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(54) Title: METHOD OF TREATING PSORIASIS WITH IL-15 ANTAGONIST

(57) Abstract: The present invention provides methods of treating diseases, wherein the number of keratinocytes is increased, in a mammal comprising administering an IL-15-specific antagonist. The invention further provides methods of identifying compounds useful in the treatment of such diseases.



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TITLE

METHOD OF TREATING PSORIASIS WITH IL-15 ANTAGONIST

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of treating a disease in a mammal wherein the number of keratinocytes is increased. In particular, the invention relates to the administration of a compound that antagonizes the activity of interleukin-15 and thus increases apoptosis of keratinocytes.

BACKGROUND OF THE INVENTION10 A. Interleukin-15 (IL-15)

IL-15, a T-cell growth factor was first reported by Grabstein et al. (Science 264:965, 1994), as a secreted cytokine in simian kidney epithelial cell supernatants that supported proliferation of the IL-2-dependent mouse T-cell line CTLL-2. 15 Grabstein et al. isolated cDNA clones encoding human (SEQ ID NO:8) and simian (SEQ ID NO:7) IL-15. The cDNA clones encode a 162 amino acid precursor polypeptide. The precursor polypeptide includes a 48 amino acid leader sequence, and is processed to a 114 amino acid mature protein. The mature amino acid sequences 20 of human (residues 49-162 of SEQ ID NO:8) and simian IL-15 (residues 49-162 of SEQ ID NO:7) are approximately 96% identical.

IL-15 shares certain biological activities with IL-2, including activation of T cells, induction of lymphokine activated killer cell (LAK) activity, natural killer cell (NK) 25 activity, and cytotoxic T lymphocyte (CTL) activity, and costimulation of B cell proliferation and differentiation. These shared activities are due, at least in part, to the use of common receptor signaling subunits.

The IL-15 receptor complex includes three subunits, 30 designated α , β , and γ . IL-15 signals through the β and γ subunits, which are also part of the IL-2 receptor complex. The γ subunit is also a component of the IL-4, IL-7, and IL-9 receptors. The IL-15 specific α subunit (IL-15R α , as described in U.S. Patent No. 5,591,630) is required for high affinity

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binding of IL-15, and differences in the expression patterns of IL-15R α and IL-2R α (the cognate IL-2 receptor α subunit) contribute to the distinct in vivo roles of IL-15 and IL-2.

IL-15 has been implicated in the maintenance of chronic
5 inflammatory immune responses (Alleva et al., J. Immunol. 159:2941, 1997), and has been reported to inhibit lymphocyte and hepatocyte apoptosis both in vitro and in vivo (Bulfone-Paus, Nat. Med. 3:1124, 1997). IL-15 mRNA is reportedly expressed in keratinocytes and dendritic cells in the skin (Blauvelt et al.,
10 J. Invest. Dermatol. 106:1047, 1996).

B. Diseases wherein the number of keratinocytes is increased

Numerous diseases are known, wherein the number of keratinocytes is increased, leading to epidermal hyperplasia, which is
15 responsible for at least some of the related disease symptoms. Among these there is a number of chronic diseases for which hardly any cure is available, such as psoriasis and atopic dermatitis as well as conditions like chronic hand eczema, contact dermatitis, viral warts (HPV associated), cutaneous T cell
20 lymphoma, impaired wound healing, such as impaired wound healing due to diabetes, where currently available treatment options are limited and/or unsatisfactory. There is thus a need for the development of more effective management strategies for these diseases and for pharmaceutical compositions which could be
25 employed in this context.

Psoriasis for example is a common chronic inflammatory hyperproliferative skin disease that presents as plaques, scaling and thickening of the skin and inflammation. Psoriasis has a prevalence of up to 2% in many populations. As many as 7 million
30 people in the U.S. suffer from some form of psoriasis, with 100,000 of these having severe symptoms. Psoriasis is characterized by keratinocyte (KC) hyperproliferation and by profound changes in KC maturation and turnover rate (McKay and Leigh, Clin. Dermatol. 13:105, 1995; Bergstresser and Taylor, Br.
35 J. Dermatol. 96:503, 1977). Keratinocytes are major constituents of the epidermis and the epithelial lining of mucosal tissue.

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Keratinocytes undergo terminal differentiation to corneocytes, as well as apoptosis, during their outgrowth from keratinocyte stem cells. Control of keratinocyte growth is mediated by a complex network of cytokines and growth factors (reviewed in Luger and Schwartz, J. Invest. Dermatol. 95:1005, 1990), some of which are produced by the KC themselves. An abnormal resistance of psoriatic KC to apoptosis (Wrone Smith et al., Am. J. Pathol. 151:1321, 1997) may contribute to the epidermal hyperplasia of psoriasis (akanthosis, thickened stratum corneum), and methotrexate, an established antipsoriatic agent, has been reported to induce apoptosis in KC (Heenen et al., Arch. Dermatol. Res. 290:240, 1998). The underlying defect in control of the apoptotic machinery of psoriatic KC is, however, not understood. According to the evolving "T-cell hypothesis" of psoriasis (reviewed in Bos and De Rie, Immunology Today 20:40, 1999) the pathogenesis of psoriasis is a result of abnormal regulation at the level of T cell-keratinocyte interaction.

Psoriasis is a mild nuisance for some patients, and almost totally disabling for others. The severity of the disease may be evaluated by the psoriasis area and severity index (PASI, Nickoloff et al., Am. J. Pathol. 144:820, 1994) There is, unfortunately, no cure for this chronic disease. Some psoriasis symptoms may be controlled by the use of moisturizing and lubricating agents, topical ointments, oral medications including retinoids cyclosporine, and methotrexate, phototherapy, or by combinations or rotations of these therapies. In addition to problems with efficacy, some of these treatments can cause unwanted side effects, e.g., some of the oral medications can cause liver and kidney toxicity, and phototherapy can enhance the risk of both melanoma and nonmelanoma skin cancer.

Molecular targets that are being studied for the development of additional agents for the treatment of psoriasis include IL-8, CD3, CD4, CD11a, tryptase, and the CD2 receptor. There is, however, a continuing need for improved methods of treating psoriasis and for improved methods of identifying compounds useful in the treatment of psoriasis.

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SUMMARY OF THE INVENTION

The present invention provides a method of treating a disease in a mammal wherein the number of keratinocytes is increased comprising administering an effective amount of an IL-15-specific antagonist. The present invention further includes the use of an effective amount of an IL-15-specific antagonist for preparing a pharmaceutical composition for increasing apoptosis of keratinocytes in a mammal suffering from a disease wherein the number of keratinocytes is increased.

According to a preferred embodiment of the present invention, the method is used to treat psoriasis, atopic dermatitis, chronic hand eczema, contact dermatitis, viral warts, cutaneous T cell lymphoma and impaired wound healing, such as impaired wound healing due to diabetes.

In some preferred embodiments the antagonist is selected from the group consisting of IL-15 antibodies, IL-15 muteins, IL-15 conjugates, IL-15 antisense nucleic acids, IL-15 ribozymes, and small molecules targeting IL-15. In some preferred embodiments the antagonist is selected from the group consisting of IL-15 receptor, IL-15 receptor subunits or fragments, IL-15 receptor antibodies, IL-15 receptor antisense nucleic acids, IL-15 receptor ribozymes, and small molecules targeting the IL-15 receptor.

According to a specifically preferred embodiment of the invention, the IL-15 receptor subunit is a soluble form of the α chain of the IL-15 receptor. Soluble IL-15 receptor polypeptides comprise all or part of the extracellular domain but generally lack a transmembrane domain that would cause retention of the polypeptide at the cell surface. Soluble polypeptides may include part of the transmembrane domain and/or all or part of the cytoplasmic domain as long as the polypeptide is secreted from the cell in which it is produced. In some preferred embodiments of the invention the antagonist is a multimeric form of soluble IL-15 receptor.

The multimers may be covalently-linked or non-covalently-linked dimers, trimers, tetramers or higher order multimers. The

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multimers may be linked, for example, by disulfide bonds between cysteine residues or via covalent or non-covalent interactions between peptide moieties fused to the IL-15 receptor polypeptides. Such peptides may be peptide linkers (spacers) or peptides that
5 have the property of promoting multimerization. Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233. Polypeptides derived from antibodies, including heavy chains, light chains and Fc domains, are particularly useful in promoting multimerization of IL-15
10 receptor polypeptides (see e.g. Ashkenazi et al., Proc. Natl. Acad. Sci. USA, 88:10535, 1991; Byrn et al., Nature 344:677, 1990; Hollenbaugh and Aruffo, Current Protocols in Immunology, Suppl. 4, pages 10.19.1-10.19.11, 1992; WO 93/10151, U.S. 5,457,035; and Baum et al., EMBO J., 13:3992, 1994). Another
15 method from preparing multimers involves use of a leucine zipper domain (see e.g. Landschulz et al., Science 240:1759, 1988; WO 94/10308; Hoppe et al., FEBS Lett., 344:191, 1994; and Fanslow et al., Semin. Immunol., 6:267, 1994).

The antagonist is preferably administered as a composition
20 comprising a pharmaceutically acceptable carrier. In some embodiments the antagonist is administered systemically; in some embodiments the antagonist is administered locally. In a particularly preferred embodiment of the invention the mammal is a human.

25 The invention also provides a method of identifying a compound useful in the treatment of diseases, wherein the number of keratinocytes is increased, comprising: (a) including a test compound in an assay system which gives a detectable response in the presence of IL-15 activity, (b) determining whether the test
30 compound inhibits IL-15 activity, and (c) repeating steps (a) and (b) until a compound that inhibits IL-15 activity is identified, wherein the compound that inhibits IL-15 activity is identified as a compound useful in the treatment of diseases, wherein the number of keratinocytes is increased.

35 In some preferred embodiments the assay system measures the binding of IL-15 to the IL-15 receptor or to at least one subunit

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thereof. In some more preferred embodiments the detectable response is IL-15-mediated proliferation. In some more preferred embodiments the detectable response is IL-15 signaling activity. A most preferred embodiment further comprises the step of
5 administering the compound identified as an IL-15 inhibitor to a non-human animal model of psoriasis and determining whether the compound alleviates one or more symptoms of psoriasis, wherein the compound that alleviates one or more symptoms of psoriasis is identified as a compound useful in the treatment of psoriasis.

10 The present invention further provides a method of increasing keratinocyte apoptosis in a mammal in need of such treatment comprising administering an effective amount of an IL-15-specific antagonist. In a preferred embodiment the mammal has psoriasis. In some preferred embodiments the antagonist is
15 selected from the group consisting of IL-15 antibodies, IL-15 muteins, IL-15 conjugates, IL-15 antisense nucleic acids, IL-15 ribozymes, and small molecules targeting IL-15. In some preferred embodiments the antagonist is selected from the group consisting of IL-15 receptor, IL-15 receptor subunits or fragments, IL-15
20 receptor antibodies, IL-15 receptor antisense nucleic acids, IL-15 receptor ribozymes, and small molecules targeting the IL-15 receptor.

