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(54) Title: COLLAGEN-BASED METHODS AND FORMULATIONS FOR THE TREATMENT OF IMMUNE SYSTEM-MEDIATED DISEASES

(57) Abstract

The invention provides novel methods and compositions for the treatment of immune system-mediated diseases, including rheumatoid arthritis. The subject compositions comprise one or more different types of collagen or collagen derivatives and a mucosa binding structure. Specific combinations of collagen and/or collagen derivatives may be used to treat specific immune system-mediated diseases. The collagen(s) and/or collagen(s) derivatives used in the subject compositions may be either obtained from natural sources or produced by recombinant genetic engineering techniques and/or chemical modification means. Another aspect of the invention is to provide methods for treating immune system-mediated diseases by administering an effective amount of the subject collagen-containing compositions. The methods of the invention involve the oral administration of a collagen or collagens found in a specific tissue so as to induce the suppression (immunological tolerance) of inflammation against the tissue from which the collagen is found to occur in nature. The methods of the invention include the administration of the subject collagen(s) and/or collagen derivative(s) containing compositions into the intestines so as to induce immune tolerance, e.g., oral administration.

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COLLAGEN-BASED METHODS AND FORMULATIONS FOR THE TREATMENT OF IMMUNE SYSTEM-MEDIATED DISEASES

1. FIELD OF THE INVENTION

The invention is in the field of autoimmune disease and other immune system-mediated disease treatments employing collagen and/or derivatives thereof.

2. RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application Serial No. 08/370,388, which was filed on January 10, 1995.

3. BACKGROUND OF THE INVENTION

While the immune system is essential for fighting off infections, the immune response to infections (foreign antigens) and the immune response to molecules produced in the body (autologous antigens) may result in numerous diseases, *i.e.*, immune system-mediated diseases. Immune system-mediated diseases may be either B cell-mediated (*i.e.*, antibody-mediated) or T cell-mediated. Additionally, immune system-mediated diseases may be caused by immune complexes formed between antibodies and antigens (either foreign or autologous). Many immune system-mediated diseases involve an undesirable inflammatory response, *e.g.*, diseases which include rheumatoid arthritis, chronic hepatitis, Crohn's Disease, psoriasis, vasculitis, and the like.

Existing therapies for immune system-mediated diseases, particularly immune system-mediated diseases resulting in an undesirable inflammatory response, such as rheumatoid arthritis, are inadequate. Most immune system-mediated diseases are chronic conditions which require the prolonged administration of drugs. Accordingly, it is important to employ relatively non-toxic drugs. However, many compounds used for

the treatment of autoimmune diseases, *e.g.*. steroids and non-steroidal anti-inflammatory compounds, have significant toxic side effects that become apparent after long-term use. Additionally, immunosuppressive drugs have been used to treat autoimmune responses. Such immunosuppressive drugs, *e.g.*, cyclosporin A and azathioprine, are relatively non-specific and have the adverse effect of weakening the entire immune system, thereby leaving a patient susceptible to infectious disease.

The oral administration of compounds has been shown to induce immune tolerance with respect to the ingested compound and compounds structurally related to the ingested compound. It has been suggested that the phenomenon of oral tolerance induction be adapted as a method of treating autoimmune disease. PCT publication WO 95/10301 describes the use of oral ingested cholera toxin conjugates to decrease a delayed hypersensitivity reaction and control experimental autoimmune encephalitis in mouse models.

In view of the shortcomings of existing techniques for treating chronic immune system-mediated diseases, it is of interest to provide new methods and compositions for the treatment of immune system-mediated diseases. Methods described herein employ one or more collagens and/or collagen derivatives so as to reduce or eliminate an immune response that is important for the pathogenesis of a given immune system-mediated disease. The methods and formulations designed to induce tolerance to antigens involved in the disease process.

4. SUMMARY OF THE INVENTION

The subject invention provides novel methods and compositions for the treatment of immune system-mediated diseases, including rheumatoid arthritis. The subject compositions comprise one or more different types of collagen or collagen derivatives. Specific combinations of collagen and/or collagen derivatives may be used to treat specific immune system-mediated diseases. The collagen(s) and/or collagen(s) derivatives used in the subject compositions may be either obtained from natural sources or produced by recombinant genetic engineering techniques.

Another aspect of the invention is to provide methods for treating immune system-mediated diseases by administering an effective amount of the subject collagen-

containing compositions. The methods of the invention include the administration of the subject collagen(s) and/or collagen derivative(s) containing compositions into the intestine so as to induce immune tolerance, *i.e.*, oral administration.

Another aspect of the invention is to provide the subject collagen and/or collagen derivative containing compositions formulated for administration to a patient. Preferred formulations of the invention are designed for the release of collagen(s) and/or collagen derivatives in the intestine so as to contact intestinal lymphoid tissue. In a preferred embodiment of the invention the subject compositions are formulated for oral administration.

Another aspect of the invention is to provide collagen derivatives that comprise at least one tolerance inducing epitope, and preferably a plurality of tolerance inducing epitopes. Such collagen derivatives may comprise a plurality of different collagen-derived tolerance inducing epitopes and are referred to herein as tolerance epitope polypeptides. Tolerance epitope polypeptides may also comprise amino acid resolve sequences derived from non-collagenous proteins.

Another aspect of the invention is to provide mucosa binding collagen conjugates for treating immune system-mediated diseases. The mucosa binding collagen conjugates of the invention comprise one or more collagen molecules linked to a mucosa binding molecule. A variety of different collagen and/or collagen derivatives may be used as the collagen component of the mucosa binding collagen conjugates of the invention. A variety of mucosa binding molecules may be used as the mucosa binding molecule component of the mucosa binding collagen conjugates of the invention suitable mucosa binding components include mucosa binding structures derived from bacterial toxins, bacterial fimbriae, viral attachment proteins, and plant lectins. A particularly preferred mucosa binding components are the β subunit of cholera toxin and the β subunit of *E. coli* heat labile enterotoxin.

Another aspect of the invention is to provide methods of treating immune system mediated diseases by administering an effective amount of a mucosa binding collagen conjugate. Yet another aspect of the invention is to provide formulations for use in the treatment of immune system-mediated diseases, wherein the formulation comprise a

mucosa binding collagen conjugate of the invention. Preferably, the formulations are adapted for oral administration.

5. DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The invention involves the use of one or more different collagens and/or collagen derivatives to induce oral tolerance in a specific population of immune cells so as to provide a method of treating immune system-mediated diseases. Methods of using various collagens and/or collagen derivatives to treat immune system-mediated diseases and compositions for use in such methods are provided herein. The methods of the invention involve the oral administration of a collagen or collagens found in a specific tissue so as to induce the suppression (immunological tolerance) of inflammation against the tissue (or tissues) from which the collagen is found to occur in nature. The correspondence between a specific collagen type and its (e.g. tissue type) may be found in various art references, including Fukai, *et al.* Methods of Enzymology, 245:3-28 (1994). Collagen (and collagen derivative) containing compositions for use in such methods are also provided. The invention also provides many different types of mucosa binding collagen conjugates and methods for their use in treating immune system-mediated diseases.

One aspect of the invention is to use minor collagens, *i.e.*, comparatively rare collagens found to be naturally associated with major collagens, to induce immune tolerance so as to reduce the severity of an undesirable immune response, thereby providing the basis of a treatment for arthritis and related immune system-mediated diseases; however, the invention also provides for the use of major collagens to induce immune tolerance. Cartilage contains diverse species of collagen that provide the major extracellular scaffolding for the surrounding tissue. Data has been found to suggest that type II collagen, a major collagen, may have the capacity to suppress inflammation in cartilage. The subject invention is based, in part, upon the realization that minor collagen(s) naturally found to occur in combination with major collagen(s) may be important in inducing immune tolerance useful in treating immune system-mediated diseases such as rheumatoid arthritis. Accordingly, one aspect of the invention is to use minor collagens, either alone or in combination with major collagens, to induce immune

tolerance that results in amelioration of the disease process in several immune system-mediated diseases.

The subject invention provides a variety of collagen and collagen derivative containing compositions. Numerous different types of collagen are known to the person of ordinary skill in the art. At present nineteen different types of collagen have been discovered. A detailed description of structure and biological functions of the various different types of naturally occurring collagens can be found, among other places, in Ayad *et al.*, The Extracellular Matrix Facts Book, Academic Press, San Diego, CA; Burgeson, R. E., and Nimmi, "Collagen types: Molecular Structure and Tissue Distribution," Clin. Orthop., 282:250-272 (1992); Kielty, C. M., et al., "The Collagen Family: Structure, Assembly And Organization In The Extracellular Matrix." In Connective Tissue And Its Heritable Disorders, Molecular Genetics, And Medical Aspects, Royce, P. M. and Steinmann, B., Eds., Wiley-Liss, NY, pp. 103-147 (1993).

Type I collagen is the major fibrillar collagen of bone and skin. Type I collagen is a heterotrimeric molecule comprising two α1(I) chains and one α2(I) chain. Details on preparing purified type I collagen can be found, among other places, in Miller, E. J., and Rhodes, R. K., Methods In Enzymology, 82:33-64 (1982), Academic Press.

Type II collagen is a homotrimeric collagen comprising three identical α1(II) chains. Purified Type II collagen may be prepared by, among other methods, the procedure described in Miller, E. J., and Rhodes, R.K. Methods In Enzymology, 82:33-64 (1982), Academic Press.

Type III collagen is a major fibrillar collagen found in skin and vascular tissues. Type III collagen is a homotrimeric collagen comprising three identical α1(III) chains. Methods for producing type III collagen can be found in, among other places, Byers, et al., Biochemistry, 13:5243-5248 (1974) and Miller E. J. and Miller, R. K., Methods in Enzymology, 82:33-64 (1982), Academic Press.

Type IV collagen is found in basement membranes in the form of a sheet rather than fibrils. Type IV collagen molecules are composed of three different alpha chains derived from six different genes, *i.e.*, type IV collagen comprises three of α1(IV), α2(IV), α3(IV), α4(IV), α5(IV), and α6(IV) chains. The particulars are expressed in a tissue-specific manner. Type IV collagen may be purified by, among other methods,

the procedures described in Furuto *et al.*, D. K., and Miller, E. J., Methods in Enzymology, 144:41-61 (1987), Academic Press.

Type V collagen is a fibrillar collagen found in bones, tendon, cornea, skin, and blood vessels. Type V collagen exists in both homotrimeric and heterotrimeric forms. One type of Type V collagen is a heterotrimer of two α1(V) chains and α2(V). Another type of type V collagen is a heterotrimer of α1(V), α2(V), and α3(V). Yet another type of type V collagen is a homotrimer of α1(V). Methods for purifying type V collagen can be found, among other places, in Elstrow, S. F., and Weiss, J. B., Collagen Rel. Res., 3:181-193 (1983) and Abedin, *et al.*, Biosci. Rep., 2:493-502 (1982).

Type VI collagen has a small triple helical region and a large non-collagenous remainder portion. Type VI collagen is found in many connective tissues. Type VI collagen is a heterotrimer comprising α1(VI), α2(VI), and α3(VI) chains. Descriptions of how to purify type IV collagen can be found, among other places, in Wu, *et al.*, Biochem. J., 248:373-381 (1987), and Kielty, *et al.*, J. Cell Sci., 99:797-807.

Type VII collagen is a fibrillar collagen found in particular epithelial tissues. Type VII is a homotrimeric molecule of three α1(VII) chains. Descriptions of how to purify type VII collagen can be found in, among other places, Lundstrom, *et al.*, J. Biol. Chem., 261:9042-9048 (1986), and Bentz, *et al.*, Proc. Natl. Acad. Sci. U.S.A., 80:3168-3172 (1983).

Type VIII collagen can be found in Descemet's membrane in the cornea. Type VIII collagen is a heterotrimer comprising two α1(VIII) and one α2(VIII) chain. Methods for the purification of type VIII collagen can be found, among other places, in Benya and Badilla, J. Biol. Chem., 261:4160-4169 (1986), and Kapoor, *et al.*, Biochemistry, 25:3930-3937 (1986).

Type IX collagen is a fibril associated collagen which can be found in cartilage and vitreous humor. Type IX collagen is a heterotrimeric molecule comprising α1(IX), α2(IX), and α3 (IX) chains. Procedures for purifying type IX collagen can be found, among other places, in Duance, *et al.*, Biochem., J., 221:885-889 (1984), Ayad *et al.*, Biochem. J., 262:753-761 (1989), Grant, *et al.*, The Control of Tissue Damage, Glauert, A. M., Ed., El Sevier, Amsterdam, pp. 3-28 (1988).

