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(54) Title: T-CELL DEATH-INDUCING EPITOPES

(57) Abstract: Cell death-inducing epitopes and polypeptides containing same. Also disclosed are compounds for inducing death of activated T-cells, a method of producing antibodies to the epitopes, a method of identifying compounds that bind to the epitopes, a method of inducing death of activated T cells, and pharmaceutical compositions containing the compounds.

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T-CELL DEATH-INDUCING EPITOPES

RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 60/570,161, filed on May 11, 2004, the contents of which are incorporated by reference in its entirety.

BACKGROUND

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Control of unwanted immune responses is critical in treating autoimmune diseases, transplant rejection, allergic diseases, and T-cell-derived cancers. The activity of overly aggressive T-cells can be contained by immunosuppression or by induction of immunological tolerance. Apoptosis is believed to be involved in maintaining proper functions of the immune system and removing unwanted cells, such as overly aggressive T-cells (Kabelitz et al. (1993) Immunol Today 14, 338-340; and Raff (1992) Nature 356, 397-399).

SUMMARY

This invention relates to T-cell death-inducing epitopes. The epitopes can be used for, among others, selecting compounds that bind to the epitopes. Such compounds are useful in treating diseases involving overly aggressive T-cells. Examples of such diseases include autoimmune diseases, transplant rejection, allergic diseases, and T-cell-derived cancers.

According to a first aspect, the present invention provides an isolated polypeptide comprising X_1-X_2-X_3-X_4-X_5, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_1 is Tyr, Trp, His, or Met;

X_2 is Asp;

X_3 is Ser, Phe, Pro, Glu, or His;

X_4 is any amino acid; and

X_5 is Pro, Tyr, His, or Trp; and

wherein X_1-X_2-X_3-X_4-X_5 is not Tyr-Asp-Phe-Leu-Pro.

According to a second aspect, the present invention provides an isolated polypeptide comprising X_6-X_7-X_8-X_9-X_{10}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_6 is Asp;

X_7 is Tyr, Met, Asn, Trp, or Phe;

X_8 is Phe or Leu;

X_9 is Pro; and

X_{10} is Glu; and

wherein X_6-X_7-X_8-X_9-X_{10} is not Asp-Phe-Leu-Pro-Glu.

According to a third aspect, the present invention provides an isolated polypeptide comprising X_{11}-X_{12}-X_{13}-X_{14}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_{11} is Pro;

X_{12} is Met;

X_{13} is Glu or Ser; and

X_{14} is Ile.

According to a fourth aspect, the present invention provides an antibody that binds to the polypeptide of the invention and induces death of activated T-cells.

According to a fifth aspect, the present invention provides a method of producing an antibody, the method comprising administering to a subject an effective amount of the polypeptide of the invention.

According to a sixth aspect, the present invention provides a method of identifying a candidate compound for inducing death of activated T-cells, the method comprising contacting a compound with the polypeptide of the invention, wherein binding of the compound to the polypeptide indicates that the compound is a candidate for inducing death of activated T-cells.

According to a seventh aspect, the present invention provides a method of inducing death of activated T-cells, the method comprising contacting activated T-cells with the antibody according to the fourth aspect.

According to an eighth aspect, the present invention provides a pharmaceutical composition, comprising the polypeptide of the invention and a pharmaceutically acceptable carrier.

According to a ninth aspect, the present invention provides a pharmaceutical composition, comprising the antibody of the invention and a pharmaceutically acceptable carrier.

According to a tenth aspect, the present invention provides an antibody when prepared by the method according to the invention.

According to an eleventh aspect, the present invention provides a method of treating an autoimmune disease, transplant rejection, an allergic disease, or a T-cell-derived cancer said method comprising the step of administering to a subject an isolated polypeptide according to the invention, an antibody according to the invention or a pharmaceutical composition according to the eighth or ninth aspects.

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According to a twelfth aspect, the present invention provides a use of an isolated polypeptide according to the invention, an antibody according the invention or a pharmaceutical composition according to the eighth or ninth aspects for the preparation of a medicament for the treatment of an autoimmune disease, transplant rejection, an allergic disease, or a T-cell-derived cancer.

According to a thirteenth aspect, the present invention provides an isolated polypeptide comprising X_1-X_2-X_3-X_4-X_5, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_1 is Tyr, Trp, His, or Met;

X_2 is Asp;

X_3 is Ser, Phe, Pro, Glu, or His;

X_4 is any amino acid; and

X_5 is Pro, Tyr, His, or Trp, and

wherein the polypeptide is 5-15 amino acids in length.

According to a fourteenth aspect, the present invention provides an isolated polypeptide comprising X_6-X_7-X_8-X_9-X_{10}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_6 is Asp;

X_7 is Tyr, Met, Asn, Trp, or Phe;

X_8 is Phe or Leu;

X_9 is Pro; and

X_{10} is Glu, and

wherein the polypeptide is 5-15 amino acids in length.

