



(11) (21) (C) **2,142,879**
(86) 1993/09/01
(87) 1994/03/17
(45) 2000/10/31

(72) Williams, David A., US

(72) Clark, Steven C., US

(73) GENETICS INSTITUTE, INC., US

(51) Int.Cl.⁶ A61K 38/19

(30) 1992/09/02 (07/941,372) US

(54) **METHODE DE TRAITEMENT DES LESIONS ET DE LA
DEPLETION DES CELLULES**

(54) **METHOD OF TREATING CELL DAMAGE OR DEPLETION**

(57) Procédé permettant de restaurer des populations cellulaires endommagées ou diminuées, selon lequel le patient est traité à l'aide de cytokines, en particulier IL-11 et IL-6.

(57) A method of restoring damaged or depleted cell populations by treating the patient with cytokines, particularly IL-11 and IL-6.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 37/02	A1	(11) International Publication Number: WO 94/05318 (43) International Publication Date: 17 March 1994 (17.03.94)
(21) International Application Number: PCT/US93/08247 (22) International Filing Date: 1 September 1993 (01.09.93) (30) Priority data: 07/941,372 2 September 1992 (02.09.92) US (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 Cambridge Park Drive, Cambridge, MA 02140 (US). (72) Inventors: WILLIAMS, David, A. ; 8751 North Moore Road, Indianapolis, IN 46278 (US). CLARK, Steven, C. ; 122 Johnson Road, Winchester, MA 01890 (US). (74) Agents: BAK, Mary, E. et al.; Howson & Howson, Spring House Corporate Center, P.O. Box 457, Spring House, PA 19477 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: METHOD OF TREATING CELL DAMAGE OR DEPLETION (57) Abstract A method of restoring damaged or depleted cell populations by treating the patient with cytokines, particularly IL-11 and IL-6.		

METHOD OF TREATING CELL DAMAGE OR DEPLETION

5

Field of the Invention

This invention provides a method of treating patients having disorders characterized by cell damage or destruction, and more specifically, a method of using a cytokine to regenerate populations of certain cells, particularly gut cells.

Background of the Invention

Certain mammalian cells in their normal state are characterized by rapid division and proliferation in the body, e.g., small intestinal epithelial cells, sperm cells, hair and skin cells, and hepatocyte or liver cells. Damage to, or depletion of, these cells can result due to certain diseases, infections, exposure to therapeutic agents and treatments, exposure to other chemical or biological agents, and injury or trauma.

For example, the use of chemotherapy and radiation therapy for the treatment of cancer and for the preparation of patients for bone marrow transplantation is toxic to the small intestinal (gut) epithelial cells. In fact, the small intestine is one of the organs most damaged by this therapy. Similarly damaged by such therapy are skin cells, hair cells and sperm cells. This cell damage, particularly to the gut cells, is the cause of significant mortality and morbidity in cancer patients undergoing therapy. Previously, such toxicity has been avoided by limiting the amount of chemotherapy or radiation administered to the patient. For example, gut cell toxicity has been diminished with radiation therapy

by both decreasing the amount of radiation and giving the total dose subdivided into fractions (called 'fractionation' therapy). However, the reduced amount of therapy also has an adverse effect on the spread and growth of the cancer against which it is directed.

Certain autoimmune diseases of the gut, such as Crohn's disease and ulcerative colitis, also have been known to damage the small intestinal cells lining the gut, causing major morbidity and mortality in patients so afflicted. Treatment of autoimmune diseases of the gut include chemotherapy and immune suppression, both of which have serious side effects, among them additional damage to the rapidly dividing gut cells.

Skin and hair cell populations may also be damaged by autoimmune diseases, burns, and alopecia. Sperm cell populations are damaged by oligospermia. Hepatocytes are also damaged by radiation, chemotherapy, and physical trauma.

Damage to gut cells, and to other cells which grow rapidly in a normal healthy mammal can also be the result of trauma or injury to the area, or shock. Exposure to certain industrial and household chemicals, among other agents, can also severely damage normal healthy populations of these cells.

There is a need in the art for methods for treating cell damage, particularly gut cell damage caused by disease or adverse effects of chemotherapeutic and radiation treatment, exposure to other damaging agents or trauma in mammals, particularly humans.

Summary of the Invention

In one aspect, this invention provides a method for treating patients having damaged or depleted cell populations selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the

large intestines and stomach, skin cells, hair cells, sperm cells and liver epithelial cells (hepatocytes). This method includes administering to the patient an effective amount of a selected hematopoietic growth factor or cytokine.

In another aspect, the invention provides a method of treating a patient undergoing chemotherapy or radiation therapy which involves administering a selected cytokine simultaneously with, or subsequently to, the initiation of chemotherapy or radiation treatment. The treatment with the cytokine is continued until a healthy cell population selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, skin cells, hair cells, sperm cells, and hepatocytes is restored.

In another aspect, the invention provides a method of restoring healthy cell populations selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, skin cells, hair cells, sperm cells, and liver epithelial cells in patients suffering from autoimmune conditions by administering to such patients effective amounts of a selected cytokine, particularly IL-11. It is anticipated that these cytokine treatments may be made in combination with currently known and used immunosuppressive therapies.

One or more cytokines alone or in combination useful in these methods may be selected from among interleukin-11, interleukin-6, leukemia inhibitory factor (LIF), Oncostatin M, and ciliary neurotrophic factor (CNTF). Additional cytokines which may be useful in this method include Interleukin-1, Interleukin-3, Interleukin-12 (also known as natural killer cell stimulatory factor) and GM-CSF.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

5 Fig. 1 is a graph illustrating the survival of control and IL-11 treated mice following combined chemotherapy and radiation treatment, as described in Example 5.

10 Fig. 2 is a bar graph illustrating the effect of IL-11 on proliferation of IEC-6 cells, as described in Example 9 below.

Detailed Description of the Invention

The invention provides a method of using a selected cytokine for the treatment of damaged or
15 depleted cell populations, particularly those that are normally rapidly dividing populations. These include, but are not limited to, small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, liver epithelial cells (hepatocytes), skin
20 cells, hair cells, and sperm cells.

The methods of the present invention, which involve the administration of one or more selected cytokines, are useful in restoring populations of these cells regardless of the source or cause of the damage to,
25 or depletion of, the cell population. Cytokines are regulatory proteins that deliver signals between cells of the immune system, and have regulatory effects on cells of the hematopoietic and immune systems. One preferred cytokine for use in treating damaged cell populations is
30 IL-11, a mammalian cytokine, which has been known to be useful in the treatment of selected diseases of the bone marrow and for directly or indirectly stimulating the production or function of B cells. IL-11 is described in

detail in International Application, PCT/US90/06803,
published May, 30, 1991.

The cloned human IL-11 sequence illustrated in
Table I [SEQ ID NO:1 and 2], was deposited with the ATCC,
12301 Parklawn Drive, Rockville, Maryland on March 30,
1990 under ATCC No. 68284. Further, as described in the
examples below, IL-11 may also be produced recombinantly
as a fusion protein with another protein. Table II [SEQ
ID NO:3 and 4] provides such a fusion sequence with *E.*
coli thioredoxin. These sequences enable the production
of IL-11 in a variety of host cells by resort to now
conventional genetic engineering techniques.

IL-11 may also be obtained from certain cell
lines. Human cell lines have been identified as sources
of at least one species of IL-11 as in, for example, the
human lung fibroblast cell line, MRC-5 (ATCC Accession
Number CCL 171), and the human trophoblastic cell line,
TPA30-1, (ATCC Accession Number CRL 1583). Other human
sources for IL-11 may also be available. Additional
information regarding the production of recombinant IL-11
and the isolation of IL-11 obtained from cell sources is
provided in the above referenced International
Application, PCT/US90/06803.

Not only is IL-11 useful in the methods of
treating and restoring the cell populations above-
described, but also, other cytokines are considered to be
useful in the same methods. Certain cytokines which are
characterized by having common signal transduction
pathways with those of IL-11, (i.e., they transduce
through gp130) are anticipated to be useful in the
treatment of patients having cell damage to the selected
cell populations in the same manner as is IL-11, and/or
in combination with IL-11. See, N. Y. Ip et al., Cell,
69:1121-1132 (1992).

Another preferred cytokine for use in this invention which shares common biological activities with IL-11 is Interleukin-6 (IL-6), which is described in detail in PCT patent application WO88/00206, published
5 January 14, 1988.

Still another cytokine sharing the IL-11 signal transduction pathway is Leukemia Inhibitory Factor (LIF), also known as Cholinergic Differentiation Factor (CDF), and is described in detail in PCT patent application
10 WO90/02183, published March 8, 1990. Another cytokine characterized in this way is Oncostatin M (OSM), described in detail in European patent application No. 290,949, published November 12, 1988. Additionally, Ciliary Neurotrophic Factor (CNTF) shares this signal
15 transduction pathway, and is anticipated to be useful in the methods of restoring these cell populations. CNTF is described in detail in PCT patent application WO9104316, published April 4, 1991.

It is further anticipated that other cytokines
20 are likely to be useful in the methods of this invention, either in place of, or in combination with, IL-11, IL-6, and/or one or more of the above disclosed cytokines. One additional cytokine useful in a therapeutic method or combination pharmaceutical preparation according to this
25 invention is Interleukin-1 (IL-1). IL-1 is described in detail in European patent application No. 456,332, published November 13, 1991. Another useful cytokine is known as Natural Killer Cell Stimulatory Factor (NKSF), also termed Interleukin-12 (IL-12). This cytokine is
30 described in detail in PCT patent application WO9205256, published April 2, 1992.

Other cytokines which may be useful in these methods of restoring the cell populations selected from small intestinal epithelial cells, epithelial cells
35 lining the large intestines and stomach, skin cells, hair

cells, and sperm cells include Interleukin-3 (IL-3), and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). GM-CSF is described in detail in PCT patent application WO8600639, published January 30, 1986, [see, 5 also, European Patent Application No. 281,069, published September 7, 1988]. IL-3 is described in detail in United States Patent No. 4,959,455, issued September 25, 1990.

For additional general information on these 10 cytokines, see also, F. Takatsuki et al., Cancer Res., 50:2885-2890 (1990); D. P. Gearing et al., Science, 255:1434-1437 (1992); G. Damia et al., Cancer Res., 52:4082-4089 (1992).

For use in the methods of treatment disclosed 15 in this invention, the above-described cytokines or biologically active fragments thereof may be prepared by genetic engineering techniques, as disclosed in the above-incorporated references. Moreover, in addition to recombinant techniques, the cytokine polypeptides 20 described above may also be produced by known conventional chemical synthesis. Methods for constructing the polypeptides useful in the present invention by synthetic means are known to those of skill in the art. The synthetically constructed cytokine 25 polypeptide sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with the natural cytokine polypeptides are anticipated to possess biological activities in common therewith. Such synthetically constructed 30 cytokine polypeptide sequences or fragments thereof which duplicate or partially duplicate the functionality thereof may also be used in the method of this invention. Thus, they may be employed as biologically active or immunological substitutes for the natural, purified 35 cytokines useful in the present invention.