Type X collagen is a homotrimeric compound of XI(x) chain. Type X collagen has been isolated from, among other tissues, hypertrophic cartilage found in growth plates.

Type XI collagen can be found in cartilaginous tissues associated with type II and type IX collagens, as well as other locations in the body. Type XI collagen is a heterotrimeric molecule comprising α1(XI), α2(XI), and α3(XI) chains. Methods for purifying type XI collagen can be found, among other places, in Grant *et al.*, In The Control of Tissue Damage, Glauert, A. M., Ed., El Savier, Amsterdam, pp. 3-28 (1988).

Type XII collagen is a fibril associated collagen found primarily associated with Type I collagen. Type XII collagen is a homotrimeric molecule comprising three α1(XII) chains. Methods for purifying type XII collagen and variants thereof can be found, among other places, in Dublet *et al.*, J. Biol. Chem., 264:13150-13156 (1989), Lundstrum *et al.*, J. Biol. Chem., 267:20087-20092 (1992), Watt *et al.*, J. Biol. Chem., 267:20093-20099 (1992).

Type XIII is a non-fibrillar collagen found, among other places, in skin, intestine, bone, cartilage, and striated muscle. A detailed description of the type XIII collagen may be found, among other places, in Juvonen, *et al.* J. Biol. Chem., 267:24700-24707 (1992).

Type XIV is a fibril associated collagen. Type XIV collagen is a homotrimeric molecule comprising three α1(XIV) chains. Methods for isolating type XIV collagen can be found, among other places, in Aubert-Foucher, *et al.*, J. Biol. Chem., 266:19759-19764 (1992) and Watt, *et al.*, J. Biol. Chem., 267:20093-20099 (1992).

Type XV collagen is homologous in structure to type XVIII collagen. Information about the structure and isolation of type XV collagen can be found, among other places, in Myers *et al.*, Proc. Natl. Aca. Sci. USA, 89: 10144-10148 (1992), Huebner *et al.*, Genomics, 14: 220-224 (1992), Kivirikko *et al.*, J. Biol. Chem., 269:4773-4779 (1994), and Muragaki, J. Biol. Chem., 264:4042-4046 (1994).

Type XVII collagen is a fibril associated collagen, found in skin, lung fibroblast, keratinocytes, and elsewhere. Information on the structure of type XVI collagen and the gene encoding type XVI can be found, among elsewhere, in Pan *et al.*, Proc. Natl.

Acad. Sci. U.S.A., 1989:6565-6569 (1992), and Yamaguchi, *et al.*, J. Biochem., 112:856-863 (1992).

Type XVIII collagen is homologous in structure to type XV collagen and can be isolated from the liver. Descriptions of the structures and isolation of type XVIII collagen can be found, among other places, in Rehn *et al.*, Proc. Natl. Acad. Sci USA, 91:4234-4238 (1994), Oh *et al.*, Proc. Natl. Acad. Sci USA, 91:4229-4233 (1994), Rehn *et al.*, J. Biol. Chem., 269: 13924-13935 (1994), and Oh *et al.*, Genomics, 19:994-999 (1994).

The above-collagen types may also be obtained by recombinant expression techniques, as described generally in U.S. Patent No. 5,405,757, PCT-published patent applications WO 93/07889 and WO94/16570, and related patents and applications.

Another aspect of the invention is to provide mucosa binding collagen conjugates. The subject mucosa binding collagen conjugates comprise a collagen component and a mucosa binding component. The two components may be linked directly to one another or may be linked to one another through one or more intermediary linking molecules. Mucosa binding collagen conjugates may be used to treat a variety of immune system-mediated diseases. The treatment is effected through the induction of immune tolerance to one or more epitopes on the collagen component of the mucosa binding collagen conjugates.

The collagen component of the subject mucosa binding collagen conjugates may comprise one or more collagen or collagen derivative molecules. The collagen molecules of the collagen component of the mucosa binding collagen conjugates may be the same or different from one another. The collagen/collagen derivative molecules of the collagen component may be linked to one another, either directly or through linker intermediates. Alternatively, the collagen/collagen derivatives of the collagen component are not necessarily linked to each other, but may be linked directly to the mucosa binding component of the subject mucosa binding collagen conjugates.

The mucosa binding component comprises one or more molecules capable of specifically binding to the mucosa cells of a patient for treatment. A variety of different molecules may serve as the mucosa binding component. These mucosa binding molecules may be derived from the mucosa binding structures of bacterial toxins,

bacterial fimbriae, viral attachment proteins and plant lectins. The use of mucosa binding structures from bacterial toxins is preferred. Particularly preferred are the β subunits of cholera toxin and the β subunits of the heat-labile enterotoxin of *E. coli*. The β subunits of cholera toxin and the β subunits of the heat-labile enterotoxin of *E. coli* have the property of specifically binding to ganglioside GM, in mucosal cells. When toxins are used as mucosa binding structures, the toxin is preferably modified so as to significantly remove of destroy the cytotoxic properties of the toxin. Inactivation of cytotoxic properties my be accomplished in a number of ways well known to the person of ordinary skill the art including, for example, denaturation, mutation, and the like. Other molecules that specifically bind to ganglioside GM_1 may be used as the mucosa binding component of the mucosa binding collagen conjugates of the invention. The mucosa binding molecules may be covalently joined to one another, either directly or indirectly, by means of an intermediary linker molecule(s). Alternatively, the mucosa binding molecules that form the mucosa binding component may associate with another by means of intermolecular attractive forces. For example, the β subunits of cholera toxin form a 5 molecule "ring" structure with allowed to associate. Examples of suitable toxin molecules include subunits S2, S3, S4 and/or S5 of *Bordatella pertussis* toxin, diphtheria toxin, diphtheria toxin β fragment, shiga toxin, shiga-like toxins, shiga toxin β subunit, shiga-like toxin β subunit, cholera toxin, *E. coli* heat-labile toxin. Examples of suitable bacterial fimbriae include *E. coli* K88, K99, 987P, F41, CFA/I, CFA/II, (CS1, CS2 and/or CS3), CFA/IV (CS4, CS5 and/or CS6), P fimbriae, *Vibrio cholera* toxin co-regulated pili (TCP), mucus sensitive hemagglatin (MSHA), fucose-sensitive hemagglatinin (FSITH), *B. pertussis* filamentous hemagglutinin and the like. Examples of suitable viral attachment proteins include Influenza hemagglutinin, Sendai Virus hemagglatin. Examples of suitable lectin include both plant lectins and animal lectins, soluble lactose-binding lectins, selecting, collecting, helix pomatin hemagglutinin, concanavalin A, Wheat-germ agglutinin, Phytohemagglutinin, aurin ricin. Immunoglobins that are specific for mucosal cell antigens may also be used as mucosal binding components.

