. Scire, C.T. Bonanno, and A. Salerno. 1998. Development of hapten-induced IL-4-producing CD4+ T lymphocytes requires early IL-4 production by alphabeta T lymphocytes carrying invariant V(alpha)14 TCR alpha chains. *Int Immunol* 10, no. 4:413.
- 15 5. Ptak, W., and P.W. Askenase. 1992. Gamma delta T cells assist alpha beta T cells in adoptive transfer of contact sensitivity. *J Immunol* 149, no. 11:3503.
6. Yokozeki, H., K. Watanabe, K. Igawa, Y. Miyazaki, I. Katayama, and K. Nishioka. 2001. Gammadelta T cells assist alphabeta T cells in the adoptive transfer of contact hypersensitivity to para-phenylenediamine. *Clin Exp Immunol* 125, no.
20 3:351.
7. Medzhitov, R., and C. Janeway, Jr. 2000. Innate immune recognition: mechanisms and pathways. *Immunol Rev* 173:89.

8. Watanabe, N., K. Ikuta, S. Fagarasan, S. Yazumi, T. Chiba, and T. Honjo. 2000. Migration and differentiation of autoreactive B-1 cells induced by activated gamma/delta T cells in antierythrocyte immunoglobulin transgenic mice. *J Exp Med* 192, no. 11:1577.
- 5 9. Ishii, N., K. Takahashi, H. Nakajima, S. Tanaka, and P.W. Askenase. 1994. DNFB contact sensitivity (CS) in BALB/c and C3H/He mice: requirement for early occurring, early-acting, antigen-specific, CS-initiating cells with an unusual phenotype (Thy-1+, CD5+, CD3-, CD4-, CD8-, sIg-, B220+, MHC class II-, CD23+, IL-2R-, IL-3R+, Mel-14-, Pgp-1+, J11d+, MAC-1+, LFA-1+, and Fc gamma RII+). *J Invest Dermatol* 102, no. 3:321.
- 10 10. Askenase, P.W. 2001. Yes T cells, but three different T cells (alphabeta, gammadelta and NK T cells), and also B-1 cells mediate contact sensitivity. *Clin Exp Immunol* 125, no. 3:345.
11. Porcelli, S.A., and R.L. Modlin. 1999. The CD1 system: antigen-presenting
15 molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* 17:297.
12. Godfrey, D.I., K.J. Hammond, L.D. Poulton, M.J. Smyth, and A.G. Baxter. 2000. NKT cells: facts, functions and fallacies. *Immunol Today* 21, no. 11:573.
13. Baxter, A.G., S.J. Kinder, K.J.L. Hammond, R. Scollay, and D.I. Godfrey. 1999. Association between $\alpha\beta$ TCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt
20 mice. *Diabetes* 46:572.
14. Wilson, S.B., S.C. Kent, K.T. Patton, T. Orban, R.A. Jackson, M. Exley, S. Porcelli, D.A. Schatz, M.A. Atkinson, S.P. Balk, J.L. Strominger, and D.A. Hafler.

1998. Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. *Nature* 391, no. 6663:177.
15. Naumov, Y.N., K.S. Bahjat, R. Gausling, R. Abraham, M.A. Exley, Y. Koezuka, S.B. Balk, J.L. Strominger, M. Clare-Salzer, and S.B. Wilson. 2001. 5 Activation of CD1d-restricted T cells protects NOD mice from developing diabetes by regulating dendritic cell subsets. *Proc Natl Acad Sci U S A* 98, no. 24:13838.
16. Cui, J., T. Shin, T. Kawano, H. Sato, E. Kondo, I. Toura, Y. Kaneko, H. Koseki, M. Kanno, and M. Taniguchi. 1997. Requirement for V α 14 NKT cells in IL-12-mediated rejection of tumors. *Science* 278:1623.
- 10 17. Smyth, M.J., K.Y.T. Thia, S.E.A. Street, E. Cretney, J.A. Trapani, M. Taniguchi, K. Tetsu, S.B. Pelikan, N.Y. Crowe, and D.I. Godfrey. 2000. Differential Tumor Surveillance by Natural Killer (NK) and NKT Cells. *J. Exp. Med.* 191, no. 4:661.
18. Kitamura, H., K. Iwakabe, T. Yahata, S. Nishimura, A. Ohta, Y. Ohmi, M. Sato, K. Takeda, K. Okumura, L. Van Kaer, T. Kawano, M. Taniguchi, and T. 15 Nishimura. 1999. The natural killer T (NKT) cell ligand alpha-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)- 12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J Exp Me* 189, no. 7:1121.
19. Tomura, M., W.-G. Yu, H.-J. Ahn, M. Yamashita, Y.-F. Yang, S. Ono, T. 20 Hamaoka, T. Kawano, M. Taniguchi, Y. Koezuka, and H. Fujiwara. 1999. A Novel Function of Valpha14+CD4+NKT Cells: Stimulation of IL-12 Production by Antigen-Presenting Cells in the Innate Immune System. *J. Immunol* 163:93.

