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(54) Title: NOVEL BETA INTEGRIN SUBUNIT (57) Abstract The cDNA encoding a novel beta integrin subunit, called the beta-8 integrin subunit, has been isolated from rabbit and human tissue and sequenced. Provided herein is nucleic acid sequence of the beta-8 integrin subunit useful as a diagnostic and in the recombinant preparation of beta-8 integrin subunit and alpha-beta integrin complexes. The beta-8 integrin subunit is used in the preparation and purification of antibodies thereto, and in diagnostic assays.		

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NOVEL BETA INTEGRIN SUBUNIT
BACKGROUND OF THE INVENTION

Field of the Invention

5 This invention relates to the isolation of a novel beta integrin subunit polypeptide and to the DNA encoding this polypeptide. The invention further relates to methods for preparing variants of this beta integrin subunit.

Description of Background and Related Art

10 The integrins are a family of plasma-membrane bound cell surface proteins. Many integrins exist; some are cell-type specific while others are present in several types of cells. At least one integrin has been found in nearly every animal cell type that has been evaluated (Hemler, Ann. Rev. Immunol., 8:365 [1990]; Albelda et al., FASEB J., 4:2868 [1990]) suggesting they may be ubiquitous in the animal kingdom.

Each integrin consists of an alpha polypeptide subunit and a beta polypeptide subunit.
15 The subunits are non-covalently bound in such a way as to confer ligand-binding activity on the complex as a whole. Both types of subunits have three domains: 1) a cytoplasmic domain, which is the carboxyl terminus of the polypeptide, 2) a membrane-spanning or trans-membrane domain, which is located near the carboxyl terminus and serves to anchor the polypeptide into the plasma membrane, and 3) an extracellular domain which binds ligand.
20 Several alpha subunits are known, (see Ruoslahti, J. Clin. Invest., 87:1 [1991]), and three human beta subunits have been known for some time. Recently, three additional human beta subunits have been identified, cloned and sequenced, and named beta-4 (Suzuki et al., EMBO J., 9:757 [1990]; Hogervorst et al., EMBO J., 9:765 [1990]), beta-5 (McLean et al., J. Biol. Chem., 265:17126 [1990]; (Ramaswamy et al., EMBO J., 9:1561 [1990]) and
25 beta-6 (Sheppard et al., 265:11502 [1990]). A seventh beta subunit, beta-7, has just been reported (Yuan et al., Internat'l. Immunol., 2:1097 [1990]). Alpha and beta subunits can associate with each other in a variety of combinations thus creating a large number of distinct integrins (see Albelda et al., *supra* and Helmer, *supra*).

30 The human beta integrin subunits that have been identified to date share an amino acid sequence identity of around 32-56 percent and show conservation of about 50 cysteine residues (Yuan et al., *supra*). The beta-1 integrin subunit has an 82-90 percent homology between human, chicken, frog, and mouse (Hemler, *supra*), suggesting a highly conserved functional role for this subunit.

35 The integrins are known to mediate cell adhesion. One of the ways this mediation can occur is via the integrin binding to the extracellular matrix. Certain integrins have been shown to bind to such extracellular matrix proteins as fibronectin, fibrin, laminin, some collagens, tenascin, vitronectin, and von Willebrand factor (Ruoslahti, *supra*). A second way in which integrins can mediate cell adhesion is through cell-cell binding. For example, one

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integrin glycoprotein, IIb/IIIa, located on the surface of platelets, promotes the binding of platelets to each other via mediator molecules, especially fibrinogen. Other integrins have been shown to have a role in mediating the attachment of circulating leukocytes to tissue, thus removing them from circulation, which is important in tissue repair (Ruoslahti, *supra*).

5 It has been observed that the extracellular matrix surrounding many tumorigenic cells is less extensive than that observed for normal, non-tumorigenic cells, leading to enhanced mobility of tumor cells as compared to normal cells (Ruoslahti, *supra*). It has been shown that the integrin alpha-5/beta-1, as well as certain other integrins, have an altered level of expression in certain tumor cells (Plantefaber *et al.*, Cell, 56:281 [1989]).

10 Recent research has suggested that some integrins may have a role in mediating the movement of neurons along glial fibers during development and differentiation of the cerebellum (Hatten, Trends. Neurol. Sci., 13:179 [1990]; Sanes, Ann. Rev. Neurosci., 12:491 [1989]).

15 There is a current and continuing need in the art to identify new integrin subunits and to identify their biological role(s), including their roles in mediating cell-cell adhesion, cell mobility, and cell adhesion to the extracellular matrix.

Accordingly, it is an object of this invention to identify a novel beta subunit integrin polypeptide that shares some common structural features with beta integrin subunits 1-7.

20 It is another object to provide nucleic acid encoding the novel beta integrin subunit polypeptide and to use this nucleic acid to produce the polypeptide in recombinant cell culture for research or diagnostic use, or for potential therapeutic use in certain neurological or immunological disorders and with certain tumors and tumorigenic cells.

It is a further object to provide derivatives and modified forms of the novel beta integrin subunit, including amino acid sequence variants and covalent derivatives thereof.

25 It is an additional object to prepare immunogens for raising antibodies against the novel beta integrin subunit, as well as to obtain antibodies capable of binding to the beta integrin subunit. It is a further object to prepare immunogens for raising antibodies against a complex comprising a novel beta integrin subunit which is associated with an alpha integrin subunit. It is preferable that the antibodies raised against these novel immunogens will not bind to
30 beta integrin subunits 1-7.

These and other objects of the invention will be apparent to the ordinary artisan upon consideration of the specification as a whole.

SUMMARY OF THE INVENTION

35 These objects are accomplished, in one aspect, by providing an isolated novel beta integrin subunit polypeptide that is related structurally to beta integrin subunits 1-7. This polypeptide is hereafter termed beta-8 integrin subunit, and includes N-terminal and C-terminal fragments thereof.

In another aspect, the invention provides a composition comprising the beta-8 integrin subunit that is free of contaminating polypeptides of the animal species from which the beta-8 integrin subunit is derived. In other aspects, the beta-8 integrin subunit provided is associated with an alpha integrin subunit.

5 The beta-8 integrin subunit or fragments thereof (which also may be synthesized by chemical methods) are fused (by recombinant expression or *in vitro* covalent methods) to an immunogenic polypeptide and this fusion polypeptide, in turn, is used to immunize an animal to raise antibodies against a beta-8 integrin subunit epitope. Anti-beta-8 integrin subunit antibodies are recovered from the serum of immunized animals. Alternatively, monoclonal
10 antibodies are prepared from cells of the immunized animal in conventional fashion. Antibodies identified by routine screening will bind to beta-8 integrin subunit but will not substantially cross-react with any other known beta integrin subunit, including beta integrin subunits 1-7.

15 Immobilized anti-beta-8 integrin subunit antibodies are useful particularly in the diagnosis (*in vitro* or *in vivo*) or purification of the beta-8 integrin subunit, e.g. a mixture of beta-8 integrin subunit is passed over a column to which the antibodies are bound.

Substitutional, deletional, or insertional variants of the beta-8 integrin subunit are prepared by *in vitro* or recombinant methods and screened for immuno-crossreactivity with the beta-8 integrin subunit and for beta-8 integrin subunit antagonist or agonist activity.

20 The beta-8 integrin subunit also is derivatized *in vitro* to prepare immobilized beta-8 integrin subunit and labeled beta-8 integrin subunit, particularly for purposes of diagnosis of beta-8 integrin subunit or its antibodies, or for affinity purification of beta-8 integrin subunit antibodies.

25 The beta-8 integrin subunit, its derivatives, or its antibodies are formulated into physiologically acceptable vehicles, especially for therapeutic use. Such vehicles include sustained-release formulations of the beta-8 integrin subunit.

30 In still other aspects, the invention provides an isolated nucleic acid molecule encoding the beta-8 integrin subunit, labeled or unlabeled, and a nucleic acid sequence that is complementary, or hybridizes under stringent conditions to, a nucleic acid sequence encoding the beta-8 integrin subunit, excluding nucleic acid sequences complementary to nucleic acid sequences encoding beta integrin subunits 1-7, i.e., those known beta integrin subunits that are not the beta-8 integrin subunit.

35 In addition, the invention provides a replicable vector comprising the nucleic acid molecule encoding the beta-8 integrin subunit operable linked to control sequences recognized by a host transformed by the vector; host cells transformed with the vector; and a method of using a nucleic acid molecule encoding the beta-8 integrin subunit to effect the production

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The nucleic acid sequence is also useful in hybridization assays for beta-8 integrin subunit nucleic acid.

In further embodiments, the invention provides a method for producing beta-8 integrin subunit comprising inserting into the DNA of a cell containing the nucleic acid encoding the beta-8 integrin subunit a transcription modulatory element in sufficient proximity and orientation to the beta-8 integrin subunit nucleic acid to influence transcription thereof, with an optional further step comprising culturing the cell containing the transcription modulatory element and the beta-8 integrin subunit nucleic acid.