The separate collagen compound and mucosa binding component of the mucosa binding components may be linked to one another by a variety of means. The two

components may be coupled together by means of chemical cross-linking agents such as N-succinimidyl (3-(2-pyridyl-dithio) propionate, dimethyl-3,3'-dithiobispropionimidate, 2-iminothiolane, N-succinimidyl-(4 azidophenyl)-1, 3-dithioprionate, ethyl-4-azidophenyl-1, 4-dithiobutyrimidate, diethyl malonimidate, 2-iminothiolane, N,N'-p-phenylenedimaleimide, and the like. Alternatively, the mucosa binding collagen conjugates of the invention may be single polypeptides that are fusion protein formed between the collagen component and the mucosa binding molecule component. These fusion proteins may be produced by conventional *in vitro* genetic engineering techniques. Another aspect of the invention is to provide polynucleotide sequences encoding mucosa binding collagen conjugate fusion proteins. Yet another aspect of the invention to provide host cells for the recombinant production of mucosa binding collagen conjugate fusion proteins.

The mucosa binding molecule components may be obtained by purifying from their naturally occurring source organism. Alternatively, the mucosa binding molecule components may be produced by recombinant DNA techniques. Methods of purifying mucosa binding molecules, or alternatively, recombinantly producing such molecules suitable for use in the invention are well known to the person of ordinary skill in the art. For example, the recombinant production of cholera toxin β subunit has been described in Sanchez and Holmgren, Proc. Natl. Acad. Sci. (USA), 86:481-485 (1989) and production of the β subunit of e. coli heat labile enterotoxin has been described in Hirst et al., Proc. Natl. Acad. Sci. (USA), 81:7752-7756 (1984).

The compositions of the invention comprise one or more purified collagens and/or collagen derivatives. The subject compositions comprise specific combinations of collagens and/or collagen(s) derivatives are provided. Table 1 provides a list of collagen types (indicated by Arabic numeral rather than Roman numerals) that may be used to treat specific diseases. Each of the collagen types listed as being useful for treating a specific disease, either alone or in a composition combining one or more collagen molecules that are suitable for treating the disease of interest. For example, by reference to Table 1, it can be seen that rheumatoid arthritis may be treated by compositions comprising type I collagen, type II collagen, type III collagen, type V collagen, type VI collagen, type IX collagen, type X collagen, type XI collagen, type XII

collagen, type XIV collagen, or any combination thereof comprising two or more of the collagens indicated as being suitable for the treatment of a specific disease condition. Thus, by referring to Table 1, it can be seen that the invention provides compositions for the treatment of rheumatoid arthritis comprising at least one compound selected from the group consisting of type I collagen, type II collagen, type III collagen, type V collagen, type VI collagen, type IX collagen, type X collagen, type XI collagen, type XII collagen and type IV collagen. Collagen derivatives corresponding to a given collagen type may also be used in addition to or in place of a specific collagen type indicated in the compositions of Table 1. Thus, for example, a composition for the treatment of rheumatoid arthritis, as indicated in Table 1, may comprise type II collagen, a type II collagen derivative, multiple different type II collagen derivatives, or a mixture of type II collagen and type II collagen derivatives.

In a preferred embodiment of the invention for treating rheumatoid arthritis, the subject compositions comprise type IX collagen and/or type IX collagen derivatives thereof, either with or without type II collagen and/or derivatives of type II collagen. In another embodiment, the invention provides compositions that comprise type XI collagen and/or derivatives thereof, either with or without type II collagen and/or derivatives thereof. In another preferred embodiment of the invention for treating rheumatoid arthritis, the invention consists of compositions comprising type IX collagen and/or derivatives thereof and type XI collagen, either with or without type II collagen and/or derivatives thereof. In another preferred embodiment of the invention for treating rheumatoid arthritis, the subject compositions comprise type II collagen derivatives.

TABLE 1

TABLE OF COLLAGEN TYPES AND INDICATIONS

INDICATION	COLLAGEN TYPE																		
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19
ankylosing spondylitis and other spondylarthropaties	X	X	X		X	X			X		X								
autoimmune hearing disease		X		X					X		X								
autoimmune liver disease	X		X															X	
blistering diseases of the skin				X			X												

INDICATION	COLLAGEN TYPE																	
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19
bulous pemphigoid																	X		
bulous diseases of skin (*e.g.* pemphigus)				X			X												
bursitis	X	X	X		X				X		X				X				
cartilage inflammation due to bacterial or viral disease (*e.g.* Lyme disease)		X							X		X								
chronic erosive inflammatory osteoarthritis	X	X	X						X		X								
Crohn's Disease			X	X			X					X		X					
diseases found in cornea (Descemet's membrane)	X	X		X				X											
endothelial cell related diseases	X		X	X				X					X		X			X	
epidermolysis bulosa							X												
Goodpasture's Syndrome				X															
hepatitis (*e.g.* chronic hepatitis)	X		X		X	X						X		X				X	
idiopathic membranous glomerulonephritis				X															
inflammation of blood vessels	X		X	X				X											
inflammation of ligaments	X		X		X	X						X		X					
inflammation of heart valves	X		X	X															
inflammation of tendons	X		X		X	X						X		X					
inflammatory diseases of the muscles	X		X	X									X		X				
kidney fibrosis (*e.g.* nephritis)	X		X	X											X			X	
lung fibrosis	X		X	X	X	X	X					X	X	X				X	
lupus				X	X														
osteoarthritis	X	X	X						X		X								
granulomitis (parasitic) diseases	X	X	X	X	X	X	X		X		X							X	
polychondritis		X							X		X								
psoriasis				X			X						X						
pulmonary fibrosis	X		X	X															
reactive arthritis		X							X		X								

INDICATION	COLLAGEN TYPE																	
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19
rheumatoid arthritis (including juvenile rheumatoid arthritis)	X	X	X		X	X			X	X	X	X		X					
localized sclerosis	X		X		X	X	X					X		X					
systemic fibrotic diseases (*e.g.* scleroderma)	X		X		X	X	X					X		X					
uterine disease (e.g. fibroids)	X		X	X			X						X				X		
vasculitis diseases				X			X												

Similar to the collagen and collagen derivative containing compositions of the invention, the mucosa binding collagen conjugates of the invention may be adapted for the treatment of specific immune system-mediated diseases. The mucosa binding collagen conjugates may be adapted by selecting the collagen component of the mucosa binding collagen conjugate in accordance with the information in Table 1.

