AGONISTS AND ANTAGONISTS OF HUMAN INTERLEUKIN-10

BACKGROUND OF THE INVENTION

This invention relates to agonists and antagonists of human interleukin-10, and to compositions and methods for making and using them. These agonists and antagonists are produced by introducing amino acid substitutions or deletions at the carboxyl and/or amino terminus of mature human interleukin-10.

Interleukin 10 (IL-10) is a cytokine capable of mediating a number of actions or effects. IL-10 has been isolated from both mouse and human cells and is involved in controlling the immune responses of different classes or subsets of $CD4^+$ T helper (Th) cells. These Th cells can be divided into different subsets that are distinguished by their cytokine production profiles. Two of these subsets are called Th1 and Th2 cells.

Th1 T cell clones produce interleukin-2 (IL-2) and gamma interferon (IFN-γ), whereas Th2 cell clones secrete IL-10, interleukin-4 (IL-4) and interleukin-5 (IL-5), generally following activation by antigens or mitogenic lectins. Both classes of Th cell clones also produce cytokines such as tumor necrosis factor-α (TNF-α), interleukin-3 (IL-3), and granulocyte-macrophage colony stimulating factor (GM-CSF). A third category of Th cells (Th0) produces IL-2, IFN-γ, IL-4, IL-5, TNF-α, IL-3 and GM-CSF simultaneously.

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The different cytokine production patterns of Th1 and Th2 cells in part reflect their roles in response to various pathogens. For example, Th1 cells are involved in successful cell-mediated responses to a variety of intracellular pathogens. They are also involved in delayed-type hypersensitivity reactions. Th2 cells are associated with humoral responses, which are characterized by antibody production. In most situations, the immune system develops the Th response that is most effective to eliminate a particular antigen or pathogen, but this is not always the case.

For example, leishmaniasis is characterized by a defective Th1 response. This defect can be demonstrated using *in vitro* assays such as an assay described by Clerici *et al.* [*J. Clin. Invest.* *84*:1892 (1989)]. Through the use of one such *in vitro* assay, it has been shown that the Th1 response defect is attributable to endogenous levels of IL-10, because Th1 function can be restored in the *in vitro* assay by the addition of neutralizing antibodies against IL-10.

Because leishmaniasis and other diseases are characterized by defective Th responses attributable to the inappropriate action of endogenous IL-10, there is a need for agonists and antagonists of IL-10 to treat such diseases.

SUMMARY OF THE INVENTION

The present invention fills this need by providing compositions and methods for providing or inhibiting the biological activity of human IL-10.

More particularly, this invention provides antagonists of human IL-10 which comprise mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues.

The amino acid sequences of three such embodiments are defined in the Sequence Listing by SEQ ID NOs: 1, 2 and 3.

The present invention further provides nucleic acids encoding an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues. Recombinant vectors comprising such nucleic acids and host cells comprising such recombinant vectors are also provided by this invention.

This invention still further provides a method for producing an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues, which method comprises culturing one of the above-mentioned host cells under conditions in which the nucleic acid encoding the antagonist is expressed.

This invention still further provides methods for inhibiting the biological activity of IL-10 comprising contacting cells bearing receptors for IL-10 with an effective amount of an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues.

The present invention still further provides agonists of human IL-10 which comprise mature human IL-10 modified by deletion of from one to eleven of the amino-terminal amino acid residues.

Nucleic acids encoding such agonists, recombinant vectors and transformed host cells comprising such nucleic acids, methods for making the antagonists, and pharmaceutical compositions comprising one or more of the IL-10 agonists or antagonists and a pharmaceutically acceptable carrier are also provided by this invention.

DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirety by reference.

The antagonists of this invention are useful for treating diseases such as leishmaniasis which are characterized by a defective Th1 response attributable to endogenous IL-10. They may also be useful for treatment of diseases related to IL-10-mediated immunosuppression or overproduction of IL-10, such as B-cell lymphomas. Moreover, the antagonists are useful for studies elucidating the mechanism of action of IL-10 and for rational drug design, because they display strong receptor binding which is uncoupled from effector function. Immobilized on a solid support, the antagonists can be used for the affinity purification of soluble forms of the IL-10 receptor, in which the transmembrane region has been deleted.

The Epstein Bar Virus (EBV) viral IL-10 protein (BCRFI, or vIL-10) also possesses the biological activity of IL-10 and presumably binds to IL-10 receptors. Expression of vIL-10 activity by EBV presumably confers some survival advantage to the virus in terms of its ability to infect, replicate and/or maintain itself within a host. The ability of vIL-10 to down-regulate IFN-γ production by both T cells and NK cells, together with its B-cell viability enhancing effects, suggests that vIL-10 can suppress antiviral immunity while at the same time enhancing the potential of EBV to transform human B cells.

The IL-10 antagonists of this invention may therefore also be useful for effectively boosting antiviral immunity against EBV, and possibly other viruses. For more on the potential uses of IL-10 antagonists, see, e.g., Howard *et al.*, *J. Clin. Immunol. 12*:239 (1992).