In still further embodiments, the invention provides a cell comprising the nucleic acid encoding the beta-8 integrin subunit and an exogenous transcription modulatory element in sufficient proximity and orientation to the beta-8 integrin subunit nucleic acid to influence transcription thereof; and a host cell containing the nucleic acid encoding the beta-8 integrin subunit operably linked to exogenous control sequences recognized by the host cell.

Still further is provided a method for obtaining cells having increased or decreased transcription of the nucleic acid encoding the beta-8 integrin subunit comprising:

- (a) providing cells containing the beta-8 integrin subunit nucleic acid;
- (b) introducing into the cells a transcription modulating element; and
- (c) screening the cells for a cell in which the transcription of the beta-8 integrin subunit nucleic acid is increased or decreased.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-E (SEQ. ID. NO. 3) depicts the complete nucleotide sequence of the rabbit beta-8 integrin subunit. The sequence contains an untranslated 5' region and a portion of the 3' untranslated region. The presumed ATG translation start codon is indicated. The predicted amino acid sequence is shown below the nucleotide sequence.

Figure 2A-H (SEQ. ID. NO. 4) depicts the complete nucleotide sequence of the beta-8 integrin subunit from the human osteosarcoma cell line MG-63 described in the examples. The sequence contains an untranslated 5' region and a 3' untranslated region. The presumed ATG translation start codon is indicated. The predicted amino acid sequence is shown below the nucleotide sequence.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims:

The term "beta-8 integrin subunit" or " β -8 integrin subunit" is defined as a polypeptide having a qualitative biological activity in common with the beta-8 integrin subunit of Fig. 1A-E or Fig. 2A-H, and which has at least 75 % amino acid sequence identity with the beta-8

integrin subunit of Fig. 1A-E or Fig. 2A-H. Preferably the beta-8 integrin subunit comprises at least 5 amino acid residues.

Identity or homology with respect to a beta-8 integrin subunit is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in Fig. 1A-E or Fig. 2A-H, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. Neither N- nor C-terminal extensions nor insertions shall be construed as reducing identity or homology.

Beta-8 integrin subunit qualitative biological activity is defined as either 1) immunological cross-reactivity with at least one epitope of the beta-8 integrin subunit, or 2) the possession of at least one adhesive, regulatory or effector function qualitatively in common with the beta-8 integrin subunit.

Immunologically cross-reactive as used herein means that the candidate polypeptide is capable of competitively inhibiting the qualitative biological activity of the beta-8 integrin subunit having this activity with polyclonal antibodies or antisera raised against the known active analogue. Such antibodies and antisera are prepared in conventional fashion by injecting an animal such as a goat or rabbit, for example, subcutaneously with the known native beta-8 integrin subunit in complete Freund's adjuvant, followed by booster intraperitoneal or subcutaneous injection in incomplete Freund's.

Included within the scope of the beta-8 integrin subunit as that term is used herein are beta-8 integrin subunits having the translated or translated mature amino acid sequences of the rabbit or human beta-8 integrin subunits as set forth in Fig. 1A-E or Fig. 2A-H, deglycosylated or unglycosylated derivative of the beta-8 integrin subunit, homologous amino acid sequence variants of the sequence of Fig. 1A-E or Fig. 2A-H, and homologous *in vitro*-generated variants and derivatives of the beta-8 integrin subunit, which are capable of exhibiting a biological activity in common with the beta-8 integrin subunit of Fig. 1A-E or Fig. 2A-H. While native beta-8 integrin subunit is a membrane-bound polypeptide, soluble forms, such as those form lacking a functional transmembrane domain, are also included within this definition.

Also included within the scope of this invention is a polypeptide or protein encoded by the human beta-8 integrin subunit nucleotide sequence set forth in Fig. 2A-H; fragments thereof having 15 and preferably 30 amino acid residues; fragments thereof having greater than about 5 residues comprising an immune epitope or other biologically active site of the beta-8 integrin subunit; amino acid sequence variants of said Fig. 1A-E or Fig. 2A-H sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, said Fig. 1A-E or Fig. 2A-H sequence or its fragment as defined above; and/or amino acid sequence variants of said Fig. 1A-E or Fig. 2A-H sequence or its fragment as defined above wherein an amino acid residue of said Fig. 1A-E or Fig. 2A-H sequence or fragment thereof has been substituted

by another residue, including predetermined mutations by, e.g., site-directed or PCR mutagenesis, and other animal species of the beta-8 integrin subunit such as rabbit, rat, porcine, non-human primate, equine, murine, and ovine beta-8 integrin subunit and alleles and other naturally occurring variants of the foregoing and human sequences; and derivatives of the beta-8 integrin subunit or its fragments as defined above wherein the beta-8 integrin subunit or its fragments have been covalently modified by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid. Such fragments and variants exclude any polypeptide heretofore identified, including any known beta integrin subunits of any animal species or any known fragment of such beta integrin subunits including beta integrin subunits 1-7. Beta-8 integrin subunit amino acid sequence variants generally will share at least about 75% (preferably >80%, more preferably >85%) sequence identity with the translated sequence shown in Fig. 1A-E or Fig. 2A-H.

The preferred beta-8 integrin subunit is human beta-8 integrin subunit. The human beta-8 integrin shares a polypeptide sequence identity of about 35%, 32%, 34%, 31%, 34%, 37% and 31% with known human beta integrins subunits 1-7, respectively.

"Isolated" beta-8 integrin subunit nucleic acid or polypeptide is beta-8 integrin subunit nucleic acid or polypeptide that is identified and separated from contaminant nucleic acids or polypeptides present in the animal or human source of the beta-8 integrin nucleic acid or polypeptide. The nucleic acid or polypeptide may be labeled for diagnostic and probe purposes, using a label as described and defined further below in the discussion of diagnostic assays. The isolated beta-8 integrin subunit may be associated with any alpha integrin subunit to form a complex, as for example in an alpha-v/beta-8 complex. Associated complexes comprising truncated forms of the beta-8 integrin subunit and/or the alpha integrin subunit are also contemplated by this invention.

Beta-8 integrin subunit "nucleic acid" is defined as RNA or DNA containing greater than ten (10) bases that encodes a beta-8 integrin subunit, is complementary to nucleic acid sequence encoding the beta-8 integrin subunit, hybridizes to such nucleic acid and remains stably bound to it under stringent conditions, or encodes a polypeptide sharing at least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the translated amino acid sequence shown in Figures 1A-E or 2A-H. Preferably the DNA which hybridizes to the nucleic acid of Fig. 1A-E or Fig. 2A-H contains at least 20, more preferably 40, and even more preferably 60 bases. Most preferably, the hybridizing DNA or RNA contains 45 or even more preferably 90 bases. Such hybridizing or complementary nucleic acid, however, is defined further as being novel and unobvious over any prior art nucleic acid including that which encodes, hybridizes under stringent conditions, or is complementary to nucleic acid encoding a known beta integrin subunit, including beta integrin subunits 1-7.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1%

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NaDodSO₄ at 50°C, or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are commercially available, are publicly available on an unrestricted basis, or can be constructed from such available plasmids in

accord with published procedures. In addition, other equivalent plasmids are known in the art and will be apparent to the ordinary artisan.

"Restriction Enzyme Digestion" of DNA refers to catalytic cleavage of the DNA with an enzyme that acts only at certain locations in the DNA. Such enzymes are called restriction endonucleases, and the sites for which each is specific is called a restriction site. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements as established by the enzyme suppliers are used. Restriction enzymes commonly are designated by abbreviations composed of a capital letter followed by other letters representing the microorganism from which each restriction enzyme originally was obtained and then a number designating the particular enzyme. In general, about 1 μ g of plasmid or DNA fragment is used with about 1-2 units of enzyme in about 20 μ l of buffer solution. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation of about 1 hour at 37°C is ordinarily used, but may vary in accordance with the supplier's instructions. After incubation, protein or polypeptide is removed by extraction with phenol and chloroform, and the digested nucleic acid is recovered from the aqueous fraction by precipitation with ethanol. Digestion with a restriction enzyme may be followed with bacterial alkaline phosphatase hydrolysis of the terminal 5' phosphates to prevent the two restriction cleaved ends of a DNA fragment from "circularizing" or forming a closed loop that would impede insertion of another DNA fragment at the restriction site. Unless otherwise stated, digestion of plasmids is not followed by 5' terminal dephosphorylation. Procedures and reagents for dephosphorylation are conventional as described in sections 1.56-1.61 of Sambrook *et al.* (Molecular Cloning: A Laboratory Manual New York: Cold Spring Harbor Laboratory Press, 1989).

"Recovery" or "isolation" of a given fragment of DNA from a restriction digest means separation of the digest on polyacrylamide or agarose gel by electrophoresis, identification of the fragment of interest by comparison of its mobility versus that of marker DNA fragments of known molecular weight, removal of the gel section containing the desired fragment, and separation of the gel from DNA. This procedure is known generally. For example, see Lawn *et al.*, Nucleic Acids Res., **9**: 6103-6114 (1981), and Goeddel *et al.*, Nucleic Acids Res. **8**: 4057 (1980).