20. Toura, I., T. Kawano, Y. Akutsu, T. Nakayama, T. Ochiai, and M. Taniguchi
1999. Cutting edge: inhibition of experimental tumor metastasis by dendritic cells
pulsed with alpha-galactosylceramide. *J Immunol* 163, no. 5:2387.
21. Zeng, Z.-H., A.R. Castano, B.W. Segelke, E.A. Stura, P.A. Peterson, and I.A.
5 Wilson. 1997. Crystal structure of mouse CD1:an MHC-like fold with a large
hydrophobic binding groove. *Science* 277, no. 5324:339.
22. Naidenko, O.V., J.K. Maher, W.A. Ernst, T. Sakai, R.L. Modlin, and M.
Kronenberg. 1999. Binding and Antigen Presentation of Ceramide-containing
glycolipids by Soluble Mouse and Human CD1d Molecules. *J. Exp. Med.* 190, no.
10 8:1069.
23. Joyce, S., A.S. Woods, J.W. Yewdell, J.R. Bennink, A.D. De Silva, A.
Boesteanu, S.P. Balk, R.J. Cotter, and R.R. Brutkiewicz. 1998. Natural ligand of
mouse CD1d1: cellular glycosylphosphatidylinositol. *Science* 279, no. 5356:1541.
24. Brossay, L., and M. Kronenberg. 1999. Highly conserved antigen-presenting
15 function of CD1d molecules. *Immunogenetics* 50, no. 3-4:146.
25. Sonoda, K.H., M. Exley, S. Snapper, S.P. Balk, and J. Stein-Streilein. 1999.
CD1-reactive natural killer T cells are required for development of systemic
tolerance through an immune-privileged site [see comments]. *J Exp Med* 190, no.
9:1215.
- 20 26. Exley, M.A., N.J. Bigley, O. Cheng, S.M. Tahir, S.T. Smiley, Q.L. Carter, H.F.
Stills, M.J. Grusby, Y. Koezuka, M. Taniguchi, and S.P. Balk. 2001. CD1d-reactive T
cell activation leads to amelioration of disease caused by diabetogenic
encephalomyocarditis virus. *J Leukoc Biol* 69, no. 5:713.

27. Smiley, S.T., M.H. Kaplan, and M.J. Grusby. 1997. Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. *Science* 275:977.
28. Kawano, T., J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, H. Koseki, and M. Taniguchi. 1997. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosceramides. *Science* 278:1626.
29. Gorski, J., M. Yassai, X. Zhu, B. Kissela, B. Kissella, C. Keever, and N. Flomenberg. 1994. Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. *J Immunol* 152, no. 10:5109.
30. Bacci, S., P. Alard, R. Dai, T. Nakamura, and J.W. Streilein. 1997. High and low doses of haptens dictate whether dermal or epidermal antigen-presenting cells promote contact hypersensitivity. *Eur J Immunol* 27, no. 2:442.
31. Gerlini, G., H.P. Hefti, M. Kleinhans, B.J. Nickoloff, G. Burg, and F.O. Nestle. 2001. Cd1d is expressed on dermal dendritic cells and monocyte-derived dendritic cells. *J Invest Dermatol* 117, no. 3:576.
32. Bendelac, A., M.N. Rivera, H.-S. Park, and J.H. Roark. 1997. Mouse CD1-Specific NK1 T Cells: Development, Specificity, and Function. *Annual Review of Immunology* 15:535.
33. Hopkin, J.M. 1997. Mechanisms of enhanced prevalence of asthma and atopy in developed countries. *Curr Opin Immunol* 9, no. 6:788.