Three representative embodiments of the mutant IL-10 antagonists of this invention are disclosed in the Example below. In one embodiment, the lysine residue at position 157 of the sequence of mature human IL-10 is replaced by a glutamic acid residue (SEQ ID NO: 1). In other embodiments, three (SEQ ID NO: 2) or four (SEQ ID NO: 3) amino acid residues are deleted from the carboxyl terminus of human IL-10. These antagonists are referred to below as the K157E, CΔ3 and CΔ4 antagonists, respectively.

As used herein, the term "mature human IL-10" is defined as a protein lacking a leader sequence which (a) has an amino acid sequence substantially identical to the sequence defined by SEQ ID NO: 4 and (b) has biological activity that is common to native IL-10. This includes natural allelic variants and other variants having one or more conservative amino acid substitutions [Grantham, *Science 185*:862 (1974)] that do not substantially impair biological activity. Such conservative substitutions involve groups of synonymous amino acids, e.g., as described in U.S. patent No. 5,017,691 to Lee *et al.*

It will be understood that although the foregoing embodiments are presently preferred, other modifications of the carboxyl terminus of human IL-10 can be made to produce other antagonists. For example, it may be possible to produce an effective antagonist by replacing the lysine residue at position 157 with an aspartic acid residue, instead of with the glutamic acid residue. As used herein, the term "acidic amino acid residue" is therefore defined to include both aspartic acid and glutamic acid residues.

More or less extensive deletions can also be made. One or more of the amino acid residues including about the 12 carboxyl-terminal residues can be deleted. Preferably, about the 8 terminal residues are deleted and, more preferably, the 3 or 4 terminal residues.

Surprisingly, it has been found that up to 11 amino acid residues can also be deleted from the amino terminus of mature human IL-10. These truncated variants, which may have different pharmacokinetic properties compared to IL-10 itself, possess the biological activity of mature human IL-10, as measured in an MC/9 mast cell stimulation assay described below. Therefore, they are useful for the treatment of any indication susceptible to treatment by IL-10 itself. They are also useful for some of the purposes described above for the antagonists of the invention, such as affinity purification.

Because they possess the biological activity of human IL-10 but have shortened amino acid sequences, these variants are also referred to herein as "agonists of human IL-10."

It is believed, however, that the cysteine residue at position 12 is essential for biological activity. In fact, deletion of the first 12 residues including this cysteine residue produced a variant which had no biological activity. Therefore, deletions at the amino terminus are limited to deletion of one or more of the first 11 residues.

Such amino-terminal deletions can be combined with the above-mentioned carboxyl-terminal modifications to produce antagonists having the characteristics described below, but possibly different pharmacokinetic properties. These antagonists are also a part of this invention.

Nucleic acids encoding the IL-10 agonists and antagonists are also a part of this invention. Of course those skilled in the art are well aware that, due to the degeneracy of the genetic code, there are many different nucleic acids that could encode each of the agonists and antagonists. The particular codons used can be selected for convenient construction and optimal expression in prokaryotic or eukaryotic systems.

Preferably, nucleic acids encoding the agonists and antagonists are made using the polymerase chain reaction (PCR) [Saiki *et al.*, *Science 239*:487 (1988)], as exemplified by Daugherty *et al.* [*Nucleic Acids Res. 19*:2471 (1991)] to modify cDNA encoding human IL-10. Such cDNA is well known in the art and can be prepared using standard methods, as described, e.g., in International Patent Application Publication No. WO 91/00349. Clones comprising sequences encoding human IL-10 have also been deposited with the American Type Culture Collection (ATCC), Rockville, Maryland, under Accession Numbers 68191 and 68192.

Alternatively, the DNA can be modified using well known techniques of site-directed mutagenesis. See, e.g., Gillman *et al.*, *Gene 8*:81 (1979); Roberts *et al.*, *Nature 328*:731 (1987) or Innis (Ed.), 1990, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, New York, NY.

The nucleic acids of the present invention can also be chemically synthesized using, e.g., the phosphoramidite solid support method of Matteucci *et al.* [*J. Am. Chem. Soc. 103*:3185 (1981)], the method of Yoo *et al.* [*J. Biol. Chem. 764*:17078 (1989)], or other well known methods.

Recombinant vectors comprising the foregoing nucleic acids are also a part of this invention, as are host cells transformed with such vectors, and methods for making the agonists and antagonists.

Insertion of DNA encoding one of the agonists and antagonists into one of the many known expression vectors is easily accomplished when the termini of both the DNAs and the vector comprise compatible restriction sites. If this cannot be done, it may be necessary to modify the termini of the DNAs and/or vector by digesting back single-stranded DNA overhangs generated by restriction endonuclease cleavage to produce blunt ends, or to achieve the same result by filling in the single-stranded termini with an appropriate DNA polymerase.

Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the termini. Such linkers may comprise specific oligonucleotide sequences that define desired restriction sites. The cleaved vector and the DNA fragments may also be modified if required by homopolymeric tailing or PCR.

The antagonists of this invention are characterized by human IL-10 receptor binding affinities that are similar to that of human IL-10 itself but are essentially devoid of biological activity. Preferably they will have less than about 10% of the biological of human IL-10 in a standard assay, more preferably less than about 1%.

The antagonists typically produce at least about 25% inhibition of a biological activity of IL-10 in cells bearing IL-10 receptors. Preferably, the degree of inhibition will be at least about 50% and, more preferably, at least about 75%. The actual degree of inhibition may vary with the particular biological activity measured.