According to a fifteenth aspect, the present invention provides an isolated polypeptide comprising X_{11}-X_{12}-X_{13}-X_{14}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_{11} is Pro or Ala;

X_{12} is Met;

X_{13} is Glu or Ser; and

X_{14} is Ile, and

wherein X_{11}-X_{12}-X_{13}-X_{14} is not Ala-Met-Glu-Ile.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

In a further aspect, the invention features a three-dimensional conformation of an isolated epitope. Binding of a ligand to the epitope on activated T-cells induces death of the cells. Such an epitope is represented by:

(1) X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:1), where

X_1 is Tyr, Trp, His, or Met;

X_2 is Asp;

X_3 is Ser, Phe, Pro, Glu, or His;

X_4 is any amino acid that naturally occurring in animals; and

X_5 is Pro, Tyr, His, or Trp;

(2) X_6-X_7-X_8-X_9-X_{10} (SEQ ID NO:2), where

X_6 is Asp;

X_7 is Tyr, Met, Asn, Trp, or Phe;

X_8 is Phe or Leu;

X_9 is Pro; and

X_{10} is Glu; or

(3) X_{11}-X_{12}-X_{13}-X_{14} (SEQ ID NO:3), where

X_{11} is Pro;

X_{12} is Met;

X_{13} is Glu or Ser; and

X_{14} is Ile.

Any of those epitopes described above can be, e.g., a polypeptide, an interacting region of two polypeptides, a carbohydrate moiety, a glycoprotein, or any conformational, functional equivalent thereof.

In another aspect, the invention features an isolated polypeptide containing X_1-X_2-X_3-X_4-X_5, X_6-X_7-X_8-X_9-X_{10}, or X_{11}-X_{12}-X_{13}-X_{14}. Binding of a ligand to the polypeptide on activated T-cells induces death of the cells. In one embodiment, the polypeptide contains 4 to 400 amino acids (e.g., any integer between 4 and 400, inclusive). For example, the polypeptide can be X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:1), X_6-X_7-X_8-X_9-X_{10} (SEQ ID NO:2), X_{11}-X_{12}-X_{13}-X_{14} (SEQ ID NO:3), or any of SEQ ID NOs:4, 6-18, and 20-22.

An "isolated epitope" or "isolated polypeptide" refers to an epitope or polypeptide substantially free from naturally associated molecules, i.e., it is at least 75% (e.g., any number between 75% and 100%, inclusive) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. An isolated epitope or polypeptide of the invention can be purified from a natural source, produced by recombinant DNA techniques, or by chemical methods.

In still another aspect, the invention features a novel compound that binds to one of the above-described epitopes. The compound can be any kind of molecule, including antibodies such as monoclonal antibodies. A compound of the invention can be used for detecting an epitope of the invention and for inducing death of activated T-cells.

Also within the scope of the invention is a method of producing antibodies. The method involves administering to a subject an effective amount of one of the above-described epitopes (e.g., polypeptides). The antibodies can be used for detecting an epitope of the invention or for inducing death of activated T-cells.

The invention also features a method of identifying a candidate compound (e.g., a monoclonal antibody) for inducing death of activated T-cells. The method involves contacting a test compound with an epitope of the invention and determining whether the test compound binds to the epitope. If the test compound binds to the epitope, it is a candidate for inducing death of activated T-cells.

The invention further features a method of inducing death of activated T-cells by contacting activated T-cells with a compound of the invention.

In yet another aspect, the invention features a pharmaceutical composition containing a pharmaceutically acceptable carrier and (1) an epitope of the invention such as a polypeptide, or (2) a compound that binds to the epitope.

The invention provides compositions and methods for treating diseases involving overly aggressive T-cells such as autoimmune diseases, transplant rejection, allergic diseases, and T-cell-derived cancers. The details of one or more embodiments of the invention are set forth in the accompanying description below. Other features, objects, and advantages of the invention will be apparent from the detailed description.

DETAILED DESCRIPTION

This invention is based on the unexpected discovery that activated T-cells can be induced to undergo apoptosis and be depleted by engagement of new T-cell death-inducing epitopes. Depletion of activated T-cells are particularly useful for treating conditions associated with an excessive or unwanted T-cell-mediated immune response or T-cell proliferation. For example, depletion of activated T-cells can result in reduction or elimination of undesirable T-cell activity or proliferation related to autoimmune diseases, transplant rejection, allergic diseases, or T-cell-derived cancers.