The antagonist is preferably administered as a composition comprising a pharmaceutically acceptable carrier. In some
25 embodiments the antagonist is administered systemically; in some embodiments the antagonist is administered locally. In a particularly preferred embodiment of the invention the mammal is a human.

These and other aspects of the present invention will become
30 evident upon reference to the following drawings and detailed description.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results of RT-PCR for IL-15 and IL-15R α using cDNA from primary KC and HaCaT cells incubated for 24 hours with medium alone or medium supplemented with IFN- γ (10^3 U/ml) or IL-15 (10 ng/ml). β -actin expression was used to normalize the cDNA amount. The control samples did not contain cDNA.

Figure 2 shows a FACS analysis of cell surface IL-15 and IL-15R α on keratinocytes. Isotype matched antibody served as a negative control. Figure 2A is IL-15 on primary foreskin KC; Figure 2B is IL-15R α on primary foreskin KC. Figure 2C is IL-15 on HaCaT cells; Figure 2D is IL-15R α on HaCaT cells.

Figure 3 shows inhibition by IL-15 of methylcellulose and anti-Fas induced KC apoptosis as detected by TUNEL staining. For Figure 3A primary KC cells and HaCaT cells were incubated with MC alone or in combination with 20 ng/ml IL-15 for 24, 48, or 72 hours. Cells were harvested, and the number of apoptotic cells was analyzed by TUNEL staining of cytopins. The % inhibition represents the percent inhibition by IL-15 of MC-induced apoptosis as compared to MC alone. For Figure 3B KC apoptosis was induced by an anti-Fas antibody. Cells were incubated with anti-Fas in the presence or absence of IL-15 (at 10 or 50 ng/ml) for 24, 48, or 72 hours. The % inhibition represents the percent inhibition by IL-15 of anti-Fas-induced apoptosis as compared to anti-Fas alone. An isotype-matched control antibody or incubation with the crosslinking antibody alone did not induce apoptosis above baseline levels.

Figure 4 shows immunohistochemical staining for IL-15 cytokine and IL-15 binding sites on skin biopsies from psoriatic patients and healthy controls. The figure shows representative staining patterns from a total of 10 biopsies from healthy controls and 12 biopsies from psoriatic patients.

Figures 4A and B show IL-15 staining in normal skin and psoriatic plaques. Hypertrophic scar tissue served as a positive control, and sections processed without primary antibody served as a negative control. In Figures 4A and B: (1) refers to a normal epidermal layer not containing IL-15; (2) refers to the

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subepidermal layer containing IL-15; and (3) refers to an increased epidermal layer containing IL-15.

Figures 4C, D and E show IL-15 receptor expression in normal skin and psoriatic plaques, as detected using the IL-15-IgG2b FP. Peripheral blood mononuclear cells (PBMC), which express IL-15R after activation, were used as positive controls. Specificity was shown by blocking the IL-15 tail, which completely prevented binding of the fusion protein (4C). As a negative control, sections were stained with IgG2b. In Figure 4E: (1) refers to a normal epidermal layer, comprising keratinocytes expressing the IL-15 receptor; and (2) refers to a psoriatic epidermal layer showing increased expression of the IL-15 receptor.

Figure 5 shows steady state mRNA levels, as determined by RT-PCR, for IL-15 and IL-15R α in psoriatic skin (patient biopsies 1-10) and in normal skin (patient biopsies 1-6). β -actin expression was used to normalize cDNA amounts. For the negative control the PCR was performed in the absence of cDNA.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the use of IL-15-specific antagonists for the treatment of a disease in a mammal wherein the number of keratinocytes is increased, preferably a mammalian skin disorder, and to methods of identifying compounds useful in the treatment of such diseases by identifying inhibitors of IL-15 activity. Without wishing to be bound to a theory, the utility of IL-15-specific antagonists for the treatment of these diseases is likely due, at least in part, to the ability of IL-15 to inhibit keratinocyte apoptosis. It is likely that additional mechanisms are also involved. IL-15 is a potent chemoattractant and inhibitor of lymphocyte apoptosis as well as a T cell growth factor, so KC-derived IL-15 may contribute to T cell accumulation, proliferation, and survival in psoriatic plaques. IL-15 may also play a role in the abnormal influx of neutrophils into inflammatory tissue, since IL-15 activates neutrophils, enhances their phagocytic activity, and inhibits their apoptosis in vitro.

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As exemplified below, primary foreskin KC as well as the human HaCaT cell line (a model for hyperproliferative skin disease) transcribe mRNA for IL-15 and the IL-15R α chain, and express the cytokine as well as the cognate high affinity
 5 receptor on the cell surface. IL-15 is not a KC growth factor in vitro, but IL-15 strongly inhibits both methylcellulose and anti-Fas induced KC apoptosis.

For example, IL-15 binding sites and IL-15R α mRNA levels are strongly upregulated in psoriatic plaques and IL-15 cytokine
 10 shows a different distribution pattern in psoriatic plaques as compared to uninvolved or normal skin.

A. Abbreviations Used in the Specification

	" BrUdr"	is bromodeoxyuridine.
15	" DMEM"	is Dulbecco's Modified Eagle Medium, a commercially available cell culture medium.
	" EGF"	is epidermal growth factor.
	" ELISA"	is enzyme-linked immunosorbent assay.
	" FACS"	is fluorescence activated cell sorter.
20	" FCS"	is fetal calf serum.
	" FP"	is fusion protein.
	" IL-15-IgG2b FP"	is a fusion protein consisting of human IL-15 and murine IgG2b.
	" IFN- γ "	is interferon-gamma.
25	" IL-15"	is interleukin-15.
	" IL-15R"	is interleukin-15 receptor; " IL-15R α " is the high affinity alpha subunit of interleukin-15 receptor.
30	" KBM"	is keratinocyte basal medium, a commercially available cell culture medium for the culture of keratinocytes.
	" KC"	is keratinocyte.
	" MC"	is methylcellulose.
	" PBMC"	are peripheral blood mononuclear cells.
35	" IFN- γ "	is interferon-gamma.
	" PASI"	is psoriasis area and severity index.

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"RT-PCR" is the combined method including reverse transcription and polymerase chain reaction.

"SCID" is severe combined immunodeficiency.

5 B. IL-15 Antagonists

An "IL-15-specific antagonist" is any compound or composition that specifically antagonizes the activity of interleukin-15, i.e., prevents IL-15 from transducing a signal through the β or γ subunits of the IL-15 receptor complex, thereby antagonizing IL-15's biological activity. Methods of analyzing whether or not IL-15 is able to transduce a signal through the β or γ subunits of the IL-15 receptor complex are well known to the person skilled in the art. One of the methods that can be used to identify inhibition of signal transduction by antagonists directly acting on the IL-15 receptor/IL-15 interaction is the CTLL-2 proliferation assay of Gillis and Smith, Nature, 268: 154, 1977.

Methods for analyzing whether or not the number of keratinocytes is increased are also well known in the art. An increased number of keratinocytes can be determined by simple immuno-histological analysis of tissue layers. More complex analyses based on double staining (KI67 and Tunel staining) are also known, which allow to determine whether proliferation of keratinocytes is increased and/or whether apoptosis is decreased (see for example Bulfone-Paus et al., Nature Med., 3:1124, 1997; and Botchkarev et al., Am. J. Pathol., 153:785, 1998).

The term IL-15-specific antagonist thus includes, inter alia, compounds and compositions that block or downregulate the production, modification, transport, or secretion of IL-15 or IL-15 receptor subunits, compounds and compositions that interfere with the interaction between IL-15 and subunits of the IL-15 receptor complex, and compounds and compositions that interfere with IL-15 signaling events. Several antagonists of IL-15, including IL-15 muteins, IL-15 conjugates, and IL-15 antibodies are described in U.S. Patent No. 5,795,966.

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Antagonists according to the invention include muteins of mature, or native, IL-15, wherein the IL-15 has been substituted at one or more amino acid residues or regions that play a role in binding to the β or γ subunits of the IL-15 receptor complex.

5 Typically, such muteins are created by additions, deletions, or substitutions at or near key positions, for example Asp⁵⁶ or Gln¹⁵⁶ of simian and human IL-15 as shown in SEQ ID NOs:7 and 8. The Asp⁵⁶ residue is believed to affect binding with the β -subunit and the Gln¹⁵⁶ residue is believed to affect binding with the γ

10 subunit of the IL-15 receptor complex. Preferably, a mutein of IL-15 is used wherein at least one of the amino acid residues Asp⁵⁶ or Gln¹⁵⁶ of IL-15 (mature simian IL-15 having the sequence of amino acid residues 49-162 shown in SEQ ID NO:7 or mature human IL-15 having the sequence of amino acid residues 49-162

15 shown in SEQ ID NO:8) is deleted or substituted with a different naturally-occurring amino acid residue. Any combination of substitutions and/or deletions can be made. For example, Asp⁵⁶ can be deleted while Gln¹⁵⁶ is substituted with any other amino acid, or both Asp⁵⁶ and Gln¹⁵⁶ are each substituted with the same

20 or different amino acid moiety. Further, Asp⁵⁶ can be substituted with any amino acid while Gln¹⁵⁶ is deleted. Generally, substitution muteins are preferred, and more preferred are those that do not severely affect the natural folding of the IL-15 molecule. Substitution muteins preferably include those wherein

25 Asp⁵⁶ is substituted by serine or cysteine; or wherein Gln¹⁵⁶ is substituted by serine or cysteine, or wherein both Asp⁵⁶ and Gln¹⁵⁶ are each substituted with a serine or cysteine. Examples of deletion muteins include those wherein Asp⁵⁶ and Gln¹⁵⁶ of mature IL-15 are both deleted; wherein only Asp⁵⁶ is deleted; or wherein

30 only Gln¹⁵⁶ is deleted. It is possible that other amino acid residues in the region of either Asp⁵⁶ and Gln¹⁵⁶ can be substituted or deleted and still have an effect of preventing signal transduction through either or both of the β or γ subunits of the IL-15 receptor complex. Therefore, the invention further

35 encompasses muteins wherein amino acid residues within the region of Asp⁵⁶ and Gln¹⁵⁶ are either substituted or deleted, and that

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possess IL-15 antagonist activity. IL-15 muteins are typically prepared using the methods of recombinant DNA technology and protein purification which are known to those having ordinary skill in the art.

5 According to a further embodiment of the present invention, the IL-15 receptor or subunits or fragments thereof is used as an IL-15 antagonist. It is especially preferred to use a soluble form of the α subunit of the IL-15 receptor as an IL-15 antagonist, since this subunit has specificity only for IL-15.