The agonists and antagonists can also be chemically synthesized by a suitable method such as by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. Chemically synthesized polypeptides are preferably prepared by solid

phase peptide synthesis as described, e.g., by Merrifield [*J. Am. Chem. Soc. 85*:2149 (1963); *Science 232*:341 (1986)] and Atherton *et al.* (*Solid Phase Peptide Synthesis: A Practical Approach*, 1989, IRL Press, Oxford).

However produced, the agonists and antagonists can be purified, e.g., using HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution and/or other well known methods.

Pharmaceutical compositions can be prepared by admixing one or more of the IL-10 agonists or antagonists, or a pharmaceutically acceptable salt thereof, and a physiologically acceptable carrier.

Useful pharmaceutical carriers can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient. Sterile water, alcohol; fats, waxes, and inert solids may be included in a carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agents) may also be incorporated into the pharmaceutical composition. Generally, compositions useful for parenteral administration of such drugs are well known; e.g. *Remington's Pharmaceutical Science*, 18th Ed. (Mack Publishing Company, Easton, PA, 1990). Single-dose packaging will often be preferred, e.g., in sterile form.

Administration of the agonists and antagonists is preferably parenteral by intraperitoneal, intravenous, subcutaneous or intramuscular injection or infusion, or by any other acceptable systemic method. Alternatively, the antagonists may be administered by an implantable or injectable drug delivery system [see, e.g., Urquhart *et al.*, Ann. Rev. Pharmacol. Toxicol. *24*:199 (1984); Lewis, Ed., *Controlled Release of Pesticides and Pharmaceuticals*, 1981, Plenum Press, New York, New York; U.S. patents Nos. 3,773,919 and 3,270,960]. Oral administration may also be carried out, using

well known formulations which protect the antagonists from gastrointestinal proteases. See also Langer, *Science 249*:1527 (1990).

The agonists and antagonists can also be delivered by standard gene therapy techniques, including, e.g., direct nucleic acid injection into tissues, the use of recombinant viral vectors or liposomes and implantation of transfected cells. See, e.g., Rosenberg, *J. Clin. Oncol. 10*:180 (1992).

The agonists and antagonists can be administered alone or in combination with one or more of the other agents commonly used to treat conditions characterized by a defective Th response. For example, drugs such as interleukin-12 (IL-12) or gamma interferon (IFN-γ) can be co-administered with the antagonists. Insulin, cyclosporin, prednisone or azathioprine can be co-administered with the agonists, e.g., if they are used to replace IL-10 for the treatment or prevention of insulin-dependent diabetes mellitus (see co-pending U.S. application Serial No. 07/955,523, filed October 1, 1992).

Such co-administration of one or more other agents can be concomitant (together with) or sequential (before or after) administration of the agonist or antagonist. All of the administered agents should be present in the patient at sufficient levels to be therapeutically effective. Typically, if a second agent is administered within about the half-life of the first agent, the two agents are considered to be co-administered.

Determination of the appropriate dosage of an agonist or antagonist for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages that are less than optimum. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily

dosage may be divided and administered in portions during the day if desired.

An effective amount will be a dose that produces a demonstrable improvement in one or more clinical parameters and/or a statistically significantly improved response in one or more of the known Th functions, some of which such as IL-2 production are described above. This response can be measured *in vitro* using blood cells taken from the patient, e.g., as described by Clerici *et al.*, *supra*. Such an *in vitro* assay can be carried out prior to the onset of therapy, to provide a reference baseline to which an improved response can be compared.

The actual amount and frequency of administration of the agonists and antagonists and the pharmaceutically acceptable salts thereof for a particular patient will be regulated according to the judgment of the attending clinician, taking into account such factors as age, condition and size of the patient and severity of the symptom(s) being treated.

EXAMPLES

The present invention can be illustrated by the following examples. Unless otherwise specified, percentages given below for solids in solid mixtures, liquids in liquids, and solids in liquids are on a wt/wt, vol/vol and wt/vol basis, respectively.

Reagents and General Methods

Restriction endonucleases were obtained from Boerhinger Mannheim (Indianapolis, IN), while a DNA ligation kit was purchased from Takara Biochem., Inc. (Berkeley, CA). Taq polymerase and Pfu polymerase were obtained from Stratagene (La Jolla, CA). Recombinant human IL-10 (hIL-10) was produced by standard methods in Chinese hamster ovary (CHO) cells, essentially as described by Tsujimoto *et al.* [*J.*

Biochem. 106:23 (1989)]. Tissue culture medium, fetal bovine serum, and glutamine were purchased from Gibco-BRL (Gaithersburg, MD). Oligonucleotide primers were synthesized by standard methods using an Applied Biosystems 380A, 380B or 394 DNA Synthesizer (Foster City, CA).

Standard recombinant DNA methods were carried out essentially as described by Sambrook *et al.* in *Molecular Cloning: A Laboratory Manual*, 2d Edition, 1989, Cold Spring Harbor Laboratory Press, Plainview, New York.

Transfection

Transient expression was carried out as follows. COS cells (ATCC CRL 1651) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 6 mM glutamine, and penicillin/streptomycin. Transfection was carried out by electroporation using a BioRad GENE PULSER® (Richmond, CA).