Accordingly, the invention features a three-dimensional conformation of an isolated epitope. Binding of a ligand to the epitope on activated T-cells induces death of the cells. The epitope is represented by X_1-X_2-X_3-X_4-X_5, X_6-X_7-X_8-X_9-X_{10}, or X_{11}-X_{12}-X_{13}-X_{14}. The three-dimensional conformation of X_1-X_2-X_3-X_4-X_5, X_6-X_7-X_8-X_9-X_{10}, or X_{11}-X_{12}-X_{13}-X_{14} can be determined, e.g., using computer modeling programs as described in Duggan et al., (1995) J Med Chem. 38:3332-41 and Toogood (2002) J Med Chem. 45: 1543-57. Epitopes of conformational, functional equivalence can be designed in accordance with the three-dimensional conformation of X_1-X_2-X_3-X_4-X_5, X_6-X_7-X_8-X_9-X_{10}, or X_{11}-X_{12}-X_{13}-X_{14}, prepared using methods known in the art, and tested for their abilities to be involved in induction of death of activated T-cells by methods such as that described in the example below. See, e.g., Barbas et al. (2001) Phage display. A laboratory manual. CSHL Press; Parmley et al. (1998) Gene 73, 305-318; Scott et al. (1990) Science 249, 386-390; U.S. Patent Application No. 20030049252 A1; WO 03/013603 A1; Osborne (1996) Curr Opin Immunol 8:245-248; Lin et al. (1997) J. Immunol. 158, 598-603; Zhang et al. (1995) Nature 377, 348-350; Lai et al. (1995) Eur J Immunol 25, 3243-3248; Mollereau et al. (1996) J Immunol 156, 3184-3190; and Gribben et al. (1995) Proc Natl Acad Sci USA 92, 811-815.

As used herein, an "activated T-cell" is a T-cell having a higher frequency, rate, or extent of proliferation than that of a non-activated T-cell. "Death" of a cell includes programmed cell death, i.e., apoptosis. "Induction of cell death" by an agent occurs when a population of cells treated with the agent exhibits a higher death rate compared to an untreated cell population. For example, the percentage of in vitro activated T-cells undergoing apoptosis is about doubled when treated with monoclonal antibodies m128-9F9, m152-15A7, or m166-43B6 compared to that of untreated cells, as determined by annexin V staining and FACS analysis (see the example below).

The invention also features an isolated polypeptide containing X_1-X_2-X_3-X_4-X_5, X_6-X_7-X_8-X_9-X_{10}, or X_{11}-X_{12}-X_{13}-X_{14}. The polypeptide can be used for identifying compounds that induce death of activated T-cells. Binding of such a compound to the polypeptide expressed on the surface of activated T-cells induces cell death. Further, free polypeptides (i.e., those not expressed on the cell surface) can inhibit unwanted cell death by competing for endogenous death-inducing ligands with the cell-surface polypeptides. The length or sequence of the polypeptide may vary for these uses. A polypeptide of the invention can be obtained, e.g., as an isolated T-cell surface protein, a synthetic polypeptide, or a recombinant polypeptide. To prepare a recombinant polypeptide, a nucleic acid encoding it can be linked to another nucleic acid encoding a fusion partner, e.g., Glutathione-S-Transferase (GST), 6x-His epitope tag, or M13 Gene 3 protein. The resultant fusion nucleic acid expresses in suitable host cells a fusion protein that can be isolated by standard methods. The isolated fusion protein can be further treated, e.g., by enzymatic digestion, to remove the fusion partner and obtain the recombinant polypeptide of this invention.

An epitope of the invention or a polypeptide of the invention can be used to generate antibodies in animals (for production of antibodies) or humans (for treatment of diseases). Methods of making monoclonal and polyclonal antibodies and fragments thereof in animals are known in the art. See, for example, Harlow and Lane, (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. The term "antibody" includes intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, Fv, scFv (single chain antibody), and dAb (domain antibody; Ward, et. al. (1989) Nature, 341, 544). These antibodies can be used for detecting the epitope, e.g., in identifying a compound that binds to the epitope (see below). The antibodies that are capable of inducing death of activated T-cells are also useful for treating diseases such as autoimmune diseases, transplant rejection, allergic diseases, or T-cell-derived cancers. In general, an epitope of the invention, e.g., a polypeptide, can be coupled to a carrier protein, such as KLH, mixed with an adjuvant, and injected into a host animal. Antibodies produced in that animal can then be purified by peptide affinity chromatography. Commonly employed host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Useful human adjuvants include BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies, heterogeneous populations of antibody molecules, are present in the sera of the immunized subjects. Monoclonal antibodies, homogeneous populations of antibodies to a particular antigen, can be prepared using standard hybridoma technology (see, for example, Kohler et al. (1975) Nature 256, 495; Kohler et al. (1976) Eur J Immunol 6, 511; Kohler et al. (1976) Eur J Immunol 6, 292; and Hammerling et al. (1981) Monoclonal Antibodies and T Cell Hybridomas, Elsevier, N.Y.). In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al. (1975) Nature 256, 495 and U.S. Patent No. 4,376,110; the human B-cell hybridoma technique (Kosbor et al. (1983) Immunol Today 4, 72; Cole et al. (1983) Proc Natl Acad Sci USA 80, 2026, and the EBV-hybridoma technique (Cole et al. (1983) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridoma producing the monoclonal antibodies of the invention may be cultivated in vitro or in vivo. The ability to produce high titers of monoclonal antibodies in vivo makes it a particularly useful method of production.

