Disclosed herein is a method of treating certain ocular and other diseases with an anti-IL-23p19 antibody.
IL23p19 ANTIBODY INHIBITOR FOR TREATING OCULAR AND OTHER CONDITIONS

CROSS-REFERENCE

This application claims the benefit of U.S. Provisional Patent Application Serial Number 61/355,466, filed on June 16, 2010, the entire disclosure of which is incorporated herein by this specific reference.

Disclosed herein are methods for treating certain ocular diseases, such as keratoconjunctivitis sicca, ocular cicatricial pemphigoid, blepharitis, ocular allergy, ocular infection, and diminished corneal sensitivity, and for treating certain systemic diseases, such as Stevens-Johnson disease and graft versus host disease, by administering to a subject an antibody that binds the p19 subunit of IL-23.

DETAILED DESCRIPTION OF THE INVENTION

IL23p19

Interleukin (IL)-12 is a secreted heterodimeric cytokine comprised of 2 disulfide-linked glycosylated protein subunits, designated p35 and p40 for their approximate molecular weights. IL-12 is produced primarily by antigen-presenting cells and drives cell-mediated immunity by binding to a two-chain receptor complex that is expressed on the surface of T cells or natural killer (NK) cells. The IL-12 receptor beta-1 (IL-12Rβ1) chain binds to the p40 subunit of IL-12, providing the primary interaction between IL-12 and its receptor. It is IL-12p35 ligation of the second receptor chain, IL-12Rβ2, that confers intracellular signaling (e.g., STAT4 phosphorylation) and activation of the receptor-bearing cell.

It was discovered that the p40 protein subunit of IL-12 can also associate with a separate protein subunit, designated p19, to form a novel cytokine, IL-23. IL-23 also signals through a two-chain receptor complex. Since the p40 subunit is shared between IL-12 and IL-23, it follows that the IL-12Rβ1 chain is also shared between IL-12 and IL-23. It is the IL-23p19 ligation of the second component of the IL-23 receptor complex, IL-23R, that confers IL-23 specific intracellular signaling (e.g., STAT3 phosphorylation) and subsequent IL-17 production by T cells. The biological functions of IL-23 are distinct from those of IL-12, despite
their structural similarity.

20090240036 (IL23p19 describes all Ab)

**KLK-1 3 Antibodies**


Antibodies useful in the method of the invention include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, and functional fragments thereof.

**Polyclonal Antibodies**

Polyclonal antibodies may be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (especially when synthetic peptides are used) to a protein that is immunogenic in the species to be immunized. For example, the antigen can be conjugated to keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, e.g., maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R₁N=C=NR, where R and R𝑖 are different alkyl groups.

Animals can be immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to
14 days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

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Monoclonal Antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567, the disclosure of which is incorporated herein by reference).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection, Manassas, Va., USA. Human myeloma and mouse-human
heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); and Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980).

Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal e.g., by i.p. injection of the cells into mice.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of


The DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy chain and light chain constant domain (CH and CL) sequences for the homologous murine sequences (U.S. Pat. No. 4,816,567, the disclosure of which is incorporated herein by reference; and Morrison, et al., *Proc. Natl. Acad. Sci. USA*, 81:6851 (1984)), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide). The non-immunoglobulin polypeptide sequences can substitute for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

**Human and Humanized Antibodies**

The anti-IL-23p19 antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins
(recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import " residues, which are typically taken from an "import " variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al, Science, 239:1 534-1 536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized " antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity and HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. According to the so-called "best-fit " method, the
sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims et al., J. Immunol. 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high binding affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Various forms of a humanized anti-IL-23p19 antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are
capable, upon immunization, of producing a full repertoire of human antibodies in
the absence of endogenous immunoglobulin production. For example, it has been
described that the homozygous deletion of the antibody heavy-chain joining region
(JH) gene in chimeric and germ-line mutant mice results in complete inhibition of
dependent antibody production. Transfer of the human germ-line
immunoglobulin gene array into such germ-line mutant mice will result in the
5,545,806, 5,569,825, 5,591,669; U.S. Pat. No. 5,545,807; and WO 97/17852 (the
disclosures of the foregoing patent references are incorporated by reference herein).

Alternatively, phage display technology (McCafferty et al., Nature 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from
unimmunized donors. According to this technique, antibody V domain genes are
cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a
single-stranded DNA copy of the phage genome, selections based on the
functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the
properties of the B-cell. Phage display can be performed in a variety of formats,
reviewed in, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in
Structural Biology 3:564-571 (1993). Several sources of V-gene segments can be
diverse array of anti-oxazolone antibodies from a small random combinatorial
library of V genes derived from the spleens of immunized mice. A repertoire of V
genes from unimmunized human donors can be constructed and antibodies to a
diverse array of antigens (including self-antigens) can be isolated essentially
following the techniques described by Marks et al., J. Mol. Biol. 222:581-597
5,565,332 and 5,573,905.
As discussed above, human antibodies may also be generated by in vitro activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275, incorporated herein by reference). Such linear antibody fragments may be monospecific or bispecific.

Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:1 07-1 17 (1992); and Brennan et al., *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter et al., *Bio/Technology* 10: 163-1 67 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased in vivo half-life comprising a salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/1 6185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458, the disclosures of which are incorporated by reference. Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus, they are suitable for reduced nonspecific binding during in vivo use. sFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody ", e.g., as described in U.S. Pat. No. 5,641,870 for example, the disclosure of which is incorporated by reference. Such linear antibody fragments may be monospecific or bispecific.

Bispecific Antibodies
Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of IL-23p19. Other such antibodies may combine a IL-23p19 binding site with a binding site for another polypeptide. Alternatively, an anti-IL-23p19 antibody arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD3), or Fc receptors for IgG (FcyR), such as FcyRI (CD64), FcyRII (CD32) and FcyRIII (CD16), so as to focus and localize cellular defense mechanisms to the IL-23p19-expressing and/or binding cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express and/or bind IL-23p19. These antibodies possess a IL-23p19 binding arm and an arm which binds the cytotoxic agent (e.g., saporin, anti-interferon-a, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g., F(ab')2 bispecific antibodies).


Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., Nature 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, the disclosure of which is incorporated by reference, and in


According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3
regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology* 121:210 (1986).

According to another approach described in U.S. Pat. No. 5,731,168 (incorporated herein by reference), the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities " of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.
Bispecific antibodies include cross-linked or "heteroconjugate " antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')2 fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')2 molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two
different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., _Proc. Natl. Acad. Sci. USA_ 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a \( V_H \) connected to a \( V_L \) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the \( V_H \) and \( V_L \) domains of one fragment are forced to pair with the complementary \( V_L \) and \( V_H \) domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., _J. Immunol._, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., _J. Immunol._ 147:60 (1991).

**Heteroconjugate Antibodies**

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Pat. No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptopbutyrimidate and those disclosed, for example, in U.S. Pat. No. 4,676,980.

**Multivalent Antibodies**

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression
of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)n-VD2-(X2)n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH 1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated herein comprise a light chain variable domain and, optionally, further comprise a CL domain.

Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, e.g., so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies may also be prepared using heterobifunctional cross-linkers as described in Wolff et al., Cancer Research 53:2560-2565 (1993).
Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., *Anti-Cancer Drug Design* 3:219-230 (1989). To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope " refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.
Methods of treatment

The method of the invention comprises administering an anti-IL-23p19 antibody to treat ocular diseases such as keratoconjunctivitis sicca, ocular cicatricial pemphigoid, blepharitis, ocular allergy, or ocular infection, and systemic diseases such as Stevens-Johnson disease, and graft versus host disease.

In one embodiment, the method of the invention may be used to treat keratoconjunctivitis sicca. Keratoconjunctivitis sicca, as used here, refers to dry eye disease, which a patient experiences as chronic dryness of the cornea and conjunctiva. Other symptoms include a sandy-gritty feeling in the eye, burning, irritation, or a foreign-body sensation. Patients suffering from dry eye disease complain of mild to severe symptoms, and those with severe symptoms may experience constant and disabling eye irritation, and develop ocular surface epithelial disease and sight-threatening sterile or microbial corneal ulceration. Although the discomfort of keratoconjunctivitis sicca is often associated with ocular inflammation, it need not be; the only criteria is that the cause of the patient's discomfort is a deficiency in the quantity or quality of tears. This distinguishes the dryness of keratoconjunctivitis sicca from the discomfort - sometimes perceived as dryness - in patients with other ocular disease. The method of the invention includes administering the compositions of the invention to alleviate symptoms of keratoconjunctivitis sicca, such as inflammation and dryness, as well as to correct the physiological changes associated with dry eye, such as increased proinflammatory cytokine expression, inflammatory cell infiltration, decreased tear production and goblet cell number.

In another embodiment, the method of the invention may be used to treat a patient suffering from diminished corneal sensitivity caused by refractive surgery, such as laser-assisted in situ keratomileusis (LASIK), or other trauma to the eye. Such trauma severs the corneal nerves, resulting in a state of nerve injury; this leads to discomfort, often perceived as dryness, despite that a patient may have normal tear production. Corneal sensitivity, as measured by a Cochet-Bonnet esthesiometer, usually returns to normal after a period of several months, but one can administer IL-10 to a patient to hasten that recovery.

In another embodiment, the method of the invention may be used to treat a patient suffering from ocular allergy, such as atopic keratoconjunctivitis (allergic inflammation of the eye) or vernal keratoconjunctivitis (seasonal inflammation of
the eye, usually the result of allergy). In another embodiment, the method of the invention may be used to treat conjunctivitis or keratoconjunctivitis of whatever cause, such as bacterial or viral infection. In another embodiment, the method of the invention may be used to treat ocular symptoms of cicatricial pemphigoid. In another embodiment, the method of the invention may be used to treat uveitis, including anterior, intermediate, and posterior uveitis, and panuveitis.