Cells were detached from culture dishes by trypsin-EDTA treatment and suspended in fresh culture medium. About 5 x 10^6 cells in a volume of 250 µl were mixed with 5 µg of plasmid DNA and then electroporated, with voltage and capacitance set at 0.2 volts and 960 mFD, respectively.

Following electroporation, the cells were transferred into 10 cm culture dishes and cultured at 37°C in 5% CO_2 for 6 hours in 10 ml of serum-containing DMEM. After the cells had attached to the dishes, the medium was removed by aspiration and replaced with serum-free medium. Seventy-two hours later, the conditioned medium was harvested for analysis.

Preparation of Antagonists

Reconstruction of Wild-type Human IL-10 cDNA and Expression Vectors

To facilitate expression and manipulation, the coding region of hIL-10 cDNA was generated by PCR using a pCDSRα-based hIL-10 vector [Vieira *et al.*, *Proc. Natl. Acad. Sci.* USA *88*:1172 (1991); sequence deposited in GenBank under Accession No. M57627] as a template, although other known sources of the cDNA could have been used.

A Kozak vertebrate consensus translational initiator [Kozak, *Nucleic Acids Res. 20*:8125 (1987)] was introduced into a 5' primer designated B1789CC (SEQ ID NO: 5). A *Pst*I site and an *Eco*RI site were added to 5' primer B1789CC and to a 3' primer designated A1715CC (SEQ ID NO: 6), respectively.

Using the above-mentioned primers, the hIL-10 cDNA was subjected to PCR in a 50 µl volume reaction mixture with a 50 µl paraffin oil overlay, in a 0.5 ml Eppendorf tube. The reaction mixture typically contained 26.5 µl of H_2O, 5 µl of Taq (*Thermus aquaticus*) DNA polymerase buffer [final concentrations in the reaction: 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin], 200 µM dNTPs, 60 ng of template DNA, 10 pmoles each of 5' primer B1789CC and 3' primer A1715CC, and 0.5 µl of Taq polymerase (2 units).

The reaction was carried out in a PHC-1 Thermocycler (Techne, Princeton, NJ) with 30 cycles of 95°C, 2 minutes for denaturation; 42°C, 2 minutes for annealing; and 70°C, 1 minute for synthesis. At the end of the 30th cycle, the reaction mixture was incubated another 9 minutes at 72°C for extension.

The PCR mixture was subjected to electrophoresis in a 1.2% agarose Tris-acetate gel containing 0.5 µg/ml ethidium bromide. DNA fragments having the expected sizes were

excised from the gel and purified using a GENECLEAN® Kit (La Jolla, CA). Following recovery from the gel, the product DNA was digested with *Pst*I and *Eco*RI, isolated by gel electrophoresis and GENECLEAN® treatment, and cloned as a *Pst*I/*Eco*RI restriction fragment into expression vector pDSRG (ATCC 68233) and subsequently transferred into expression vector pSV.Sport (Gibco-BRL, Gaithersburg, MD).

The hIL-10 cDNA-containing vectors were propagated in *E. coli* strain DH5α (Gibco-BRL), and the sequence of the DNA was verified by DNA sequencing. The pSV.Sport-based hIL-10 expression vector was used for COS transfection as well as for construction of mutant hIL-10 vectors.

The resynthesized hIL-10 cDNA retained a unique *Bgl*II site and a unique *Bst*EII site, both of which were present in the wild-type cDNA. These two internal restriction sites, the relative positions of which are shown schematically below, were later used to generate mutant hIL-10 cDNAs by cassette replacement.

*Pst*I ------- *Bgl*II ------- *Bst*EII ---------- *Eco*RI

Carboxyl-terminal Modifications

To generate C-terminal mutant antagonists of hIL-10, mutant cDNA fragments corresponding to the *Bst*EII/*Eco*RI region of wild-type hIL-10 cDNA were synthesized by PCR and used to replace the corresponding region in the pSV.Sport hIL-10 DNA described above.

The K157E, CΔ3 and CΔ4 mutant antagonists of human IL-10 were produced by PCR using oligonucleotide primers complementary to the sequence of the resynthesized hIL-10 cDNA described above, with designated mutations pre-introduced in the 3'-end primers.

A 5' primer designated B3351CC having an amino acid sequence defined by SEQ ID NO: 7 was used to produce three mutant antagonists. The sequence of this 5' primer was complementary to a human IL-10 cDNA internal sequence encompassing a unique *Bst*EII restriction site of the wild-type hIL-10 cDNA. The 3' primers used to make the antagonists had sequences complementary to the 3' end sequence of hIL-10 encoding cDNA. These primers, followed by the SEQ ID NOs defining their sequences, were as follows:

Mutant	Primer	SEQ ID NO.
K157E	C3481CC	8
CΔ3	C3482CC	9
CΔ4	B3350CC	10

Using the above-mentioned primers, human IL-10 cDNA was subjected to PCR in a 50 µl volume reaction mixture with a 50 µl paraffin oil overlay, in a 0.5 ml Eppendorf tube. The reaction mixture typically contained 26.5 µl of H_2O, 5 µl of pfu DNA polymerase buffer [final concentrations in the reaction: 20 mM Tris-HCl, pH 8.2, 10 mM KCl, 2 mM $MgCl_2$, 6 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, and 10 µg/ml nuclease-free bovine serum albumin (BSA)], 200 µM dNTPs, 40 ng of template DNA, 10 pmoles each of 5' primer B3351CC and one of the 3' primers, and 0.5 µl of pfu polymerase (2.5 units).