10 Antagonists according to the invention can also be conjugated to one or more chemical groups that interfere with the IL-15/IL-15 receptor interaction. The mature IL-15 polypeptides disclosed herein (mature simian IL-15 having the sequence of amino acids 49-162 of SEQ ID NO:7 and mature human IL-15 having
15 the sequence of amino acid residues 49-162 shown in SEQ ID NO:8), as well as the IL-15 muteins, may for example be modified by forming covalent or aggregative conjugates with other chemical moieties. Such moieties can include PEG, mPEG, dextran, PVP, PVA, polyamino acids such as poly-L-lysine or polyhistidine, albumin
20 and gelatin at specific sites on the IL-15 molecule that can interfere with binding of IL-15 to the β or γ subunits of the IL-15 receptor complex, while maintaining the high affinity of IL-15 for the IL-15R α subunit. Additionally, IL-15 can be specifically glycosylated at sites that can interfere with binding of IL-15
25 to the β or γ subunits of the IL-15 receptor complex, while maintaining the high affinity of IL-15 for the IL-15R α subunit. Preferred groups for conjugation are PEG, dextran, and PVP. Most preferred for use in the invention is PEG, wherein the molecular weight of the PEG is preferably between about 1,000 to about
30 20,000. A molecular weight of about 5000 is preferred for use in conjugating IL-15, although PEG molecules of other weights would be suitable as well. A variety of forms of PEG are suitable for use in the invention. For example, PEG can be used in the form of succinimidyl succinate PEG (SS-PEG) which provides an ester
35 linkage that is susceptible to hydrolytic cleavage in vivo, succinimidyl carbonate PEG (SC-PEG) which provides a urethane

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linkage and is stable against hydrolytic cleavage in vivo, succinimidyl propionate PEG (SPA-PEG) provides an ether bond that is stable in vivo, vinyl sulfone PEG (VS-PEG) and maleimide PEG (Mal-PEG) all of which are commercially available. In general, 5 SS-PEG, SC-PEG and SPA-PEG react specifically with lysine residues in the polypeptide, whereas VS-PEG and Mal-PEG each react with free cysteine residues. However, Mal-PEG is prone to react with lysine residues at alkaline pH. SC-PEG and VS-PEG are preferred, and SC-PEG is most preferred due to its in vivo 10 stability and specificity for lysine residues.

The PEG moieties can be bonded to IL-15 in strategic sites to take advantage of PEG's large molecular size. As described above, PEG moieties can be bonded to IL-15 by utilizing lysine or cysteine residues naturally occurring in the protein or by 15 site-specific PEGylation. One method of site specific PEGylation is through methods of protein engineering wherein cysteine or lysine residues are introduced into IL-15 at specific amino acid locations. The large molecular size of the PEG chain(s) conjugated to IL-15 is believed to block the region of IL-15 that 20 binds to the β and/or γ subunits but not the α subunit of the IL-15 receptor complex. Conjugations can be made by a simple addition reaction wherein PEG is added to a basic solution containing IL-15. Typically, PEGylation is carried out at either (1) about pH 9.0 and at molar ratios of SC-PEG to lysine residue 25 of approximately 1:1 to 100:1, or greater; or (2) at about pH 7.0 and at molar ratios of VS-PEG to cysteine residue of approximately 1:1 to 100:1, or greater.

Characterization of the conjugated PEGylated IL-15 molecules can be performed by SDS-PAGE on a 4-20% gradient polyacrylamide 30 gel. Conventional silver staining may be employed, or conventional Western blotting techniques can be utilized for highly PEGylated proteins that are not visualized easily by silver staining. Purification of the PEGylated IL-15 molecules can be performed using size exclusion chromatography, dialysis, 35 ultrafiltration or affinity purification.

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The extent of modification and heterogeneity of PEGylated IL-15 can be determined using conventional matrix assisted laser desorption ionization mass spectrometry (MALDI). Since human IL-15 has a molecular weight of about 13,000 and by using PEG having
5 a molecular weight of 5000, MALDI indicates that preparations weighing 13,000 are unPEGylated, those weighing 18,000 indicate that 1 molecule of IL-15 is bonded to one PEG molecule; those weighing 23,000 signify that one IL-15 molecule is bound with two PEG molecules, etc.. Other advantages of conjugating chains of
10 PEG to proteins are known in the art. For example, PEG can prolong the circulation half-lives (see, e.g. Delgado et al., Crit. Rev. Ther. Drug Carr. Syst., 9:249, 1992), enhance solubility (see, e.g., Katre et al., Proc. Natl. Acad. Sci USA, 84:1487, 1987) and reduce immunogenicity (see, e.g., Katre, N.
15 V., Immunol. 144:209, 1990) of some proteins in vivo.

Antagonists according to the invention also include monoclonal antibodies that immunoreact with mature IL-15 and prevent signal transduction through the IL-15 receptor complex. The term "antibodies" is herein intended to include polyclonal
20 antibodies, monoclonal antibodies, antibody fragments such as F(ab')₂ and Fab fragments, recombinantly produced binding partners, humanized, anti-idiotypic, chimeric, single chain antibodies, transgenic, and transgenic human antibodies, and epitope-binding fragments thereof. The specificity and affinity
25 of an antibody is readily determined by one having ordinary skill in the art.

Antagonists according to the invention also include IL-15 antisense nucleic acids, IL-15 ribozymes, small molecules targeting IL-15, IL-15 receptor antibodies, IL-15 receptor
30 antisense nucleic acids, IL-15 receptor ribozymes, and small molecules targeting the IL-15 receptor.

Antisense approaches involve the design of DNA or RNA oligonucleotides that are complementary to the target DNA or RNA. A sequence "complementary" to a portion of a DNA or RNA means a
35 sequence having sufficient complementarity to be able to hybridize with the DNA or RNA, forming a stable double-stranded

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duplex or triple-stranded triplex. Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes or triplexes that block or inhibit expression by one of several means, including enhanced
5 degradation of the mRNA by RNase H, inhibition of splicing, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of IL-15 or IL-15 receptor proteins. Oligonucleotides which are believed to inhibit expression of IL-
10 15 by promoting triplex formation have been described in U.S. 5,874,566.

The ability of an oligonucleotide to hybridize to its target depends on both the degree of complementarity and the length of the antisense nucleic acid. Antisense nucleic acids are typically
15 at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (Cancer Res. 48:2659, 1988) and van der Krol et al. (BioTechniques 6:958,
20 1988). Generally, the longer the hybridizing nucleic acid, the more base mismatches it may contain and still form a stable duplex or triplex. The antisense nucleic acids may be complementary to the coding region of the target or complementary to an untranslated or untranscribed region. For example,
25 oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, are generally effective at inhibiting translation. Sequences complementary to the 3' untranslated sequences of mRNAs have also been shown effective in inhibiting
30 translation of certain mRNAs.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphodiester backbone, for example,
35 to improve stability of the molecule, hybridization, etc. Oligonucleotides with resistant sugar linkages are stable in vivo

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(i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of oligonucleotides include those
5 oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents
10 or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Oligonucleotides for use in the invention may be synthesized by standard methods known in the art, e.g. by use of an automated
15 DNA synthesizer. As an example, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al., Nucl. Acids Res. 16:3209, 1988.

A number of methods have been developed for delivering antisense DNA or RNA to cells including, for example,
20 lipofection, CaPO_4 -mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. Antisense molecules can be injected directly, administered systemically, or administered by way of a recombinant DNA construct in which the antisense oligonucleotide is placed under
25 the control of regulatory sequences such that the oligonucleotide is expressed within target cells.

Ribozyme molecules designed to catalytically cleave IL-15 or IL-15 receptor subunit mRNA transcripts may also be used to inhibit IL-15 activity. The term ribozyme is intended to include
30 hammerhead ribozymes, RNA endoribonucleases, and other classes of ribozymes.

IL-15-specific antagonists may be identified using any assay system that gives a detectable response in the presence of IL-15 activity. Test compounds are added to the assay system to
35 determine whether the compound has inhibits IL-15 activity. The test compounds that may be screened and identified in accordance

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with the invention include, but are not limited to, peptides, soluble peptides, and fragments thereof, antibodies and fragments thereof, nucleic acids, peptidomimetics and other small organic and inorganic compounds. In some preferred embodiments the test compounds are present in a combinatorial chemistry-derived molecular library of natural products or synthetic chemicals including peptides, antibodies, nucleic acids, or small molecules. Computer modeling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can inhibit IL-15 activity.

One preferred screening assay is based upon the CTLL-2 proliferation assay described by Gillis and Smith, Nature 268:154, 1977, in which antagonist activity is detected as the inhibition of IL-15-mediated cell proliferation. In another preferred screening assay, antagonists are detected as compounds that interfere with the binding of radiolabeled IL-15 to cells or cell fractions containing the IL-15 receptor complex, or to IL-15 receptor subunits. In other preferred screening assays, antagonists are detected as compounds that interfere with IL-15 signaling processes. Because of the relationship between IL-15 and psoriasis, methods for the identification of IL-15-specific antagonists are also useful as methods for the identification of compounds useful in the treatment of psoriasis. IL-15-specific antagonists can be identified and characterized in vitro (e.g., in cultured cells that respond to IL-15), ex vivo (e.g., in organ cultures of psoriatic and healthy human skin), and in vivo (e.g., in animal models of psoriasis and IL-15 knockouts) using the methods exemplified below as well as the variations and additional methods known in the art.

C. Therapeutic Methods

The disclosed methods are used to increase keratinocyte apoptosis and to treat any disease wherein the number of keratinocytes is increased. For the purposes of this disclosure, the term "disease" is used in its broadest sense and is intended to encompass states that may be referred to as sickness, illness,

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medical condition, ailment, affliction, malady, ill health, infirmity, abnormality or medical disorder.

The methods according to the present invention can be tested in in vivo animal models for the desired prophylactic or
5 therapeutic activity, as well as to determine the optimal therapeutic dosage, prior to administration to humans.

The amount of a particular IL-15-specific antagonist that will be effective in a particular method of treatment depends upon the age and body weight of the patient, the severity of the
10 condition to be treated, the desired duration of treatment, the method of administration, and other parameters. Effective dosages are determined by a physician or other qualified medical professional. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration
15 can be calculated according to art-accepted practices. Typical effective dosages are about 0.01 mg/kg to about 100 mg/kg body weight. In some preferred embodiments the dosage is about 0.1-50 mg/kg; in some preferred embodiments the dosage is about 0.5-10 mg/kg. The dosage for local administration is typically lower
20 than for systemic administration. Some embodiments include a single administration of antagonist; in some embodiments the soluble antagonist is administered as multiple doses over one or more days. For a given patient, the antagonist may be administered for a longer or shorter duration at the advice of
25 the physician or other professional. Treatment may be continued for as long as symptoms persist, which may include long term treatment in some patients.