Modifications in the protein, peptide or DNA sequences of these cytokines or active fragments thereof may also produce proteins which may be employed in the methods of this invention. Such modified cytokines can
5 be made by one skilled in the art using known techniques. Modifications of interest in the cytokine sequences, e.g., the IL-11 sequence, may include the replacement, insertion or deletion of one or more selected amino acid residues in the coding sequences. Mutagenic techniques
10 for such replacement, insertion or deletion are well known to one skilled in the art. [See, e.g., United States Patent No. 4,518,584].

Other specific mutations of the sequences of the cytokine polypeptides which may be useful
15 therapeutically as described herein may involve, e.g., the insertion of one or more glycosylation sites. An asparagine-linked glycosylation recognition site can be inserted into the sequence by the deletion, substitution or addition of amino acids into the peptide sequence or
20 nucleotides into the DNA sequence. Such changes may be made at any site of the molecule that is modified by addition of O-linked carbohydrate. Expression of such altered nucleotide or peptide sequences produces variants which may be glycosylated at those sites.

25 Additional analogs and derivatives of the sequence of the selected cytokine which would be expected to retain or prolong its activity in whole or in part, and which are expected to be useful in the present method, may also be easily made by one of skill in the
30 art. One such modification may be the attachment of polyethylene glycol (PEG) onto existing lysine residues in the cytokine sequence or the insertion of one or more lysine residues or other amino acid residues that can react with PEG or PEG derivatives into the sequence by

conventional techniques to enable the attachment of PEG moieties.

Additional analogs of these selected cytokines may also be characterized by allelic variations in the DNA sequences encoding them, or induced variations in the DNA sequences encoding them. It is anticipated that all analogs disclosed in the above-referenced publications, including those characterized by DNA sequences capable of hybridizing to the disclosed cytokine sequences under stringent hybridization conditions or non-stringent conditions [Sambrook et al, Molecular Cloning. A Laboratory Manual., 2d edit., Cold Spring Harbor Laboratory, New York (1989)] will be similarly useful in this invention.

Also considered useful in these methods are fusion molecules, prepared by fusing the sequence or a biologically active fragment of the sequence of one cytokine to another cytokine or proteinaceous therapeutic agent, e.g., IL-11 fused to IL-6 [see, e.g., methods for fusion described in PCT Patent Application No. WO92/04455, published March 19, 1992]. Alternatively, combinations of the cytokines may be administered together according to the method.

Thus, where in the description of the methods of this invention a particular cytokine is mentioned by name, it is understood by those of skill in the art that the named cytokine encompasses the protein produced by the sequences presently disclosed in the art, e.g., for IL-11, the sequences of Table I and Table II, as well as proteins characterized by the modifications described above yet which retain substantially similar activity in restoring the cell populations of one or more of the cell populations identified herein.

The present invention thus involves treating patients having damaged or depleted cell populations

selected from small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, liver epithelial cells, skin cells, hair cells, and sperm cells. The treatment involves administering an effective
5 amount of a selected cytokine in a pharmaceutical carrier. This treatment enables the restoration or regeneration of the damaged or depleted cell population primarily by the stimulation, direct or indirect, of any undamaged stem cells. The stem cells are stimulated to
10 differentiate into the cell population which had been damaged or depleted.

Cytotoxic agents used in bone marrow transplantation and cancer therapy affect rapidly proliferating cells in both the bone marrow and small
15 intestine, leading to severe and often dose-limiting toxicities. The present invention overcomes this problem as is exemplified below in the treatment of small intestinal epithelial cells (gut cells) damaged by chemotherapy or radiation therapy with IL-11 and/or IL-6
20 as the selected cytokine. In Examples 4-9, analysis of small intestinal mucosa demonstrated rapid recovery of villi length and increased proliferative activity within the crypt cells of IL-11-treated or IL-6-treated mice compared to control mice. For example, in these
25 examples, with mice, IL-11 and/or IL-6 had a positive effect on mouse survival after exposure to 5-fluorouracil and irradiation without an effect on peripheral neutrophil (white blood cell) counts. Further, these studies provided evidence of rapid recovery of the small
30 intestinal mucosa. Thus, IL-11 and IL-6 have positive effects on the recovery of several tissues where dose-limiting toxicities following cytoablative therapies occur.

Where damage to, or depletion of, gut cells or
35 other cell populations is caused by therapy, the

treatment of the present invention may occur simultaneously with, or sequentially after, the therapy, e.g., chemotherapy or radiation. For example, effective amounts of IL-11 alone, IL-6 alone, another
5 cytokine alone, or a combination of cytokines, may be administered in a suitable pharmaceutical carrier.

Preferably, treatment begins concurrently with or shortly after the chemotherapy or radiation therapy is begun and is continued until the level of the gut cells
10 or other cells is returned to acceptable levels. However, the selected cytokine, e.g., IL-11, IL-6 or combination of cytokines, may be administered for a suitable period of time prior to the beginning of chemotherapy or radiation therapy to improve the efficacy
15 with which the cytokine, e.g., IL-11 or IL-6, stimulates the stem cell which differentiates into the mature gut cell.

The invention also involves methods for treating patients afflicted with damaged or depleted cell
20 populations selected from small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, liver epithelial cells, skin cells, hair cells, and sperm cells, where the damage or depletion is caused by autoimmune disease. For example, Crohn's disease,
25 damages and depletes the population of normally rapidly dividing gut cells. Other autoimmune diseases may similarly affect the cells of the large intestine or stomach, liver, skin and hair, and sperm cells. The present invention also involves treating such conditions
30 by administering effective doses of a selected cytokine, e.g., IL-11, IL-6 or a combination of cytokines.

Similarly, infection, trauma or shock can damage or deplete normal populations of gut cells, as well as the other cells mentioned herein, thereby

requiring the administration of effective amounts of one or more cytokines, particularly IL-11 or IL-6.

In one embodiment of the present invention, a selected cytokine, such as IL-11 or IL-6, obtained by
5 recombinant expression or prepared synthetically and purified to homogeneity, is combined with a pharmaceutical carrier suitable for internal administration. Purification is performed using conventional techniques (see, e.g., PCT/US90/06803 and
10 the examples below).

Suitable pharmaceutically acceptable carriers facilitate administration of the cytokine, e.g., IL-11, IL-6, and are well known in the art. Exemplary carriers include sterile saline, lactose, sucrose, calcium
15 phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil, and water. Additionally, the carrier or diluent includes a time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax. In addition, slow release polymer
20 formulations can be used. Suitable sustain-release matrices contain the active ingredient in a mixture with one or more of the following: sodium bentonite, ethylcellulose, stearic acid, calcium stearate, adipic acid, fumeric acid, polyethylene glycol, deacetylated
25 chitin, and cellulose acetate. Suitable preservatives and/or stabilizers may be included.

Alternatively, the selected cytokine, e.g., IL-11, IL-6, can be combined with other conventional agents useful in alleviating the symptoms associated with
30 chemotherapy, such as antiemetics, anti-oxidants, and other hematopoietic growth factors.

The therapeutic method of the present invention may also include co-administration or combination of a selected cytokine with other human factors known to those
35 of skill in the art. Exemplary cytokines or

hematopoietins for such use include those cytokines specifically referenced above. Growth factors, such as B cell growth factor, B cell differentiation factor, or eosinophil differentiation factors may also prove useful in co-administration with these cytokines. Other agents for co-administration may include other pharmaceutically effective chemical agents and drugs, e.g., such as agents to control infection. The dosage recited below would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by conventional methods.

Cytotoxic agents used in bone marrow transplantation and cancer therapy affect rapidly proliferating cells in both the bone marrow and small intestine, leading to severe and often dose-limiting toxicities. In the following Examples 4-9, analysis of small intestinal mucosa demonstrated rapid recovery of villi length and increased proliferative activity within the crypt cells of IL-11-treated or IL-6-treated mice compared to control mice. Thus, IL-11 and IL-6 have positive effects on the recovery of several tissues where dose-limiting toxicities following cytoablative therapies occur.

Without wishing to be bound by theory, the inventors believe that treatment of damage to, or depletion of, the normally rapidly dividing gut epithelial cells with IL-11, IL-6 or another cytokine, provides two important advantages to current methods of dealing with gut toxicity. First, the cytokine, e.g., IL-11, IL-6, improves the integrity of the gut by stimulating the stem cells to restore a healthy cell population, thereby preventing entry of bacteria and fungi into the blood of the treated patient. IL-11 or IL-6 treatment of patients can thereby reduce the morbidity and mortality associated with chemotherapy and

radiation treatment-induced gut damage. Additionally, IL-11, IL-6 or other cytokines identified herein may allow increased amounts of chemotherapy and radiation therapy to be used in cancer treatments, a highly
5 desirable effect, since this may improve the survival rates of patients with certain cancers which are currently fatal.

Similarly, in the treatment of autoimmune diseases of the small intestine, a cytokine, such as IL-
10 11 or IL-6, is expected restore the cell population thereby improving healing and reducing morbidity and mortality without the deleterious side effects of previous therapies.

The treatment of a patient with a selected
15 cytokine, such as IL-11, IL-6, or combination of cytokines is anticipated to have the same effects on other cell populations, e.g., skin, hair, sperm cells, epithelial linings of stomach and large intestines, and liver epithelial cells, which are damaged or depleted by
20 disease, infection, shock or trauma. The cytokine is theorized to restore healthy populations by stimulating stem cells into differentiating into mature cell populations.

In the treatment of any of these conditions
25 resulting in damage to, or depletion of, the cell population, the cytokine, e.g., IL-11, IL-6, can be administered by any suitable route, but is preferably administered systemically, i.e., parenterally. Of the parental routes, subcutaneous and intraperitoneal are
30 preferred. With chemotherapy, intravenous administration may be desired.

A suitable treatment regimen for patients undergoing chemotherapy or radiation, or for patients who have already sustained cell damage or depletion due to
35 trauma or disease, may be determined by the attending

physician based upon such factors as the patient's age, sex, weight, and general health. Generally, a suitable dose of a cytokine, e.g., IL-11, ranges between about 1 μ g/kg body weight and about 1000 μ g/kg body weight. If
5 desirable, these doses can be adjusted to units. A unit is conventionally described as the concentration of polypeptide which leads to half maximal stimulation in a suitable assay, e.g., for IL-11, the T1165 assay described in PCT/US90/06803. Another suitable dose may
10 be in the range of between about 10 μ g/kg and about 1000 μ g/kg, and more preferably about 100 μ g/kg and about 500 μ g/kg, of cytokine, e.g., IL-11, per kg of body weight. These doses may be administered daily for between 1 day and 6 months, or for as long as is deemed necessary,
15 depending on the nature of the cell damage or depletion.

The dosages of the other cytokines described herein may be similar or adjusted downwardly depending on the toxicity of the selected cytokine. For example, IL-1 would be administered in a dosage range of about 10ng/kg
20 body weight to about 1 μ g/kg body weight due to its toxicity. The adjustment of the dosages is well within the skill of the art based on the known toxicities of the cytokines useful in this invention.