The reaction was carried out in a PHC-1 Thermocycler (Techne, Princeton, NJ) with 22 cycles of: 94°C, 2 minutes for denaturation; 50°C, 2 minutes for annealing; and 72°C, 2 minutes for synthesis. At the end of the 22nd cycle, the reaction mixture was incubated another 7.5 minutes at 72°C for extension.

The PCR mixture was processed by phenol-$CHCl_3$ extraction and ethanol precipitation and then digested sequentially with *Bst*EII and *Eco*RI. The restriction digestion products were subjected to electrophoresis in a 1% agarose/Tris-acetate gel containing 0.5 μg/ml ethidium bromide. DNA fragments having the expected sizes were excised from the gel and recovered by phenol-$CHCl_3$ extraction and ethanol precipitation.

Following recovery from the gel, the *Bst*EII/*Eco*RI restriction fragments of the hIL-10 mutants were used to replace the corresponding region of the wild-type hIL-10 DNA in the pSV.Sport vector. The pSV.Sport-based hIL-10 mutant cDNAs were propagated in *E. coli* strain DH5α and verified by DNA sequencing. The same expression vectors were used to transfect COS cells as described above.

Amino-terminal Modifications

To generate N-terminal variants of human IL-10, modified cDNA fragments corresponding to the *Pst*I/*Bgl*II region of the wild-type hIL-10 cDNA were synthesized by PCR using pairs of primers without a DNA template. The resulting fragments were used to replace the corresponding region of the wild-type hIL-10 DNA in the pSV.Sport vector. Variants were thus produced in which 7 (variant NΔ7), 10 (variant NΔ10), 11 (variant NΔ11) or 12 (variant NΔ12) residues were deleted from the N-terminus of wild-type hIL-10. The pairs of primers used to make each variant, followed by the SEQ ID NOs defining their sequences, were as follows:

Variant	Primer (end)	SEQ ID NO.
NΔ7	C3352CC (5')	11
	C3355CC (3')	12
NΔ10	C3353CC (5')	13
	C3354CC (3')	14
NΔ11	C3483CC (5')	15
	C3485CC (3')	16
NΔ12	C3484CC (5')	17
	C3486CC (3')	18

PCR was carried out using 10 pmoles of each primer of the indicated primer pairs, as described above for synthesis of the C-terminal mutant antagonists. The PCR mixture was processed by phenol-$CHCl_3$ extraction and ethanol precipitation and then digested sequentially with *Bgl*II and *Pst*I. The restriction digestion products were subjected to electrophoresis in an agarose gel as described above, and DNA fragments having the expected sizes were excised from the gel and recovered by phenol-$CHCl_3$ extraction and ethanol precipitation.

Following recovery from the gel, the *Pst*I/*Bgl*II restriction fragments of the hIL-10 variants were used to replace the corresponding region of the wild-type hIL-10 DNA in the pSV.Sport vector, after excision of that region by *Pst*I/*Bgl*II digestion and ligation of the replacement fragment. The pSV.Sport-based hIL-10 mutant cDNAs were propagated, verified and used as described above.

The resulting NΔ7, NΔ10, NΔ11 and NΔ12 variants had amino acid sequences defined by residues 8-160, 11-160, 12-160 and 13-160, respectively, of the sequence of SEQ ID NO: 4.

Human IL-10 Antagonists Having N-terminal Modifications

Antagonists having modifications at both the amino and carboxyl termini can readily be prepared by combining the foregoing methods. For example, an NΔ7/K157E antagonist can be made by producing pSV.Sport containing cDNA encoding the K157E antagonist, by carrying out PCR using 5' primer B3351CC and 3' primer C3481CC as described above. Following preparation and isolation of the NΔ7 variant fragment using 5' primer C3352CC and 3' primer C3355CC as described above, the *PstI/BglII* restriction fragment of the variant is used to replace the corresponding region of the K157E mutant DNA in the pSV.Sport vector, after excision of that region by *PstI/BglI* digestion and ligation of the replacement fragment.

Metabolic Labeling

COS cells were transfected as described above with expression vector pSV.Sport bearing cDNA inserts encoding human IL-10; antagonists K157E, CΔ3 or CΔ4; or agonist variants NΔ7, NΔ10, NΔ11 or NΔ12. The cells were then incubated in 10 cm culture dishes in serum-containing culture medium for 48 to 72 hours. Following this incubation, the culture dishes were washed twice with phosphate-buffered saline (PBS) and incubated at 37°C in 5% CO_2 for 30 minutes, with 8 ml/dish of methionine-free DMEM medium supplemented with dialyzed FBS and glutamine. The medium in each dish was removed by aspiration and replaced with 500 µl of methionine-free medium containing 250-300 µCi of ^{35}S-methionine (DuPont NEN, Boston, MA; specific activity 43.3 mCi/ml).

The cells were incubated at 37°C in 5% CO_2 for 5 hours, after which 10 µl of 1.5 mg/ml L-methionine stock solution was added to the dishes and a 30 minute chase was carried out. The labeled conditioned medium was collected and

subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis [SDS PAGE; Laemmli, *Nature* 227:680 (1970)] in 10-20% gels under non-reducing conditions, and the gels were dried and autoradiographed using standard methods and Kodak XAR film.