The antagonist is typically administered in the form of a pharmaceutical composition comprising an effective amount of
30 antagonist and one or more pharmacologically acceptable carriers. Pharmaceutically acceptable carriers include (e.g., Tris-HCl, acetate, phosphate), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, fillers, adjuvants, excipients, and vehicles which are pharmaceutically
35 acceptable for the route of administration, and may be aqueous or oleaginous suspensions formulated using suitable dispersing,

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wetting, and suspending agents. Pharmaceutically acceptable carriers are generally sterile and free of pyrogenic agents, and may include water, oils, solvents, salts, sugars and other carbohydrates, emulsifying agents, buffering agents, antimicrobial agents, and chelating agents. The particular pharmaceutically acceptable carrier and the ratio of active compound to carrier are determined by the solubility and chemical properties of the composition, the mode of administration, and standard pharmaceutical practice. The compositions used in the invention can contain an IL-15-specific antagonist complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of an IL-15-specific antagonist. An IL-15-specific antagonist can also be conjugated to antibodies against tissue-specific receptors, ligands or antigens, or coupled to ligands of tissue-specific receptors.

The antagonist is administered to the patient in a manner appropriate to the extent and severity of the symptoms. Thus, for example, the antagonist, or pharmaceutical compositions thereof, may be administered by intravenous, transdermal, intradermal, intraperitoneal, intracisternal, intramuscular, intranasal, inhalation, epidural, oral, topical, subcutaneous, intracavity, sustained release from implants, peristaltic routes, infusion routes, or by any other suitable technique.

In certain embodiments of the claimed invention, the treatment comprises treating the mammal with one or more further therapeutic agents. The further therapeutic agent(s) may be administered prior to, concurrently with, or following the administration of the antagonist.

The methods of the present invention may be used as a first line treatment of diseases wherein the number of keratinocytes

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is increased or as an adjuvant strategy in the treatment of such diseases.

5 **EXAMPLES**

The following examples are intended to illustrate particular embodiments and not to limit the scope of the invention.

EXAMPLE 1

10 **Materials and Methods**

A. Keratinocyte (KC) Cell Culture

Human epidermal cell suspensions were obtained from normal donors undergoing foreskin surgery. KC were propagated in serum free
15 medium (KGM, BioWhittaker, Heidelberg, Germany) with the following supplements: 0.1 ng/ml epidermal growth factor, 0.5 µg/ml hydrocortisone, 5 µg/ml insulin, 7.5 mg/ml bovine pituitary extract, 50 µg/ml gentamycin, 50 ng/ml amphotericin B and 0.15 mM calcium. Keratinocytes were passaged by dissociating the
20 monolayer with trypsin-EDTA (0.025% (w/v) trypsin, 0.01% (w/v) EDTA). For the experiments described below, cells were derived from the third to fifth passage grown to subconfluency as a monolayer.

The human keratinocyte cell line HaCaT (Boukamp et al., *J. Cell. Biol.* 106:761, kindly provided by N. Fusenig) was cultured
25 in DMEM with 5% (v/v) FCS and antibiotics at 37° C with 5% CO₂. Spontaneously transformed immortal HaCaT cells are a hyperproliferative KC cell line exhibiting a psoriatic keratin expression pattern. Psoriatic KC and HaCaT both express keratin
30 17 that can be upregulated by IFN-γ (Bonnekoh et al., *J. Invest. Dermatol.* 104:58, 1995).

B. KC Proliferation Assay

10⁴ cells/well were seeded in 96-well microtiter plates with
35 0, 5, 10, 20 and 50 ng/ml human recombinant IL-15 (Genzyme Corporation, Cambridge, MA, USA) in quintuplicate assays. IFN-γ

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(10 ng/ml, Genzyme Corporation, Cambridge, MA, USA), which showed the expected suppression of the baseline KC proliferation, was used as internal assay control. The rate of DNA synthesis was determined after 24 and 48 hours by incorporation of [³H]-thymidine (1 µCi/well) for the last 4 hours. Cells were harvested and radioactivity was measured by scintillation counting. To normalize the baseline proliferation (counts per minute) from different experiments the proliferation after IL-15 stimulation was converted to a percentage, by setting medium proliferation without addition of IL-15 or IFN-γ to 100%.

C. Induction of Apoptosis by Anti-Fas

Semiconfluent keratinocyte cultures were incubated for 24 hours in the presence or absence of human recombinant IFN-γ (10³ U/ml), to promote anti-Fas induced apoptosis (see, e.g., Matsue et al., Arch Dermatol. Res. 287:315, 1995). The cells were washed twice with PBS and once with culture medium, and treated with 1 µg/ml anti-Fas antibody (Clone SM1/1; Bender Med Systems, Boehringer Ingelheim, Heidelberg, FRG) for 2 hours at 37° C. After washing twice with PBS, the cells were treated with a crosslinking goat-anti-mouse-IgG antibody (SIGMA, Deisenhofen, FRG) in the presence or absence of human recombinant IL-15 (10 and 50 ng/ml). The cells were then cultured for 24, 48 or 72 hours at 37° C. As a control, cells were incubated with an irrelevant, isotype matched IgG2a antibody (anti HIV p24) or with the crosslinking antibody alone. Detached cells were recovered by collecting the supernatant and adherent cells were harvested by trypsinization. All cells were then used for viability tests and apoptosis detection.

D. Induction of Apoptosis in Methylcellulose (MC)

Induction of apoptosis in semisolid medium was performed essentially as previously described (Wrone Smith et al., Am. J. Pathol. 151:1321, 1997; Sadik et al., J. Biol. Chem. 269:16067, 1994). Briefly, sterile 1.68% methylcellulose (MG 4000, SERVA, Heidelberg, Germany) was prepared by autoclaving 3.37 g

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methylcellulose and a magnetic stir bar in a 250 ml glass bottle. 100 ml of keratinocyte basal medium (KBM-medium) was heated to 60°C, added to the methylcellulose powder, and stirred at room temperature for 20 minutes. An additional 100 ml of serum-free
5 KBM medium was added and the mixture was stirred at 4°C for 1 hour. Aliquots of the methylcellulose solution were centrifuged at 10,000 rpm for 90 minutes at 4°C. Freshly trypsinized HaCaT cells or human keratinocytes were resuspended in 1.68% methylcellulose solution at 10^6 cells/ml, with or without the
10 addition of human recombinant IL-15 (20 ng/ml), in a 50 ml conical tube. The cells were incubated at 37°C for 24, 48, or 72 hours in a humidified 5% CO₂ atmosphere. The cells were recovered from suspension by repeated dilution of the methylcellulose by KBM medium followed by centrifugation.

15

E. Detection of Apoptosis

KC were harvested at various time points and cytopins of 10^4 cells were prepared, air-dried, fixed and stained for apoptotic cell death (TUNEL, as described in Bulfone-Paus, Nat.
20 Med. 3:1124, 1997) using an apoptosis-detection kit (ApopTag, Oncor, Gaithersburg, MD) according to the manufacturer's instructions. Cell nuclei were counterstained with HOECHST 33342 dye (SIGMA). Apoptotic and non-apoptotic cells were counted in 10 microscopic fields (approximately 700 cells) and the
25 percentage of apoptosis was calculated. Apoptosis was also detected by FACS staining of propidium iodide inclusion, as described in Nicoletti et al., J. Immunol Methods 139:271, 1991.

F. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

30 RNA was extracted from cells using the RNA Clean reagent (AGS GmbH, Heidelberg, FRG) according to the manufacturer's instructions. A 5 µg aliquot of total cellular RNA was reverse transcribed using random hexanucleotides as primers and SUPERScript II preamplification kit (Gibco BRL, Karlsruhe, FRG).
35 cDNA was amplified in a 20 µl PCR reaction mixture containing 250 µM of each dNTPs, 200 nM primers, 2 µl 10-fold PCR buffer (Perkin

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Elmer/Cetus, Emeryville) and 1 U Taq DNA polymerase (Amplitaq, Perkin Elmer). The following primers were used to amplify the various targets:

- 5 human IL-15 R α sense 5' GCC AGC GCC ACC CTC CAC AGT AA 3'
(SEQ ID NO:1);
human IL-15 R α antisense 5' GCC AGC GGG GGA GTT TGC CTT GAC
3' (SEQ ID NO:2);
human IL-15 sense 5' GGC TTT GAG TAA TGA GAA TTT CGA 3'
10 (SEQ ID NO:3);
human IL-15 antisense 5' ATC AGT TGC AAT CAA GAA GTG TTG 3'
(SEQ ID NO:4);
human β -actin sense 5' GTG GGG CGC CCC AGG CAC CA 3' (SEQ
ID NO:5); and
15 human β -actin antisense 5' CTC CTT AAT GTC ACG CAC GAT TTC
3' (SEQ ID NO:6).

Samples were amplified in a DNA thermocycler for 35 cycles. Each cycle consisted of denaturation at 94° C for 1 minute,
20 annealing at 60° C for 2 minutes, and extension at 72° C for 2
minutes. Aliquots of PCR products were electrophoresed on a 1.5%
agarose gel and visualized by ethidium bromide staining. β -actin
message expression was used to normalize cDNA amounts. A mock PCR
(without cDNA) was performed for all experiments, to exclude the
25 possibility of contamination.

G. Immunohistology

Skin biopsies of psoriatic plaques and of uninvolved skin were obtained from 12 psoriasis patients. Control biopsies were
30 obtained from 10 normal, healthy donors. The biopsy samples were
immediately snap frozen in liquid nitrogen and stored at -80° C.
10 μ m cryostat sections were air dried, fixed in acetone (-20°
C, 10 minutes), and stained with mouse anti-IL-15 or biotinylated
IL-15-IgG2b fusion protein, following a standard ABC technique
35 as previously described (Paus et al., J. Invest. Dermatol. 111:7,
1998). Controls were prepared by omitting the primary Ab or by

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using biotinylated murine IgG2b (Clone 49.2; Pharmingen, Hamburg, FRG). To verify the specificity of the IL-15-IgG2b binding to the IL-15R, additional staining was performed on concanavalin A-activated human peripheral blood T cells after stimulation with IL-2 (10 ng/ml for 24 hours). IL-15 binding to these cells was blocked by preincubation of the IL-15-IgG2b fusion protein with anti-IL-15 Ab (M111, Genzyme, Alzenau, FRG).

H. Fluorescence Activated Cell Sorter (FACS) Staining

Expression of the IL-15 receptor α subunit or surface IL-15 cytokine on primary KC or HaCaT was analyzed on unstimulated cells and on cells activated with IFN- γ (10^3 U/ml) for 24 hours. The IL-15 R α was detected using mouse anti-huIL-15R α (M116, provided by Immunex Corporation, Seattle, WA, USA). IL-15 surface expression was detected with a mouse-anti huIL-15 Ab (M111, Genzyme) followed by FITC-labeled goat-anti mouse IgG (Southern Biotechnology Inc., Birmingham, USA). Isotype-matched antibodies were used as controls and the fluorescence was analyzed with a FACScan (Becton-Dickinson, Heidelberg, FRG).

I. Statistical Analysis

Results were calculated as mean values $\pm$ SD unless stated otherwise. The Mann-Whitney U test was used to determine the level of difference between different treatment groups, or between patients and healthy donors (*p < 0.05).

EXAMPLE 2

Keratinocytes Express IL-15 and IL-15 Receptor α Chain

The following experiments show that, in vitro, normal and transformed human KC transcribe the genes encoding IL-15 and its high affinity receptor chain α , and express IL-15 and its high affinity receptor chain α on the cell surface.