When used to treat autoimmune conditions, the
25 cytokine composition, e.g., IL-11, may be formulated to contain other agents useful in alleviating the symptoms of these conditions, including e.g., prednisone, cyclosporine, cyclophosphamide, and azathioprine, as well as other known agents.

30 Also, where treatment is directed to skin and hair cells, a pharmaceutical preparation may be prepared using agents which are conventional for topically administering therapeutics to the skin and hair, e.g., for systemic or local or topical administration.
35 Suitable pharmaceutical carriers for a topical

16

composition of the present invention may include several conventional ingredients of creams, lotions, gels or ointments. Such conventional ingredients are included in skin creams or oils for topical administration for
5 treating a variety of diseases of the skin. Such compositions may be used as drug delivery systems to transmit the IL-11 through the skin or to facilitate the absorption of the IL-11 into the skin or onto a rash or other skin eruption. [See, e.g., U.S. Patent 3,981,996;
10 U.S. Patent 4,731,241; U.S. Patent 4,164,563; U.S. Patent 3,924,004; U.S. Patent 3,888,995; U.S. Patent 3,592,930; and U.S. Patent 4,753,958].

The following examples illustrate the production of IL-11 and fusion proteins thereof (Examples
15 1-3) and methods of the present invention employing IL-11 or IL-6 as the selected cytokine, and gut cell populations damaged and depleted by chemotherapy as the model rapidly growing cell population (Example 4-9). However, these examples do not limit the scope of the
20 invention.

EXAMPLE 1 - HUMAN IL-11

The isolation and cloning of human IL-11 is described in detail in published PCT Application No. US90/06803 and now known to the art. The full sequence
25 for human IL-11 was determined and is shown in Table I below. This protein is characterized by the sequence of Table I [SEQ ID NO:1 and 2].

Table I

30 5'AGCTGGGAAGGGTTAAAGGCCCGGCTCCCTGCCCCCTGCCCTGG

(1)
GGAACCCCT GGCCCTGCGGGGAC ATG AAC TGT GTT TGC CGC
M N C V C R

[illegible]

18

TCTTATTTATTTATTTATTTTCGGTACTGGGGGCGAAACAGCCAGGTG
ATCCCCCTGCCTTTAGCTCCCCCTAGTTAGAGACAGTCCTTCCGTGA
GGCTGGGGGGCATCTGTGCCTTATTTATACTTATTTATTTTCAGGAGC
GGGGGTGGGCTCCTGGGTCCCCGAGGAGGAGGGAGCTGGGGTCCCGG
5 ATTCTTGTGTCCACAGACTTCTGCCCTGGCTCCTCCCCCTCGAGGCC
TGGGCAGGAATACATACTATTTATTTAAGAGCTC

EXAMPLE 2 - THIOREDOXIN-IL-11 FUSION MOLECULE

IL-11 was also prepared in a fusion molecule for use in the method of the present invention. The
10 fusion molecule contained *E. coli* thioredoxin and recombinant IL-11, obtained as described in PCT Application No. US90/06803 [see also Paul et al, Proc. Natl. Acad. Sci. U.S.A., 87:7512-7516 (1990) and PCT Patent publication W091/07495, published May 30, 1991].

15 The *E. coli* thioredoxin (*trxA*) gene was cloned based on its published sequence [Lim et al, J. Bacteriol., 163:311-316 (1985)] and employed to construct various related *E. coli* expression plasmids using standard DNA manipulation techniques, described
20 extensively by Sambrook, Fritsch and Maniatis, Molecular Cloning. A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). (Nucleotides 2242-2568 of Table II encode the *E. coli* thioredoxin protein.)

25 An expression plasmid pALtrxA-781 was constructed containing the *E. coli* *trxA* gene without fusion to another sequence. This plasmid, which directs the accumulation of >10% of the total cell protein as thioredoxin in an *E. coli* host strain GI724, was further
30 manipulated to provide for the construction of a *trxA*/IL-11 fusion sequence, resulting in the expression vector, pALtrxA/EK/IL-11ΔPro-581.

The entire sequence of the plasmid expression vector, pALtrxA/EK/IL-11 Δ Pro-581 [SEQ ID NO:3 and SEQ ID NO:4], is illustrated in Table II and contains the following principal features:

5 Nucleotides 1-2060 contain DNA sequences originating from the plasmid pUC-18 [Norrande et al, Gene, 26: 101-106 (1983)] including sequences containing the gene for β -lactamase which confers resistance to the antibiotic ampicillin in host *E. coli* strains, and a
10 colE1-derived origin of replication. Nucleotides 2061-2221 contain DNA sequences for the major leftward promoter (pL) of bacteriophage λ [Sanger et al, J. Mol. Biol., 162:729-773 (1982)], including three operator sequences, O_L1, O_L2, and O_L3. The operators are the
15 binding sites for λ cI repressor protein, intracellular levels of which control the amount of transcription initiation from pL. Nucleotides 2222-2241 contain a strong ribosome binding sequence derived from that of gene 10 of bacteriophage T7 [Dunn and Studier, J. Mol. Biol., 166:477-535 (1983)].
20

 Nucleotides 2242-2568 contain a DNA sequence encoding the *E. coli* thioredoxin protein [Lim et al, J. Bacteriol., 163:311-316 (1985)]. There is no translation termination codon at the end of the thioredoxin coding
25 sequence in this plasmid.

 Nucleotides 2569-2583 contain a DNA sequence encoding the amino acid sequence for a short, hydrophilic, flexible spacer peptide "--GSGSG--". Nucleotides 2584-2598 provide DNA sequence encoding the
30 amino acid sequence for the cleavage recognition site of enterokinase (EC 3.4.4.8), "--DDDDK--" [Maroux et al, J. Biol. Chem., 246:5031-5039 (1971)].

 Nucleotides 2599-3132 contain a DNA sequence encoding the amino acid sequence of a modified form of
35 mature human IL-11 [Paul et al, Proc. Natl. Acad. Sci.

USA, 87:7512-7516 (1990)]; the N-terminal prolyl-residue normally found in the natural protein has been deleted. Thus, these nucleotides encode IL-11 beginning with amino acid #2 of the mature native sequence. The sequence
 5 includes a translation termination codon at the 3'-end of the IL-11 sequence.

Nucleotides 3133-3159 provide a "Linker" DNA sequence containing restriction endonuclease sites. Nucleotides 3160-3232 provide a transcription termination
 10 sequence based on that of the *E. coli* aspA gene [Takagi et al, Nucl. Acids Res., 13:2063-2074 (1985)]. Nucleotides 3233-3632 are DNA sequences derived from pUC-18.

Table II

15 pALtrxA/EK/IL-11ΔPro-581
 SEQ ID NO:3 and SEQ ID NO:4

	GACGAAAGGG	CCTCGTGATA	CGCCTATTTT	TATAGGTTAA	40
	TGTCATGATA	ATAATGGTTT	CTTAGACGTC	AGGTGGCACT	80
	TTTCGGGGAA	ATGTGCGCGG	AACCCCTATT	TGTTTATTTT	120
20	TCTAAATACA	TTCAAATATG	TATCCGCTCA	TGAGACAATA	160
	ACCCTGATAA	ATGCTTCAAT	AATATTGAAA	AAGGAAGAGT	200
	ATGAGTATTC	AACATTTCCG	TGTCGCCCTT	ATTCCCTTTT	240
	TTGCGGCATT	TTGCCTTCCT	GTTTTTGCTC	ACCCAGAAAC	280
	GCTGGTGAAA	GTAAAAGATG	CTGAAGATCA	GTTGGGTGCA	320
25	CGAGTGGGTT	ACATCGAACT	GGATCTCAAC	AGCGGTAAGA	360
	TCCTTGAGAG	TTTTCGCCCC	GAAGAACGTT	TTCCAATGAT	400
	GAGCACTTTT	AAAGTTCTGC	TATGTGGCGC	GGTATTATCC	440
	CGTATTGACG	CCGGGCAAGA	GCAACTCGGT	CGCCGCATAC	480
	ACTATTCTCA	GAATGACTTG	GTTGAGTACT	CACCAGTCAC	520
30	AGAAAAGCAT	CTTACGGATG	GCATGACAGT	AAGAGAATTA	560
	TGCAGTGCTG	CCATAACCAT	GAGTGATAAC	ACTGCGGCCA	600
	ACTTACTTCT	GACAACGATC	GGAGGACCGA	AGGAGCTAAC	640
	CGCTTTTTTG	CACAACATGG	GGGATCATGT	AACTCGCCTT	680
	GATCGTTGGG	AACCGGAGCT	GAATGAAGCC	ATACCAAACG	720
35	ACGAGCGTGA	CACCACGATG	CCTGTAGCAA	TGGCAACAAC	760

21

GTTGCGCAAA CTATTAAGT GCGAACTACT TACTCTAGCT 800
TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG 840
TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG 880
GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT 920
5 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT 960
CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC 1000
TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC 1040
TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT 1080
CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT 1120
10 TAAAAGGATC TAGGTGAAGA TCCTTTTGA TAATCTCATG 1160
ACCAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT 1200
CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC 1240
TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA 1280
CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC 1320
15 TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC 1360
GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA 1400
GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC 1440
TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG 1480
CGATAAGTCG TGTCTTACCG GGTGACTC AAGACGATAG 1520
20 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT 1560
CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA 1600
ACTGAGATAC CTACAGCGTG AGCATTGAGA AAGCGCCACG 1640
CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG 1680
GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG 1720
25 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC 1760
CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG 1800
GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT 1840
TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG 1880
TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA 1920
30 TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG 1960
AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA 2000
GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC 2040
CGATTCATTA ATGCAGAATT GATCTCTCAC CTACCAAACA 2080
ATGCCCCCCT GCAAAAATA AATTCATATA AAAAACATAC 2120
35 AGATAACCAT CTGCGGTGAT AAATTATCTC TGGCGGTGTT 2160

22

	GACATAAATA	CCACTGGCGG	TGATACTGAG	CACATCAGCA	2200
	GGACGCACTG	ACCACCATGA	ATTCAAGAAG	GAGATATACA	2240
	T ATG AGC GAT AAA ATT ATT CAC CTG ACT GAC GAC	2274			
	Met Ser Asp Lys Ile Ile His Leu Thr Asp Asp				
5	1	5	10		
	AGT TTT GAC ACG GAT GTA CTC AAA GCG GAC GGG	2307			
	Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly				
	15	20			
10	GCG ATC CTC GTC GAT TTC TGG GCA GAG TGG TGC	2340			
	Ala Ile Leu Val Asp Phe Trp Ala Glu Trp Cys				
	25	30			
	GGT CCG TGC AAA ATG ATC GCC CCG ATT CTG GAT	2373			
	Gly Pro Cys Lys Met Ile Ala Pro Ile Leu Asp				
	35	40			
15	GAA ATC GCT GAC GAA TAT CAG GGC AAA CTG ACC	2406			
	Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr				
	45	50	55		
	GTT GCA AAA CTG AAC ATC GAT CAA AAC CCT GGC	2439			
	Val Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly				
20	60	65			
	ACT GCG CCG AAA TAT GGC ATC CGT GGT ATC CCG	2472			
	Thr Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro				
	70	75			
25	ACT CTG CTG CTG TTC AAA AAC GGT GAA GTG GCG	2505			
	Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala				
	80	85			
	GCA ACC AAA GTG GGT GCA CTG TCT AAA GGT CAG	2538			
	Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln				
	90	95			
30	TTG AAA GAG TTC CTC GAC GCT AAC CTG GCC GGT	2571			
	Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala Gly				
	100	105	110		
	TCT GGT TCT GGT GAT GAC GAT GAC AAA GGT CCA	2604			
	Ser Gly Ser Gly Asp Asp Asp Asp Lys Gly Pro				
35	115	120			
	CCA CCA GGT CCA CCT CGA GTT TCC CCA GAC CCT	2637			
	Pro Pro Gly Pro Pro Arg Val Ser Pro Asp Pro				
	125	130			