The autoradiography revealed distinct labeled bands for human IL-10; antagonists K157E, CΔ3 and CΔ4; and for agonists NΔ7 and NΔ10, all of which migrated with apparent molecular weights of about 16 to 18 kilodaltons. Under identical transfection and cell culture conditions, all three human IL-10 carboxyl-terminal mutant antagonists were expressed at somewhat reduced levels -- about 2 to 4 fold less than that of IL-10. Expression of the amino-terminal agonist variant NΔ7 was comparable to that of IL-10, while the NΔ10 variant was expressed at a level about 4 fold less than the level of IL-10. Expression of variants NΔ11 and NΔ12 was too low to be detected by this method.

ELISA Analysis

To further quantify the levels of the mutant antagonists and human IL-10 in the COS cell conditioned media, an enzyme-linked immunosorbent assay (ELISA) was carried out essentialy as described by Abrams *et al.* [*Immunol. Rev. 127*:5 (1992)]. Two monoclonal antibodies specific for different epitopes on human IL-10, designated 9D7 and 12G8, were prepared by standard methods and used as the capture and detection agents, respectively. Serially-diluted conditioned media were tested in this assay using the purified recombinant human IL-10 as a standard. The detection limit of this assay was about 1 ng/ml, and IL-10 levels in the range of 100 to 300 ng/ml were typically measured in culture media following a 72-hour incubation.

It was thereby found that the relative levels of the IL-10 and the antagonists correlated well with the results obtained by metabolic labeling, suggesting that the epitopes recognized by the monoclonal antibodies used were not in the mutated regions. In a typical assay, expression levels measured for human IL-10, K157E, CΔ3, CΔ4, NΔ7, NΔ10, NΔ11 and NΔ12 were 133, 80, 63, 48, 139, 28, 23 and 6.5 ng/ml, respectively.

Bioassays

Human IL-10 and the representative IL-10 mutant antagonists were examined for activity using mouse mast cells and human peripheral mononuclear cells (PBMCs).

A mast cell stimulation assay was performed essentially as described by O'Garra *et al.* [*Int. Immunol.* 2:821 (1990) and Thompson-Snipes *et al.* [*J. Exp. Med.* 173:507 (1991)]. Briefly, 5 x 10^3 MC/9 cells (ATCC CRL 8306) per well in 100 µl of assay medium [RPMI-1640 containing 10% fetal bovine serum (FBS), 50 µM β-mercaptoethanol, 2 mM glutamine and penicillin/streptomycin] in a 96-well microtiter plate were treated for 48 hours with varying amounts of human IL-10 or one of the IL-10 antagonists. Twenty-five microliters of 5 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, St. Louis, MO) were then added to each well, and the plate was incubated for 3-5 hours. The cells were then lysed using 10% SDS with 10 mM HCl, and absorbance was measured at 570 nm.

The human IL-10 and variants NΔ7, NΔ10 and NΔ11 were active in this assay, but no activity was observed for variant NΔ12. None of the carboxyl-terminal mutant antagonists was active, even when tested at concentrations up to 375 ng/ml (about 100 times the amount of IL-10 which produced strong activity).

To measure inhibition by the IL-10 antagonists of cytokine synthesis induced by lipopolysaccharide (LPS), human peripheral mononuclear cells (PBMC) were obtained from healthy donors and isolated by FICOLL® gradient centrifugation [Boyum, *Scand. J. Clin. Lab. Invest.* Suppl:77 (1966)]. Aliquots of the PBMCs were transferred to wells (10^5 cells/well in 200 µl of RPMI-1640 medium containing 5% FBS, penicillin/streptomycin, non-essential amino acids, sodium pyruvate and 2 mM glutamine) of 96-well microtiter plates.

Human IL-10 was added to some of the wells at a fixed 100 pM concentration, with or without a 100-fold molar excess (10 nM) of an IL-10 antagonist (as measured by ELISA). This was followed immediately by the addition of LPS (Sigma) to each well, to a final concentration of 80 ng/ml. Positive and negative IL-10 controls were incubated in parallel, using medium conditioned by COS cells transfected with an IL-10-expressing vector or plasmid pSV.Sport, respectively. The latter control conditioned medium was used as a diluent for all samples. All determinations were performed in duplicate and confirmed in followup assays, using different cell batches.

The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 24 hours, after which the supernatant fluids were collected and stored at -20°C for later analysis. Levels of IL-6, IL-1α and TNFα were measured in the collected samples using ELISA kits (R & D Systems, Minneapolis, MN), according to the manufacturer's instructions.

All of the antagonists were found to reverse the inhibitory activity of the IL-10 on cytokine synthesis in this assay, as is shown in Table 1.

Table 1

Sample	Percent Residual IL-10 Activity* IL-6	IL-1α	TNF-α
Buffer	100	100	100
Antibody	0	0	0
K157E	21	27	51
CΔ3	12	13	39
CΔ4	19	27	61

* The inhibitory effect of human IL-10 on synthesis of the indicated cytokines was measured in the presence of control buffer, a saturating amount of a neutralizing anti-IL-10 monoclonal antibody, and 100-fold molar excesses of the three IL-10 antagonists.