34. Akdis, C.A., M. Akdis, A. Trautmann, and K. Blaser. 2000. Immune regulation in atopic dermatitis. *Curr Opin Immunol* 12, no. 6:641.
35. Girolomoni, G., S. Sebastiani, C. Albanesi, and A. Cavani. 2001. T-cell subpopulations in the development of atopic and contact allergy. *Curr Opin Immunol* 13, no. 6:733.
36. Apostolou, I., Y. Takahama, C. Belmant, T. Kawano, M. Huerre, G. Marchal, J. Cui, M. Taniguchi, H. Nakauchi, J.J. Fournie, P. Kourilsky, and G. Gachelin. 1999. Murine natural killer T(NKT) cells [correction of natural killer cells] contribute to the granulomatous reaction caused by mycobacterial cell walls. *Proc Natl Acad Sci U S A* 96, no. 9:5141.
37. Mempel, M., B. Flageul, F. Suarez, C. Ronet, L. Dubertret, P. Kourilsky, G. Gachelin, and P. Musette. 2000. Comparison of the T cell patterns in leprous and cutaneous sarcoid granulomas. Presence of Valpha24-invariant natural killer T cell in T- cell-reactive leprosy together with a highly biased T cell receptor Valpha repertoire. *Am J Pathol* 157, no. 2:509.
38. Mempel, M., C. Ronet, F. Suarez, M. Gilleron, G. Puzo, L. Van Kaer, A. Lehuen, P. Kourilsky, and G. Gachelin. 2002. Natural Killer T Cells Restricted by the Monomorphic MHC Class Ib CD1d1 Molecules Behave Like Inflammatory Cells. *Journal of Immunology* 168:365.
39. Sebastiani, S., P. Allavena, C. Albanesi, F. Nasorri, G. Bianchi, C. Traidl, S. Sozzani, G. Girolomoni, and A. Cavani. 2001. Chemokine receptor expression and function in CD4+ T lymphocytes with regulatory activity. *J Immunol* 166, no. 2:996.

40. Cavani, A., F. Nasorri, C. Prezzi, S. Sebastiani, C. Albanesi, and G. Girolomoni. 2000. Human CD4⁺ T lymphocytes with remarkable regulatory functions on dendritic cells and nickel-specific Th1 immune responses. *J Invest Dermatol* 114, no. 2:295.
- 5 41. Bendelac, A., R.D. Hunziker, and O. Lantz. 1996. Increased interleukin 4 and immunoglobulin E production in transgenic mice overexpressing NK1 T cells. *Journ of Experimental Medicine* 184:1285.
42. Mendiratta, S.K., W.D. Martin, S. Hong, A. Boesteanu, S. Joyce, and L. Van Kaer. 1997. CD1d1 mutant mice are deficient in natural T cells that promptly
10 produce IL-4. *Immunity* 6:469.
43. Wilson, S.B., S.C. Kent, H.F. Horton, A.A. Hill, P.L. Bollyky, D.A. Hafler, J.L. Strominger, and M.C. Byrne. 2000. Multiple differences in gene expression in regulatory V α 24J α Q T cells from identical twins discordant for type I diabetes. *Proc Natl Acad Sci U S A* 97, no. 13:7411.
- 15 44. Yang, O.O., F.K. Racke, P.T. Nguyen, R. Gausling, M.E. Severino, H.F. Horton, M.C. Byrne, J.L. Strominger, and S.B. Wilson. 2000. CD1d on Myeloid Dendritic Cells Stimulates Cytokine Secretion and Cytolytic Activity of V α 24J α Q T Cells: A Feedback Mechanism for Immune Regulation. *J. Immunol.* 165, no. 7:3756.
- 20 45. Exley, M., J. Garcia, S.P. Balk, and S. Porcelli. 1997. Requirements for CD1d Recognition by Human Invariant V α 24⁺ CD4-CD8⁻ T Cells. *The Journal of Experimental Medicine* 186:1.

46. Kadowaki, N., S. Antonenko, S. Ho, M.C. Rissoan, V. Soumelis, S.A. Porcell
L.L. Lanier, and Y.J. Liu. 2001. Distinct cytokine profiles of neonatal natural killer
cells after expansion with subsets of dendritic cells. *J Exp Med* 193, no. 10:1221.
47. Ikarashi, Y., R. Mikami, A. Bendelac, M. Terme, N. Chaput, M. Terada, T.
5 Turz, E. Angevin, F.A. Lemonnier, H. Wakasugi, and L. Zitvogel. 2001. Dendritic
Cell Maturation Overrides H-2D-mediated Natural Killer T (NKT) Cell Inhibition:
Critical Role for B7 in CD1d-dependent NKT Cell Interferon gamma Production.
Journal of Experimental Medicine 194, no. 8:1179.
48. Rissoan, M.C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. de Waal
10 Malefyt, and Y.J. Liu. 1999. Reciprocal control of T helper cell and dendritic cell
differentiation [see comments]. *Science* 283, no. 5405:1183.
49. Shreedhar, V., A.M. Moodycliffe, S.E. Ullrich, C. Bucana, M.L. Kripke, and I
Flores-Romo. 1999. Dendritic cells require T cells for functional maturation in vivo
Immunity 11, no. 5:625.