Purified collagens for use in the methods and compositions of the invention subject inventions may be isolated from animal or human tissues; however, the use of human collagens in the subject compositions and methods is preferred when the subject to be treated is a human. In addition to obtaining collagens for use in the subject methods and compositions from natural sources, collagens for use in the methods and compositions of the invention may be produced by recombinant DNA technology. A description of how to produce type II by recombinant DNA technology technique can be found, among other places, in PCT-published patent applications WO 93/07889 and WO94/16570, which are herein incorporated by reference. The recombinant production techniques described in these PCT publications may readily be adapted so as to produce many different types of collagen, human or otherwise, by recombinant DNA techniques. Preferably, human collagens produced by recombinant DNA technology are used in compositions of the invention. Recombinant DNA technology avoids ethical and economic problems associated with obtaining adequate quantities of human tissue.

In addition to employing collagen obtained directly from natural sources, the methods and compositions of the invention may comprise many different types of collagen derivatives. Collagen derivatives may vary from naturally occurring collagens

in several respects. Collagen derivatives may be non-glycosylated or glycosylated differently than naturally-occurring collagens. Desired glycosylation patterns may be produced by a variety of methods, including direct chemical modification and enzymatically catalyzed glycosylation and deglycosylation reactions. Desired glycosylation patterns may also be produced by inhibiting or deleting enzymes necessary for producing the naturally-occurring glycosylation patterns found on collagens. Collagen derivatives have a glycosylation pattern that differs from a corresponding naturally-occurring collagen, but are otherwise essentially the same in structure (except for the amount of proline and/or lysine residues that have been hydroxylated) are referred to herein as "variably glycosylated collagens." Collagen derivatives also include various fragments of naturally-occurring collagens. Such collagen derivatives also include various fragments of naturally-occurring collagens. Such collagen fragments may be produced by, among other methods, chemically or enzymatically cleaving one or more peptide bands. Collagen derivatives may also contain one or more amino acid residue differences as compared with corresponding amino acid residue positions is a naturally-occurring collagen. Collagen derivatives containing such amino acid residue substitutions may be produced by a variety of methods including genetic engineering techniques and by *in vitro* peptide synthesis. Additional collagen derivatives may be produced by varying the amount of hydroxylysines and/or hydroxyprolines present in a given molecule, by the varied expression of lysine hydroxylases, and/or proline hydroxylases, wherein the hydroxylase genes (recombinant or otherwise) are also expressed in a host cell for the expression of recombinant collagen, or derivatives thereof. Collagen derivatives for use in the compositions and methods of the invention have the capacity to reduce the undesired immune reaction in the immune system-mediated disease of interest when administered to a patent either alone or as part of a composition of the invention, including compositions comprising a plurality of collagen and/or collagen derivatives. Collagen derivatives suitable for use in those methods and compositions of the invention for the treatment of rheumatoid arthritis may be determined by employing *in vitro* tests of T cell from rheumatoid arthritis patients, wherein the tests could determine if a given collagen derivative can stimulate suppressor T cells, induce clonal anergy, or induce other forms of immune tolerance. Methods of

measuring antigen tolerance induction are well known to persons of ordinary skill in the art and can be found, for example, in Paul, W. E., editor, Fundamental Immunology, 2nd Ed., Raven Press: New York (1994). Similarly, collagen derivatives for use in the treatment of other immune system-mediated disorders may be determined to be suitable for use in treating a given immune system-mediated disease by *in vitro* tests on immune system cell isolated from patients suffering from the disease of interest.

A collagen molecule, collagen derivative or mucosa binding collagen conjugate suitable for use in the methods of the invention comprises at least one tolerance inducing epitope. The term "tolerance inducing epitope" as used herein refers to epitopes on collagen that have the property of inducing immune tolerance with respect to a pathogenic immune response in an immune system-mediated disease, independent of other portions of the collagen molecule from which the "tolerance-inducing epitope" was derived. Individual collagen molecules may comprise one or more tolerance inducing epitopes. Additionally, the tolerance-inducing epitope or epitopes on a given collagen molecule may vary in accordance with the specific immune system-mediated disease to be treated by the collagen.

The invention also provides for collagen derivatives consisting of amino acid residue sequences that comprise one or more collagen tolerance-inducing epitopes; such novel polypeptides are referred to herein as "tolerance epitope polypeptides." Tolerance epitope polypeptides may comprise a plurality of identical tolerance-inducing epitopes. Tolerance epitope polypeptides of the invention may also comprise one or more non-identical tolerance-inducing epitopes derived from the same or different collagen molecules. Embodiments of the subject tolerance epitope polypeptides include polypeptides in which the different epitopes are arranged in a regular repeating pattern and polypeptides in which the various tolerance-inducing epitopes are arranged randomly. Tolerance epitope polypeptides comprise a plurality of tolerance-inducing epitopes; while tolerance epitope polypeptides may be of virtually any size, the person of ordinary skill in the art will appreciate that certain technical problems arise in synthesizing and using very large polypeptides, e.g., solubility, stability, *etc.* Accordingly, the tolerance epitope polypeptides of the invention preferably comprise the 500 tolerance inducing epitopes, more preferably in the range of to 250 tolerance-

inducing epitopes and still more preferably in the range of 50 to 100 tolerance-inducing epitopes. Tolerance-inducing epitopes may be glycosylated or non-glycosylated, depending upon whether the carbohydrate matrix are considered to be part of a tolerance-inducing epitope. Tolerance epitope polypeptides may be produced by conventional genetic engineering techniques such as those described, for example, in Goeddel Gene Expression Technology, Methods in Enzymology Volume 185, Academic Press (1991). Additionally, tolerance epitope polypeptides may comprise one or more non-collagen derived amino acid residue sequences, whereby the collagen derived tolerance-inducing epitope region on the tolerance epitope polypeptide serves as a carrier for the non-collagen derived amino acid sequence so that immune tolerance can also be induced against the non-collagen derived amino acid residue sequence. In other words, some embodiments of the subject tolerance epitope polypeptides are polypeptides that act as "carriers" for inducing immune tolerance against non-collagen proteins that may be involved in the pathogenesis of immune system mediated diseases. For example, the subject invention provides tolerance epitope polypeptides that comprise regions of myelin basic protein that have been shown to be important in the development of the pathogenic immune response in multiple sclerosis and multiple tolerance inducing epitopes.