"Southern blot analysis" is a method by which the presence of DNA sequences in a restriction endonuclease digest of DNA or DNA-containing composition is confirmed by hybridization to a known, labeled oligonucleotide or DNA fragment. Southern analysis typically comprises electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane supports for analysis with a radiolabeled, biotinylated or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook *et al.*, *supra*.

"Northern analysis" is a method used to identify RNA sequences that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ^{32}P , or by biotinylation, or with an enzyme. The RNA to be analyzed is usually electrophoretically separated on an agarose
5 or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., *supra*.

"Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments. To ligate the DNA fragments together, the ends of the DNA fragments must
10 be compatible with each other. In some cases, the ends will be directly compatible after endonuclease digestion. However, it may be necessary to first convert the staggered ends commonly produced after endonuclease digestion to blunt ends to make them compatible for ligation. To blunt the ends, the DNA is treated in a suitable buffer for at least 15 minutes at 15°C with about 10 units of the Klenow fragment of DNA polymerase I or T4 DNA
15 polymerase in the presence of the four deoxyribonucleotide triphosphates. The DNA is then purified by phenol-chloroform extraction and ethanol precipitation. The DNA fragments that are to be ligated together are put in solution in about equimolar amounts. The solution will also contain ATP, ligase buffer, and a ligase such as T4 DNA ligase at about 10 units per $0.5\ \mu\text{g}$ of DNA. If the DNA is to be ligated into a vector, the vector is first linearized by digestion
20 with the appropriate restriction endonuclease(s). The linearized fragment is then treated with bacterial alkaline phosphatase, or calf intestinal phosphatase to prevent self-ligation during the ligation step.

"Preparation" of DNA from cells means isolating the plasmid DNA from a culture of the host cells. Commonly used methods for DNA preparation are the large and small scale
25 plasmid preparations described in sections 1.25-1.33 of Sambrook et al., *supra*. After preparation of the DNA, it can be purified by methods well known in the art such as that described in section 1.40 of Sambrook et al., *supra*.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or
30 phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published 4 May 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., *Nucl. Acids Res.*, 14: 5399-5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally
35 refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar

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in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, Cold Spring Harbor Symp. Quant. Biol., 51: 263 (1987); Erlich, ed., PCR Technology, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

II. Suitable Methods for Practicing the Invention

1. Preparation of Native Beta-8 Integrin Subunit and Variants

A. Isolation of DNA Encoding Beta-8 Integrin Subunit

The DNA encoding the beta-8 integrin subunit may be obtained from any cDNA library prepared from tissue believed to possess the beta-8 integrin subunit mRNA and to express it at a detectable level. The beta-8 integrin subunit gene may also be obtained from a genomic library. Libraries are screened with probes designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind to the beta-8 integrin subunit; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of the beta-8 integrin subunit cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a similar gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or a similar gene; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, *supra*.

An alternative means to isolate the gene encoding beta-8 integrin is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook *et al.*, *supra*. This method requires the use of oligonucleotide probes that will hybridize to the beta-8 integrin. Strategies for selection of oligonucleotides are described below.

Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Engels *et al.* (Agnew. Chem. Int. Ed. Engl., 28: 716-734 [1989]). These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or alternatively, if the target amino acid sequence is known, one may infer

potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues, preferably
5 mammalian placental, fetal, brain, and carcinoma cell lines. More preferably, human or rabbit placental, fetal, brain, and carcinoma cell line cDNA libraries are screened with the oligonucleotide probes.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide
10 sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of other beta integrin subunits. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is not known. The oligonucleotide must be labeled such that it can be detected upon
15 hybridization to DNA in the library being screened. The preferred method of labeling is to use 32-P labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Of particular interest is the beta-8 integrin subunit nucleic acid that encodes a full-length polypeptide. In some preferred embodiments, the nucleic acid sequence includes the
20 native beta-8 integrin subunit signal sequence. Nucleic acid having all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and, if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have
25 been reverse-transcribed into cDNA.

B. Amino Acid Sequence Variants of the Beta-8 Integrin Subunit

Amino acid sequence variants of the beta-8 integrin subunit are prepared by introducing appropriate nucleotide changes into the beta-8 integrin subunit DNA, or by *in vitro* synthesis
30 of the desired beta-8 integrin polypeptide. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence shown for the human beta-8 integrin subunit in Figure 2A-H. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. Excluded from the scope of this invention are beta-8
35 variants or polypeptide sequences that are not novel and unobvious over the prior art. The amino acid changes also may alter post-translational processes of the beta-8 integrin subunit, such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, and/or altering the intra-cellular location of the beta-8 integrin

subunit by inserting, deleting, or otherwise affecting the leader sequence of the native beta-8 integrin subunit.

In designing amino acid sequence variants of beta-8 integrin subunits, the location of the mutation site and the nature of the mutation will depend on the beta-8 integrin subunit characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3.

A useful method for identification of certain residues or regions of the beta-8 integrin subunit polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (Science, 244: 1081-1085 [1989]). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed beta-8 integrin subunit variants are screened for the optimal combination of desired activity.

In general, the regions of the beta-8 integrin subunit molecule preferred for alterations are the regions that are highly conserved with respect to other known beta integrin subunits. For example, the region between amino acids 118 and 180 of the human beta-8 integrin (numbered according to Figure 2A-H) is highly homologous with other beta integrins, and thus is a preferred region for mutations. Another preferred region is the region between amino acids 240 and 275. Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues, and typically are contiguous. Deletions may be introduced into regions of low homology between the beta-8 integrin subunit and other beta integrin subunits to modify the activity of the beta-8 integrin subunit. Deletions from the beta-8 integrin subunit in areas of substantial homology with any other beta integrin subunits will be more likely to modify the biological activity of the beta-8 integrin subunit more significantly. The number of consecutive deletions will be selected so as to preserve the tertiary structure of beta-8 integrin in the affected domain, e.g., beta-pleated sheet or alpha helix.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the beta-8 integrin sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, most preferably 1 to 3. Examples of terminal insertions include the beta-8 integrin subunit with an N-terminal methionyl residue, an artifact of the direct expression of beta-8 integrin in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the beta-8 integrin subunit molecule to facilitate the secretion of the mature beta-8 integrin subunit from recombinant host cells. Such signal sequences generally will be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include STII or lpp for *E. coli*, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

Other insertional variants of the beta-8 integrin subunit include the fusion to the N- or C-terminus of the beta-8 integrin subunit of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published 6 April 1989.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the beta-8 integrin subunit molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s) of the beta-8 integrin subunit, and sites where the amino acids found in the beta-8 integrin subunit from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity.

Other sites of interest are those in which particular residues of the beta-8 integrins obtained from various species are identical. These positions may be important for the biological activity of the beta-8 integrin subunit. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

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Table 1

	<u>Original Residue</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
5	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
10	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro	pro
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe; norleucine	leu
15	Leu (L)	norleucine; ile; val; met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
20	Phe (F)	leu; val; ile; ala	leu
	Pro (P)	gly	gly
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr	tyr
25	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in function or immunological identity of the beta-8 integrin subunit are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

- 35 (1) hydrophobic: norleucine, met, ala, val, leu, ile;
 (2) neutral hydrophilic: cys, ser, thr;
 (3) acidic: asp, glu;
 (4) basic: asn, gln, his, lys, arg;
 (5) residues that influence chain orientation: gly, pro; and
 40 (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another. Such substituted residues may be introduced into regions of the beta-8 integrin subunit that are homologous with other integrins, or, more preferably, into the non-homologous regions of the molecule.

In one embodiment of the invention, it is desirable to inactivate one or more protease cleavage sites that are present in the molecule. These sites are identified by inspection of the encoded amino acid sequence. Where protease cleavage sites are identified, they are rendered inactive to proteolytic cleavage by substituting the targeted residue with another residue, preferably a basic residue such as glutamine or a hydrophobic residue such as serine; by deleting the residue; or by inserting a prolyl residue immediately after the residue.

In another embodiment, any methionyl residues other than the starting methionyl residue of the signal sequence, or any residue located within about three residues N- or C-terminal to each such methionyl residue, is substituted by another residue (preferably in accord with Table 1) or deleted. Alternatively, about 1-3 residues are inserted adjacent to such sites.

Any cysteine residues not involved in maintaining the proper conformation of beta-8 integrin also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

DNA encoding amino acid sequence variants of the beta-8 integrin subunit is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the beta-8 integrin subunit.

Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of beta-8 integrin DNA. This technique is well known in the art as described by Adelman *et al.*, DNA, 2: 183 (1983). Briefly, the beta-8 integrin subunit DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the beta-8 integrin subunit. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the beta-8 integrin subunit DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea *et al.* (Proc. Natl. Acad. Sci. USA, 75: 5765 [1978]).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the beta-8 integrin subunit, and the other strand (the original template) encodes the native, unaltered sequence of the beta-8 integrin subunit. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion. After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

DNA encoding beta-8 integrin subunit mutants with more than one amino acid to be substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If, however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

5 The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis
10 as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

15 PCR mutagenesis is also suitable for making amino acid variants of beta-8 integrin. While the following discussion refers to DNA, it is understood that the technique also finds application with RNA. The PCR technique generally refers to the following procedure (see Erlich, *supra*, the chapter by R. Higuchi, p. 61-70): When small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the
20 corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of
25 the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the
30 position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using
35 standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

In a specific example of PCR mutagenesis, template plasmid DNA (1 μ g) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide tri-phosphates and is included in the GeneAmp® kits (obtained from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA), and 25 pmole of each oligonucleotide primer, to a final volume of 50 μ l. The reaction mixture is overlaid with 35 μ l mineral oil. The reaction is denatured for 5 minutes at 100°C, placed briefly on ice, and then 1 μ l *Thermus aquaticus* (Taq) DNA polymerase (5 units/ μ l, purchased from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows:

2 min. 55°C,
30 sec. 72°C, then 19 cycles of the following:
30 sec. 94°C,
30 sec. 55°C, and
30 sec. 72°C.

At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells *et al.* (*Gene*, 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the beta-8 integrin subunit DNA to be mutated. The codon(s) in the beta-8 integrin subunit DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the beta-8 integrin subunit DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated beta-8 integrin subunit DNA sequence.

C. Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding native or variant beta-8 integrin subunit is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on 1) whether it is to be used for DNA amplification or for DNA expression, 2) the size of the DNA to be inserted into the vector, and 3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) Signal Sequence Component

In general, the signal sequence may be a component of the vector, or it may be a part of the beta-8 integrin subunit DNA that is inserted into the vector. The native pro beta-8 integrin subunit DNA encodes a signal sequence at the amino terminus (5' end of the DNA) of the polypeptide that is cleaved during post-translational processing of the polypeptide to form the mature beta-8 integrin subunit polypeptide. Native beta-8 integrin subunit is not however secreted from the cell as it contains a membrane anchoring domain near the carboxyl terminus of the polypeptide. Thus, to form a secreted version of beta-8 integrin subunit, the carboxyl terminal domain of the molecule, including the membrane anchoring domain, is ordinarily deleted. This truncated variant beta-8 integrin subunit polypeptide may be secreted from the cell, provided that the DNA encoding the truncated variant retains the amino terminal signal sequence.

Included within the scope of this invention are beta-8 integrin subunits with the native signal sequence deleted and replaced with a heterologous signal sequence. The heterologous signal sequence selected should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell.

For prokaryotic host cells that do not recognize and process the native beta-8 integrin subunit signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native beta-8 integrin subunit signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

(ii) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the

vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of the beta-8 integrin subunit DNA. However, the recovery of genomic DNA encoding the beta-8 integrin subunit is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the beta-8 integrin subunit DNA.

(iii) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern *et al.*, J. Molec. Appl. Genet., 1: 327 [1982]), mycophenolic acid (Mulligan *et al.*, Science, 209: 1422 [1980]) or hygromycin (Sugden *et al.*, Mol. Cell. Biol., 5: 410-413 [1985]). The three examples given above employ

bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the beta-8 integrin subunit nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the beta-8 integrin subunit. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the beta-8 integrin subunit are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the beta-8 integrin subunit. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the beta-8 integrin subunit, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, Nature, 282: 39 [1979]; Kingsman *et al.*, Gene, 7: 141 [1979]; or Tschemper *et al.*, Gene, 10: 157 [1980]). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85: 12 [1977]). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

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(iv) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the beta-8 integrin subunit nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as the beta-8 integrin subunit, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the beta-8 integrin subunit by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native beta-8 integrin subunit promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the beta-8 integrin subunit DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed beta-8 integrin subunit as compared to the native beta-8 integrin subunit promoter.

Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter systems (Chang *et al.*, Nature, 275: 615 [1978]; and Goeddel *et al.*, Nature, 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the tac promoter (deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80: 21-25 [1983]). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding the beta-8 integrin subunit (Siebenlist *et al.*, Cell, 20: 269 [1980]) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the beta-8 integrin subunit.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255: 2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7: 149 [1968]; and Holland, Biochemistry, 17: 4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with

nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

5 Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition
10 of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Beta-8 integrin subunit transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma
15 virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the beta-8 integrin subunit sequence, provided such promoters are compatible with the host cell systems.

20 The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209: 1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78: 7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment.
25 Greenaway *et al.*, Gene, 18: 355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295: 503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297: 598-601 (1982) on expression of human β -interferon cDNA in
30 mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79: 5166-5170 (1982) on expression of the human interferon β 1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79: 6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and
35 mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(v) Enhancer Element Component

Transcription of a DNA encoding the beta-8 integrin subunit of this invention by higher

eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins *et al.*, Proc. Natl. Acad. Sci. USA, **78**: 993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., **3**: 1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, **33**: 729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., **4**: 1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, **297**: 17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the beta-8 integrin subunit DNA, but is preferably located at a site 5' from the promoter.

15

(vi) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the beta-8 integrin subunit. The 3' untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., **9**: 309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, **65**: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the beta-8 integrin subunit. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by

the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of the beta-8 integrin subunit that have beta-8 integrin subunit-like activity.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the beta-8 integrin subunit in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, **293**: 620-625 [1981]; Mantei *et al.*, Nature, **281**: 40-46 [1979]; Levinson *et al.*; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the beta-8 integrin subunit is pRK5 (EP pub. no. 307,247).

D. Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli*, *Bacilli* such as *B. subtilis*, *Pseudomonas* species such as *P. aeruginosa*, *Salmonella typhimurium*, or *Serratia marcescans*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* χ 1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. Alternatively, *in vitro* methods of cloning, e.g. PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing beta-8 integrin subunit DNA. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, **290**: 140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., **737** (1983)], *Yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, **76**: 5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., **112**: 284-289 (1983); Tilburn *et al.*, Gene, **26**: 205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, **81**: 1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., **4**: 475-479 (1985)].

Suitable host cells for the expression of glycosylated beta-8 integrin subunit polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding

permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, **6**: 47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, **315**: 592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the beta-8 integrin DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding beta-8 integrin is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the beta-8 integrin DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., **1**: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., **36**: 59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, **77**: 4216 [1980]); mouse sertoli cells (TM4, Mather, Biol. Reprod., **23**: 243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., **383**: 44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media

modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, **23**: 315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook *et al.*, *supra*, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., **130**: 946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), **76**: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or by protoplast fusion may also be used.

E. Culturing the Host Cells

Prokaryotic cells used to produce the beta-8 integrin subunit polypeptide of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the beta-8 integrin subunit of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., **58**: 44 (1979), Barnes and Sato, Anal. Biochem., **102**: 255 (1980), U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195 or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as GentamycinTM drug), trace elements (defined as inorganic compounds usually present at final concentrations

in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the
5 ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells that are within a host animal.

It is further envisioned that the beta-8 integrin subunit of this invention may be produced by homologous recombination, or with recombinant production methods utilizing
10 control elements introduced into cells already containing DNA encoding the beta-8 integrin subunit currently in use in the field. For example, a powerful promoter/enhancer element, a suppressor, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired beta-8 integrin subunit. The control element does not encode the
15 beta-8 integrin subunit of this invention, but the DNA is present in the host cell genome. One next screens for cells making the beta-8 integrin subunit of this invention, or increased or decreased levels of expression, as desired.

F. Detecting Gene Amplification/Expression

20 Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77: 5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes,
25 particularly ³²P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA
30 hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to
35 quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels,

and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., **75**: 734-738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native beta-8 integrin subunit polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further in Section 4 below.

G. Purification of The Beta-8 Integrin Subunit Polypeptide

The beta-8 integrin subunit preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal.

When the beta-8 integrin subunit is produced in cultured cells which normally produce alpha integrin subunits (such as 293 cells), frequently the alpha and beta subunits will be found associated in a complex. This complex may be found in the membrane or in the cytoplasm of the cell, or in the cell culture medium if it is a truncated beta-8 integrin subunit which is expressed with a functional signal sequence. The alpha and beta subunits may be separated from each other for purposes of purification by treating the complex with EDTA, EGTA, or other calcium and cation chelator. When the alpha/beta-8 integrin complex is disrupted, some changes in tertiary conformation of either subunit may result, and these changes may affect the biological activity of either subunit. It may be possible to avoid formation of the complex by selecting host cells which do not produce an alpha subunit, or by transfecting host cells with beta-8 integrin fragments.

When the beta-8 integrin subunit is expressed in a recombinant cell other than one of human origin, the beta-8 integrin subunit is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the beta-8 integrin subunit from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the beta-8 integrin subunit. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The membrane and soluble protein fractions are then separated. The beta-8 integrin subunit may then be purified from the soluble protein fraction and from the membrane fraction of the culture lysate, depending on whether the beta-8 integrin subunit is membrane bound. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

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Beta-8 integrin subunit variants in which residues have been deleted, inserted or substituted are recovered in the same fashion as the native beta-8 integrin subunit, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a beta-8 integrin subunit fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-beta-8 integrin subunit column can be employed to absorb the beta-8 integrin subunit variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native beta-8 integrin subunit may require modification to account for changes in the character of the beta-8 integrin subunit or its variants upon expression in recombinant cell culture.