23

	CGG	GCC	GAG	CTG	GAC	AGC	ACC	GTG	CTC	CTG	ACC	2670
	Arg	Ala	Glu	Leu	Asp	Ser	Thr	Val	Leu	Leu	Thr	
			135					140				
5	CGC	TCT	CTC	CTG	GCG	GAC	ACG	CGG	CAG	CTG	GCT	2703
	Arg	Ser	Leu	Leu	Ala	Asp	Thr	Arg	Gln	Leu	Ala	
		145					150					
	GCA	CAG	CTG	AGG	GAC	AAA	TTC	CCA	GCT	GAC	GGG	2736
	Ala	Gln	Leu	Arg	Asp	Lys	Phe	Pro	Ala	Asp	Gly	
	155					160					165	
10	GAC	CAC	AAC	CTG	GAT	TCC	CTG	CCC	ACC	CTG	GCC	2769
	Asp	His	Asn	Leu	Asp	Ser	Leu	Pro	Thr	Leu	Ala	
					170					175		
	ATG	AGT	GCG	GGG	GCA	CTG	GGA	GCT	CTA	CAG	CTC	2802
15	Met	Ser	Ala	Gly	Ala	Leu	Gly	Ala	Leu	Gln	Leu	
				180					185			
	CCA	GGT	GTG	CTG	ACA	AGG	CTG	CGA	GCG	GAC	CTA	2835
	Pro	Gly	Val	Leu	Thr	Arg	Leu	Arg	Ala	Asp	Leu	
			190					195				
	CTG	TCC	TAC	CTG	CGG	CAC	GTG	CAG	TGG	CTG	CGC	2868
20	Leu	Ser	Tyr	Leu	Arg	His	Val	Gln	Trp	Leu	Arg	
		200					205					
	CGG	GCA	GGT	GGC	TCT	TCC	CTG	AAG	ACC	CTG	GAG	2901
	Arg	Ala	Gly	Gly	Ser	Ser	Leu	Lys	Thr	Leu	Glu	
	210					215					220	
25	CCC	GAG	CTG	GGC	ACC	CTG	CAG	GCC	CGA	CTG	GAC	2934
	Pro	Glu	Leu	Gly	Thr	Leu	Gln	Ala	Arg	Leu	Asp	
					225					230		
	CGG	CTG	CTG	CGC	CGG	CTG	CAG	CTC	CTG	ATG	TCC	2967
30	Arg	Leu	Leu	Arg	Arg	Leu	Gln	Leu	Leu	Met	Ser	
				235					240			
	CGC	CTG	GCC	CTG	CCC	CAG	CCA	CCC	CCG	GAC	CCG	3000
	Arg	Leu	Ala	Leu	Pro	Gln	Pro	Pro	Pro	Asp	Pro	
			245					250				
	CCG	GCG	CCC	CCG	CTG	GCG	CCC	CCC	TCC	TCA	GCC	3033
35	Pro	Ala	Pro	Pro	Leu	Ala	Pro	Pro	Ser	Ser	Ala	
		255					260					
	TGG	GGG	GGC	ATC	AGG	GCC	GCC	CAC	GCC	ATC	CTG	3066
	Trp	Gly	Gly	Ile	Arg	Ala	Ala	His	Ala	Ile	Leu	
	265					270					275	

24

	GGG GGG CTG CAC CTG ACA CTT GAC TGG GCC GTG	3099
	Gly GLy Leu His Leu Thr Leu Asp Trp Ala Val	
	280 285	
5	AGG GGA CTG CTG CTG CTG AAG ACT CGG CTG TGA	3132
	Arg Gly Leu Leu Leu Leu Lys Thr Arg Leu	
	290 295	
	AAGCTTATCG ATACCGTCGA CCTGCAGTAA TCGTACAGGG	3172
	TAGTACAAAT AAAAAAGGCA CGTCAGATGA CGTGCCTTTT	3212
	TTCTTGTGAG CAGTAAGCTT GGCACCTGGCC GTCGTTTTAC	3252
10	AACGTCGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA	3292
	TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT	3332
	AGCGAAGAGG CCCGCACCGA TCGCCCTTCC CAACAGTTGC	3372
	GCAGCCTGAA TGGCGAATGG CGCCTGATGC GGTATTTTCT	3412
	CCTTACGCAT CTGTGCGGTA TTTCACACCG CATATATGGT	3452
15	GCACTCTCAG TACAATCTGC TCTGATGCCG CATAGTTAAG	3492
	CCAGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA	3532
	CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG	3572
	TGACCGTCTC CGGGAGCTGC ATGTGTCAGA GGTTTTCACC	3612
	GTCATCACCG AAACGCGCGA	3632

20 As described in Example 3 below, when cultured under the appropriate conditions in a suitable *E. coli* host strain, can direct the production of high levels (approximately 10% of the total cellular protein) of a thioredoxin-IL-11 fusion protein. By contrast, when not

25 fused to thioredoxin, IL-11 accumulated to only 0.2% of the total cellular protein when expressed in an analogous host/vector system.

EXAMPLE 3 - EXPRESSION OF A FUSION PROTEIN

30 A thioredoxin-IL-11 fusion protein was produced according to the following protocol using the plasmid constructed as described in Example 2.

pALtrxA/EK/IL-11 Δ Pro-581 (SEQ ID NO: 3) was transformed into the *E. coli* host strain GI724 (F^- , lacI^q, lacP^{L8}, ampC:: λ cI⁺) by the procedure of Dagert and Ehrlich, Gene,

6: 23 (1979). The untransformed host strain *E. coli* GI724 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland on January 31, 1991 under ATCC No. 55151 for patent purposes pursuant to applicable laws and regulations.

Transformants were selected on 1.5% w/v agar plates containing IMC medium, which is composed of M9 medium [Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory, New York (1972)] supplemented with 0.5% w/v glucose, 0.2% w/v casamino acids and 100 µg/ml ampicillin.

GI724 contains a copy of the wild-type λ cI repressor gene stably integrated into the chromosome at the ampC locus, where it has been placed under the transcriptional control of Salmonella typhimurium trp promoter/operator sequences. In GI724, λ cI protein is made only during growth in tryptophan-free media, such as minimal media or a minimal medium supplemented with casamino acids such as IMC, described above. Addition of tryptophan to a culture of GI724 will repress the trp promoter and turn off synthesis of λ cI, gradually causing the induction of transcription from pL promoters if they are present in the cell.

GI724 transformed with pALtrxA/EK/IL-11 Δ Pro-581 [SEQ ID NO:3 and SEQ ID NO:4] was grown at 37°C to an A₅₅₀ of 0.5 in IMC medium. Tryptophan was added to a final concentration of 100 µg/ml and the culture incubated for a further 4 hours. During this time thioredoxin-IL-11 fusion protein accumulated to approximately 10% of the total cell protein.

All of the fusion protein was found to be in the soluble cellular fraction, and was purified as follows. Cells were lysed in a French pressure cell at 20,000 psi in 50 mM HEPES pH 8.0, 1 mM phenylmethylsulfonyl fluoride. The lysate was clarified

by centrifugation at 15,000 x g for 30 minutes and the supernatant loaded onto a QAE-Toyopearl* column. The flow-through fractions were discarded and the fusion protein eluted with 50 mM HEPES pH 8.0, 100 mM NaCl. The
5 eluate was adjusted to 2M NaCl and loaded onto a column of phenyl-Toyopearl. The flow-through fractions were again discarded and the fusion protein eluted with 50 mM HEPES pH 8.0, 0.5 M NaCl.

The fusion protein was then dialyzed against 25
10 mM HEPES pH 8.0 and was >80% pure at this stage. By T1165 bioassay [Paul et al, cited above], the purified thioredoxin-IL-11 protein exhibited an activity of 8×10^5 U/mg. This value agrees closely on a molar basis with the activity of 2×10^6 U/mg found for COS cell-derived
15 IL-11 in the same assay. One milligram of the fusion protein was cleaved at 37°C for 20 hours with 1000 units of bovine enterokinase [Leipnieks and Light, J. Biol. Chem., 254:1677-1683 (1979)] in 1 ml 10mM Tris-Cl (pH 8.0)/10mM CaCl_2 . IL-11 could be recovered from the
20 reaction products by passing them over a QAE-Toyopearl column in 25 mM HEPES pH 8.0, where IL-11 was found in the flow-through fractions. Uncleaved fusion protein, thioredoxin and enterokinase remained bound on the column.

25 EXAMPLE 4 - TREATMENT OF IRRADIATED MICE

The IL-11 used in the tests below was obtained from Genetics Institute, Inc., Cambridge, MA and was prepared in *E. coli* essentially as described in the examples above. The IL-11 (140 µg/ml) was then mixed
30 with 10 mM Tris buffer, to a pH of about 8.0. The level of endotoxin in this *in vivo* grade formulation is about 1.4 U/mg of protein. The preparation also contains about 10% molar hydroxylmate and about 3 ng/ml (0.002%) thioredoxin. Eight-ten week old C3H/HeJ [Jackson Labs]

* Trademark

mice were administered intraperitoneally (i.p.) 150 mg/kg 5-fluorouracil (5-FU) diluted in Hanks Balanced Salt Solution (HBSS) containing 0.024 M Hepes buffer [both Gibco], three days prior to sublethal irradiation.

- 5 Irradiation consisted of 6.0 Gys TBI delivered by Siemens 250 Kvp X-ray therapy machine, filtered with 1.0 mm Cu, giving half value layer of 2.1 mm Cu at 50 cm SSD, and with a dose rate of 78.13 (cGy/min). On the same day as the irradiation dose was given, mice were administered
- 10 the above-described recombinant *in vivo* grade human IL-11 [Genetics Institute] at a divided dose (twice/day) of 250 micrograms/kg/day. These divided doses were given in 0.2 ml volumes subcutaneously in HBSS with Hepes and 0.1% bovine serum albumin (BSA) [Boehringer-Mannheim].
- 15 Control animals received the same volume of HBSS and BSA without IL-11. Treatment was continued for 9 to 18 days post-irradiation or until animals died.