Similar assays performed with varying amounts of the IL-10 mutant antagonists in the absence of IL-10 showed that none of antagonists had cytokine synthesis inhibitory activity. No inhibitory activity could be detected with any of the antagonists, at concentrations up to and including 100 pM.

To examine the effect of the IL-10 antagonists on T cell activity, a mixed lymphocyte response (MLR) assay was performed. Human PBMC's were isolated as described above. Stimulator PBMCs were prepared by treating the cells with 50 mg/ml mitomycin C (Sigma, St. Louis, MO) for 20 minutes at 37°C.

About 1 x 10^5 each of responder PBMCs and stimulator cells were mixed in each well of a 96-well microtiter dish, along with varying amounts of human IL-10 or one of the K157E, CΔ3 or CΔ4 antagonists, in a total volume of 200 µl (in triplicate). The cells were incubated at 37°C with 5% CO_2

for 6 days, after which the cultures were pulsed with 1 μCi of tritiated thymidine ([^{3}H]-TdR; 15.6 Ci/mmol, NEN, Boston, MA) per well for 16 hours. Lysates were harvested onto a filter using a 96-well cell harvester (Skatron, Inc., Sterline, VA) and counted in a β-counter (Pharmacia LKB Nuclear Inc., Gaithersburg, MD).

It was found that the antagonists were unable to inhibit MLR at a 1 ng/ml concentration. In contrast, human IL-10 produced 82% inhibition of MLR at that concentration.

Receptor Binding Assays

Purified human IL-10 (about 99% pure) was radioiodinated by the ENZYMOBEAD® method (BioRad, Richmond, CA), following the manufacturer's instructions. Approximately 4 x 10^5 transfected COS cells expressing human IL-10 receptor cDNA were pelleted by centrifugation at 200 x *g* for 10 minutes, washed in binding buffer (PBS, 10% fetal calf serum, 0.1% NaN_3), and resuspended in 200 μl of binding buffer containing [^{125}I]-human IL-10 (specific radioactivity 225 μCi/μg) at a concentration of 150 pM, with serially diluted conditioned medium from COS cells expressing cDNA encoding human IL-10 or one of the mutant antagonists of the invention.

After incubation at 4°C for two hours, the cells were centrifuged at 200 x *g* for 10 minutes at the same temperature. The supernatants were then removed, and each cell pellet was resuspended in 100 μl of binding buffer without labeled IL-10, layered over 200 μl of 10% glycerol in binding buffer in elongated microcentrifuge tubes, centrifuged at 200 x *g* for 10 minutes at 4°C, and quick frozen in liquid nitrogen. The cell pellets were then cut into counting tubes and counted in a CLINIGAMMA® 1272 counter (Pharmacia LKB). Non-specific binding was determined by performing the

binding in the presence of 500 to 1000-fold molar excess unlabeled human IL-10.

The results are shown in Table 2, where it can be seen that all of the IL-10 antagonists were almost as effective as IL-10 itself in receptor binding competition.

Table 2

Inhibition of Radiolabeled IL-10 Binding*

Sample	IC_{50} (pM)
Human IL-10	100
K157E	136 ± 65
CΔ3	172 ± 28
CΔ4	120 ± 9

* Data shown, which are the averages from 2 independent assays, are the concentrations of unlabeled human IL-10 or the indicated IL-10 antagonists which produced a 50% inhibition of radiolabeled human IL-10 binding to cellular receptors.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will become apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Schering Corporation

(ii) TITLE OF INVENTION: Agonists and Antagonists of Human Interleukin-10

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Schering-Plough Corporation

(B) STREET: One Giralda Farms

(C) CITY: Madison

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07940

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Macintosh 7.1

(D) SOFTWARE: Microsoft Word 5.1a

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA: U.S. Patent Application Serial No. 08/098,943

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Lunn, Paul, G.

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 160 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1               5                  10                  15
Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30
Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45
Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
```

```
    50                  55                  60
Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80
Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95
Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110
Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125
Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140
Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Glu Ile Arg Asn
145                 150                 155                 160
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1               5                   10                  15
Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30
Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45
Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60
```

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65 70 75 80
Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
85 90 95
Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
100 105 110
Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
115 120 125
Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
130 135 140
Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys
145 150 155

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 156 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1 5 10 15
Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
20 25 30
Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
35 40 45
Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
50 55 60

```
Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80
Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95
Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110
Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125
Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140
Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 160 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1               5                  10                  15
Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30
Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45
Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60
```

```
Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80
Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95
Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110
Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125
Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140
Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
GTCGACTGCA GCCGCCACCA TGCACAGCTC AGCACTGCTC TGTTGCCTGG TCCTCCTGAC  60
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACGTCGAATT CTCAGTTTCG TATCTTCATT GTCATGTAGG C 41

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGACTTTAAG GGTTACCTGG GTTGCCAAGC CTTGTCTGAG ATGATCCAGT TTTATCTAGA 60
GGAGGTGATG CCCCAAGCTG AGAAC 85

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGCTGAATTC AGTTTCGTAT CTCCATTGTC ATGTAGGCTT CTATGTAGT 49

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGCTGAATTC ACTTCATTGT CATGTAGGCT TCTATGTAGT 40

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AGCTGAATTC ACATTGTCAT GTAGGCTTCT ATGTAGT 37

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 91 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GACGACGGTG GCTGCAGCCG CGACCATGCA CAGCTCAGCA CTGCTCTGTT GCCTGGTCCT 60
CCTGACTGGG GTGAGGGCCT CTGAGAACAG C 91