H. Covalent Modifications of Beta-8 Integrin Subunit Polypeptides

Covalent modifications of beta-8 integrin subunit polypeptides are included within the scope of this invention. Both native beta-8 integrin subunits and amino acid sequence variants of the beta-8 integrin subunit may be covalently modified. One type of covalent modification included within the scope of this invention is a beta-8 integrin subunit polypeptide fragment. Beta-8 integrin subunit fragments having up to about 40 amino acid residues may be conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length beta-8 integrin subunit polypeptide or beta-8 integrin subunit variant polypeptide. Other types of covalent modifications of the beta-8 integrin subunit or fragments thereof are introduced into the molecule by reacting targeted amino acid residues of the beta-8 integrin subunit or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteiny residues most commonly are reacted with α -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteiny residues also are derivatized by reaction with bromotrifluoroacetone, α -bromo- β -(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α -amino-containing residues include imidoesters such as methyl picolinimide; pyridoxal phosphate; pyridoxal; chloroborohydride; 5 trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline 10 conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidazole and tetranitromethane 15 are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides ($R'-N=C=N-R'$), where R and R' are different alkyl groups, such as 1- 20 cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginy and glutaminy residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking beta-8 integrin to a water-insoluble support matrix or surface for use in the method for purifying anti-beta-8 25 integrin subunit antibodies, and vice versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p- 30 azidophenyl)dithio]propioimide yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

35 Glutaminy and asparaginy residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the beta-8 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in native beta-8, and/or adding one or more glycosylation sites that are not present in the native beta-8 polypeptide.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the beta-8 integrin polypeptide is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native beta-8 integrin subunit sequence (for O-linked glycosylation sites). For ease, the beta-8 integrin subunit amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the beta-8 integrin subunit polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above under the heading of "Amino Acid Sequence Variants of Beta-8 Integrin Subunit Polypeptide".

Another means of increasing the number of carbohydrate moieties on the beta-8 polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- and O- linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin and Wriston (CRC Crit. Rev. Biochem., pp. 259-306 [1981]).

Removal of carbohydrate moieties present on the native beta-8 integrin subunit polypeptide may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin *et al.* (Arch. Biochem. Biophys., 259:52 [1987]) and by Edge *et al.* (Anal. Biochem., 118:131 [1981]). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo- glycosidases as described by Thotakura *et al.* (Meth. Enzymol., 138:350 [1987]).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.* (J. Biol. Chem., 257:3105 [1982]). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of the beta-8 integrin subunit comprises linking the beta-8 integrin subunit polypeptide to various nonproteinaceous polymers, e.g. polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The beta-8 integrin subunit also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacrylate] microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., Ed., (1980).

Beta-8 integrin subunit preparations are also useful in generating antibodies, as standards in assays for the beta-8 integrin subunit (e.g. by labeling the beta-8 integrin subunit for use as a standard in a radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

Since it is often difficult to predict in advance the characteristics of a variant beta-8 integrin subunit, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example, a change in the immunological character of the beta-8 integrin subunit molecule, such as affinity for a given antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity observed for native beta-8 integrin subunit in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

3. Therapeutic Compositions and Administration of Beta-8 Integrin

Therapeutic formulations of beta-8 integrin subunit are prepared for storage by mixing beta-8 integrin subunit having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra),
5 in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as
10 polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

15 The beta-8 integrin subunit to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The beta-8 integrin subunit ordinarily will be stored in lyophilized form or in solution.

20 Therapeutic beta-8 integrin subunit compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of beta-8 integrin subunit or beta-8 integrin subunit antibody administration is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, or by sustained
25 release systems as noted below. The beta-8 integrin subunit is administered continuously by infusion or by bolus injection. Beta-8 integrin subunit antibody is administered in the same fashion, or by administration into the blood stream or lymph.

Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release
30 matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22: 547-556 [1983]), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, J. Biomed. Mater. Res., 15: 167-277 [1981] and Langer, Chem. Tech., 12: 98-105 [1982]), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release beta-8
35 integrin compositions also include liposomally entrapped beta-8 integrin subunit. Liposomes containing beta-8 integrin subunit are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688-3692 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949;

EP 142,641; Japanese patent application 83-118008; U.S. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal beta-8 integrin subunit therapy.

5 Another use of the present invention comprises incorporating beta-8 integrin subunit polypeptide into formed articles. Such articles can be used in modulating cellular migration, such as the migration of granule cells into the molecular layer of the cerebellum during differentiation of brain cells. In addition, cell migration during wound healing or tumor invasion may be modulated with these articles.

10 An effective amount of beta-8 integrin subunit to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 1 μ g/kg to up to 100 mg/kg or more,
15 depending on the factors mentioned above. Typically, the clinician will administer the beta-8 integrin subunit until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

4. Beta-8 Integrin Subunit Antibody Preparation

20 Polyclonal antibodies to the beta-8 integrin subunit generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the beta-8 integrin subunit and an adjuvant. It may be useful to conjugate the beta-8 integrin subunit or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or
25 soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N} = \text{C} = \text{NR}$, where R and R^1 are different alkyl groups.

Animals are immunized against the immunogenic conjugates or derivatives by
30 combining 1 mg or 1 μ g of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for anti-beta-8 integrin titer. Animals are boosted
35 until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same beta-8 integrin subunit, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

Monoclonal antibodies are prepared by recovering spleen cells from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The monoclonal antibody preferably does not cross-react with other known beta integrin subunit polypeptides.

5. Uses of Beta-8 Integrin and its Antibodies

The nucleic acid encoding the beta-8 integrin subunit may be used as a diagnostic for tissue specific typing. For example, such procedures as *in situ* hybridization, and northern and Southern blotting, and PCR analysis may be used to determine whether DNA and/or RNA encoding the beta-8 integrin subunit are present in the cell type(s) being evaluated. In particular, the nucleic acid may be useful as a specific probe for certain types of tumor cells such as, for example, brain tumor, glioma, other tumor cells that are neuronal in origin, and certain other non-neuronal carcinomas such as adenocarcinoma, osteocarcinoma, and lung carcinoma. Probes based on the nucleic acid or polypeptide sequences of the beta-8 integrin subunit may also be used to locate additional, novel, beta integrin subunits.

Isolated beta-8 integrin subunit polypeptide may be used in quantitative diagnostic assays as a standard or control against which samples containing unknown quantities of beta-8 integrin subunit may be compared.

Beta-8 integrin subunit antibodies are useful in diagnostic assays for beta-8 integrin expression in specific cells or tissues. The antibodies are labeled in the same fashion as the beta-8 integrin subunit described above and/or are immobilized on an insoluble matrix.

Beta-8 integrin subunit antibodies also are useful for the affinity purification of the beta-8 integrin subunit from recombinant cell culture or natural sources. The beta-8 integrin subunit antibodies that do not detectably cross-react with other beta integrin subunits can be used to purify beta-8 integrin subunit free from other known beta integrins.

Suitable diagnostic assays for the beta-8 integrin subunit and its antibodies are well known *per se*. Such assays include competitive and sandwich assays, and steric inhibition assays. Competitive and sandwich methods employ a phase-separation step as an integral part of the method while steric inhibition assays are conducted in a single reaction mixture. Fundamentally, the same procedures are used for the assay of the beta-8 integrin subunit and for substances that bind the beta-8 integrin subunit, although certain methods will be favored depending upon the molecular weight of the substance being assayed. Therefore, the substance to be tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or antibody, and proteins that bind to the analyte are denominated binding partners, whether they be antibodies, cell surface receptors, or antigens.

Analytical methods for the beta-8 integrin subunit or its antibodies all use one or more of the following reagents: labeled analyte analogue, immobilized analyte analogue, labeled

binding partner, immobilized binding partner and steric conjugates. The labeled reagents also are known as "tracers."

The label used (and this is also useful to label beta-8 integrin subunit nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalently to proteins or polypeptides. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like may be used to tag the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. Nos. 3,940,475 (fluorimetry) and 3,645,090 (enzymes); Hunter *et al.*, Nature, **144**: 945 (1962); David *et al.*, Biochemistry, **13**: 1014-1021 (1974); Pain *et al.*, J. Immunol. Methods, **40**: 219-230 (1981); and Nygren, J. Histochem. and Cytochem., **30**: 407-412 (1982). Preferred labels herein are enzymes such as horseradish peroxidase and alkaline phosphatase. The conjugation of such label, including the enzymes, to the antibody is a standard manipulative procedure for one of ordinary skill in immunoassay techniques. See, for example, O'Sullivan *et al.*, "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in Methods in Enzymology, ed. J.J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, New York, 1981), pp. 147-166. Such bonding methods are suitable for use with beta-8 integrin or its antibodies, all of which are proteinaceous.

Immobilization of reagents is required for certain assay methods. Immobilization entails separating the binding partner from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the binding partner or analyte analogue before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich *et al.*, U.S. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the partner or analogue afterward, e.g., by immunoprecipitation.