In addition, techniques developed for the production of "chimeric antibodies" can be used. See, e.g., Morrison et al. (1984) Proc Natl Acad Sci USA 81, 6851; Neuberger et al. (1984) Nature 312, 604; and Takeda et al. (1984) Nature 314:452. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778 and 4,704,692) can be adapted to produce a phage library of single chain Fv antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge. Moreover, antibody fragments can be generated by known techniques. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments that can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments.

Antibodies can also be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; and Oxford Molecular, Palo Alto, Calif.). Fully human antibodies, such as those expressed in transgenic animals are also features of the invention (see, e.g., Green et al. (1994) Nature Genetics 7, 13; and U.S. Patent Nos. 5,545,806 and 5,569,825).

The invention further features a novel compound that binds to an epitope of the invention and induces death of activated T-cells. Such a compound can be designed, e.g., using computer modeling programs, according to the three-dimensional conformation of the epitope, and synthesized using methods known in the art. It can also be identified by library screening as described below.

The test compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art. Such libraries include: peptide libraries, peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone that is resistant to enzymatic degradation), spatially addressable parallel solid phase or solution phase libraries, synthetic libraries obtained by deconvolution or affinity chromatography selection, the "one-bead one-compound" libraries, and antibody libraries. See, e.g., Zuckermann et al. (1994) J Med Chem 37, 2678-85; Lam (1997) Anticancer Drug Des 12, 145; Lam et al. (1991) Nature 354, 82; Houghten et al. (1991) Nature 354, 84; and Songyang et al. (1993) Cell 72, 767.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example, in: DeWitt et al. (1993) PNAS USA 90, 6909; Erb et al. (1994) PNAS USA 91, 11422; Zuckermann et al. (1994) J Med Chem 37, 2678; Cho et al. (1993) Science 261, 1303; Carrell et al. (1994) Angew Chem Int Ed Engl 33, 2059; Carell et al. (1994) Angew Chem Int Ed Engl 33, 2061; and Gallop et al. (1994) J Med Chem 37,1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) Biotechniques 13, 412-421), or on beads (Lam (1991) Nature 354, 82-84), chips (Fodor (1993) Nature 364, 555-556), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent No. 5,223,409), plasmids (Cull et al. (1992) PNAS USA 89, 1865-1869), or phages (Scott and Smith (1990) Science 249, 386-390; Devlin (1990) Science 249, 404-406; Cwirla et al. (1990) PNAS USA 87, 6378-6382; Felici (1991) J Mol Biol 222, 301-310; and U.S. Patent No. 5,223,409).

To identify a candidate compound for inducing death of activated T-cells, an epitope of the invention is contacted with a test compound, and the binding of the compound to the epitope is evaluated. If the compound binds to the epitope, it is a candidate for inducing death of activated T-cells.

The screening assay can be conducted in a variety of ways. For example, one method involves anchoring the epitope (or an epitope-containing molecule, e.g., a polypeptide or a fusion protein) or the test compound onto a solid phase and detecting an epitope-test compound complex formed on the solid phase at the end of the reaction. In practice, microtiter plates may conveniently be utilized as the solid phase. The anchor component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the anchor component and drying the plates. Alternatively, an immobilized antibody (e.g., a monoclonal antibody) specific for the anchor component may be used to immobilize the anchor component to the solid surface. The non-anchor component is added to the solid surface coated with the anchor component. After the reaction is complete, unbound fraction of the non-anchor components is removed (e.g., by washing) under conditions such that any complexes formed remain immobilized on the solid surface. Detection of these complexes can be accomplished in a number of ways. Where the non-anchor component is pre-labeled, detection of the label immobilized on the solid surface indicates that complexes were formed. Where the non-anchor component is not pre-labeled, an indirect label can be used to detect complexes formed on the surface, e.g., using an antibody specific for the non-anchor component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, the reaction can be conducted in a liquid phase. The complexes are separated from unbound components, e.g., using an immobilized antibody specific for the epitope (or the epitope-containing molecule) or the test compound. The complexes are then detected, e.g., using a labeled antibody specific for the other component.

The candidate compound can be validated by ascertaining its ability to induce death of activated T-cells, using the method described in the example below, or any other method know in the art. The validated compound can be used for inducing death of activated T-cells and for treating diseases such as autoimmune diseases, transplant rejection, allergic diseases, or T-cell-derived cancers.