IL-15 and IL-15R α expression were analyzed, by RT-PCR, on primary foreskin KC from different donors and on the HaCaT cell line. mRNA transcription was analyzed in KC treated with IL-15

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(10 ng/ml), IFN- γ (10^3 U/ml), or left untreated for 24 hours. IL-15 reportedly downregulates its own high-affinity receptor chain (IL-15R α) on T cells (Kumaki et al., Eur. J. Immunol. 26:1235, 1996). IFN- γ is reportedly overexpressed in psoriatic lesions
5 (Livden et al., Arch. Dermatol. Res. 281:392, 1989), stimulates KC expression of activation markers (HLA-DR, (Kerr et al., J. Invest. Dermatol. 95:653, 1990)) and cytokine receptors (IL-1R, Groves et al., Am. J. Pathol. 145:1048, 1994), and enhances the expression of IL-15 in epithelial cells of human fetal retina
10 (Kumaki et al., Curr Eye Res. 15:876, 1996). The results are shown in Figure 1.

Unstimulated primary KC showed expression of IL-15 cytokine and receptor mRNA. Steady state-levels of the receptor transcripts were slightly downregulated by incubation with IL-15.
15 In contrast, IFN- γ upregulated mRNA levels for both IL-15 cytokine and receptor. HaCaT cells showed substantially higher baseline mRNA levels for IL-15 and IL-15R α , and neither IL-15 nor IFN- γ stimulation significantly altered the IL-15 cytokine or receptor steady state-levels.

20 Protein levels were analyzed in primary KC and in HaCaT cells. The cells were stained for surface IL-15R α and surface IL-15 cytokine expression as determined by FACS analysis. The results are shown in Figure 2.

Primary KC and HaCaT cells both expressed IL-15 cytokine as
25 well as IL-15R α protein on their cell surface. Stimulation for 24 hours with IFN- γ did not upregulate the surface expression of IL-15 cytokine or its receptor on protein level (data not shown).

To determine whether exogenous IL-15 serves as growth factor for KC, primary KC or HaCaT cells were incubated with increasing
30 concentrations of IL-15 for 24 or 48 hours, and the 3 H-thymidine incorporation was determined. The addition of IL-15 had no significant effect on proliferation of primary KC and HaCaT cells, suggesting that IL-15 stimulation alone is insufficient to induce KC proliferation in vitro.

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EXAMPLE 3**IL-15 Inhibits Keratinocyte Apoptosis**

The following experiments show that, in vitro, IL-15
5 inhibits methylcellulose and anti-Fas induced keratinocyte
apoptosis. Apoptotic KC cell death was detected using the TUNEL
method, propidium iodide staining, and light microscopy.

Primary KC and HaCaT cells were incubated in semisolid MC
medium, which induces apoptosis by inhibiting cell-cell contact
10 (Wrone-Smith, 1977). Apoptosis was detected using the TUNEL
method. After 24 hours, approximately 10% of the KC had undergone
apoptosis; after 48 hours approximately 30% of the KC had
undergone apoptosis; after 72 hours in culture, more than 60% of
the KC had undergone apoptosis. Inhibition of apoptosis by IL-15
15 was calculated as a percentage of inhibition (where MC or anti-
Fas apoptosis *without* IL-15 was set equal to 0% inhibition), so
that IL-15 effects on MC or anti-Fas induced apoptosis could be
compared. The results are shown in Figure 3A.

The addition of IL-15 (20 ng/ml) to the MC medium
20 significantly inhibited apoptosis, with the most prominent
effects seen after 24 hours (75% inhibition in primary KC and 50%
in HaCaT, compared to MC alone = 0%). After 72 hours 35%
inhibition was seen in primary KC, whereas no significant
inhibition was seen in HaCaT cells.

25 In order to address whether the apoptosis-inhibitory effects
of IL-15 reflect general down-modulatory properties of IL-15 on
human KC apoptosis, a monoclonal antibody to Fas which is
expressed by KC, was used to induce apoptosis. Fas cross-linking
induces apoptosis by oligomerization of the membrane-bound
30 receptor. KC were activated for 24 hours by IFN- γ , to maximize
Fas mediated apoptosis (Matsue, 1995). Apoptosis was detected
using the TUNEL method. The KC were then incubated with an anti-
Fas antibody, which thereafter was cross-linked by a second
antibody. This technique induced apoptosis in 10% of the KC after
35 24 hours, and in 60% of the KC after 72 hours of culture.
Simultaneous addition of IL-15 in two different concentrations

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to the cell culture significantly suppressed Fas-induced apoptosis, as shown in Figure 3B.

After 48 hours IL-15 (10 ng/ml) suppressed apoptosis by 75% as compared to controls (anti-Fas alone). The addition of a 5-fold higher dose did not further increase the anti-apoptotic effect. In HaCaT cells, apoptosis was maximally inhibited after 72 hours (60%) by 10 ng/ml IL-15. In HaCaT cells, higher concentrations of IL-15 (50 ng/ml) did exhibit stronger anti-apoptotic effects. After 24 hours, 60% apoptosis inhibition by IL-15 was seen in HaCaT cells. Inhibition was not significantly changed at 48 hours or 72 hours.

IL-15 inhibition of apoptosis was also shown using flowcytometric propidium iodide staining to detect apoptotic cell death.

In addition to TUNEL and PI staining, morphological signs of anti-Fas induced apoptosis were analyzed by light microscopy. In primary KC grown in a confluent monolayer, addition of the anti-Fas and the crosslinking antibodies induced marked morphological alterations consistent with the morphological features of apoptosis, including cell condensation and shrinkage, appearance of apoptotic bodies, and loss of adhesion. Incubation with an isotype-matched, irrelevant control antibody did not induce these alterations. Coincubation with anti-Fas and IL-15 (50 ng/ml) markedly inhibited apoptosis-associated cell detachment and shrinkage in both primary KC cells and HaCaT cells.

EXAMPLE 4

IL-15 and IL-15 Receptor Overexpression in Psoriasis

The following experiments show that IL-15 cytokine and IL-15 binding sites are overexpressed in psoriatic plaques, and that cutaneous IL-15 and IL-15 R transcription levels are higher in psoriatic plaques as compared to normal skin.

To analyze the distribution of IL-15 cytokine and IL-15 binding sites, immunohistological staining was performed on skin

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sections from normal, healthy donors as well as on sections derived from involved and uninvolved skin of patients with psoriasis vulgaris. The results are shown in Figure 4.

5 Staining of psoriatic plaques for IL-15 with a monoclonal antibody revealed high expression levels throughout the entire epidermis (from Stratum basillare, which contains epidermal stem cells, to Stratum granulosum, that is characterized by KC, which undergo cell death and cornification) as well as in the dermis below the epidermal basal membrane (Stratum papillare). In normal
10 skin from healthy donors only subepidermal expression of IL-15 was seen by immunohistology; no cytokine could be detected in the epidermis. Skin biopsies from hypertrophic scars, which overexpress IL-15, were used as positive control. Omission of the anti-IL-15 antibody was used as a negative control, to show the
15 specificity of the staining.

A fusion protein (FP) consisting of functional human IL-15 and murine IgG2b (Bulfone-Paus et al., *Nat. Med.* 3:1124, 1997) was used for the detection of binding sites. The murine antibody portion is easily detected in human tissues. Human peripheral
20 blood mononuclear cells, which express high levels of IL-15R (Bulfone-Paus et al., *Cytokine* 9:507, 1997), were used as a positive control. In vitro preincubation of the IL-15-IgG2b FP with an anti-IL-15 Ab served as negative control: no staining with the biotinylated FP was observed under these conditions. As
25 an additional control, it was shown that human cells did not bind the murine IgG2b tail of the FP, as determined by staining with biotinylated IgG2b.

Uninvolved skin from psoriatic patients and normal skin from healthy donors was found to constitutively express binding sites
30 for IL-15 on epidermal KC at very low levels. In contrast, staining of skin sections from psoriatic plaques revealed a massive overexpression of IL-15 binding sites on epidermal KC.

RT-PCR was employed to determine whether the overexpression of IL-15 cytokine and binding sites correlates with IL-15 and IL-
35 15R α gene transcription *in situ*. Steady-state levels for IL-15 α and IL-15 cytokine were analyzed in skin biopsies from 10

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psoriatic plaques and in 6 skin biopsies from healthy donors. The results are shown in Figure 5.

Transcription levels for the receptor were substantially higher in psoriatic plaques as compared to normal skin. And despite the heterogeneity in IL-15 cytokine expression between biopsies from psoriatic patients and healthy controls, the RT-PCR revealed a slightly higher expression in psoriatic plaques. These RT-PCR results are consistent with the histological findings, where psoriatic plaques also demonstrated massive overexpression of IL-15 cytokine and IL-15 binding sites. Overexpression of the IL-15 receptor and an altered distribution pattern of the IL-15 cytokine appear to contribute to the anti-apoptotic effects of IL-15-related signaling events in psoriasis.

15 **EXAMPLE 5**

Activity of IL-15-Antagonists in Cultured Cells

The efficacy of IL-15-specific antagonists for the treatment of psoriasis is demonstrated in vitro using cultured cells.

IL-15-specific antagonists are identified and characterized using a CTLL-2 cell ³H-thymidine incorporation assay, such as that described by Gillis and Smith, Nature 268:154, 1977. Serial dilutions of the antagonist or compound to be tested are made in the wells of 96-well flat bottom tissue culture plates in culture medium at a final volume of 50 µl. A sub-optimal amount of IL-15 (final concentration approx. 20-40 pg/ml) is added to the assay wells (5 µl/well). Washed CTLL-2 cells are added to the wells (approx. 2000 cells per well in 50 µl), and the plates are incubated for 24 hours at 37°C in a humidified atmosphere of 10% CO₂ in air. ³H-thymidine (0.5 µCi, 25 Ci/mMol) is added to the wells and the cultures are incubated a further five hours. The cultures are then harvested on glass fiber filters and ³H-thymidine incorporation is measured. The counts per minute (cpm) generated in the assay are converted to percent inhibition and the percent inhibition values of each titrated sample are used to calculate antagonist activity in units/ml.

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IL-15-specific antagonists are also identified and characterized using HaCaT keratinocytes, which exhibit a psoriatic phenotype and serve as a model for hyperproliferative growth (Ockenfels et al., Arch. Dermatol. Res. 287:304, 1995; Schoop et al., J. Invest. Dermatol. 112:343, 1999). The antagonist or compound to be tested is added to cultured HaCaT cells which have been stimulated with IL-15 and the effect on cell growth and survival is determined using methods such as those described in Examples 1-3.

10

EXAMPLE 6**Activity of IL-15-Specific Antagonists in a Skin Organ Culture Model**

15 The efficacy of IL-15-specific antagonists for the treatment of psoriasis is demonstrated ex vivo using a skin organ culture model. The maintenance of psoriatic lesional skin in organ culture is a valuable experimental approach for investigation the pathophysiology of psoriasis.