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Other assay methods, known as competitive or sandwich assays, are well established and widely used in the commercial diagnostics industry.

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding partner generally is insolubilized before or after the competition and then the tracer and analyte bound to the binding partner are separated from the unbound tracer and analyte. This separation is accomplished by decanting (where the binding partner was preinsolubilized) or by centrifuging (where the binding partner was precipitated after the competitive reaction). The amount of test sample analyte is inversely proportional to the amount of bound tracer as measured by the amount of marker substance. Dose-response curves with known amounts of analyte are prepared and compared with the test results to quantitatively determine the amount of analyte present in the test sample. These assays are called ELISA systems when enzymes are used as the detectable markers.

Another species of competitive assay, called a "homogeneous" assay, does not require a phase separation. Here, a conjugate of an enzyme with the analyte is prepared and used such that when anti-analyte binds to the analyte the presence of the anti-analyte modifies the enzyme activity. In this case, the beta-8 integrin subunit or its immunologically active fragments are conjugated with a bifunctional organic bridge to an enzyme such as peroxidase. Conjugates are selected for use with anti-beta-8 integrin subunit so that binding of the anti-beta-8 integrin inhibits or potentiates the enzyme activity of the label. This method *per se* is widely practiced under the name of EMIT.

Steric conjugates are used in steric hindrance methods for homogeneous assay. These conjugates are synthesized by covalently linking a low-molecular-weight hapten to a small analyte so that antibody to hapten substantially is unable to bind the conjugate at the same time as anti-analyte. Under this assay procedure the analyte present in the test sample will bind anti-analyte, thereby allowing anti-hapten to bind the conjugate, resulting in a change in the character of the conjugate hapten, e.g., a change in fluorescence when the hapten is a fluorophore.

Sandwich assays particularly are useful for the determination of beta-8 integrin subunit or beta-8 integrin subunit antibodies. In sequential sandwich assays an immobilized binding partner is used to adsorb test sample analyte, the test sample is removed as by washing, the bound analyte is used to adsorb labeled binding partner, and bound material is then separated from residual tracer. The amount of bound tracer is directly proportional to test sample analyte. In "simultaneous" sandwich assays the test sample is not separated before adding the labeled binding partner. A sequential sandwich assay using an anti-beta-8 integrin subunit monoclonal antibody as one antibody and a polyclonal anti-beta-8 integrin subunit antibody as the other is useful in testing samples for beta-8 integrin activity.

The foregoing are merely exemplary diagnostic assays for beta-8 integrin and

antibodies. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof, including the bioassays described above.

The following examples are offered by way of illustration and not by way of limitation.

5

EXAMPLES

Example 1: Cloning and Sequencing of Rabbit and Human Beta-8 Integrin

Beta-8 integrin was initially cloned from a rabbit placental cDNA library, constructed as follows: Total RNA was isolated from rabbit placental tissue by guanidium thiocyanate extraction (McDonald *et al.*, Meth. Enzymol. 152:219 [1987]). Poly(A)⁺ RNA was purified from 2 mg of total placental RNA using MAP paper (Amersham Corp.). Double-stranded cDNA was synthesized from poly(A)⁺ RNA using oligo(dT) primer. cDNA fragments larger than 2-3 kilobase pairs were purified on a 5% polyacrylamide gel and ligated to *EcoRI* linkers using standard procedures. Linkered cDNA was ligated into lambda gt10 (Stratagene) and packaged using Gigapack Gold II (Stratagene).

15 Approximately 1.6×10^8 recombinant phage of the oligo(dT) primed rabbit placental library (unamplified) were initially screened with three ³²P-labelled probes. The first probe was oligonucleotide 1, a published probe which has 54 of 97 nucleotides conserved between the beta-1, beta-2 and beta-3 integrins and is known to hybridize to both rabbit and human beta-1, beta-3, and beta-5 integrin subunits. This probe has the sequence AAGCAGAGTG
20 TGTCACGGAA CCGAGATGCC CCAGAGGGTG GCTTTGATGC CATCATGCAG GCTACAGTCT GTGATGAAAA GATTGGCTGG AGGAATG (SEQ. ID. NO. 1) (see the probe identified as intb5 in McLean *et al.*, *J. Biol. Chem.* 265:17126 [1990]). This first oligonucleotide probe is composed of the human beta-3 integrin subunit sequence that is conserved at the DNA level in all known beta integrins.

25 In addition, probes that encode the rabbit beta-1 integrin subunit and the rabbit beta-5 integrin were used to screen out positives that code for these more abundant beta integrins. The second probe was oligonucleotide 2, a beta-5 integrin subunit oligonucleotide (5'-ACGTGCGAGAAGTGCCCCACCTGCCCGGATGCTTGCAGCACCAAG-3' (SEQ. ID. NO. 2)) that hybridizes only to rabbit beta-5 subunit sequences. A third probe was a partial rabbit beta-1
30 integrin subunit cDNA.

Prehybridization and hybridization conditions were 5X SSC (SSC: 150 mM NaCl, 15 mM trisodium citrate), 0.05M sodium phosphate, pH 6.8, 5X Denhardt's solution, 10% dextran sulfate, and 20 µg/ml boiled, sheared salmon sperm DNA. Formamide was added to a final concentration of either 20% (for oligonucleotide probes) or 50% (for cDNA probes).
35 Prehybridization and hybridization were at 42°C, and the filters were washed for 30 minutes with either 2X SSC at 45°C (oligonucleotide probes) or with 0.1X SSC at 65°C (cDNA probes). The filters were exposed overnight to x-ray film with two intensifying screens at -70°C. The cDNA inserts from six phage isolates that hybridized to oligonucleotide 1, but not

to rabbit beta-1 integrin subunit cDNA or to oligonucleotide 2 were excised from the lambda gt10 vector and were subcloned into the bacteriophage M13 and the DNA was partially sequenced. Two of these clones contained sequences that overlapped. This overlapping sequence was designated Ra-1, and it was found to be similar to, but distinct from, beta integrin subunits 1-7. This Ra-1 clone was thus identified as a novel beta integrin subunit, named the beta-8 integrin subunit. The Ra-1 (beta-8 integrin subunit) clone was used to screen filters from a second, randomly primed rabbit placental cDNA library (constructed as described for the oligo (dT) library, except cDNA was primed using random hexamers (Amersham Corp.). Three additional beta-8 integrin subunit isolates were obtained from this rabbit placental library.

The full-length beta-8 integrin subunit sequence from the rabbit was inferred from two overlapping clones to be 2972 nucleotides (as shown in Figure 1A-E). This comprises an open reading frame of 2304 nucleotides (nt), a 5' untranslated region (UTR) of 651 nt, and a 3' UTR of 17 nt.

The rabbit beta-8 integrin subunit cDNA was subsequently used to screen human cDNA libraries to isolate the human homolog of the beta-8 integrin subunit. Initially, an amplified human placental cDNA library was screened as follows: Approximately 1.2×10^8 recombinant phage of a randomly primed human placental library were screened with the rabbit beta-8 integrin subunit cDNA probe at medium stringency (the same hybridization solution as described above, but containing 30% formamide) and these filters were washed with 1X SSC at 55°C. This library yielded a single clone of approximately 900 base pairs (bp) that comprised about 40% of the coding sequence of the human beta-8 integrin subunit plus a small amount of 3' UTR.

Using PCR analysis, it was determined that beta-8 integrin subunit mRNA was present in MG-63 human osteosarcoma cells. Therefore, a MG-63 cDNA library was screened with the human beta-8 integrin subunit partial cDNA (obtained from the human placental library) as a probe. The randomly primed MG-63 library was constructed using a method similar to that described above for the rabbit placental libraries, except the starting material was 5×10^8 MG-63 cells, poly(A+) was prepared using poly(U) Sepharose affinity chromatography and cDNA fragments greater than 3 kb were ligated into lambda gt10. Packaging was done using Gigapack Gold IIXL (Stratagene). This library yielded three overlapping clones, the longest of which, clone Hu-2, is 3789 bp (Figure 2A-H) and contains an open reading frame of 2307 nucleotides, a 5' UTR of 680 nucleotides and a 3' UTR of 802 nucleotides.

Both strands of the rabbit and human beta-8 integrin subunit clones were sequenced. The beta-8 integrin subunit isolates were subcloned into the Bluescript II vector (Stratagene) and double stranded DNA was sequenced using the Sequenase version 2.0 kit (U.S. Biochemical) and synthetic oligonucleotide primers. To facilitate sequence determination of long stretches of DNA, nested deletions were created by exonuclease III and mung bean

nuclease (using a kit obtained from Stratagene). The rabbit and human beta-8 integrin subunit polypeptides encoded by the cDNAs obtained are 768 (calculated molecular weight of 84,406) and 769 (calculated molecular weight of 85,631) amino acid residues, respectively as determined from translation of the cDNA sequences. These beta-8 integrin subunit amino acid sequences are very similar (89% at the amino acid level). The beta-8 integrin subunit contains 54 cysteine residues of which all but one are conserved colinearly between rabbit and human. The human beta-8 integrin subunit amino acid sequence was found to be very distinct from known beta integrins; the beta-8 integrin subunit amino acid sequence shares approximately 35%, 32%, 34%, 31%, 34%, 37%, and 31% identity with human beta integrin subunits 1 through 7, respectively.