The invention provides a method of inducing death of activated T-cells, e.g., by contacting activated T-cells with a compound of the invention in vitro, or by administering to a subject in need thereof an effective amount of a compound of the invention. Subjects to be treated can be identified as having or being at risk for acquiring a condition characterized by an excessive or unwanted T-cell-mediated immune response, e.g., patients suffering from autoimmune diseases, transplant rejection, allergic diseases, or T-cell-derived cancers. This method can be performed alone or in conjunction with other drugs or therapy.

The term “treating” is defined as administration of a composition to a subject with the purpose to cure, alleviate, relieve, remedy, prevent, or ameliorate a disorder, the symptom of the disorder, the disease state secondary to the disorder, or the predisposition toward the disorder. An “effective amount” is an amount of the composition that is capable of producing a medically desirable result, e.g., as described above, in a treated subject.

Exemplary diseases to be treated include, but are not limited to, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, and psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, type I diabetes, inflammatory bowel diseases, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves’ disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, interstitial lung fibrosis, graft-versus-host disease, cases of transplantation (including transplantation using allogeneic or xenogeneic tissues) such as bone marrow transplantation, liver transplantation, or the transplantation of any organ or tissue, allergies such as atopic allergy, AIDS, and T-cell neoplasms such as leukemias or lymphomas.

In one in vivo approach, a therapeutic composition (e.g., a composition containing an epitope of the invention, a polypeptide of the invention, or a compound of the invention) is administered to the subject. Generally, the epitope, the polypeptide, or the compound is suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected or implanted subcutaneously, intramuscularly, intrathecally, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily.

The dosage required depends on the choice of the route of administration; the nature of the formulation; the nature of the subject's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100.0 mg/kg. Wide variations in the needed dosage are to be expected in view of the variety of compositions available and the different efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Encapsulation of the composition in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Also within the scope of this invention is a pharmaceutical composition that contains a pharmaceutically acceptable carrier and an effective amount of a compound of the invention. The pharmaceutical composition can be used to treat diseases described above. The pharmaceutically acceptable carrier includes a solvent, a dispersion medium, a coating, an antibacterial and antifungal agent, and an isotonic and absorption delaying agent.

The pharmaceutical composition of the invention can be formulated into dosage forms for different administration routes utilizing conventional methods. For example, it can be formulated in a capsule, a gel seal, or a tablet for oral administration. Capsules can contain any standard pharmaceutically acceptable materials such as gelatin or cellulose. Tablets can be formulated in accordance with conventional procedures by compressing mixtures of the composition with a solid carrier and a lubricant. Examples of solid carriers include starch and sugar bentonite. The composition can also be administered in a form of a hard shell tablet or a capsule containing a binder, e.g., lactose or mannitol, a conventional filler, and a tableting agent. The pharmaceutical composition can be administered via the parenteral route. Examples of parenteral dosage forms include aqueous solutions, isotonic saline or 5% glucose of the active agent, or other well-known pharmaceutically acceptable excipient. Cyclodextrins, or other solubilizing agents well known to those familiar with the art, can be utilized as pharmaceutical excipients for delivery of the therapeutic agent.

The efficacy of a composition of this invention can be evaluated both in vitro and in vivo. See, e.g., the examples below. Briefly, the composition can be tested for its ability to induce death of activated T-cells in vitro. For in vivo studies, the composition can be injected into an animal (e.g., a mouse model) and its therapeutic effects are then accessed. Based on the results, an appropriate dosage range and administration route can be determined.

The specific example below is to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications recited herein are hereby incorporated by reference in their entirety.

Preparation of a mouse spleen cell suspension

Mouse spleen was immersed in 8 ml of Hank's balanced salt solution (HBSS), gently minced with a sterile cover slip, transferred to a 15 ml centrifuge tube (Costar), and spun at 200 x g for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in the residual buffer by gently tapping the wall. The contaminating red blood cells (RBC) were lysed by addition of 1 ml of RBC lysis buffer (0.6 M NH_4Cl, 0.17 M Tris-base, pH 7.65), followed by a 2 min incubation at room temperature and rapid quenching with 9 ml of HBSS. The cells were pelleted at 200 x g for 5 minutes, washed twice, and resuspended in RPMI medium. The concentration and viability of the cells in the mixture were determined with a hemocytometer (Cambridge Scientific Inc.) and Trypan blue exclusion.