- Hematologic analysis of leukocyte cell counts and platelet counts were performed on tail vein bleeds on
- 20 a Coulter Counter Model ZM [Coulter Electronics] using a 100 micron aperture for leukocyte determinations and a 50 micron aperture for platelet determinations. Red blood cells were lysed using Zapglobin* [Coulter] according to manufacturer's recommendations. Blood smears were
- 25 stained with Wright-Giemsa using standard methods and examined at 100X for differential analysis. The absolute numbers of neutrophils, lymphocytes, monocytes, and eosinophils in the peripheral blood was calculated by multiplying the total leukocyte counts with the
- 30 percentage of leukocytes obtained on the differential. Peripheral blood hematocrits were performed by spinning capillary tubes for five minutes in a Clay-Adams hematocrit centrifuge.

* Trademark

Whole dead mice (dying in the course of the experiment or by sacrifice) were fixed in 10% buffered formalin overnight. One femur/mouse was fixed in Bouin's solution. Tissues from each organ (liver, spleen, kidney, small intestine mesentery, abdominal wall, lung, heart, testes, and femur) were embedded in paraffin wax using standard techniques and four micron sections were cut and stained with hematoxylin and eosin. For analysis of small intestinal crypts, ten independent measurements of villus height, crypt depth, and metaphases/crypt were made in each section of small intestine using an objective-mounted micrometer.

Results are expressed in Table III below as the mean +/- SD unless otherwise stated. The probability of significant differences when two related groups were compared was determined using a two-tailed Student t-test. The probability of significant differences when multiple treatments were examined was determined by analysis of variance followed by Student-Newman-Keuls multiple range tests to define the unique subsets within the study.

Table III

Effect of IL-11 on Endogenous Infection
Combined Modality Model

5	Mouse No.	Day Post- Irradiat. Examined ¹	Diarrhea ²	Hepatic Bacterial Foci	
				Microscopic ⁴	Macroscopic ³
10	BSA				
	1	5	+	0	+++
	2	8	-	19	++
	3	5	+	21	+++
	4	6	-	79	+++
15	5	9	-	119	+++
20	IL-11				
	1	9	+	0	+
	2	4	+	0	-
	3	9	-	14	++
	4	9	-	3	+
	5	9	-	0	-

¹ All animals sacrificed at day 9, other days represent day of death.

25 ²+ = present at day of death
- = no diarrhea

³ Surface foci present on fixed liver.

30 ⁴ Microscopic foci present on examination of randomly chosen histologic sections; +<10 foci/section; ++ 10-50; +++>50.

In three separate experiments, all control mice died between day 3 and day 10 after irradiation, while only 3/13 (23%) of IL-11 treated mice died (on days 4, 9, 10 post-irradiation). In experiment 1, all control
5 animals died by day 9. Animals were autopsied on the day of death or (in the treated group) on day 9 by sacrificing remaining animals (day of examination listed in Table III). At autopsy, 4/5 mice in the control group had macroscopic infection foci in the liver compared to
10 2/5 of the IL-11 treated mice. In addition, the foci present in IL-11 treated mice were present in fewer numbers and smaller in size (Table III). These foci subsequently were demonstrated to contain *E. coli* bacteria by identification using microbiological
15 analysis. Microscopically many foci (12-129/random section) were found within the liver from control mice, while fewer (6-21/section) were demonstrated in IL-11 treated mice (Table III). Similar bacterial foci were also seen in the mesentery and spleen of animals.

20 Surprisingly, these differences in mortality and the presence of bacterial foci in organs of mice were not associated with differences in peripheral leukocyte counts or absolute neutrophil counts as shown by data in Table IV.

Table IV

Effect of rhIL-11 on Peripheral Blood Counts in Mice
Combined Modality Model

5	Day ¹	Treatment	WBC X 10 ³ /mm ³	Platelet 10 ³ /mm ³
10	Day 1	BSA	5.56±1.80 (5)	894.7±168.3 (5)
		IL-11	4.89±0.16 (5)	770.3±192.6 (5)
	Day 3	BSA	0.49±0.16 (10)	294.8±43.1 (10)
		IL-11	0.64±0.31 (10)	454.1±115.5 (10)
	Day 4	BSA	0.43±0.04 (10)	237.4±109.6 (10)
		IL-11	0.49±0.14 (10)	337.3±143.2 (10)
15	Day 5	BSA	0.30±0.06 (9)	126.6±55.1 (10)
		IL-11	0.31±0.07 (11)	171.9±76.9 (11)
	Day 6	BSA	0.40±0.24 (9)	134.4±80.7 (9)
		IL-11	0.47±0.22 (12)	257.9±195.2 (12)
20	Day 8	BSA	1.19±0.13 (2)	236.1±18.2 (2)
		IL-11	0.72±0.23 (7)	365.6±256.1 (7)
	Day 9	BSA	1.39±0.32 (2)	269.0±100.8 (2)
		IL-11	1.06±0.45 (9)	248.8±92.4 (9)

¹ Post-irradiation

() number of animals

* p < 0.001 compared to BSA group

Since *E. coli* are a known resident organism of the small intestine, the increase in bacterial infection and mortality in the control animals probably reflects gut toxicity from irradiation and chemotherapy.

5 Histologic section of the small intestine and morphometric quantitation of the length of the small intestine villi confirmed extensive damage in control mice as shown by data in Tables VA and VB. In contrast, IL-11 treatment was associated with almost complete
10 preservation of villi length (Table VA). In addition, IL-11 treated mice demonstrated near normal numbers of mitotic crypt cells, a further indication of stimulation of proliferation of crypt progenitor or stem cells.

Table VA

15 Effect of IL-11 of Murine Gut Epithelium
Combined Modality Mode

		C Crypt Depth ¹	V Villi Length	C C+V x 100%
20	Normal (2)	84.1±21.1	477.7±99.8	15.4±5.9
	Day 5			
	BSA(5)	122.8±29.5	253.7±79.7	33.5±8.2
	IL-11(5)	117.1±14.5	512.8±6.7**	19.5±6.7**
25	Day 9			
	BSA(2)	124.6±40.7	330.1±92.1	27.3±1.0
	IL-11(2)	98.5±7.0	405.9±84.3	19.9±4.4

() number of animals

30 ¹ = in microns

* = p < 0.01 compared to BSA group

** = p < 0.02 compared to BSA group

Table VB

Effect of IL-11 of Murine Gut Epithelium
Combined Modality Mode

5		Crypt Cir.	Mitoses/ Crypt	Mitoses/ 100 μ crypt
	Normal (2)	162.3 $\pm$ 11.6	1.8 $\pm$ 0.4	1.07 $\pm$ 0.14
	Day 5			
	BSA (5)	853.4 $\pm$ 62.2	0.9 $\pm$ 0.4	0.10 $\pm$ 0.04
10	IL-11 (5)	928.2 $\pm$ 104.4	2.0 $\pm$ 0.5*	0.22 $\pm$ 0.06*
	Day 9			
	BSA (2)	830.0 $\pm$ 28.2	1.1 $\pm$ 0	0.13 $\pm$ 0
	IL-11 (2)	957.5 $\pm$ 10.4	2.2 $\pm$ 0.6	0.23 $\pm$ 0.06

15 () number of animals

1 = in microns

* = p < 0.01 compared to BSA group

** = p < 0.02 compared to BSA group

20 These data demonstrate that the administration
of IL-11 in vivo has marked positive effects on the
recovery of small intestinal crypt epithelial cells from
the combined cytotoxic effects of radiation and
chemotherapy.

25 EXAMPLE 5 - SURVIVAL OF MICE FOLLOWING COMBINED
CHEMOTHERAPY/RADIATION

Eight-ten week old C3H/HeJ mice [Jackson
Laboratory, Bar Harbor, ME] were given 5-fluorouracil (5-
FU) (diluted in Hanks Balanced Salt Solution (HBSS)
containing 0.025M Hepes) [G. R. Siber et al, Cancer Res.,
30 40, 3430-3436 (1980)] at 150mg/kg body weight I.P.

threedays prior to sublethal irradiation (6.0 Gys total body irradiation delivered from a Siemens 250 KVP X-ray therapy machine, filtered with 1.0 mm Cu, giving a half value layer of 2.1 mm Cu at 50cm SSD, and with a dose rate of 78.13 cGy/min). No bone marrow infusions were given to these animals.

Recombinant human IL-11 (rhIL-11) [provided by Genetics Institute, Cambridge, MA and prepared in *E. coli* essentially as described in the examples above] was diluted in HBSS [HBSS-Gibco] containing 0.1% BSA [wt/vol, Boehringer-Mannheim, Indianapolis, IN] and 0.025M Hepes [Gibco]. RhIL-11 (250 micrograms/kg body weight) was injected subcutaneously in 0.2 ml volumes twice per day starting on the same day as irradiation. Control mice received the same volume of HBSS/0.1% BSA (vehicle injections).

The results of these studies are provided in Fig. 1 below. As can be seen therein, following treatment with 5-FU and sublethal doses of X-irradiation (combined modality, CM, therapy), damage to the small intestine of C3H/HeJ mice is extensive and the majority of mice die within 10 days (Fig. 1). Dying animals demonstrate wasting, diarrhea, and tilting and rotating indicative of central nervous system infection. Treatment of mice with 250 micrograms/kg/day of recombinant human interleukin-11 (rhIL-11) was associated with significant increase in survival following this cytoablative therapy, in spite of no increase in peripheral neutrophil counts or bone marrow myeloid progenitors (Fig. 1).

Additional experiments in which increasing doses and an alternative source of irradiation (^{137}Cs) were administered confirmed the increase in survival in IL-11 treated mice over several dose ranges. The experiments tested radiation doses of 6.0, 7.0, 7.5 and

8.0 Gy (using ^{137}Cs source at 95.83 cGy/min) as above (all following 150 mg/kg 5-FU) and included a total of 135 mice. Survival in the control group in all experiments was 27% compared to 62% in the IL-11 treated mice (p<0.0001). IL-11 treatment was associated with a marked reduction in the number of bacterial foci in multiple organs with none detectable in many mice after sacrifice. Microbiological analysis of foci dissected from the liver of control mice uniformly demonstrated that the causative organism was *E. coli*, a common enteric organism in mice.

EXAMPLE 6 - HISTOLOGIC SECTIONS OF SMALL INTESTINE

To determine the potential source of *E. coli* organisms demonstrated in mice following CM treatment, histologic sections of the small intestine were examined in control and IL-11 treated mice sacrificed daily after irradiation.

Mice dying or sacrificed following the combined radiation/chemotherapy (as described in Example 5 above) were autopsied and tissues fixed in 10% buffered formalin overnight within 12 hours after death. Tissues from each organ (liver, spleen, kidney, small intestine and mesentery, abdominal wall, lung, heart, and femurs) were embedded in paraffin wax using standard techniques. Four-micron sections were cut and stained with hematoxylin/eosin (H&E). Ten independent measurements of villus height, crypt depth and metaphases/crypt per specimen of small intestine were made from mice sacrificed daily using an objective mounted micrometer at 200X magnification. Hepatic bacterial foci were counted both macroscopically as surface colonies and microscopically on randomly chosen liver histologic sections.