20 Full thickness punch biopsies (2-6 mm) are obtained under local anesthesia from lesional and nonlesional skin from psoriasis patients and from normal skin of healthy controls. The tissue pieces are placed into wells containing keratinocyte basal medium (KBM) and incubated at 37°C, 5% CO₂, with fresh medium provided approximately every other day. IL-15-specific antagonists or test compounds are added to the medium.

The phenotype of normal and psoriatic skin cultures, in the presence or absence of added antagonists or test compounds, is monitored in several ways, including: (1) measuring the release of cytokines, growth and/or differentiation promoting factors, proinflammatory and other mediators into the medium; (2) measuring the expression levels of adhesion molecules, growth factors, and proto-oncogenes; and (3) by histologic analysis of stained tissue sections.

35 The release of cytokines, growth and/or differentiation promoting factors, proinflammatory and other mediators into the

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medium is determined by ELISA (see, e.g., Bonifati et al., Arch. Dermatol. Res. 290:9, 1998; Ameglio et al., Arch. Dermatol. Res. 289:399, 1997), or other methods known in the art.

5 The expression levels of adhesion molecules, growth factors, and proto-oncogenes are determined by immunohistochemistry, RNA blot hybridization, RT-PCR, or other methods known in the art (see, e.g., Petzelbauer et al., J. Invest. Dermatol. 103:300, 1994; Elder et al., J. Invest. Dermatol. 95:7S, 1990).

10 For the histologic analysis, tissue pieces are fixed in 10% buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and evaluated by light microscopy. After eight days, normal skin and non-lesional psoriatic skin cultured in the absence of growth factors maintain a histological appearance very similar to that of normal skin at the time of
15 biopsy. Psoriatic skin, in contrast, maintains a number of distinct histological features, including a loss of basal cell polarization and expansion of rete ridges (see, e.g., Varani et al., Pathobiology 66:253, 1998).

20 EXAMPLE 7

Activity of IL-15-Specific Antagonists in Animal Models

The efficacy of IL-15-specific antagonists for the treatment of psoriasis is demonstrated in vivo by administering the
25 antagonist to an animal model of psoriasis. At least three animal models, which show human psoriasis-like skin disorders, are known in the art: human normal, pre-psoriatic, and psoriatic skin grafts onto SCID mice (Nickoloff et al., Am. J. Pathol. 146:580, 1995), T-cell-induced (Schön et al., Nat. Medicine 3:183, 1997),
30 and CD18 receptor knockout mice (Bullard et al., Proc. Natl. Acad. Sci USA 93:2116, 1996). The phenotype of the animals is monitored in the presence and absence of added antagonists or test compounds.

35 Animal models are used to identify agents that inhibit the activity of IL-15, to identify agents useful for increasing keratinocyte apoptosis, to identify agents useful in the

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treatment of psoriasis, and to optimize treatment regimens (including formulation, dose, route of administration, and schedule of administration) for use in humans.

IL-15 knockout mice are also used to study the effects of IL-15 on the growth, differentiation, and survival of keratinocytes in vivo. These studies include immunohistological analysis of skin sections to detect abnormal expression of cell surface markers, in vivo incorporation of BrUdr to detect growth inhibition, and detection of apoptosis levels.

The relevant disclosures of publications cited herein are specifically incorporated by reference. The examples presented above are not intended to be exhaustive or to limit the scope of the invention. The skilled artisan will understand that variations and modifications and variations are possible in light of the above teachings, and such modifications and variations are intended to be within the scope of the invention.

CLAIMS

1. Use of an effective amount of an IL-15-specific antagonist for preparing a pharmaceutical composition for increasing apoptosis of keratinocytes in a mammal suffering from a disease wherein the number of keratinocytes is increased.
2. Use of claim 1, wherein the disease is psoriasis, atopic dermatitis, chronic hand eczema, contact dermatitis, viral warts, cutaneous T cell lymphoma or impaired wound healing, such as impaired wound healing due to diabetes.
3. Use of claim 1 or 2, wherein the antagonist is selected from the group consisting of IL-15 antibodies, IL-15 muteins, IL-15 conjugates, IL-15 antisense nucleic acids, IL-15 ribozymes, and small molecules targeting IL-15.
4. Use of claim 1 or 2, wherein the antagonist is selected from the group consisting of IL-15 receptor, IL-15 receptor subunits or fragments, IL-15 receptor antibodies, IL-15 receptor antisense nucleic acids, IL-15 receptor ribozymes, and small molecules targeting the IL-15 receptor.
5. Use of claim 4, wherein the IL-15 receptor subunit is a soluble form of the α chain of the IL-15 receptor.
6. Use of one of claims 1 to 5, wherein the antagonist is administered systemically.
7. Use of one of claims 1 to 5, wherein the antagonist is administered locally.
8. Use of one of claims 1 to 7, wherein the antagonist is administered as a composition comprising a pharmaceutically acceptable carrier.

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9. Use of one of claims 1 to 8, wherein the pharmaceutical composition comprises one or more further therapeutic agents.
10. Use of one of claims 1 to 9, wherein the mammal is a human.
11. A method of treating a disease in a mammal wherein the number of keratinocytes is increased comprising administering an effective amount of an IL-15-specific antagonist.
12. The method of claim 11, wherein the disease is psoriasis, atopic dermatitis, chronic hand eczema, contact dermatitis, viral warts, cutaneous T cell lymphoma or impaired wound healing, such as impaired wound healing due to diabetes.
13. The method of claim 11 or 12 wherein the antagonist is selected from the group consisting of IL-15 antibodies, IL-15 muteins, IL-15 conjugates, IL-15 antisense nucleic acids, IL-15 ribozymes, and small molecules targeting IL-15.
14. The method of claim 11 or 12 wherein the antagonist is selected from the group consisting of IL-15 receptor, IL-15 receptor subunits or fragments, IL-15 receptor antibodies, IL-15 receptor antisense nucleic acids, IL-15 receptor ribozymes, and small molecules targeting the IL-15 receptor.
15. The method of claim 14, wherein the IL-15 receptor subunit is a soluble form of the α chain of the IL-15 receptor or a multimer thereof.
16. The method of one of claims 11 to 15, wherein the antagonist is administered systemically.

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17. The method of one of claims 11 to 15, wherein the antagonist is administered locally.
18. The method of one of claims 11 to 17, wherein the antagonist is administered as a composition comprising a pharmaceutically acceptable carrier.
19. The method of one of claims 11 to 18, wherein the pharmaceutical composition comprises one or more further therapeutic agents.
20. The method of one of claims 11 to 19, wherein the mammal is a human.
21. A method of identifying a compound useful in the treatment of diseases, wherein the number of keratinocytes is increased, comprising
 - (a) including a test compound in an assay system which gives a detectable response in the presence of IL-15 activity,
 - (b) determining whether the test compound inhibits IL-15 activity, and
 - (c) repeating steps (a) and (b) until a compound that inhibits IL-15 activity is identified, wherein the compound that inhibits IL-15 activity is identified as a compound useful in the treatment of a disease, wherein the number of keratinocytes is increased.
22. The method of claim 21, wherein the assay system measures the binding of IL-15 to the IL-15 receptor or to at least one subunit thereof.
23. The method of claim 21 or 22, wherein the detectable response is IL-15-mediated proliferation.

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24. The method of claim 21 or 22, wherein the detectable response is IL-15 signaling activity.
25. The method of one of claims 21 to 25, wherein the disease is psoriasis, atopic dermatitis, chronic hand eczema, contact dermatitis, viral warts, cutaneous T cell lymphoma or impaired wound healing, such as impaired wound healing due to diabetes.
26. The method of one of claims 21 to 25, further comprising the step of administering the compound identified in step c to a non-human animal model of psoriasis and determining whether the compound alleviates one or more symptoms of psoriasis, wherein the compound that alleviates one or more symptoms of psoriasis is identified as a compound useful in the treatment of psoriasis.