Preparation of anti-T-cell, apoptosis-inducing monoclonal antibodies

T-cell apoptosis-inducing monoclonal antibodies were generated by immunizing a mouse with Concanovalin A-activated human T-cells and screened for their abilities to bind to activated human T-cells and subsequently to induce T-cell apoptosis. The monoclonal antibodies were prepared according to the well-known cell fusion methods of Kohler and Milstein ((1976) Euro J Immunol 6, 511-519) to produce a hybridoma secreting desired antibodies. Three hybridomas generated according to these methods secreted monoclonal antibodies, designated m128-9F9, m152-15A7, and m166-43B6, respectively, that were able to induce T-cell apoptosis in vitro.

Concentrated culture supernatant of each hybridoma was spun at 20000 x g for 10 minutes, and the supernatant was diluted at a 1:1 ratio with the binding buffer (0.1 M sodium acetate, pH 5.0). A protein G column (approximately 1 ml bed volume) was washed three times with 3-5 ml of the binding buffer. The cleared culture supernatant was loaded onto the protein G column, and the flow-through was collected and reloaded to the column. The column was then washed with 6-10 ml of the binding buffer, and the bound antibody was eluted from the column with 5 ml of the elution buffer (0.1 M glycine-HCl, pH 2.8). Each fraction contained 1 ml of the eluted antibody, and the eluted fraction was adjusted to the neutral pH by mixing each 1 ml fraction with 50 microliters of 1 M Tris-HCl, pH 7.5. Fractions containing the antibody were pooled and dialyzed against 2 liters of PBS, pH 7.4 three times for three hours per dialysis. Protein concentrations in the antibody samples were determined following the procedure described by Bradford using the Bio-Rad Protein Assay (BIO-RAD, Hercules, CA).

Induction of death of activated human T-cells by monoclonal antibodies

Activated T cells (see above) were resuspended to a final concentration of 5 x 10^5 cells/ml in RPMI medium containing 5 ng/ml of IL-2, and treated with control Ig, m128-9F9, m152-15A7, or m166-43B6.

It is well known that T-cell death-inducing antibodies can be used as therapeutic agents to treat T-cell-related diseases such as transplantation rejections, autoimmune diseases, and allergy. Three monoclonal antibodies against human T-cells were generated, and the capabilities of these monoclonal antibodies to induce apoptosis of activated human T-cells were examined. Culture supernatants containing monoclonal antibodies secreted by hybridoma cell line m128-9F9, m152-15A7, or m166-43B6 were incubated with either non-activated human T-cells (Day 0) or in vitro activated human T-cells (Day7) for 6 hours. Cells were stained with annexin V after incubation, and subjected to FACS analysis. CD3-positive cells were gated to ensure counting of either in vitro activated human T-cells or resting human T-cells. The apoptotic cells were annexin V staining-positive. Table 1 summarizes the percentage of apoptotic T-cells among all of the T-cells scanned. Unexpectedly, monoclonal antibodies secreted by hybridoma cell lines m128-9F9, m152-15A7, and m166-43B6 induced death of in vitro activated human T-cells but did not affect non-activated human T-cells. This capability of inducing apoptosis of activated T-cells yet sparing the resting T-cells is a unique feature of the apoptotic pathway and is a dominating feature of therapeutic reagents targeting T-cell-mediated diseases.

Table 1 Percentage of apoptotic T-cells

	Untreated	Anti-myc	m128-9F9	Untreated	Anti-myc	m152-15A7	m166-43
Day 0	4.17	6.67	5.82	18.18	15.52	5.23	6.57
Day 7	12.63	13.36	**28.71**	24.18	23.08	**51.66**	**49.44**

Identification of T-cell death-inducing epitopes

In order to identify death-inducing epitopes recognized by monoclonal antibodies m128-9F9, m152-15A7, and m166-43B6, these monoclonal antibodies were used to screen for consensus binding sequences in a polypeptide library (Ph. D.-12™ Phage Display Peptide Library Kit, New England Biolabs, Inc.). The library contained various 12-mer peptides linked to the 406-aa M13 Gene 3 protein. 96-well microtiter plates were coated with 50 µl/well antibodies at the concentration of 10 µg/ml in 0.1 M $NaHCO_3$ (pH 8.6) coating buffer overnight at 4°C. After the wash, the plates were blocked by incubation with the blocking buffer containing 0.1 M $NaHCO_3$ (pH 8.6), 5 mg/ml BSA, 0.02% NaN_3 (150 µl/well) for at least one hour at 4°C. Plates were then incubated with fusion proteins from the polypeptide library described above at various concentrations for one hour at room temperature. After the wash with 0.5% Tween containing TBS, the bound fusion proteins were eluted with 1 mg/ml BSA containing 0.2 M Glycine-HCl (pH 2.2) buffer and neutralized with 1 M Tris-HCl (pH 9.1). The amino acid sequences of eluted fusion proteins were then determined.