Example 2: Detection of The Beta-8 Integrin Subunit mRNA Expression by PCR

The method of PCR analysis was used to detect expression of the beta-8 integrin subunit mRNA in several human cell lines. The cell lines evaluated were: 293 human kidney adenocarcinoma (ATCC number CRL 1573), MG-63 human osteosarcoma (ATCC number CRL 1427), UCLA-P3 human lung carcinoma (obtained from Dr. Donald Morton, University of California at Los Angeles) and HEL human erythroleukemia (obtained from the University of California at San Francisco Cell Culture Facility). PCR primers were derived from the human beta-8 integrin subunit nucleotide sequence. Single stranded cDNA was synthesized by priming 1 μ g of poly(A)⁺ RNA from several cell lines (prepared as described by Bodary *et al.*, *J. Biol. Chem.*, 265:5938 [1990]) with random hexanucleotides and extending with avian myoblastosis virus reverse transcriptase (cDNA synthesis kit, Amersham Corp.). One twentieth of the reaction product was amplified using the PCR GeneAmp kit, (Perkin-Elmer-Cetus), with 50 pmol of oligonucleotide primers (24mers) in a 100 μ l reaction volume, on a Perkin-Elmer-Cetus DNA thermal cycler. PCR conditions were: cycle 1, denaturation at 98°C for 2 min., annealing at 45°C for 30 sec., extension at 72°C for 30 sec.; cycles 2-5, denaturation at 98°C for 15 sec., annealing and extensions as for cycle 1, cycles 6-35, denaturation at 96°C for 30 sec., annealing and extension as for cycle 1 except that extension times were incremented by 1sec./cycle. One-tenth of the reaction product was analyzed on a 6.4% acrylamide gel. Primers (5'-TTCATCATTTTCATAGTTACATTC-3' [SEQ. ID. NO. 6] and 5'CATTAAAGTGTTTAAAAATCTTTT-3' [SEQ. ID. NO. 7]) were chosen that gave a predicted amplified DNA product of 276 bases.

The results indicated that 293, MG-63, and UCLA P-3 cells expressed beta-8 integrin subunit message, while HEL cells did not.

Example 3: Detection of Beta-8 Integrin mRNA Expression by Northern Analyses

The rabbit beta-8 integrin subunit cDNA was used as a probe in northern analyses to investigate expression of beta-8 integrin subunit mRNA in various rabbit tissues. A pregnant

rabbit was sacrificed and its tissues (whole fetus, heart, lung, pancreas, spleen, brain, ovary, bone marrow, whole blood, uterus, skeletal muscle, kidney, liver and placenta) were dissected. Total mRNA was prepared from these tissues as follows: Total RNA was obtained from one gram of each tissue by guanidium thiocyanate extraction. Poly(A)⁺ RNA was purified from 500 μ g of total RNA using oligo(dT) cellulose columns (Stratagene). One half of this poly(A)⁺ RNA was then fractionated on a denaturing 1.2% agarose gel containing 2% formaldehyde and blotted to Biotrace membrane (Gelman Sciences) overnight. The membrane was prehybridized and hybridized as for the cDNA library screening except that the formamide concentration was increased to 50% and all solutions (including wash solutions) contained 0.2% SDS. The membrane was probed with randomly primed ³²P-labelled, partial rabbit beta-8 integrin subunit, beta-1 integrin subunit, and beta-3 integrin subunit cDNAs and washed with 0.1X SSC at 60°C. The membrane was stripped before addition of new probe by washing in 0.2% SDS at 80°C with gentle agitation, for 5 minutes.

Tissues that were positive for an approximately 8 kilobase (kb) beta-8 integrin subunit message included kidney, brain, placenta, ovary and uterus. To demonstrate that this pattern of expression was specific for the beta-8 integrin subunit, the blot with the cDNA probes that encoded rabbit beta-1 integrin subunit and beta-3 integrin subunit was reprobed. In these experiments, an approximately 3.5 kb beta-1 integrin subunit message was detected in all tissues. In addition, two beta-3 integrin subunit messages of about 3.5 and 5.5 kb were detected in only uterus and bone marrow. From these and the sequencing results, the beta-8 integrin subunit appears to be a distinct and separate beta integrin subunit.

Example 4: Expression and Detection of the Human Beta-8 Integrin Subunit

The 3789 bp human beta-8 integrin subunit cDNA was subcloned into the pRK5 expression vector described in EP 07/560,482 and human embryonic kidney 293 cells were transfected with this construct using the standard calcium phosphate precipitate method (Sambrook *et al.*, *supra*). Cells were plated on day 1 at a concentration of 2×10^6 , exposed to a calcium phosphate precipitate containing 10 μ g of expression plasmid or no DNA (control cells) on day 2 and harvested on day 4 with 5 mM EDTA in Ca²⁺/Mg²⁺-free PBS (phosphate buffered saline, see section B.12 of Sambrook *et al.*, *supra*).

Cells ($1-2 \times 10^7$) were surface labelled with 1 mCi Na¹²⁵I (Amersham Corp.) and 100 μ g lactoperoxidase (Sigma) in the presence of 0.0015% H₂O₂. After labelling for 10 min. at room temperature, cells were washed once with 5 mM KI in PBS and then twice with PBS. Labelled cells were solubilized for 30 min. at 4°C in 0.5 ml of Extraction Buffer (1% Triton X-100, 20 mM Tris pH 7.5, 150 mM NaCl and 1 mM CaCl₂) with occasional vortexing. Cellular debris was then pelleted by centrifugation at 5000 x g for 5 min., and the cell extract supernatant diluted to 0.5% Triton X-100 by the addition of 0.5 ml Extraction Buffer without Triton X-100.

The beta-8 integrin subunit polypeptide was detected by immunoprecipitation using anti-peptide polyclonal antibodies specific for the beta-8 integrin subunit. The synthetic peptide H₂N-EEIKMDISKLNHETFR CNF-COOH (SEQ. ID. NO. 5), comprising C-terminal human beta-8 integrin residues predicted by the cDNA sequence was used as an immunogen. The peptide was synthesized by F-moc chemistry using a Milligen 9050 peptide synthesizer from Biosearch. For immunization, ten mg of this peptide was coupled to 20 mg of soybean trypsin inhibitor using sulfo-MBS (Pierce) as per manufacturer's specifications. Rabbits were immunized, by subcutaneous injection, with the equivalent of 200 µg of peptide at three week intervals. High titer bleeds were obtained at about two to three months after the initial immunization.

For the immunoprecipitation, four hundred microliters of the labelled cell extract was incubated with 1/100 volume of antibody (ascites, culture supernatant or antiserum) at 4°C overnight, and immunoprecipitations were carried out using Protein G Sepharose™ 4FF (Pharmacia) after first preclearing with Sepharose™ CL-4B (Pharmacia). Protein G Sepharose™ beads were washed three times with 0.05% Tween 20, 20nM Tris pH 8, 120 mM NaCl, 2 mM CaCl₂. Proteins precipitated from the labelled cell extracts were solubilized by heating in sample buffer and analyzed by separation on a 7.5% SDS-polyacrylamide gel followed by autoradiography.

The results indicated that the beta-8 integrin subunit polypeptide is expressed on the surface of transformed 293 cells but not on the surface of control 293 cells.

Example 5: Detection of the beta-8/alpha-v Complex

Human embryonic kidney 293 cells, transfected as described in the previous example, were used to demonstrate the presence of a novel beta-8/alpha-v integrin complex. These cells endogenously express various alpha integrin subunits, including alpha-v. The beta-8 integrin transfected cell proteins were prepared for immunoprecipitation as described in Example 4.

Monoclonal antibodies against several different alpha integrin subunits were used in the immunoprecipitations in order to identify with which alpha subunit the beta-8 integrin formed a complex. Mouse monoclonal antibodies against alpha-1 (antibody TS2/7, Hemler *et al.*, J. Immunol., **132**:3011 [1984]), alpha-2 (antibody P1H5, Wayner *et al.*, *supra*), alpha-3 (antibody P1B5, Wayner *et al.*, *supra*), alpha-5 (antibody P1D6, Wayner *et al.*, *supra*), alpha-v (antibody 13C2, obtained from M. Horton, Dept. of Haematology, St. Bartholomew's Hospital, London, England) and rat monoclonal antibody against alpha-6 (antibody GoH3, Sonnenberg *et al.*, J. Biol. Chem., **262**: 10376 [1987]) were tested. The immunoprecipitations were conducted as described in Example 4.

The results of these immunoprecipitations indicated that the alpha-v subunit readily associates with and forms a complex with the beta-8 integrin subunit in transfected human

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embryonic kidney 293 cells, while in 293 cells, associations were not made with alpha integrin subunits 1,2,3,5, and 6.