15

All references described herein are incorporated herein by reference.

We claim:

1. A composition that comprises (a) an agent that attenuates CD1d-restricted NK T cells responses, wherein the agent is selected from the group consisting of antagonists that bind CD1d and inhibit activation of CD1d-restricted NK T cells, agents that block CD1d-specific receptors and CD1d decoys and (b) a pharmaceutically acceptable carrier or diluent.
2. The composition of claim 1, wherein the composition is adapted for topical administration.
3. The composition of claim 1, wherein the agent is a phospholipid that binds CD1d without activating NK T cells.
4. The composition of claim 4, wherein the phospholipid is selected from the group consisting of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine (DPPE), 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Poly(ethylene glycol) 2000] (DPPE-PEG), and phosphatidyl inositol.
5. The composition of claim 1, wherein the agent is a glycolipid.
6. The composition of claim 5, wherein the glycolipid is selected from the group consisting of ceramide, α Man Cer, and β Gal Cer.
7. A method of attenuating CD1d-restricted NK T cells responses, comprising administering to a subject a composition that comprises an agent that attenuates CD1d-restricted NK T cells responses, wherein the agent is selected from the group consisting of antagonists that bind CD1d and inhibit activation of CD1d-restricted NK T cells, agents that block CD1d-specific receptors on NK T cells, and decoys and mimics.

8. The method of claim 7, wherein the CD1d-restricted NK T cells responses are associated with skin disorders due to hyperactive CD1d-restricted T cell responses and other disorders associated with ongoing CD1d-restricted immune responses.
- 5 9. The method of claim 8, wherein the skin disorders include contact dermatitis and further eczematous dermatitises, atopic dermatitis, seborrheic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinphilias, Lupus erythematosus, and Alopecia areata.
- 10 10. The method of claim 7, wherein the CD1d-restricted NK T cells responses are associated with psoriasis.
11. The method of claim 8, wherein the skin disorder is contact dermatitis.
12. The method of claim 7, wherein the composition is present in a pharmaceutical composition, moisturizing composition or a cosmetic composition.
- 15 13. The composition of claim 7, wherein the agent is a phospholipid that binds CD1d without activating NK T cells.
14. The composition of claim 13, wherein the phospholipid is selected from the group consisting of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine (DPPE) and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Poly(ethylene glycol) 2000] (DPPE-PEG).
- 20 15. The composition of claim 7, wherein the agent is a glycolipid.
16. The composition of claim 15, wherein the glycolipid is selected from the group consisting of ceramide, α Man Cer, and β Gal Cer.

17. A method of preventing or reducing a CD1d-restricted NK T cells responses, comprising administering to a subject prior to exposure to an irritant a composition that comprises an agent that attenuates CD1d-restricted NK T cells responses, wherein the agent is selected from the group consisting of antagonists that
5 bind CD1d and inhibit activation of CD1d-restricted NK T cells, agents that block CD1d-specific receptors on NK T cells, and decoys and mimics.

18. The method of claim 17, wherein the irritant is selected from the group consisting of poison ivy, poison oak, poison sumac, insecticides, dye intermediates, resins, detergents, antioxidants, rubber, latex, metals, fragrances, chemicals,
10 cosmetics, textiles, plastics, pollen, therapeutic agents, skin cleansers, industrial cleaning agents, alkalis, acids, oils, organic solvents, oxidizing agents, reducing agents, plant matter, and animal matter.

19. The method of claim 7, wherein the composition is administered by topical administration.

15 20. The method of claim 7, wherein the composition is administered by systemic administration.

21. The method of claim 20, wherein the compositions are administered to a subject with an autoimmune disease such as lupus or at high risk for spontaneously aborting pregnancies.

20 22. A method of preventing and/or inhibiting a CD1d associated skin disorder comprising administering to a subject at risk for the activated CD1d associated skin disorder, an effective amount of the compound of claim 1.

23. The method of claim 22, wherein the CD1d associated skin disorder is selected from the group consisting of contact dermatitis, eczematous dermatitises,

atopical dermatitis, seborrheic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinphilias, Lupus erythematosus, psoriasis and Alopecia areata.