Polynucleotide sequence encoding tolerance epitope polypeptides may be produced by, among other methods, *in vitro* polynucleotide synthesis and the manipulation of previously-isolated collagen-encoding polynucleotides.

Tolerance epitope polypeptides may be designed for the treatment of specific immune system-mediated diseases in accordance with the information provided in Table 1. Tolerance epitope polypeptides may comprise tolerance-inducing epitopes derived from collagen or collagens found in a specific tissue so as to induce the suppression (immunological tolerance) of inflammation against the tissue (or tissues) from which the collagen is found to occur in nature. Thus, for example, a tolerance epitope polypeptides comprising tolerance-inducing epitopes of type II, type IX, and type XI collagen may be used in place of (or in addition to) a mixture of type II, type IX, and type XI collagens.

Tolerance-inducing epitopes for a given collagen and a given immune system-mediated disease may be readily determined by a person of ordinary skill in the art of immunology. For example, certain subpopulations of lymphocytes in a person wiring from (or likely to develop) a specific immune system-mediated disease have receptors capable of binding tolerance inducing options. Wherein a putative tolerance-inducing epitopes may be obtained by making systematic deletions in a collagen molecule of interest.

Another aspect of the invention is to provide methods of treating immune system-mediated diseases. The terms "treatment" or "treating" as used herein with reference to a disease refer both to prophylaxis and to the amelioration of symptoms already present in an individual. It will be appreciated by the person of ordinary skill in the art that a treatment need not be completely effective in preventing the onset of a disease or in reducing the symptoms associated with the disease. Any reduction of the severity of symptoms, delay in the onset of symptoms, or delay in the progression of severity of symptoms is desirable to a patient. Persons at risk of developing a given immune system-mediated disease may be treated prophylactically based on any of a variety of factors suggesting the possible onset of an immune system-mediated disease, *e.g.*, family history, genetic markers, early symptoms, and the like.

Immune system-mediated diseases that may be treated by the subject methods include, but are not limited to, the diseases listed in Table 1. The subject methods of the invention comprise the step of administering an effective amount of a composition of the invention, e.g., collagens, collagen derivatives, or mucosa binding collagen conjugates. Preferred compositions for use in treating specific immune system-mediated diseases are provided in Table 1, as described in the preceding sections. In a preferred embodiment of the subject methods, the compositions administered to the subject comprise variably glycosylated collagens or mucosa binding collagen conjugates, wherein the collagen component comprises variably glycosylated collagen molecules. The compositions administered in the subject methods are administered so that the active components, *i.e.*, collagens and/or collagen derivatives, contact the lymphoid tissue of the gut, *e.g.*, Peyer's patches or other similar sites, so that immune tolerance is induced. Such administration may be effected, by among many possible methods,

through the use of formulations comprising the subjected compositions that are designed for oral administration, *i.e.*, the active components are not destroyed or inactivated in the mouth, stomach, or other portions of the digestive system prior to contacting the appropriate gut lymphoid tissue. The treatment methods of the invention may also comprise the steps of administering additional pharmaceutical compounds for the treatment of immune system-mediated diseases, such as anti-inflammatory agents and the like.

The dosage at which the subject compositions are administered may vary within a wide range and will depend on various factors such as for example the severity of the inflammation, the age of the patient, etc., and may have to be individually adjusted. As a possible range for the amount of collagen(s) and/or collagen(s) derivatives which may be administered per day may be in the range of from about 0.001 mg to about 200 mg. Preferably, the amount of collagen and/or collagen derivatives administered is low, thereby favoring the induction of immune tolerance by suppression rather than clonal anergy. The pharmaceutical compositions containing the collagen(s) and/or collagen(s) derivatives may suitably be formulated so that they provide doses within these ranges either as single dosage units or as multiple dosage units.

The optimal dosage of tolerance inducing compositions for use in the methods of the invention will vary in accordance with a number of factors. The terms "dosage" and "dose" as used herein, unless indicated otherwise, may refer not only to a single administration of a composition but may be used to refer to the total amount of a given pharmaceutical composition administered over a selected period of time and involving multiple individual administrations. Factors affecting the optimal dosage include the choice of collagen molecule or molecules (and/or collagen derivatives) administered to the patient, the specific mucosa binding molecules selected, the age of the patient, the severity of the disease, other diseases that may be present in the patient, inert components in the formulation, adjuvants, and the like. There may be considerable variation in the range of dosages that are effective in treating a given immune disorder. Different dosages of the same pharmaceutical composition may produce the desired tolerance effect by different mechanisms. Although the operation of the invention is not dependent upon a particular theory of operation, the person of ordinary skill in the art

will better understand the invention and provide additional embodiments by appreciating that there are believed to be two primary mechanisms by which oral tolerance is mediated. Oral tolerance may be mediated by active cellular suppression in which regulatory T cells that suppress the activation and proliferation of lymphocytes specific for tolerized antigen. Another mechanism of oral tolerance induction is clonal anergy in which T lymphocytes having a suitable receptor are rendered unresponsive. Generally active suppression tolerance is favored by "low" doses of a tolerizing antigen and clonal anergy is favored by comparatively "high" doses of the same tolerizing antigen. A review of the principles and techniques for oral tolerance induction can be found in Weiner *et al.*, Annual Review of Immunology: 1994, 809-835, Annual Reviews.