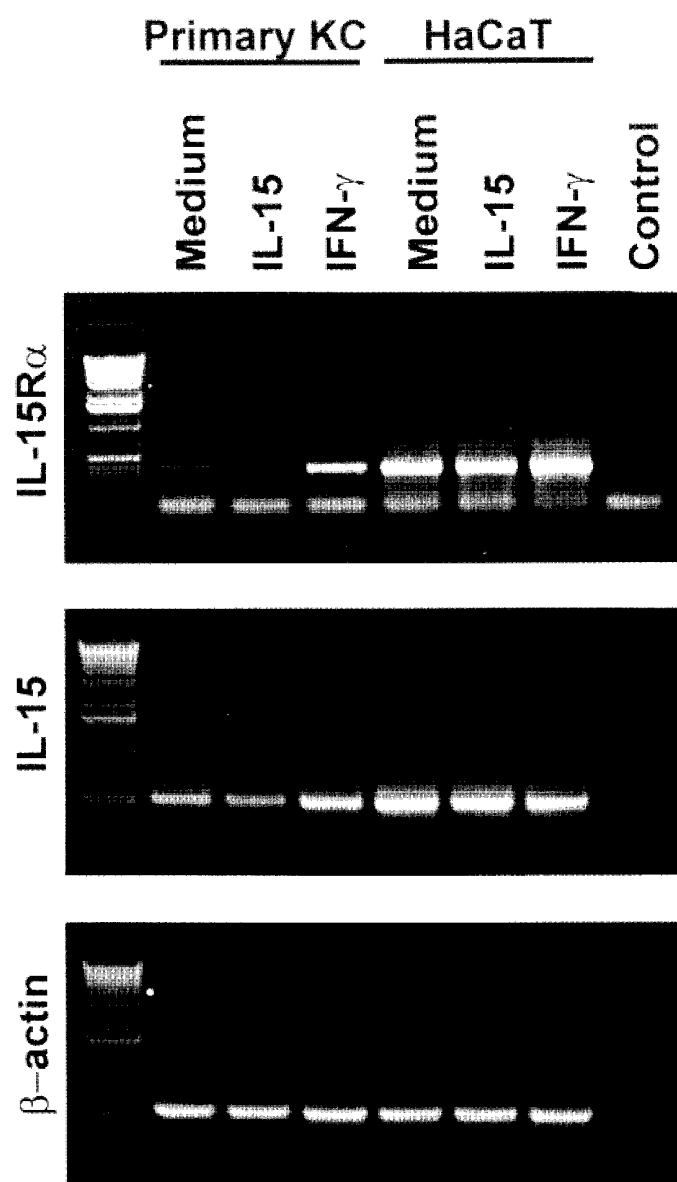


Figure 1

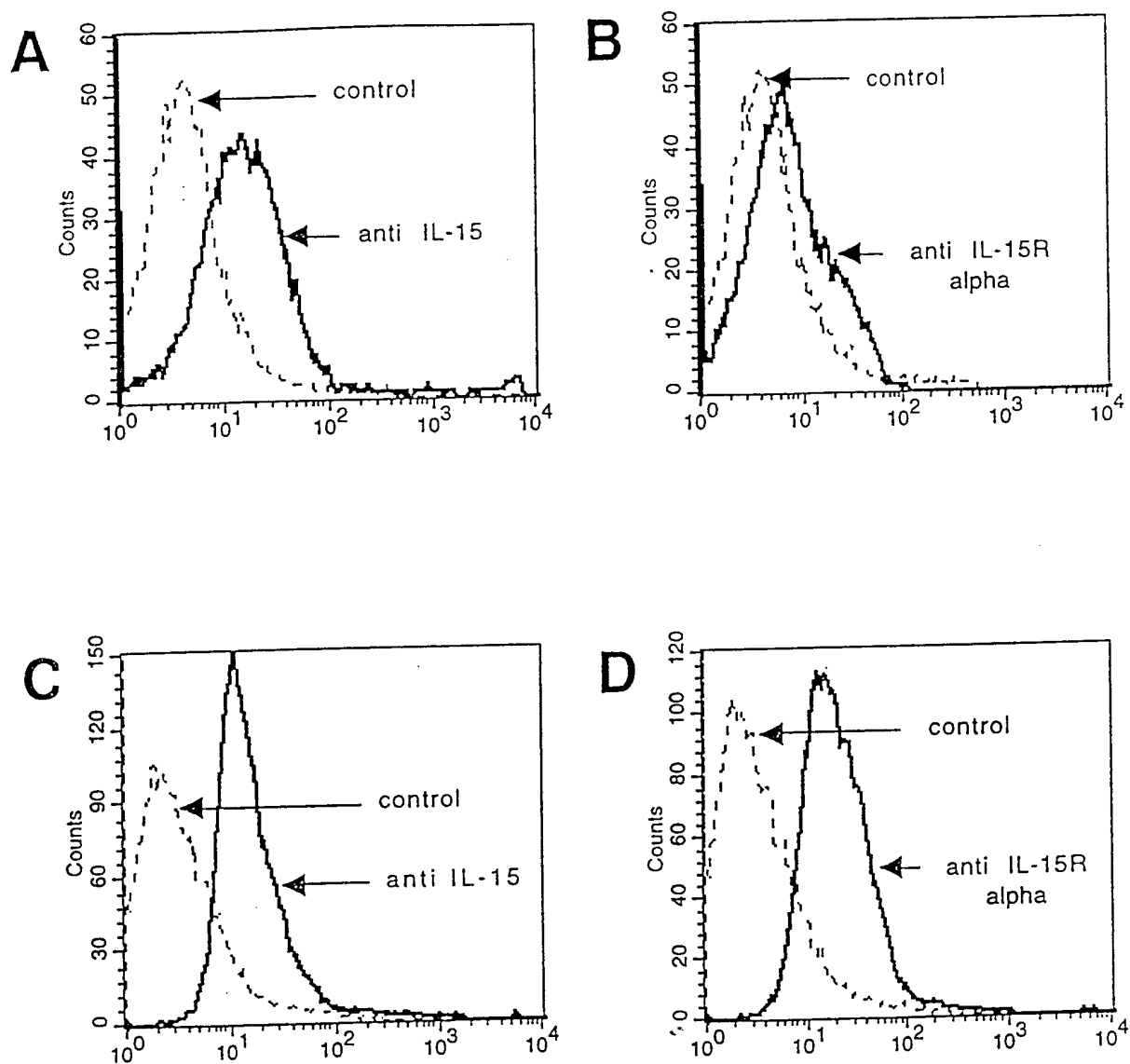


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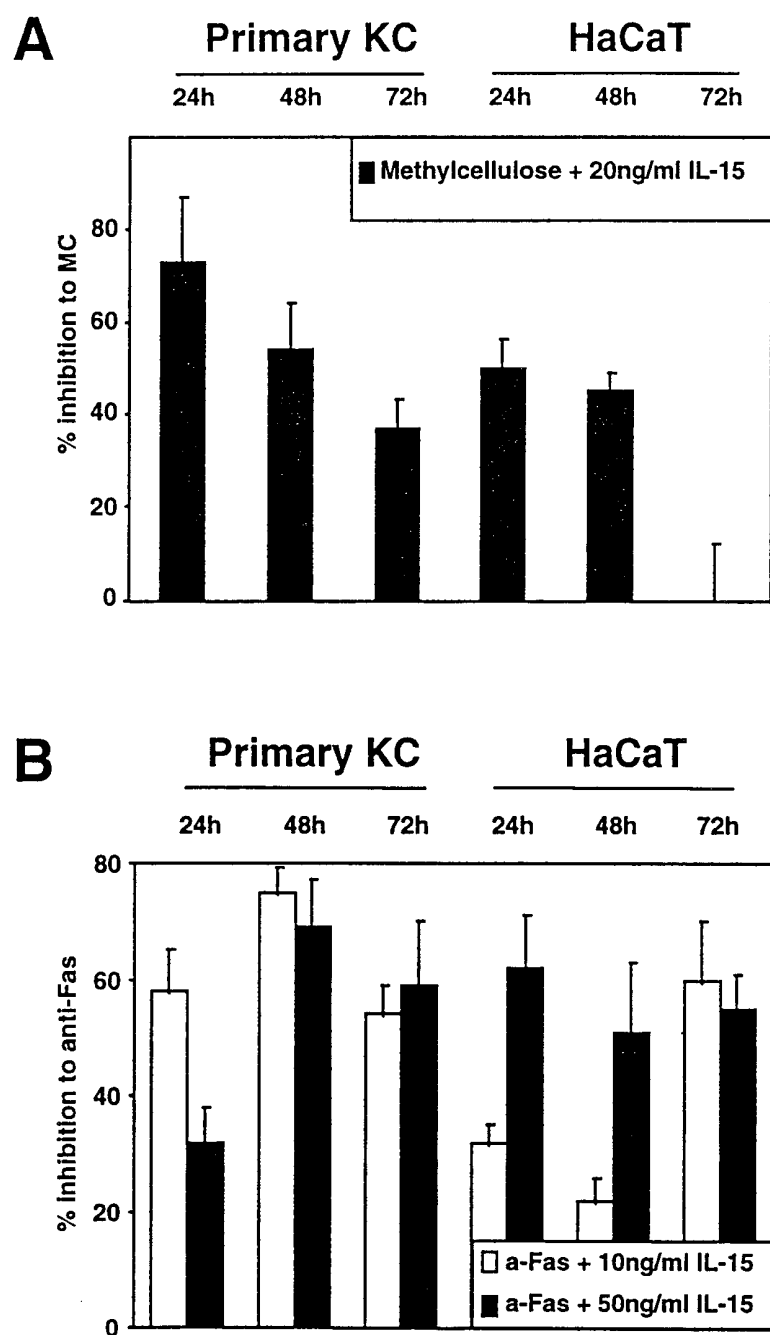


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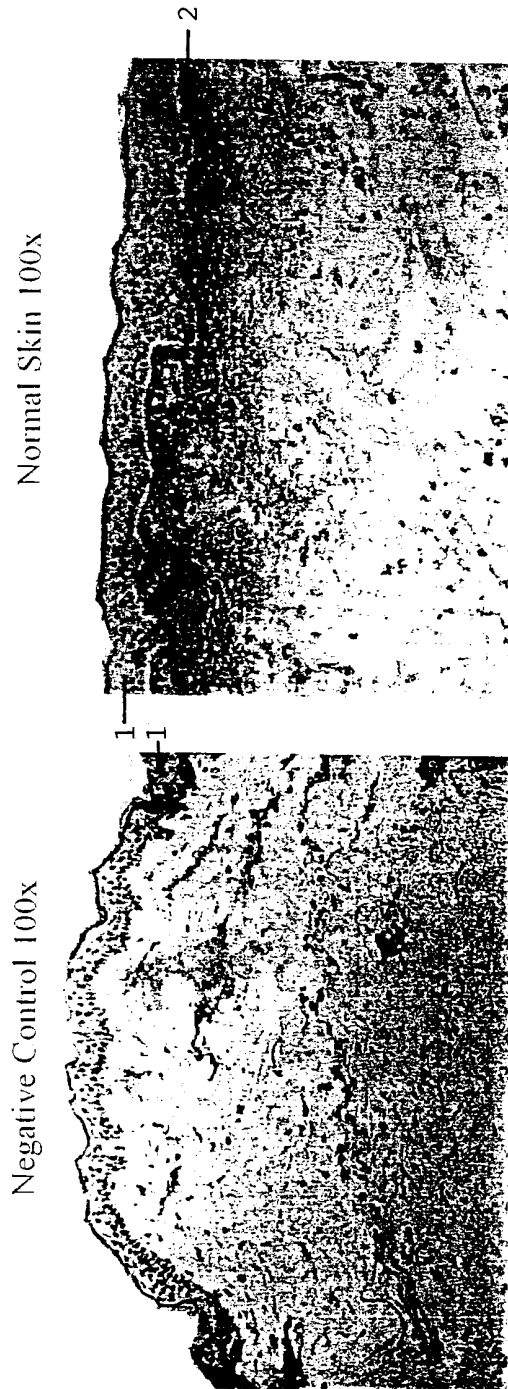