When administering the therapeutic proteins of the invention to treat conditions of the eye, the proteins may be administered by any means that locally affects the eye, meaning that they may be administered topically, by injection, or by the implantation of a substrate or device which releases the protein into the eye. The proteins may also be administered systemically.

In another embodiment, the method of the invention may be used to treat Stevens-Johnson disease and graft versus host disease. The therapeutic proteins of the invention may be administered locally to treat Stevens-Johnson disease, such as by topically apply a cream or gel containing the protein. For both Stevens-Johnson disease and graft versus host disease the proteins may also be delivered systemically.

Formulation

Therapeutic formulations of antibody according to the present invention may be prepared for storage by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine;
monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS®, or polyethylene glycol (PEG).

In relationship to any of the compositions described herein, it is preferable that an effective amount of buffer be included to maintain the pH from about 6 to about 8, preferably about 7. Buffers used are those known to those skilled in the art, and, while not intending to be limiting, some examples are acetate, borate, carbonate, citrate, and phosphate buffers. Preferably, the buffer comprises borate. An effective amount of buffer necessary for the purposes of this invention can be readily determined by a person skilled in the art without undue experimentation. In cases where the buffer comprises borate, it is preferable that the concentration of the borate buffer be about 0.6%.

In any of the compositions related described herein related to this invention, it is preferable for a tonicity agent to be used. Tonicity agents are used in ophthalmic compositions to adjust the concentration of dissolved material to the desired isotonic range. Tonicity agents are known to those skilled in the ophthalmic art, and, while not intending to be limiting, some examples include glycerin, mannitol, sorbitol, sodium chloride, and other electrolytes. Preferably, the tonicity agent is sodium chloride.

In any of the compositions related to the present invention which are described herein, it is preferable for a preservative to be used when the composition is intended for multiple use. There may also be reasons to use a preservative in single use compositions depending on the individual circumstances. The term preservative has the meaning commonly understood in the ophthalmic art. Preservatives are used to prevent bacterial contamination in multiple-use ophthalmic preparations, and, while not intending to be limiting, examples include benzalkonium chloride, stabilized oxychloro complexes (otherwise known as Purite®), phenylmercuric acetate, chlorobutanol, benzyl alcohol, parabens, and thimerosal. Preferably, the preservative is benzalkonium chloride (BAK).

Under certain circumstances, a surfactant might be used in any of the compositions related to this invention which are described herein. The term
surfactant used herein has the meaning commonly understood in the art. Surfactants are used to help solubilize the therapeutically active agent or other insoluble components of the composition, and may serve other purposes as well. Anionic, cationic, amphoteric, zwitterionic, and nonionic surfactants may all be used in this invention. For the purposes of this invention, it is preferable that a nonionic surfactant, such as polysorbates, poloxamers, alcohol ethoxylates, ethylene glycol-propylene glycol block copolymers, fatty acid amides, alkylphenol ethoxylates, or phospholipids, is used in situations where it is desirable to use a surfactant.

Another type of compound that might be used in any composition of this invention described herein is a chelating agent. The term chelating agent refers to a compound that is capable of complexing a metal, as understood by those of ordinary skill in the chemical art. Chelating agents are used in ophthalmic compositions to enhance preservative effectiveness. While not intending to be limiting, some useful chelating agents for the purposes of this invention are edetate salts, like edetate disodium, edetate calcium disodium, edetate sodium, edetate trisodium, and edetate dipotassium.

Administration

For the treatment of eye conditions, compositions of the invention are administered topically to the eye. Depending on the type and severity of the disease, about 1 pg/kg to about 50 mg/kg (e.g., 0.1 -20 mg/kg) of antibody is an initial candidate dosage for administration to the patient. A typical daily or weekly dosage might range from about 1 pg/kg to about 20 mg/kg or more.
What is claimed is

1. A method for the treatment of an ocular condition selected from keratoconjunctivitis sicca, diminished corneal sensitivity, atopic keratoconjunctivitis, vernal keratoconjunctivitis, ocular cicatricial pemphigoid, or blepharitis, the method comprising administering to a patient having the condition a composition comprising an anti-IL-23p19 antibody.

2. The method of claim 1, wherein the condition is keratoconjunctivitis sicca.

3. The method of claim 1, wherein the condition is diminished corneal sensitivity.

4. The method of claim 1, wherein the condition is atopic keratoconjunctivitis.

5. The method of claim 1, wherein the condition is vernal keratoconjunctivitis.

6. The method of claim 1, wherein the condition is ocular cicatricial pemphigoid.

7. The method of claim 1, wherein the condition is blepharitis.

8. The method of claim 1, wherein the anti-IL-23p19 antibody is a polyclonal, monoclonal, humanized, bispecific, or heteroconjugate antibody, or a functional fragment of the foregoing.

9. A method for the treatment of a patient having Stevens-Johnson disease or graft versus host disease, the method comprising administering to the patient an anti-IL-23p19 antibody.

10. The method of claim 9, wherein the anti-IL-23p19 antibody is a polyclonal, monoclonal, humanized, bispecific, or heteroconjugate antibody, or a functional fragment of the foregoing.