The subject compositions may be formulated as pharmaceutical compositions so as to be adapted for certain types of administration to mucosal surfaces, e.g., oral, topical, and inhalation. The preferred form of formulation for oral administration in a form where the collagen and/or collagen derivatives in the composition come into contact with intestinal lymphoid tissue, *e.g.*, Peyer's patches. Compositions of the invention may be administered topically, orally, intranasally, by injection or by inhalation in the form of a pharmaceutical compositions comprising a collagen(s) and/or collagen(s) derivatives in the form of the original compound or optionally in the form of a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier which may be a solid, semi-solid or liquid diluent or an ingestible capsule, and such preparations comprise a further aspect of the invention. The collagen(s) and/or collagen(s) derivatives and mucosa binding collagen conjugates may also be used with carrier material. As examples of pharmaceutical preparations may be mentioned tablets, drops such as nasal drops, preparations for topical application such as ointments, jellies, creams and suspensions, aerosols for inhalation, nasal spray, liposomes, etc. Usually the collagen(s) and/or collagen(s) derivatives will comprise between 0.05 and 99%, or between 0.1 and 99% by weight of the preparation, for example between 0.5 and 20% for preparations intended for injection and between 0.1 and 50% for preparations intended for oral administration.

To produce pharmaceutical preparations in this form of dosage units for oral application containing a compound of the invention the active ingredient may be mixed

with a solid, pulverulent carrier, for example lactose, saccharose, sorbitol, mannitol, a starch such as potato starch, corn starch, amylopectin, laminaria powder or citrus pulp powder, a cellulose derivative or gelatine and also may include lubricants such as magnesium or calcium stearate or a Carbowax® or other polyethylene glycol waxes and are compressed to form tablets or cores for dragees. If dragees are required, the cores may be coated, for example, with concentrated sugar solutions which may contain gum arabic, talc and/or titanium dioxide, or alternatively with a film forming agent dissolved in easily volatile organic solvents or mixtures of organic solvents. Dyestuffs can be added to these coatings, for example, to distinguish between different contents of active substance. For the preparation of soft gelatine capsules consisting of gelatine and, for example, glycerol as a plasticizer, or similar closed capsules, the active substance may be admixed with a Carbowax® or a suitable oil as e.g. sesame oil, olive oil, or arachis oil. Hard gelatine capsules may contain granulates of the active substance with solid, pulverulent carriers such as lactose, saccharose, sorbitol, mannitol, starches (for example) potato starch, corn starch or amylopectin), cellulose derivatives or gelatine, and may also include magnesium stearate or stearic acid as lubricants.

The compositions of the invention may also be formulated so as to provide a sustained release. By using several layers of the active drug, separated by slowly dissolving coatings sustained release tablets may be obtained. Another way of preparing sustained release tablets is to divide the dose of the active drug into granules with coatings of different thicknesses and compress the granules into tablets together with the carrier substance. The collagen(s) and/or collagen(s) derivatives and mucosa binding collagen conjugates may also be incorporated in slowly dissolving tablets made, for instance, of fat and wax substances or evenly distributed in a tablet of an insoluble substance such as a physiologically inert plastic substance.

In order to obtain dosage units of oral preparations -- tablets, capsules, etc. -- which are designed so as to prevent release of and possible decomposition of the active substance in the gastric juice, the tablets, dragees etc. may be enteric-coated, that is provided with a layer of gastric juice-resistant enteric film or coating having such properties that it is not dissolved at the acidic pH in the gastric juice. Thus, the active substance will not be released until the preparation reaches the intestines. As example

of such known enteric coatings may be mentioned cellulose acetate phthalate, hydroxypropyl-methylcellulose phthalates such as those sold under the trade names HP 55 and HP 50, and Edragit®L and Eudragit®S.

Liquid preparations for oral application may be in the form of elixirs, syrups or suspensions, for example solutions containing from about 0.1% to 20% by weight of active substance, sugar and a mixture or ethanol, water glycerol, propylene glycol and optionally aroma, saccharine and/or carboxymethylcellulose as a dispersing agent.

The term "purified" as used herein in reference to collagens denotes that the indicated molecules are present in the substantial absence of other biological macromolecules, *e.g.*, polynucleotides, proteins, and the like. The term "purified" as used herein preferably means at least 95% by weight, more preferably at least 99.8% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons can be present). The term "isolated" as used herein refers to a protein molecule separated not only from other proteins that are present in the natural source of the protein, but also from other proteins, and preferably refers to a protein found in the presence of (if anything) only a solvent, buffer, ion, or other component normally present in a solution of the same. The terms "isolated" and "purified" do not encompass proteins present in their natural source.

6. EXAMPLES

The following examples are provided for the purpose of illustrating the subject invention and should not be considered as limiting the scope of the invention.

6.1. *In Vivo* Animal Study Regarding Development Of Antibodies To Collagen Following Immunization With Collagen

Groups of 10 DBA/1mice were immunized with either human type II collagen (CII), mammalian cell derived recombinant type II collagen (rCII+), or insect cell derived recombinant type II collagen (rCII-). In each case, the collagen was dissolved in 0.01M acetic acid and then emulsized in a 1 to 1 ration with Complete Freud's Adjuvent prior to immunization. Each mouse received 100 μg of protein subcutaneously in the tail. Two groups received two doses of 100 μg rCII+ or rCII-, thereby receiving in total 200 μg of rCII+ or rCII-. In cases where 200 μg total dose

was administered, the first 100 μg dose of protein was administered as described above. The second 100 μg dose of protein was emulsified in incomplete Freud's adjuvent and administered three weeks after administration of the first dose.

The incidence of arthritis was measured and reported, as set forth in Table 2, at six weeks (following immunization).

TABLE 2

Antigen Used for Immunization	% Arthritic Mice	Antibodies to HII
rCII + (100 μg)	4/10	39 ± 19
rCII + (200 μg)	9/10	120.8 ± 40
rCII - (100 μg)	8/10	69.3 ± 39
rCII - (200 μg)	9/10	80.3 ± 39
rCII (100 μg)	9/10	117.5 ± 56

As evidenced by the data set forth in Table 2, antibodies represent the mean levels/group (expressed in units) against native human type II collagen sera collected four weeks after immunization.

6.2. *In Vivo* Animal Study Regarding Development Of Antibodies To Collagen Following Intravenous Administration Of Collagen

Ovalalbumin, human type II collagen (CII), mammalian cell derived recombinant type II collagen (rCII+), or insect cell derived recombinant type II collagen (rCII-) were administered intravenously to groups of 10 DBA/1 mice. The collagens (CII, rCII+ and rCII-) were dissolved in 0.01M acetic acid and dialyzed against PBS prior to administration. Either 33 μg or 33μg of protein was administered daily for three days such that a total of either 100 μg or 1000 μg of protein was administered to each test mouse. Four days after administration of the last dose, the mice were then immunized with CII. The incidence of arthritis at six (6) weeks after immunization is set forth at Table 3.