The polypeptide sequences bound by monoclonal antibody m128-9F9 are shown below:

WPEDSS**YDS**W**P**RG	SEQ ID NO:4
LD**YDF**L**P**ETEP	SEQ ID NO:5
TAT**WDP**D**Y**FSDS	SEQ ID NO:6
AETD**YDP**D**H**FTPG	SEQ ID NO:7
DARYS**HDP**A**W**PYG	SEQ ID NO:8
AGQK**WDP**E**W**PHSG	SEQ ID NO:9
EPN**MDP**N**W**ASPSG	SEQ ID NO:10
KSH**YDE**S**W**WYNGG	SEQ ID NO:11
YDHH**W**TNPPTQK	SEQ ID NO:12
YDHH**W**PRDDIAP	SEQ ID NO:13

A consensus polypeptide sequence of X_1-X_2-X_3-X_4-X_5 was obtained, where X_1 = Y/W/H/M, X_2 = D, X_3 = S/F/P/E/H, X_4 = any amino acid, and X_5 = P/Y/H/W.

The polypeptide sequences bound by monoclonal antibody m166-43B6 are shown below:

QDTWYP**DYFPE**S	SEQ ID NO:14
SHTLLN**DMFPE**S	SEQ ID NO:15
SPLR**DNFPE**TLW	SEQ ID NO:16
ASPYM**DNFPE**EN	SEQ ID NO:17
QLVQ**DWLPE**ESH	SEQ ID NO:18
YLDY**DFLPE**TEPP	SEQ ID NO:19

A consensus polypeptide sequence of X_6-X_7-X_8-X_9-X_{10} was obtained, where X_6 = D, X_7 = Y/M/N/W/F, X_8 = F or L, X_9 = P, and X_{10} = E.

The polypeptide sequences bound by monoclonal antibody m152-15A7 are shown below:

YTPM**PMEI**SHSA	SEQ ID NO:20
MNDKYI**PMSI**SA	SEQ ID NO:21
KIPHKTLV**PMEI**	SEQ ID NO:22
TDSA**AMEI**QTTQ	SEQ ID NO:23

A consensus polypeptide sequence of X_{11}-X_{12}-X_{13}-X_{14} was obtained, where X_{11} = P/A, X_{12} = M, X_{13} = E/S, and X_{14} = I.

ELISA assay of T-cell death-inducing epitopes recognized by monoclonal antibodies

In order to identify the specificities of the death-inducing epitopes recognized by the monoclonal antibodies described above, the sandwich ELISA was conducted. Serial dilutions (from 0.0017 fmol to 17 fmol) of the epitope-containing polypeptides were incubated with monoclonal antibodies m128-9F9, m152-15A7, or m166-43B6 pre-coated on the ELISA plates to determine their binding affinities.

96-well microtiter plates were coated with 50 μl/well antibodies at the concentration of 1 μg/ml overnight at 4°C. Plates were blocked by incubation with 0.25% of BSA in PBS (150 μl/well) for 1 hour at 37°C. Plates were then incubated with fusion proteins containing various polypeptides for 2 hours at room temperature. After being washed 4 times with PBS containing 0.05% of Tween 20 (PBST), plates were then incubated with antibodies specific for the fusion partner at 2 μg/ml for 1.5 hours at room temperature. After incubation, plates were washed 4 times with PBST. 50 μl of 1 to 3000 times diluted specific goat anti-fusion partner antibodies conjugated with alkaline phosphotase (AP) was then added to each well, and the plates were incubated for 1 hour at 37°C. Enzyme reaction was carried out by adding 50 ul of AP substrate solution (1 AP substrate tablet dissolved in 5 ml of substrate buffer). The results confirmed that all of the selected polypeptides bind specifically to their corresponding antibodies used for selection.

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the scope of the invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. An isolated polypeptide comprising X_1-X_2-X_3-X_4-X_5, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_1 is Tyr, Trp, His, or Met;

X_2 is Asp;

X_3 is Ser, Phe, Pro, Glu, or His;

X_4 is any amino acid; and

X_5 is Pro, Tyr, His, or Trp; and

wherein X_1-X_2-X_3-X_4-X_5 is not Tyr-Asp-Phe-Leu-Pro.

2. An isolated polypeptide comprising X_6-X_7-X_8-X_9-X_{10}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_6 is Asp;

X_7 is Tyr, Met, Asn, Trp, or Phe;

X_8 is Phe or Leu;

X_9 is Pro; and

X_{10} is Glu; and

wherein X_6-X_7-X_8-X_9-X_{10} is not Asp-Phe-Leu-Pro-Glu.

3. The polypeptide of claim 1 or claim 2, wherein the polypeptide is 5 to 1500 amino acids in length.

4. The polypeptide of claim 3, wherein the polypeptide is 5 to 150 amino acids in length.

5. The polypeptide of claim 1, wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 4 and 6-13.

6. The polypeptide of claim 2, wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 14-18.

7. The polypeptide of claim 1, wherein the polypeptide is X_1-X_2-X_3-X_4-X_5.

8. The polypeptide of claim 2, wherein the polypeptide is X_6-X_7-X_8-X_9-X_{10}.

9. An isolated polypeptide comprising X_{11}-X_{12}-X_{13}-X_{14}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_{11} is Pro;

X_{12} is Met;

X_{13} is Glu or Ser; and

X_{14} is Ile.