Figure 4A

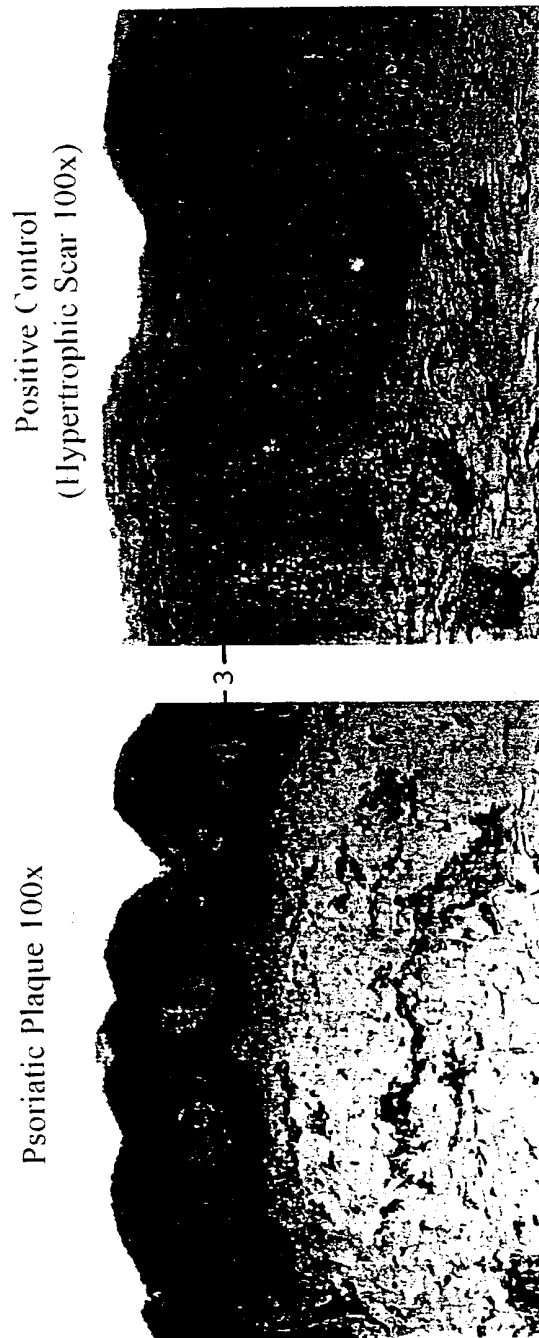


Figure 4 B

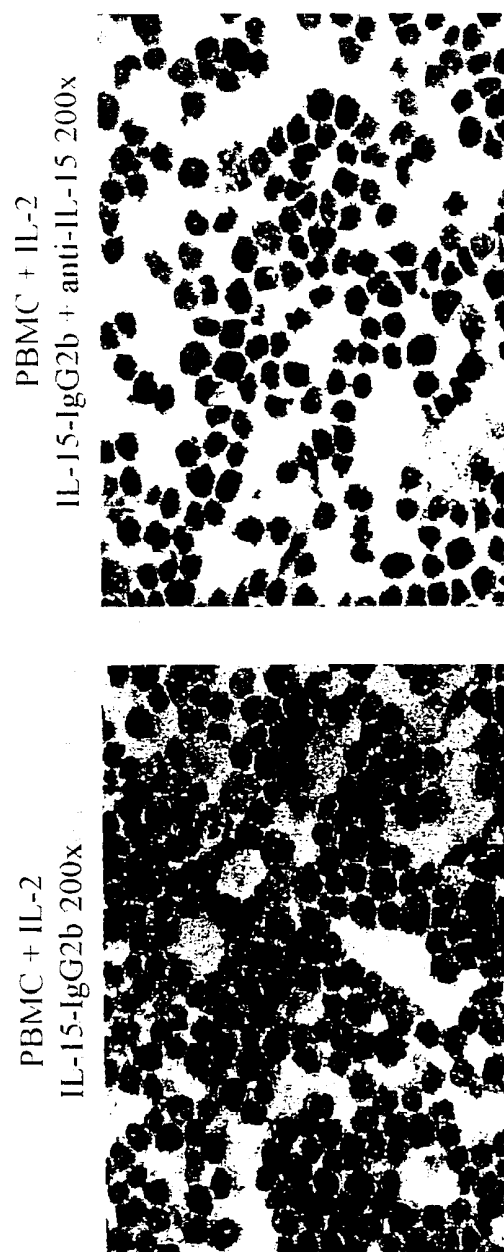


Figure 4 C

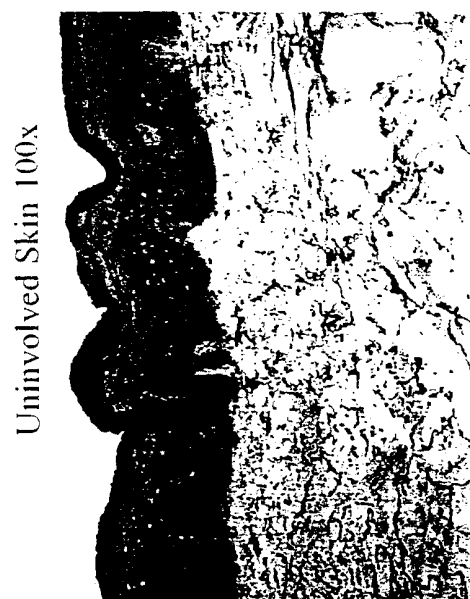


Figure 4D



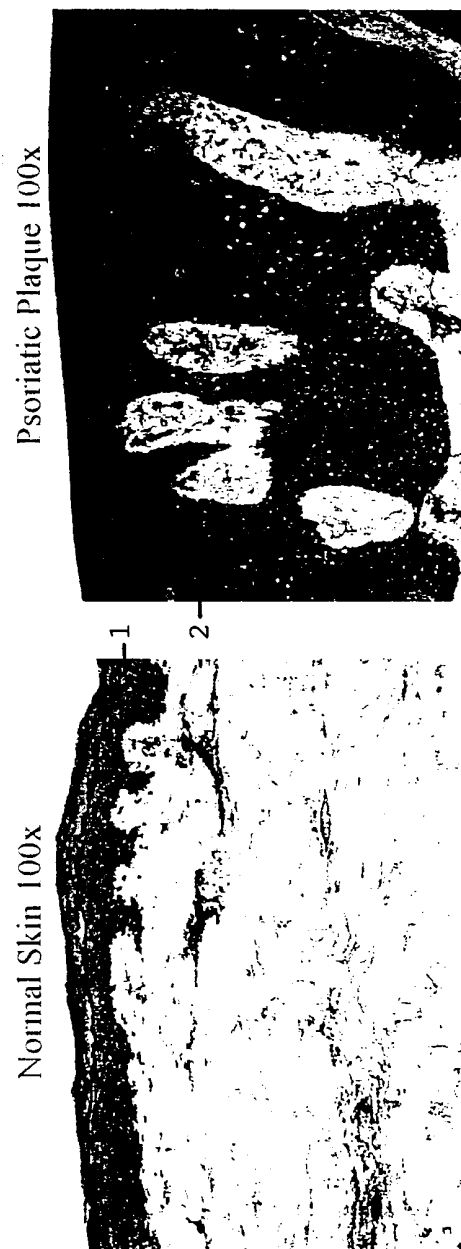


Figure 4E

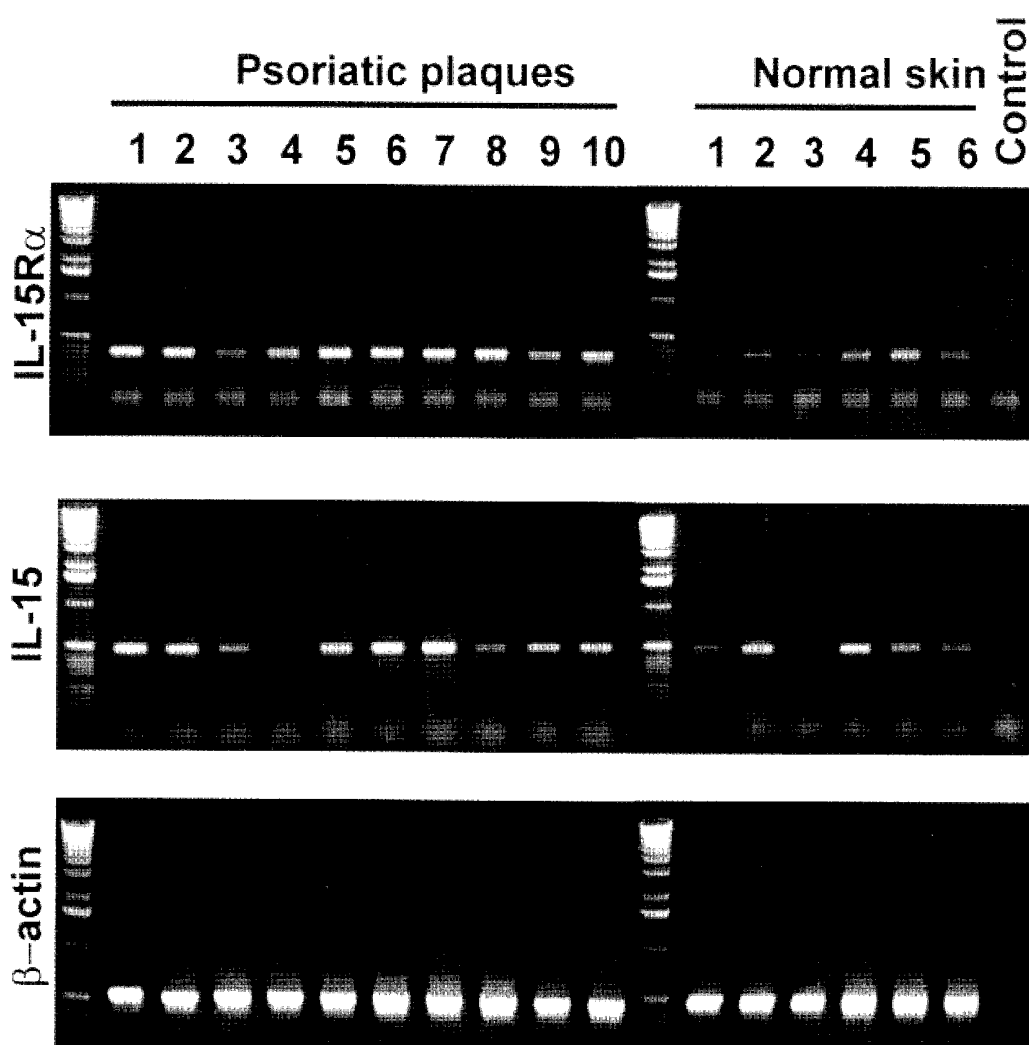


Figure 5

SEQUENCE LISTING

<110> Bulfone-Paus, Silvia
Ruckert, Rene

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/06419

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/20 A61K38/17 A61K31/70 A61K39/395 G01N33/566
C12Q1/02 A61P17/00 A61P17/06 //C07K16/24, C07K16/28,
C07K14/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, LIFESCIENCES, CANCERLIT, EMBASE, CHEM ABS Data, SCISEARCH, BIOSIS, WPI
Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LINDNER GERD ET AL: "Inhibition of chemotherapy-induced keratinocyte apoptosis in vivo by an interleukin-15-IgG fusion protein." JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 110, no. 4, April 1998 (1998-04), pages 457-458, XP000960618 ISSN: 0022-202X the whole document --- -/--	1-26

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

14 November 2000

Date of mailing of the international search report

30/11/2000

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Covone, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/06419

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WRONE-SMITH TAMARA ET AL: "Keratinocytes derived from psoriatic plaques are resistant to apoptosis compared with normal skin." AMERICAN JOURNAL OF PATHOLOGY, vol. 151, no. 5, November 1997 (1997-11), pages 1321-1329, XP000960683 ISSN: 0002-9440 abstract page 1328, right-hand column, line 2 ---	1-26
A	WO 96 26274 A (IMMUNEX CORP) 29 August 1996 (1996-08-29) cited in the application page 2, line 30 -page 3, line 22 ---	1-26
A	WO 97 41232 A (BETH ISRAEL HOSPITAL) 6 November 1997 (1997-11-06) page 8, line 23 -page 9, line 3 claims 30,37 ---	1-26
A	DE 198 23 351 A (BULFONE PAUS SILVIA DR DR ;KUNZENDORF ULRICH PROF DR (DE); KRAUSE) 10 December 1998 (1998-12-10) the whole document ---	1-16
T	RUCKERT R ET AL: "Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis?." JOURNAL OF IMMUNOLOGY, (2000 AUG 15) 165 (4) 2240-50. , XP002152746 the whole document -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/06419

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9741232 A	06-11-1997	CA 2252557 A EP 0927254 A US 6001973 A	06-11-1997 07-07-1999 14-12-1999
DE 19823351 A	10-12-1998	NONE	