TABLE 3

Antigen Administered Intravenously	% Arthritic Mice	Antibodies to HII
OVA (1000 μg)	9/10	100.8 ± 40
rCII + (1000 μg)	0/10	120 ± 5.3 $p \leq 0.0005$
rCII + (100 μg)	0/10	14.3 ± 6 $p \leq 0.0005$
rCII - (1000 μg)	0/10	14.9 ± 7 $p \leq 0.0005$
rCII - (100 μg)	0/10	8.5 ± 4 $p \leq 0.0005$
CII (1000 μg)	0/10	5.5 ± 1 $p \leq 0.0005$

Antibodies represent the mean levels/group (expressed in units) against native human type II collagen using sera collected four (4) weeks after immunization. The statistics are reported using Student's T test.

6.3. *In Vivo* Animal Study Regarding Development Of Antibodies To Collagen Following Oral Administration Of Collagen

Groups of ten to twelve DBA/1 mice were orally administered either Ovalbumin, human type II collagen (CII), mammalian cell derived recombinant type II collagen (rCII+), or insect cell derived recombinant type II collagen (rCII-). The collagens were dissolved in 0.01M acetic acid and administered four (4) times per week for two weeks for a total of eight doses. Either 10 μg or 100 μg was administered daily so that mice received a total of either 80 μg or 800 μg of protein. Three (3) days after receipt of the last dose, the mice were then immunized with CII. Table 4 provides the incidence of arthritis at five weeks after immunization.

TABLE 4

Antigen Fed	% Arthritic Mice	Antibodies to HII
OVA (800 μg)	8/12 (67%)	100.3 ± 48
rCII + (800 μg)	8/12 (67%)	53 ± 33 p ≤ .01
rCII + (80 μg)	2/10 (20%) p ≤ 0.05	45 ± 45 p ≤ .01
rCII - (1000 μg)	8/12 (67%)	91 ± 77
rCII - (100 μg)	3/12 (25%) p ≤ 0.05	63 ± 24 p ≤ .025
CII (1000 μg)	6/12 (50%)	47 ± 23 p ≤ .025

The reported statistical variance was calculated using a Fisher's Exact Test. Antibodies represent the mean levels per group (expressed in units) against native human type II collagen using sera collected four weeks after immunization and statistics are reported using Student's T test.

6.4. Human Platelet Aggregation Study

Type III recombinant collagen and human types I and III collagen were tested in blood samples from four donors and tested, using routine platelet aggregation assays. All types of collagen demonstrated platelet aggregation activity. More specifically, the percentage of aggregation of 10 μg of recombinant type III collagen is equivalent to the percent aggregation of either 0.3 μg of type III or 0.6 μg of type I collagen.

INCORPORATION BY REFERENCE

All patents, patents applications, and publications cited are incorporated herein by reference.

EQUIVALENTS

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-described makes for carrying out the invention which are obvious to those skilled in the field of immunology, biochemistry, or related fields are intended to be within the scope of the following claims.

CLAIMS

What is claimed is:

1. A compound for the treatment of an autoimmune disease, said compound comprising a collagen molecule component linked to a mucosa binding molecule component.

2. A compound according to claim 1, wherein the collagen molecule is selected from the group consisting of type II collagen, type IX collagen, and type XI collagen.

3. A compound according to claim 2, wherein the collagen molecule is a type II collagen derivative.

4. A compound according to claim 3, wherein the collagen derivative is variably glycosylated.

5. A compound according to claim 1, where the mucosa binding molecule is selected from the group of mucosa binding molecules derived from mucosa binding structures of bacterial toxins, bacterial fimbriae, viral attachment proteins, and plant lectins.

6. A compound according to claim 5, wherein the mucosa binding protein can bind to ganglioside GM_1.

7. A compound according to claim 5, wherein the binding fragment is the β subunit of cholera toxin or heat-labile enterotoxin of *E. coli*.

8. A method of treating an immune system mediated disease, said method comprising the step of administering an effective amount of a compound according to claim 1.

9. A method according to claim 8, wherein the collagen molecule is selected from the group consisting of type II collagen, type IX collagen, and type XI collagen.

10. A method according to claim 9, wherein the collagen molecule is a type II collagen derivative.

11. A method according to claim 11, wherein the collagen derivative is variably glycosylated.

12. A method according to claim 8, where the mucosa binding molecule is selected from the group of mucosa binding molecules derived from mucosa binding structures of bacterial toxins, bacterial fimbriae, viral attachment proteins, and plant lectins.

13. A method according to claim 12, wherein the mucosa binding protein can bind to ganglioside GM_1.

14. A method according to claim 12, wherein the binding fragment is the β subunit of cholera toxin or heat-labile enterotoxin of *E. coli*.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00533

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/17, 38/00, 39/00
US CL :530/356; 514/8,21; 424/184.1, 185.1
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)

U.S. : 530/356; 514/8,21; 424/184.1, 185.1

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)

APS, CAS ONLINE, MEDLINE, EMBASE, DERWENT, BIOSIS
Search terms: collagen, autoimmune disease, type I or II or IX or XI, toxin, endotoxin, lectin, ganglioside, treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US, A, 5,399,347 (TRENTHAM ET AL) 21 March 1995, entire document, especially abstract and columns 4-9.	1-14
Y	Autoimmunity, Volume 16, issued 1993, Thompson et al, "Suppression of Collagen Induced Arthritis by Oral Administration of Type II Collagen: Changes in Immune and Arthritic Responses Mediated by Active Peripheral Suppression", pages 189-199, especially page 189.	1-14
Y	Science, Volume 261, issued 24 September 1993, Trentham et al, " Effects of Oral Administration of Type II Collagen on Rheumatoid Arthritis", pages 1727-1730, especially page 1727.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 25 APRIL 1996	Date of mailing of the international search report 03 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ABDEL A. MOHAMED Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00533

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proc. Natl. Acad. Sci. USA, Volume 91, issued January 1994, Tisch et al, "Antigen-Specific Immunotherapy: Is it a Real Possibility to Combat T-Cell-Mediated Autoimmunity?", pages 437-438, especially page 438, column 1, second paragraph.	1-14