The small intestinal mucosa of control mice showed marked destruction of villus structure with shortening of the villus length, vacuolization, and pyknotic nuclear structures. In contrast, IL-11 treated mice demonstrated mild changes in morphology of the small intestinal villi. Morphometric analysis of crypt and villi length demonstrated a significant increase in the ratio of the crypt depth/villi length in IL-11 treated mice compared to control mice (Table VI below). Villus shortening was most prominent 24 hours after irradiation in both groups of mice, while remaining abnormal in surviving control mice through day 9.

Table VI

Effect of IL-11 on Small Intestine Crypt
Cell Recovery Post 5-FU and Irradiation

		CRYPT DEPTH		VILLUS LENGTH	
		BSA	IL-11	BSA	IL-11
	Day 1	76.8±9.2	71.1±4.7	398.6±45.5	479.0±31.8
20	Day 2	123.4±14.3	129.0±7.8	491.9±23.2	603.4±41.9 ²
	Day 3	154.5±10.2	141.8±19.9	468.7±47.4	533.9±24.2
	Day 4	141.5±11.9	118.5±14.0	500.2±51.3	635.0±26.9 ²
	Day 5	120.6±11.7	117.8±7.7	523.2±17.4	661.5±20.1 ¹
		CRYPT/VILLUS			
		BSA	IL-11		
25	Day 1	0.20±0.01	0.15±0.00 ¹		
	Day 2	0.25±0.01	0.22±0.00 ²		
	Day 3	0.33±0.01	0.27±0.01 ¹		
	Day 4	0.29±0.00	0.19±0.00 ¹		
30	Day 5	0.23±0.00	0.18±0.01 ¹		

¹p<0.01 vs BSA group

²p<0.05 vs BSA group

Each number represents data from 3 animals

10 crypts/animal, 10 villi/animal

EXAMPLE 7 - USE OF IL-11 OR IL-6 IN CYTOABLATIVE THERAPY

In an additional experiment, recombinant human IL-6 [Preprotech, Rocky Hill, New Jersey] was diluted in HBSS [HBSS-Gibco] containing 0.1% BSA (wt/vol) and 0.025 M HEPES. Ten C3H/HeJ mice were injected subcutaneously with recombinant human IL-6 (250 μ g/kg body weight) in 0.2 mL volumes twice per day starting on the same day as irradiation. Ten (10) mice were injected with 250 μ g/kg body weight IL-11, as described in Example 5 above.

Control mice (55) received the same volume of HBSS/0.1% BSA. The results of this study are provided in Table VII below.

Table VII

Percent Survival

Day	<u>2</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>
BSA:	100	98	95	76	55	47	42	34	32	32	31
IL11:	90	90	90	90	80	80	80	80	80	70	70
IL6:	100	100	100	100	100	80	80	80	80	80	80

Treatment of mice with 500 μ g/kg/day of recombinant human interleukin-6 resulted in a significant increase in survival following this cytoablative therapy.

EXAMPLE 8 - PCNA STAINING OF SMALL INTESTINAL CRYPTS

Since the villus length is dependent on proliferation and differentiation of crypt stem and progenitor cells [Chwalinski et al, Am J. Anat., 186, 397-406 (1989); Ijiri, et al, Br. J. Cancer, 47, 175-185 (1983); Al-Dewachi, et al, Cell Tissue Kinet., 10, 203-213 (1977)], the mitotic index of crypt cells was determined in control and IL-11 treated mice sacrificed daily after irradiation.

Proliferating cell nuclear antigen (PCNA) immunohistochemical staining was done on 2 cm jejunum sections obtained from CM mice sacrificed daily from day

1 to day 5 post irradiation (day 4-8 post 5-FU). Tissue was fixed in 10% formalin for 6-12 hours, paraffin-embedded, and sections were dried on polylysine-treated glass slides. Slides were then deparaffinized and rehydrated. Endogenous peroxidase was quenched by a 5 minute incubation in 3% hydrogen peroxide. The slides were covered with normal goat serum for 20 minutes, incubated overnight at 4°C with PC10 antibody against PCNA [1:80 Dako, Santa Barbara, CA] and then stained for 30 minutes with a biotin-conjugated goat anti-mouse antibody [Gaithersburg, MD], followed by peroxidase-conjugated streptavidin [both Kirkegaard & Perry Laboratories, Gaithersburg, MD] for 30 minutes. The enzyme was developed with 3,3'-diaminobenzidine [DAB Sigma, St. Louis, MO]. PCNA positive nuclei stained brown. The percentage of PCNA positive crypt cells and absolute number of PCNA positive cells per crypt were measured by counting 20 randomly chosen crypts/sections /mouse.

20

Table VIII

Effect of IL-11 on Proliferation of Small Intestine Crypt Cells as Quantitated by PCNA Staining

25	BSA		IL-11	
	nuclei (%)	+nuclei/crypt (%)	+ nuclei (%)	+ nuclei/crypt (%)
Day 1	17.0±6.8	3.1±1.4	18.4±9.2	5.0±1.2
Day 2	1.9±1.3	0.4±0.3	10.5±1.0 ¹	2.6±0.3 ¹
Day 3	2.4±1.7	0.8±0.5	7.6±1.9 ²	2.1±0.5 ²
30 Day 4	1.3±0.4	0.4±0.2	4.2±1.5 ²	1.3±0.4 ²
Day 5	1.7±0.4	0.6±0.1	10.5±11.3	3.3±3.4

¹p<0.01 vs BSA group

²p<0.05 vs BSA group

Each number represents data from 3 animals
20 crypts/animal, 400-600 nuclei

35

Significant increases in the number of mitoses/crypt (2.0 ± 0.5 vs 0.9 ± 0.4 , IL-11 vs control, $p < 0.001$) as well as in the number of mitoses/100 micron of epithelial basement membrane (0.22 ± 0.06 vs 0.10 ± 0.04 , $p < 0.01$) were seen on day 5 after irradiation in the IL-11 treated mice. Control mice surviving to day 9 post irradiation demonstrated slightly increased numbers of mitotic crypt cells, but the numbers were still depressed compared to normal or IL-11 treated mice.

The increase in cell cycle activity following CM treatment and IL-11 administration was further characterized by staining with PC-10 [Hall, et al, J. Pathol., 162, 285-294 (1990); Zeymer et al, Am. J. Pathol., 141, 685-690 (1992); Garcia et al, Am. J. Pathol., 134, 733-739 (1989)]. PC-10 is a monoclonal antibody directed against proliferating cell nuclear antigen (PCNA) and a member of the cyclin family of nuclear proteins (Table VIII). IL-11 administration was associated with a 2 to 5-fold increase in the number of crypt cell nuclei staining with PC-10 on days 2-4 following irradiation. Taken together, these data demonstrate that IL-11 administration to mice following severe damage to the small intestinal crypt cells hastens recovery of the villus structure due to increased proliferation of presumably the crypt progenitor cell.

EXAMPLE 9 - EFFECT OF IL-11 ON PROLIFERATION OF IEC-6 CELLS

In an effort to determine if the effect of IL-11 on the recovery of small intestinal villi seen in vivo was a direct effect of IL-11 on crypt progenitor cells, IEC-6 cells [Barnard, et al, Proc. Natl. Acad. Sci., USA, 86, 1578-1582 (1989)], a non-transformed rat jejunal crypt cell line, were incubated with increasing concentrations of IL-11 (Fig. 2).

IEC-6 cells obtained from the American Type Culture Collection [Rockville, MD; CRL 1592] were grown in Dulbecco's Modified Eagles Medium [Gibco] with 10% dialyzed fetal calf serum. Cells were studied between
5 passage 20 and 35 by incubating 5×10^4 cells/well in 96 well plates in media with and without (control) 5-400 ng/ml rhIL-11. After 72 hours incubation, the absorbance of the reduced formazan was measured at 490 nm with an ELISA plate reader using a non-radioactive cell
10 proliferation assay (Cell Titer 96AQ) [Promega, Madison, WI] according to the manufacturer's instructions. Samples were done in quadruplicate and samples compared by student t-tests. See Fig. 2 below. Results shown are mean $\pm$ S.D. * $p < 0.05$.

15 A dose response was noted when sub-confluent IEC-6 cells were incubated with IL-11, optimal activity occurring at the 50 ng/ml dose. Optimal activity with IL-11 was similar to proliferative activity induced by epidermal growth factor (EGF), a growth factor known to
20 stimulate IEC-6 cells [Baliga et al, Biochem. Int'l., 20, 161-168 (1990)]. No proliferative activity was noted when IL-11 was added to IEC-6 cells after the cells had reached confluence, suggesting there was cycle-specificity to the growth-stimulatory effect of IL-11.
25 Thus, IL-11 has proliferative effects on IEC-6 cells.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to
30 the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Genetics Institute, Inc.,
87 CambridgePark Drive,
Cambridge, MA 02140, USA
- (ii) TITLE OF INVENTION: Method of Treating Cell Damage or
Depletion
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Howson and Howson
 - (B) STREET: Spring House Corporate Cntr,
P.O. Box 457
 - (C) CITY: Spring House
 - (D) STATE: Pennsylvania
 - (E) COUNTRY: USA
 - (F) ZIP: 19477
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/941,372
 - (B) FILING DATE: 02-SEP-1992
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Bak, Mary E.
 - (B) REGISTRATION NUMBER: 31,215
 - (C) REFERENCE/DOCKET NUMBER: IND1Apct
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (215) 540-9206
 - (B) TELEFAX: (215) 540-5818

42

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 977 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 70..666

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

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1 5	
GTC GTG CTG AGC CTG TGG CCA GAT ACA GCT GTC GCC CCT GGG	138
Val Val Leu Ser Leu Trp Pro Asp Thr Ala Val Ala Pro Gly	
10 15 20	
CCA CCA CCT GGC CCC CCT CGA GTT TCC CCA GAC CCT CGG GCC	180
Pro Pro Pro Gly Pro Pro Arg Val Ser Pro Asp Pro Arg Ala	
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GAG CTG GAC AGC ACC GTG CTC CTG ACC CGC TCT CTC CTG GCG	222
Glu Leu Asp Ser Thr Val Leu Leu Thr Arg Ser Leu Leu Ala	
40 45 50	
GAC ACG CGG CAG CTG GCT GCA CAG CTG AGG GAC AAA TTC CCA	264
Asp Thr Arg Gln Leu Ala Ala Gln Leu Arg Asp Lys Phe Pro	
55 60 65	
GCT GAC GGG GAC CAC AAC CTG GAT TCC CTG CCC ACC CTG GCC	306
Ala Asp Gly Asp His Asn Leu Asp Ser Leu Pro Thr Leu Ala	
70 75	
ATG AGT GCG GGG GCA CTG GGA GCT CTA CAG CTC CCA GGT GTG	348
Met Ser Ala Gly Ala Leu Gly Ala Leu Gln Leu Pro Gly Val	
80 85 90	
CTG ACA AGG CTG CGA GCG GAC CTA CTG TCC TAC CTG CGG CAC	390
Leu Thr Arg Leu Arg Ala Asp Leu Leu Ser Tyr Leu Arg His	
95 100 105	