10. The polypeptide of claim 9, wherein the polypeptide is 4 to 1500 amino acids in length.

11. The polypeptide of claim 10, wherein the polypeptide is 4 to 150 amino acids in length.

12. The polypeptide of claim 11, wherein the polypeptide is 4 to 15 amino acids in length.

13. The polypeptide of claim 9, wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 20-22.

14. The polypeptide of claim 9, wherein the polypeptide is X_{11}-X_{12}-X_{13}-X_{14}.

15. An antibody that binds to the polypeptide of any one of claims 1 to 14 or 27 to 34 and induces death of activated T-cells.

16. The antibody of claim 15, wherein the antibody is monoclonal.

17. A method of producing an antibody, the method comprising administering to a subject an effective amount of the polypeptide of any one of claims 1 to 14 or 27 to 34.

18. A method of identifying a candidate compound for inducing death of activated T-cells, the method comprising contacting a compound with the polypeptide of any one of claims 1 to 14 or 27 to 34, wherein binding of the compound to the polypeptide indicates that the compound is a candidate for inducing death of activated T-cells.

19. The method of claim 18, wherein the compound is an antibody.

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20. The method of claim 19, wherein the antibody is a monoclonal antibody.

21. A method of inducing death of activated T-cells, the method comprising contacting activated T-cells with the antibody of any one of claims 15, 16 or 24.

22. A pharmaceutical composition, comprising the polypeptide of any one of claims 1 to 14 or 27 to 34 and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition, comprising the antibody of any one of claims 15, 16 or 24 and a pharmaceutically acceptable carrier.

24. An antibody when prepared by the method according to claim 17.

25. A method of treating an autoimmune disease, transplant rejection, an allergic disease, or a T-cell-derived cancer said method comprising the step of administering to a subject an isolated polypeptide according to any one of claims 1 to 14 or 27 to 34, an antibody according to any one of claims 15, 16 or 24 or a pharmaceutical composition according to claims 22 or claim 23.

26. Use of an isolated polypeptide according to any one of claims 1 to 14, an antibody according to any one of claims 15, 16 or 24 or a pharmaceutical composition according to claim 22 or claim 23 for the preparation of a medicament for the treatment of an autoimmune disease, transplant rejection, an allergic disease, or a T-cell-derived cancer.

27. An isolated polypeptide comprising X_1-X_2-X_3-X_4-X_5, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_1 is Tyr, Trp, His, or Met;

X_2 is Asp;

X_3 is Ser, Phe, Pro, Glu, or His;

X_4 is any amino acid; and

X_5 is Pro, Tyr, His, or Trp, and

wherein the polypeptide is 5-15 amino acids in length.

28. An isolated polypeptide comprising X_6-X_7-X_8-X_9-X_{10}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_6 is Asp;

X_7 is Tyr, Met, Asn, Trp, or Phe;

X_8 is Phe or Leu;

X_9 is Pro; and

X_{10} is Glu, and

wherein the polypeptide is 5-15 amino acids in length.

29. An isolated polypeptide comprising X_{11}-X_{12}-X_{13}-X_{14}, wherein binding of a ligand to the polypeptide on activated T-cells induces death of the cells, and

X_{11} is Pro or Ala;

X_{12} is Met;

X_{13} is Glu or Ser; and

X_{14} is Ile, and

wherein X_{11}-X_{12}-X_{13}-X_{14} is not Ala-Met-Glu-Ile.

30. The polypeptide of claim 29, wherein the polypeptide is 4 to 1500 amino acids in length.

31. The polypeptide of claim 30, wherein the polypeptide is 4 to 150 amino acids in length.

32. The polypeptide of claim 31, wherein the polypeptide is 4 to 15 amino acids in length.

33. The polypeptide of claim 29, wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 20-22.

34. The polypeptide of claim 29, wherein the polypeptide is X_{11}-X_{12}-X_{13}-X_{14}.

35. An isolated polypeptide according to any one of claims 1, 2, 27, 28 or 29; an antibody according to any one of claims 15, 16 or 24; a method of producing an antibody according to claim 17; a method of identifying a candidate compound for inducing death of activated T-cells according to claim 18; a method of inducing death of activated T-cells according to claim 21; a pharmaceutical composition according to claim 22 or claim 23; a method of treating an autoimmune disease, transplant rejection, an allergic disease, or a T-cell-derived cancer according to claim 25; or use of an isolated polypeptide according to claim 26;

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substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

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