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 90 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGCATCTCGG AGATCTCGAA GCATGTTAGG CAGGTTGCCT GGGAAGTGGG TGCAGCTGTT 60
CTCAGAGGCC CTCACCCCAG TCAGGAGGAC 90

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GACGACGGTG GCTGCAGCCG CCACCATGCA CAGCTCAGCA CTGCTCTGTT GCCTGGTCCT 60
CCTGACTGGG GTGAGGGCCA GC 82

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGCATCTCGG AGATCTCGAA GCATGTTAGG CAGGTTGCCT GGGAAGTGGG TGCAGCTGGC 60
CCTCACCCCA GTCAGGAGGA C 81

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GACGACGGTG GCTGCAGCCG CCACCATGCA CAGCTCAGCA CTGCTCTGTT GCCTGGTCCT 60
CCTGACTGGG GTGAGGGCCT GC 82

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 78 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGCATCTCGG AGATCTCGAA GCATGTTAGG CAGGTTGCCT GGGAAGTGGG TGCAGGCCCT 60
CACCCCAGTC AGGAGGAC 78

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GACGACGGTG GCTGCAGCCG CCACCATGCA CAGCTCAGCA CTGCTCTGTT GCCTGGTCCT 60
CCTGACTGGG GTGAGGGCCA C 81

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGCATCTCGG AGATCTCGAA GCATGTTAGG CAGGTTGCCT GGGAAGTGGG TGGCCCTCAC 60
CCCAGTCAGG AGGAC 75

WHAT IS CLAIMED IS:

1. An antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about12 carboxyl-terminal residues.

2. The antagonist of claim 1 in which from 1 to 11 amino acid residues have been deleted from the amino terminus.

3. The antagonist of claim 1 which has an amino acid sequence defined by SEQ ID NO: 1, 2 or 3.

4. A nucleic acid encoding an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues.

5. The nucleic acid of claim 4 which encodes an antagonist in which from 1 to 11 amino acid residues have been deleted from the amino terminus.

6. The nucleic acid of claim 4 which encodes an antagonist of human IL-10 which has an amino acid sequence defined by SEQ ID NO: 1, 2 or 3.

7. A recombinant vector comprising the nucleic acid of claim 4, which vector is capable of directing expression of the nucleic acid.

8. A host cell comprising the recombinant vector of claim 7.

* C L M S *

9. A method for producing an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues, which method comprises culturing the host cell of claim 8 under conditions in which the nucleic acid is expressed.

10. The method of claim 9 in which the nucleic acid encodes an antagonist in which from 1 to 11 amino acid residues have been deleted from the amino terminus.

11. The method of claim 9 in which the nucleic acid encodes an antagonist which has an amino acid sequence defined by SEQ ID NO: 1, 2 or 3.

12. A method for inhibiting the biological activity of human IL-10 comprising contacting cells bearing receptors for human IL-10 with an effective amount of an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues.

13. The method of claim 12 in which from 1 to 11 amino acid residues have been deleted from the amino terminus of the antagonist.

14. The method of claim 12 in which the antagonist has an amino acid sequence defined by SEQ ID NO: 1, 2 or 3.

15. An agonist of human IL-10 which comprises mature human IL-10 modified by deletion of from one to eleven of the amino-terminal amino acid residues.

* C L M S *

16. The antagonist of claim 15 in which 7, 10 or 11 amino acid residues have been deleted.

17. A nucleic acid encoding an agonist of human IL-10 which comprises mature human IL-10 modified by deletion of from one to eleven of the amino-terminal amino acid residues.

18. The nucleic acid of claim 17 which encodes an agonist in which 7, 10 or 11 amino acid residues have been deleted.

19. A recombinant vector comprising the nucleic acid of claim 17, which vector is capable of directing expression of the nucleic acid.

20. A host cell comprising the recombinant vector of claim 19.

21. A method for producing an agonist of human IL-10 which comprises mature human IL-10 modified by deletion of from one to eleven of the amino-terminal amino acid residues, which method comprises culturing the host cell of claim 20 under conditions in which the nucleic acid is expressed.

22. The method of claim 21 in which the nucleic acid encodes an agonist in which 7, 10 or 11 amino acid residues have been deleted.

23. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of (a) an agonist of human IL-10 which comprises mature human IL-10 modified by deletion of from one to eleven of the amino-terminal amino acid residues, or (b) an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues.

24. The pharmaceutical composition of claim 23 in which from 1 to 11 amino acid residues have been deleted from the amino terminus of the antagonist.

25. The pharmaceutical composition of claim 23 in which the antagonist has an amino acid sequence defined by SEQ ID NO: 1, 2 or 3.

26. The pharmaceutical composition of claim 23 in which 7, 10 or 11 amino acid residues have been deleted in the agonist.

27. The use of an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about the 12 carboxyl-terminal residues for inhibiting the biological activity of IL-10.

28. The use of an antagonist of human IL-10 which comprises mature human IL-10 modified by replacement of the lysine residue at position 157 with an acidic amino acid residue or deletion of one or more amino acid residues in the region containing about 12 carboxyl-terminal residues for the manufacture of a medicament for inhibiting the biological activity of IL-10.