43

GTG	CAG	TGG	CTG	CGC	CGG	GCA	GGT	GGC	TCT	TCC	CTG	AAG	ACC	432
Val	Gln	Trp	Leu	Arg	Arg	Ala	Gly	Gly	Ser	Ser	Leu	Lys	Thr	
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CTG	GAG	CCC	GAG	CTG	GGC	ACC	CTG	CAG	GCC	CGA	CTG	GAC	CGG	474
Leu	Glu	Pro	Glu	Leu	Gly	Thr	Leu	Gln	Ala	Arg	Leu	Asp	Arg	
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CTG	CTG	CGC	CGG	CTG	CAG	CTC	CTG	ATG	TCC	CGC	CTG	GCC	CTG	516
Leu	Leu	Arg	Arg	Leu	Gln	Leu	Leu	Met	Ser	Arg	Leu	Ala	Leu	
				140					145					
CCC	CAG	CCA	CCC	CCG	GAC	CCG	CCG	GCG	CCC	CCG	CTG	GCG	CCC	558
Pro	Gln	Pro	Pro	Pro	Asp	Pro	Pro	Ala	Pro	Pro	Leu	Ala	Pro	
150					155					160				
CCC	TCC	TCA	GCC	TGG	GGG	GGC	ATC	AGG	GCC	GCC	CAC	GCC	ATC	600
Pro	Ser	Ser	Ala	Trp	Gly	Gly	Ile	Arg	Ala	Ala	His	Ala	Ile	
	165					170					175			
CTG	GGG	GGG	CTG	CAC	CTG	ACA	CTT	GAC	TGG	GCC	GTG	AGG	GGA	642
Leu	Gly	Gly	Leu	His	Leu	Thr	Leu	Asp	Trp	Ala	Val	Arg	Gly	
		180					185					190		
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Leu	Leu	Leu	Leu	Lys	Thr	Arg	Leu							
			195											
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GACAGTCCTT	CCGTGAGGCT	GGGGGGCATC	TGTGCCTTAT	TTATACTTAT										836
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 199 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

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Pro	Asp	Thr	Ala	Val	Ala	Pro	Gly	Pro	Pro	Pro	Gly	Pro	Pro	Arg
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Val	Ser	Pro	Asp	Pro	Arg	Ala	Glu	Leu	Asp	Ser	Thr	Val	Leu	Leu
				35					40					45
Thr	Arg	Ser	Leu	Leu	Ala	Asp	Thr	Arg	Gln	Leu	Ala	Ala	Gln	Leu
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Arg	Asp	Lys	Phe	Pro	Ala	Asp	Gly	Asp	His	Asn	Leu	Asp	Ser	Leu
				65					70					75
Pro	Thr	Leu	Ala	Met	Ser	Ala	Gly	Ala	Leu	Gly	Ala	Leu	Gln	Leu
				80					85					90
Pro	Gly	Val	Leu	Thr	Arg	Leu	Arg	Ala	Asp	Leu	Leu	Ser	Tyr	Leu
				95					100					105
Arg	His	Val	Gln	Trp	Leu	Arg	Arg	Ala	Gly	Gly	Ser	Ser	Leu	Lys
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Thr	Leu	Glu	Pro	Glu	Leu	Gly	Thr	Leu	Gln	Ala	Arg	Leu	Asp	Arg
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Leu	Leu	Arg	Arg	Leu	Gln	Leu	Leu	Met	Ser	Arg	Leu	Ala	Leu	Pro
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Gln	Pro	Pro	Pro	Asp	Pro	Pro	Ala	Pro	Pro	Leu	Ala	Pro	Pro	Ser
				155					160					165
Ser	Ala	Trp	Gly	Gly	Ile	Arg	Ala	Ala	His	Ala	Ile	Leu	Gly	Gly
				170					175					180
Leu	His	Leu	Thr	Leu	Asp	Trp	Ala	Val	Arg	Gly	Leu	Leu	Leu	Leu
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Lys	Thr	Arg	Leu											

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3632 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2242..3132

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 2242..2568
- (D) OTHER INFORMATION: /product= "E. coli
thioredoxin protein"
/note= "Lim et al, J. Bacteriol., 163:311-316
(1985)"

(ix) FEATURE:

- (A) NAME/KEY: RBS
- (B) LOCATION: 2222..2241
- (D) OTHER INFORMATION: /standard_name= "ribosome
binding sequence"
/note= "Dunn and Studier, J. Mol. Biol,
166:477-535 (1983)"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 2061..2221
- (D) OTHER INFORMATION: /function= "leftward promoter
of bacteriophage lambda"
/note= "Sanger et al, J. Mol. Biol,
162:729-773 (1982)"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..2060
- (D) OTHER INFORMATION: /function= "derived from
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/note= "Norrande et al, Gene, 26:101-106
(1983)"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 2569..2583
- (D) OTHER INFORMATION: /function= "short,
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(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 2584..2598
- (D) OTHER INFORMATION: /function= "enterokinase
cleavage recognition site"
/note= "Maroux et al, J. Biol. Chem.,
246:5031-5039 (1971)"

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 2599..3132
(D) OTHER INFORMATION: /product= "modified form of
mature human IL11"
/note= "Paul et al, Proc. Natl. Acad. Sci.
USA, 87:7512-7516 (1990)"

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 3133..3159
(D) OTHER INFORMATION: /function= "linker sequence
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(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 3160..3232
(D) OTHER INFORMATION: /function= "transcription
termination sequence based on E. coli aspA"
/note= "Takagi et al, Nucl. Acids Res.,
13:2063-2074 (1985)"

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 3233..3632
(D) OTHER INFORMATION: /function= "DNA sequences
derived from pUC-18"

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

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AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA	150
TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT	200
ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT	250
TTGCCTTCCT GTTTTGTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG	300
CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC	350
AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT	400
GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTATTGACG	450
CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG	500
GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT	550

AAGAGAATTA	TGCAGTGCTG	CCATAACCAT	GAGTGATAAC	ACTGCGGCCA	600
ACTTACTTCT	GACAACGATC	GGAGGACCGA	AGGAGCTAAC	CGCTTTTTTG	650
CACAACATGG	GGGATCATGT	AACTCGCCTT	GATCGTTGGG	AACCGGAGCT	700
GAATGAAGCC	ATACCAAACG	ACGAGCGTGA	CACCACGATG	CCTGTAGCAA	750
TGGCAACAAC	GTTGCGCAAA	CTATTAACTG	GCGAACTACT	TACTCTAGCT	800
TCCCGGCAAC	AATTAATAGA	CTGGATGGAG	GCGGATAAAG	TTGCAGGACC	850
ACTTCTGCGC	TCGGCCCTTC	CGGCTGGCTG	GTTTATTGCT	GATAAATCTG	900
GAGCCGGTGA	GCGTGGGTCT	CGCGGTATCA	TTGCAGCACT	GGGGCCAGAT	950
GGTAAGCCCT	CCCGTATCGT	AGTTATCTAC	ACGACGGGGA	GTCAGGCAAC	1000
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AGCATTGGTA	ACTGTCAGAC	CAAGTTTACT	CATATATACT	TTAGATTGAT	1100
TTAAAACTTC	ATTTTTAATT	TAAAAGGATC	TAGGTGAAGA	TCCTTTTTGA	1150
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CAGACCCCGT	AGAAAAGATC	AAAGGATCTT	CTTGAGATCC	TTTTTTTCTG	1250
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GGCCACCACT	TCAAGAACTC	TGTAGCACCG	CCTACATACC	TCGCTCTGCT	1450
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ACGGGGGGTT	CGTGACACACA	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	1600
ACTGAGATAC	CTACAGCGTG	AGCATTGAGA	AAGCGCCACG	CTTCCCGAAG	1650
GGAGAAAGGC	GGACAGGTAT	CCGGTAAGCG	GCAGGGTCGG	AACAGGAGAG	1700
CGCACGAGGG	AGCTTCCAGG	GGGAAACGCC	TGGTATCTTT	ATAGTCCTGT	1750
CGGGTTTCGC	CACCTCTGAC	TTGAGCGTCG	ATTTTGTGA	TGCTCGTCAG	1800
GGGGGCGGAG	CCTATGGAAA	AACGCCAGCA	ACGCGGCCTT	TTTACGGTTC	1850
CTGGCCTTTT	GCTGGCCTTT	TGCTCACATG	TTCTTTCCTG	CGTTATCCCC	1900

TGATTCTGTG	GATAACCGTA	TTACCGCCTT	TGAGTGAGCT	GATACCGCTC	1950
GCCGCAGCCG	AACGACCGAG	CGCAGCGAGT	CAGTGAGCGA	GGAAGCGGAA	2000
GAGCGCCCAA	TACGCAAACC	GCCTCTCCCC	GCGCGTTGGC	CGATTCATTA	2050
ATGCAGAATT	GATCTCTCAC	CTACCAAACA	ATGCCCCCCT	GCAAAAAATA	2100
AATTCATATA	AAAAACATAC	AGATAACCAT	CTGCGGTGAT	AAATTATCTC	2150
TGGCGGTGTT	GACATAAATA	CCACTGGCGG	TGATACTGAG	CACATCAGCA	2200
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				Met Ser	
				1	
GAT AAA ATT ATT CAC CTG ACT GAC GAC AGT TTT GAC ACG GAT	2289				
Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp					
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GTA CTC AAA GCG GAC GGG GCG ATC CTC GTC GAT TTC TGG GCA	2331				
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala					
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GAG TGG TGC GGT CCG TGC AAA ATG ATC GCC CCG ATT CTG GAT	2373				
Glu Trp Cys Gly Pro Cys Lys Met Ile Ala Pro Ile Leu Asp					
35 40					
GAA ATC GCT GAC GAA TAT CAG GGC AAA CTG ACC GTT GCA AAA	2415				
Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys					
45 50 55					
CTG AAC ATC GAT CAA AAC CCT GGC ACT GCG CCG AAA TAT GGC	2457				
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly					
60 65 70					
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Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu					
75 80 85					
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Val Ala Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln Leu					
90 95 100					
AAA GAG TTC CTC GAC GCT AAC CTG GCC GGT TCT GGT TCT GGT	2583				
Lys Glu Phe Leu Asp Ala Asn Leu Ala Gly Ser Gly Ser Gly					
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GAT GAC GAT GAC AAA GGT CCA CCA CCA GGT CCA CCT CGA GTT	2625				
Asp Asp Asp Asp Lys Gly Pro Pro Pro Gly Pro Pro Arg Val					
115 120 125					