1/5

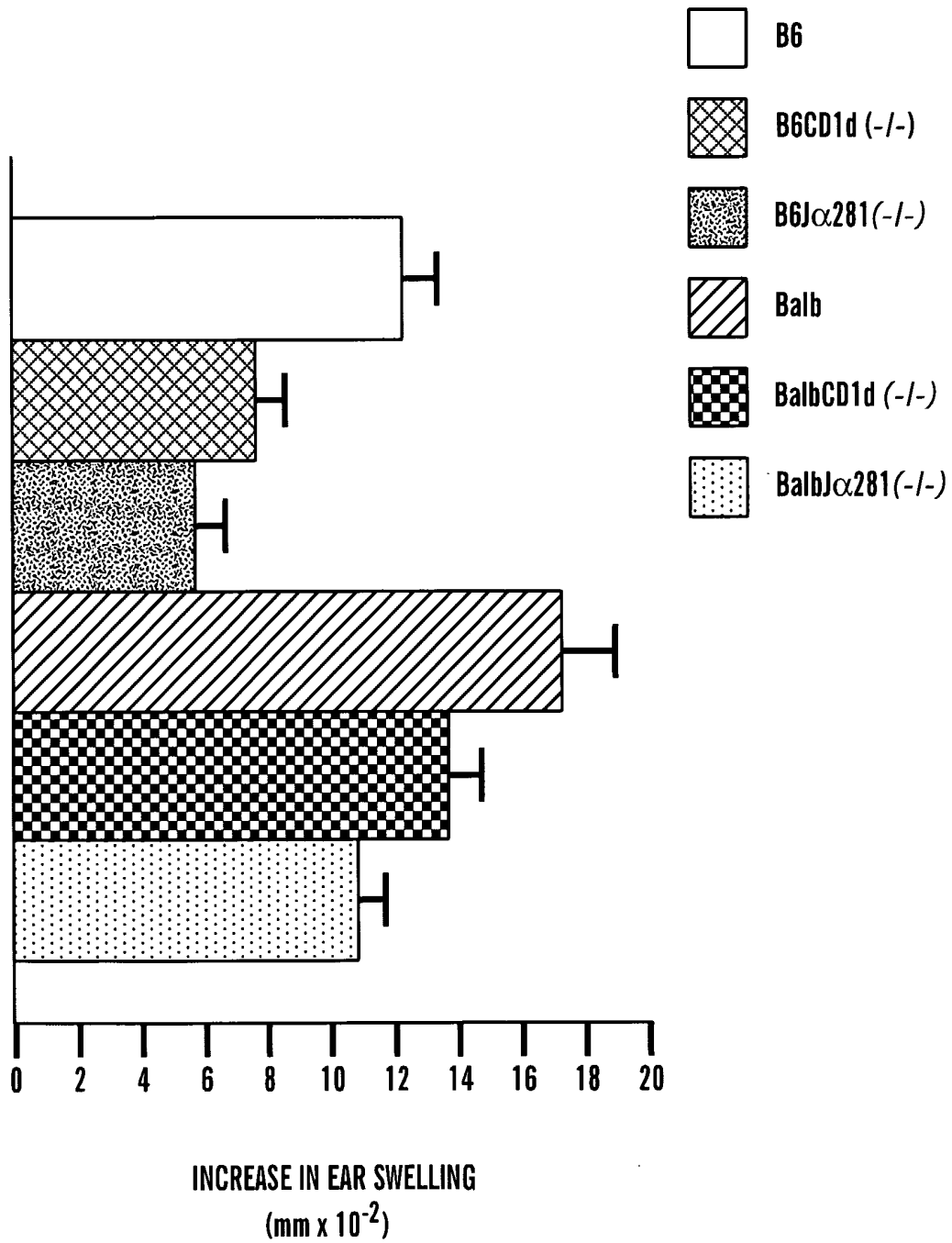


FIG. 1

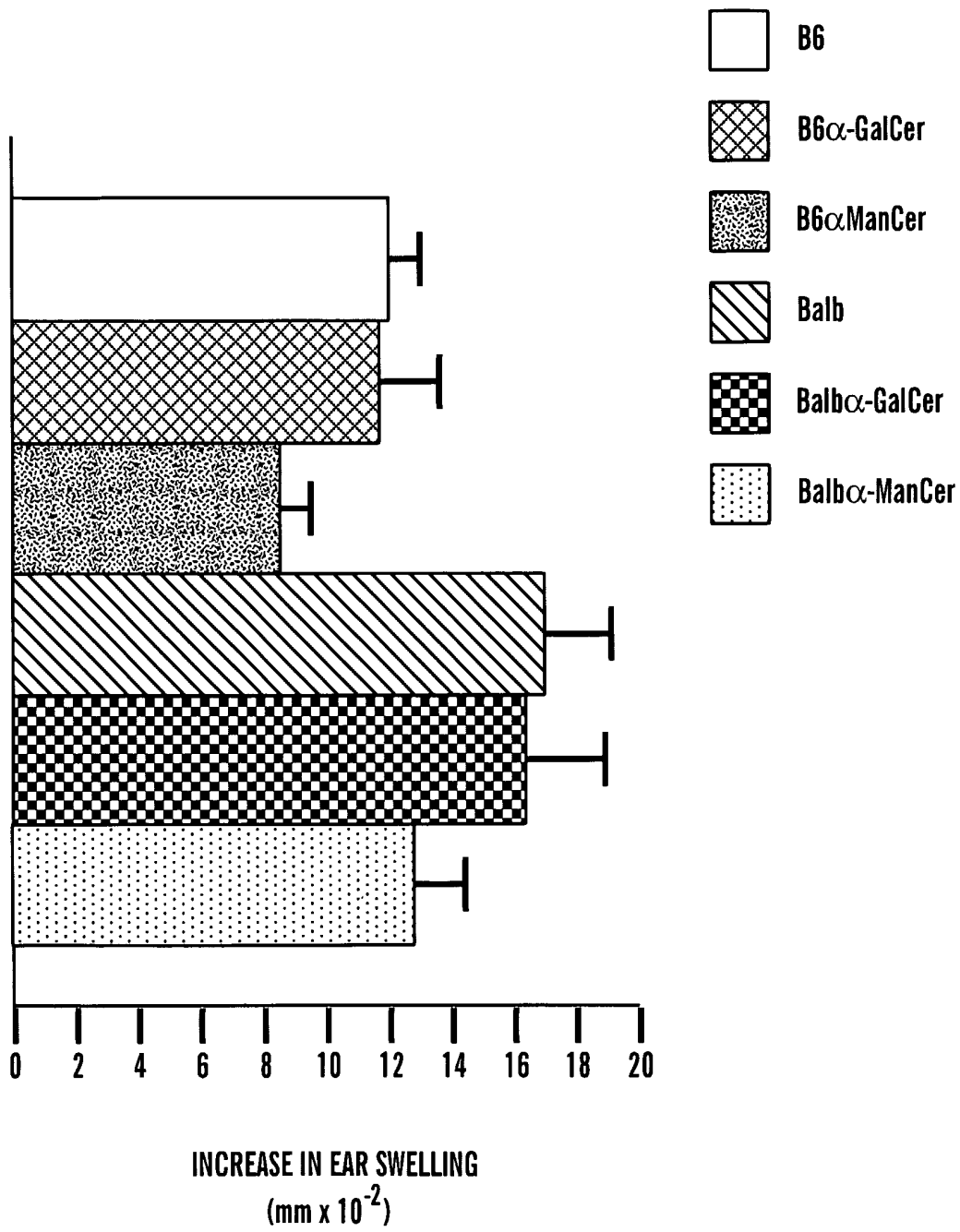


FIG. 2