TCC Ser	CCA Pro 130	GAC Asp	CCT Pro	CGG Arg	GCC Ala	GAG Glu 135	CTG Leu	GAC Asp	AGC Ser	ACC Thr	GTG Val 140	CTC Leu	CTG Leu	2667
ACC Thr	CGC Arg	TCT Ser 145	CTC Leu	CTG Leu	GCG Ala	GAC Asp	ACG Thr 150	CGG Arg	CAG Gln	CTG Leu	GCT Ala 155	GCA Ala	CAG Gln	2709
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GCG Ala 255	CCC Pro	CCG Pro	CTG Leu	GCG Ala	CCC Pro 260	CCC Pro	TCC Ser	TCA Ser	GCC Ala	TGG Trp 265	GGG Gly	GGC Gly	ATC Ile	3045
AGG Arg	GCC Ala 270	GCC Ala	CAC His	GCC Ala	ATC Ile	CTG Leu 275	GGG Gly	GGG Gly	CTG Leu	CAC His	CTG Leu 280	ACA Thr	CTT Leu	3087
GAC Asp	TGG Trp	GCC Ala 285	GTG Val	AGG Arg	GGA Gly	CTG Leu 290	CTG Leu	CTG Leu	CTG Leu	AAG Lys	ACT Thr	CGG Arg 295	CTG Leu	3129
TGAAAGCTTA TCGATACCGT CGACCTGCAG TAATCGTACA GGGTAGTACA														3179
AATAAAAAAG GCACGTCAGA TGACGTGCCT TTTTCTTGT GAGCAGTAAG														3229

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CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCTGGCG 3279
 TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC CAGCTGGCGT 3329
 AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT 3379
 GAATGGCGAA TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG 3429
 GTATTTTACA CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG 3479
 CCGCATAGTT AAGCCAGCCC CGACACCCGC CAACACCCGC TGACGCGCCC 3529
 TGACGGGCTT GTCTGCTCCC GGCATCCGCT TACAGACAAG CTGTGACCGT 3579
 CTCCGGGAGC TGCATGTGTC AGAGGTTTTC ACCGTCATCA CCGAAACGCG 3629
 CGA 3632

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 296 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met	Ser	Asp	Lys	Ile	Ile	His	Leu	Thr	Asp	Asp	Ser	Phe	Asp	Thr	1	5	10	15
Asp	Val	Leu	Lys	Ala	Asp	Gly	Ala	Ile	Leu	Val	Asp	Phe	Trp	Ala	20	25	30	
Glu	Trp	Cys	Gly	Pro	Cys	Lys	Met	Ile	Ala	Pro	Ile	Leu	Asp	Glu	35	40	45	
Ile	Ala	Asp	Glu	Tyr	Gln	Gly	Lys	Leu	Thr	Val	Ala	Lys	Leu	Asn	50	55	60	
Ile	Asp	Gln	Asn	Pro	Gly	Thr	Ala	Pro	Lys	Tyr	Gly	Ile	Arg	Gly	65	70	75	
Ile	Pro	Thr	Leu	Leu	Leu	Phe	Lys	Asn	Gly	Glu	Val	Ala	Ala	Thr	80	85	90	
Lys	Val	Gly	Ala	Leu	Ser	Lys	Gly	Gln	Leu	Lys	Glu	Phe	Leu	Asp	95	100	105	
Ala	Asn	Leu	Ala	Gly	Ser	Gly	Ser	Gly	Asp	Asp	Asp	Asp	Lys	Gly	110	115	120	

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Pro	Pro	Pro	Gly	Pro	Pro	Arg	Val	Ser	Pro	Asp	Pro	Arg	Ala	Glu
				125					130					135
Leu	Asp	Ser	Thr	Val	Leu	Leu	Thr	Arg	Ser	Leu	Leu	Ala	Asp	Thr
				140					145					150
Arg	Gln	Leu	Ala	Ala	Gln	Leu	Arg	Asp	Lys	Phe	Pro	Ala	Asp	Gly
				155					160					165
Asp	His	Asn	Leu	Asp	Ser	Leu	Pro	Thr	Leu	Ala	Met	Ser	Ala	Gly
				170					175					180
Ala	Leu	Gly	Ala	Leu	Gln	Leu	Pro	Gly	Val	Leu	Thr	Arg	Leu	Arg
				185					190					195
Ala	Asp	Leu	Leu	Ser	Tyr	Leu	Arg	His	Val	Gln	Trp	Leu	Arg	Arg
				200					205					210
Ala	Gly	Gly	Ser	Ser	Leu	Lys	Thr	Leu	Glu	Pro	Glu	Leu	Gly	Thr
				215					220					225
Leu	Gln	Ala	Arg	Leu	Asp	Arg	Leu	Leu	Arg	Arg	Leu	Gln	Leu	Leu
				230					235					240
Met	Ser	Arg	Leu	Ala	Leu	Pro	Gln	Pro	Pro	Pro	Asp	Pro	Pro	Ala
				245					250					255
Pro	Pro	Leu	Ala	Pro	Pro	Ser	Ser	Ala	Trp	Gly	Gly	Ile	Arg	Ala
				260					265					270
Ala	His	Ala	Ile	Leu	Gly	Gly	Leu	His	Leu	Thr	Leu	Asp	Trp	Ala
				275					280					285
Val	Arg	Gly	Leu	Leu	Leu	Leu	Lys	Thr	Arg	Leu				
				290					295					

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A use of a cytokine selected from the group consisting of Interleukin-11, Interleukin-6, leukemia inhibitory factor, Oncostatin M, and ciliary neurotrophic factor in the preparation of a pharmaceutical composition for stimulating cell growth in a patient having damaged or depleted cell populations selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, liver epithelial cells, skin cells, hair cells, and sperm cells.
2. The use according to claim 1 wherein said cytokine is interleukin-11.
3. The use according to claim 1 wherein said cytokine is IL-6.
4. The use according to claim 1 wherein said cell populations is small intestinal epithelial cells.

5. A use of an effective amount of a cytokine selected from the group consisting of Interleukin-11, Interleukin-6, leukemia inhibitory factor, Oncostatin M, and ciliary neurotrophic factor in a suitable pharmaceutical carrier for treating a patient having damaged or depleted cell populations.

6. A use according to claim 5 wherein said cell populations are selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the large intestines and stomach, liver epithelial cells, skin cells, hair cells, and sperm cells.

7. A use of an effective amount of a cytokine selected from the group consisting of Interleukin-11, Interleukin-6, leukemia inhibitory factor, Oncostatin M, and ciliary neurotrophic factor in a suitable pharmaceutical carrier for stimulating gut epithelial cell growth in a patient having damaged or depleted cell populations.

8. A use according to claim 5 or 6 further comprising using simultaneously or in combination with said cytokine, at least one additional cytokine.

9. A use according to claim 5 or 6 wherein said use of said cytokine to the patient begins prior to, simultaneously with, or subsequent to the administration of chemotherapy or radiation therapy.

10. The use according to claim 5 or 6 wherein said damaged or depletion is caused by an agent selected from the group consisting of disease, infection, trauma, shock, chemotherapy and radiation therapy.



11. A use of an effective amount of Interleukin-11, in a suitable pharmaceutical carrier for treating a patient having damaged or depleted cell populations.
12. The use according to claim 11 wherein said cell populations are selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the large intestine and stomach, liver epithelial cells, skin cells, hair cells and sperm cells.
13. The use according to claim 12 wherein said cell populations is small intestinal epithelial cells.
14. A use of an effective amount of Interleukin-11, in a suitable pharmaceutical carrier for the production of a medicament for treating a patient having damaged or depleted cell populations.
15. The use according to claim 14 wherein said cell populations are selected from the group consisting of small intestinal epithelial cells, epithelial cells lining the large intestine and stomach, liver epithelial cells, skin cells, hair cells and sperm cells.
16. The use according to claim 15 wherein said cell populations is small intestinal epithelial cells.

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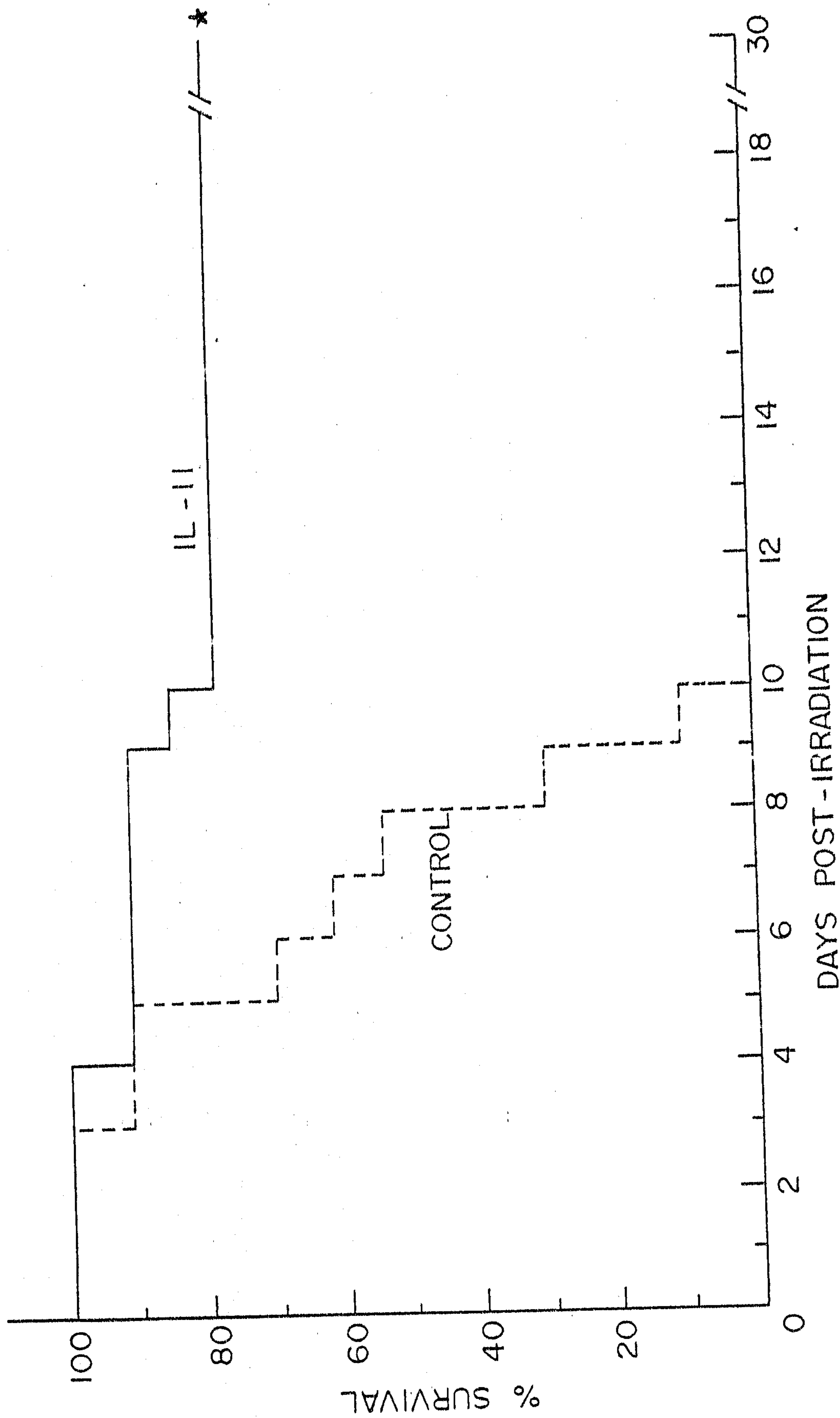


FIG. 1

* p < 0.01, STUDENT T-TEST

FIG. 2