**INHIBITION OF OXAZALONE-INDUCED CONTACT HYPERSENSITIVITY
BY A CD1d-BINDING LIPID ANTAGONIST**

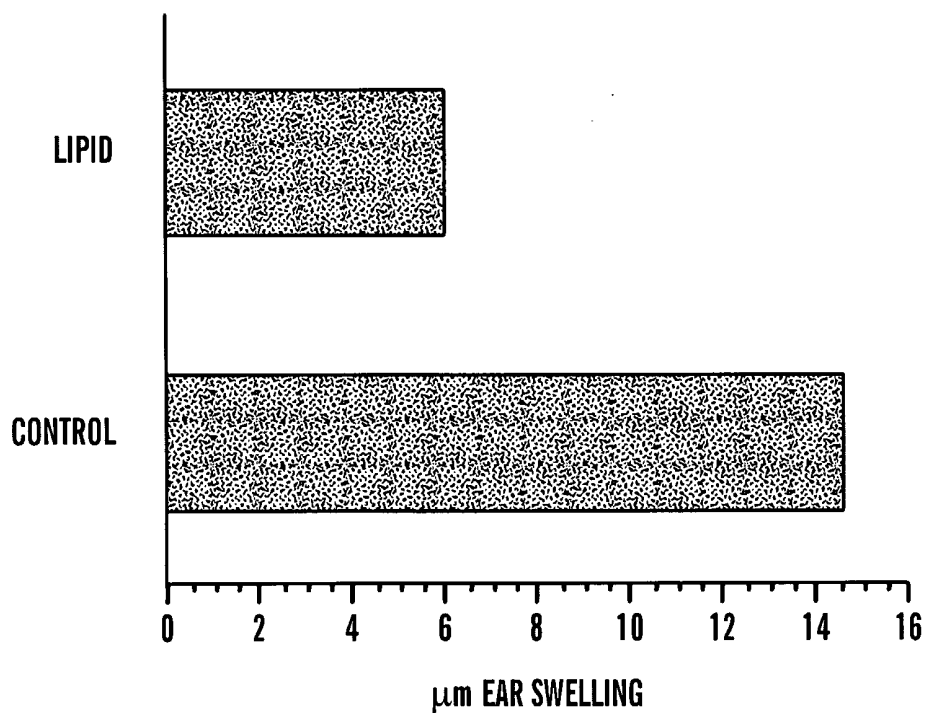


FIG. 3

4/5

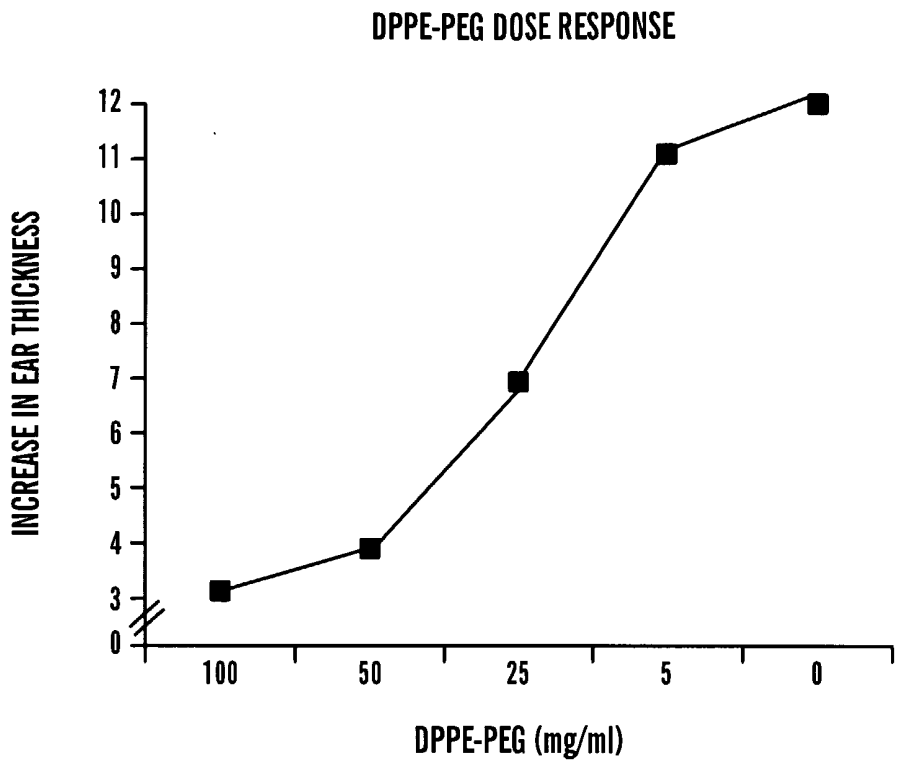


FIG. 4A

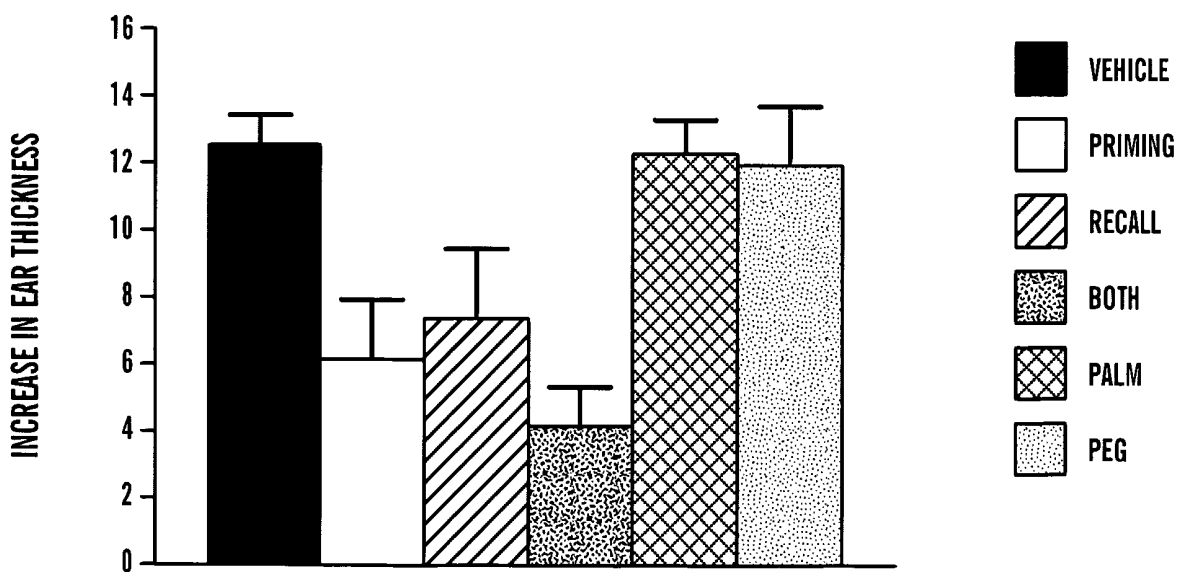


FIG. 4B

5/5

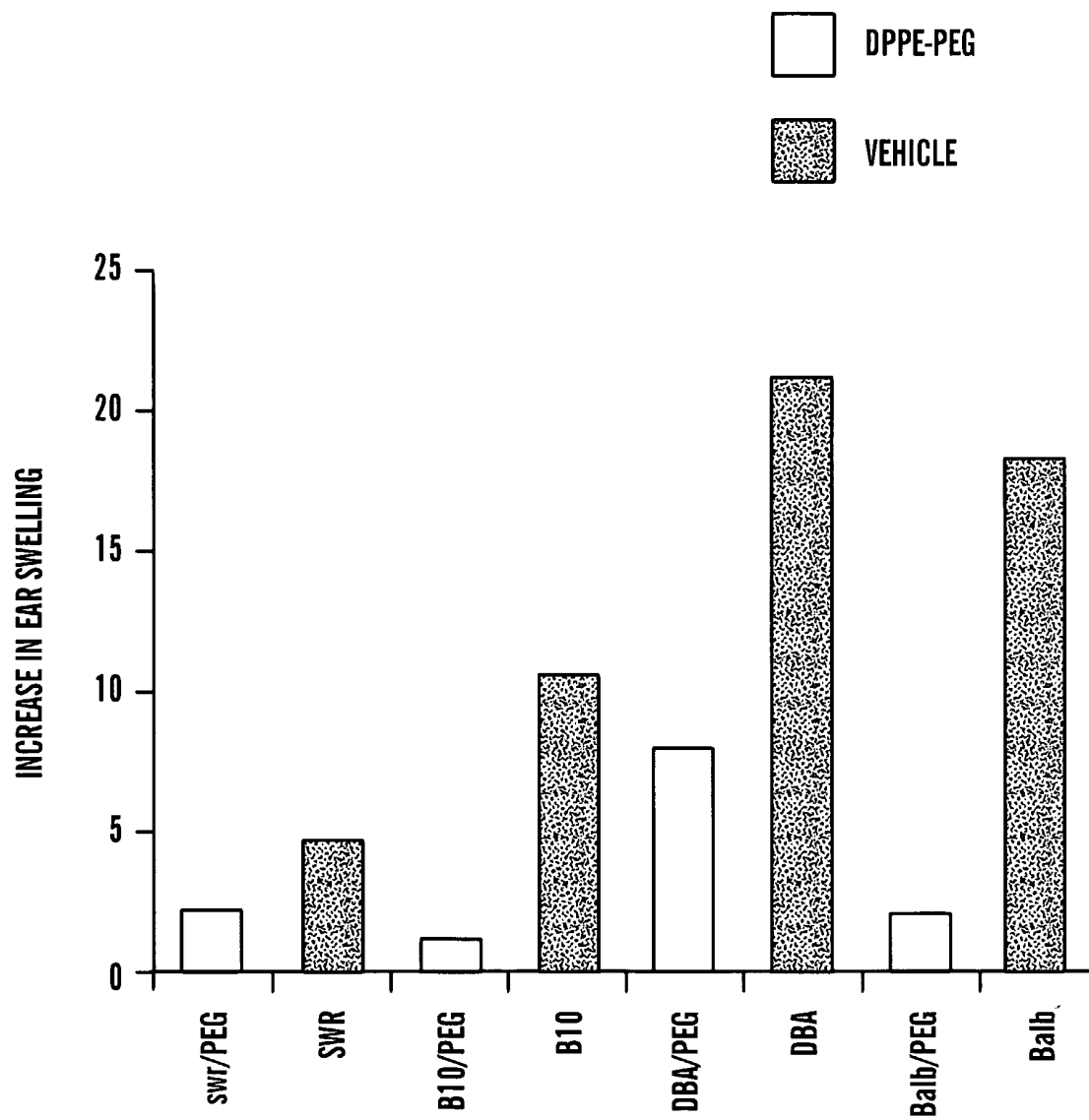


FIG. 5