-45-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Genentech, Inc.
- (ii) TITLE OF INVENTION: Novel Beta Integrin Subunit
- 10 (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Genentech, Inc.
- (B) STREET: 460 Point San Bruno Blvd
- 15 (C) CITY: South San Francisco
- (D) STATE: California
- (E) COUNTRY: USA
- (F) ZIP: 94080
- 20 (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: patin (Genentech)
- 25 (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- (B) FILING DATE: 26-FEB-1992
- (C) CLASSIFICATION:
- 30 (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: U.S.S.N. 07/670607
- (B) FILING DATE: 14-MAR-1991
- 35 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Adler, Carolyn R.
- (B) REGISTRATION NUMBER: 32,324
- (C) REFERENCE/DOCKET NUMBER: 699
- 40 (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 415/266-2614
- (B) TELEFAX: 415/952-9881
- (C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

- 45 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 97 bases
- (B) TYPE: nucleic acid
- 50 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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

55 AAGCAGAGTG TGTCACGGAA CCGAGATGCC CCAGAGGGTG GCTTTGATGC 50

CATCATGCAG GCTACAGTCT GTGATGAAAA GATTGGCTGG AGGAATG 97

60

(2) INFORMATION FOR SEQ ID NO:2:

- 65 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 45 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-46-

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

5 ACGTGCGAGA AGTGCCCCAC CTGCCCCGAT GCTTGCAGCA CCAAG 45

(2) INFORMATION FOR SEQ ID NO:3:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2972 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (iv) SEQUENCE DESCRIPTION: SEQ ID NO:3:

20 GCGCATGGCT CGCGCGGAGC CTGCGGGCGT CGGCAGGCGC TGCTAGGGCG 50
 CTCCCCGAGC CGCCTCCCCG GGCTGCTGGC GCCCAGAGCT TCCTCCCTGG 100
 25 CCCGCCGGGC GCGGGGCTGC AAGCCGGGGG ACGTGGCCTC TCCTGCCAC 150
 CTGTGGAAGG AGCTCGCGCC GATGGCTGCG CCCTCCGGCC TCGCAGGCGT 200
 30 CCCCTCCAC TAAGGCAGCA TCACCCAGCG AATGTACTTT AGGGTGGTTT 250
 CCCCCTCCCC AGCTTCGGGC TTTGTTTGGG TTTGATTGTG TTTGGCTCTC 300
 35 CGCTAAGCTG ATTTATGCAG CACAAGCCCC ATCGGCTGGC GAGAAACAAA 350
 AGCTCTTTTC TTTGTCCCGG GAGCGGGCTT GCGGAGCCCC GGCTCGCGTC 400
 GCGGACCGGG CCATCGGCCG TCGCGGGAGG CGCTGCTGGC CGAGGCCGCG 450
 45 CCGAGCTGGG AGGGCCGCGG GGGCCCTGGG ATGCCGAGCG GCGCCGGGGC 500
 CCGCGTACCT GCACCGCCTG CCGGGGAGCG CCTGCCAGGC CTGCTGGAGA 550
 50 CGTCCTAGCG GGCTCGGCCC GGGCCCCGAG GTCGCCCCGG AGGCCGAGCG 600
 CGCGTCCCCA GAGCGGCCAG GCGGCGGGCG CGGGGCGGGC CGCTGTGCAG 650
 T ATG TGC GGC TCG GCC CTG GGT CTC CCT CCC GCC GCA 687
 Met Cys Gly Ser Ala Leu Gly Leu Pro Ala
 1 5 10
 TTC GTC CGC CTG CGC AGC TGC CGG CCA GGT CCT GCG GCG 726
 Phe Val Arg Leu Arg Ser Cys Arg Pro Gly Pro Ala Ala
 15 20 25
 65 TTC CTC CGG GCG GCC TGG GTG CTC TCA CTT GTT CTC GGA 765
 Phe Leu Arg Ala Ala Trp Val Leu Ser Leu Val Leu Gly
 30 35

-47-

	CTG GGT CGA AGC GAA AAC AGT AGA TGC GCA TCC TCC CAT 804
	Leu Gly Arg Ser Glu Asn Ser Arg Cys Ala Ser Ser His
	40 45 50
5	GCT GTG TCG TGT TCC GAG TGC CTT GCG CTG GGT CCA GAC 843
	Ala Val Ser Cys Ser Glu Cys Leu Ala Leu Gly Pro Asp
	55 60
10	TGT GGA TGG TGT GTT CAC GAG GAT TTC ATC TCA GGT GGA 882
	Cys Gly Trp Cys Val His Glu Asp Phe Ile Ser Gly Gly
	65 70 75
15	CCA AGG AGT GAG CGC TGT GAT ATT GTT TCC AAT TTA ATA 921
	Pro Arg Ser Glu Arg Cys Asp Ile Val Ser Asn Leu Ile
	80 85 90
20	AGC AAA GGC TGC CCG GTG GAT TCA ATA GAA TAC CCA TCT 960
	Ser Lys Gly Cys Pro Val Asp Ser Ile Glu Tyr Pro Ser
	95 100
25	GTG CAT GTG ACA ATT CCA AGT GAA AAT GAA GTT AAT ACC 999
	Val His Val Thr Ile Pro Ser Glu Asn Glu Val Asn Thr
	105 110 115
30	CAG GTG ACA CCA GGA GAA GTG TCG ATT CAG CTG CGA CCA 1038
	Gln Val Thr Pro Gly Glu Val Ser Ile Gln Leu Arg Pro
	120 125
35	GGA GCT GCA GCT AAT TTT ATG CTG AAA ATT CAT CCG CTG 1077
	Gly Ala Ala Ala Asn Phe Met Leu Lys Ile His Pro Leu
	130 135 140
40	AAG AAA TAT CCT GTG GAT CTT TAT TAT CTG GTA GAT GTC 1116
	Lys Lys Tyr Pro Val Asp Leu Tyr Tyr Leu Val Asp Val
	145 150 155
45	TCA GCA TCA ATG CAC AAC AAT ATA GAA AAA TTA AAT TCT 1155
	Ser Ala Ser Met His Asn Asn Ile Glu Lys Leu Asn Ser
	160 165
50	GTT GGA AAT GAC TTA TCT AGA AAA ATG GCA TTT TTC TCC 1194
	Val Gly Asn Asp Leu Ser Arg Lys Met Ala Phe Phe Ser
	170 175 180
55	CGT GAC TTC CGC CTT GGG TTC GGC TCC TAT GTT GAT AAA 1233
	Arg Asp Phe Arg Leu Gly Phe Gly Ser Tyr Val Asp Lys
	185 190
60	ACA GTC TCA CCA TAC ATC AGT ATC CAC CCA GAA AGG ATT 1272
	Thr Val Ser Pro Tyr Ile Ser Ile His Pro Glu Arg Ile
	195 200 205
65	CAC AAC CAA TGC AGT GAC TAC AAC TTA GAC TGC ATG CCC 1311
	His Asn Gln Cys Ser Asp Tyr Asn Leu Asp Cys Met Pro
	210 215 220
70	CCC CAC GGA TAC ATC CAT GTG CTG TCC CTG ACG GAG AAC 1350
	Pro His Gly Tyr Ile His Val Leu Ser Leu Thr Glu Asn
	225 230
75	ATC ACG GAG TTC GAG CGA GCT GTG CAC AGA CAG AAA ATC 1389
	Ile Thr Glu Phe Glu Arg Ala Val His Arg Gln Lys Ile
	235 240 245
80	TCT GGC AAC ATC GAC ACA CCC GAA GGA GGC TTT GAT GCC 1428
	Ser Gly Asn Ile Asp Thr Pro Glu Gly Gly Phe Asp Ala
	250 255

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	ATG CTG CAG GCT GCC GTC TGC GAG AGT CAC ATC GGA TGG 1467
	Met Leu Gln Ala Ala Val Cys Glu Ser His Ile Gly Trp
	260 265 270
5	CGA AAA GAA GCT AAA AGA TTG CTG CTG GTG ATG ACG GAT 1506
	Arg Lys Glu Ala Lys Arg Leu Leu Leu Val Met Thr Asp
	275 280 285
10	CAG ACA TCT CAT CTG GCC CTT GAT AGC AAG TTG GCA GGC 1545
	Gln Thr Ser His Leu Ala Leu Asp Ser Lys Leu Ala Gly
	290 295
15	ATT GTG GTG CCG AAT GAT GGA AAT TGC CAT CTG AGA AAC 1584
	Ile Val Val Pro Asn Asp Gly Asn Cys His Leu Arg Asn
	300 305 310
20	AAC GTC TAC GTC AAG TCC ACA ACC ATG GAA CAT CCC TCA 1623
	Asn Val Tyr Val Lys Ser Thr Thr Met Glu His Pro Ser
	315 320
25	CTA GGC CAA CTT TCA GAG AAA TTA ATA GAC AAC AAC ATC 1662
	Leu Gly Gln Leu Ser Glu Lys Leu Ile Asp Asn Asn Ile
	325 330 335
30	AAT GTC ATC TTT GCA GTT CAA GGA AAA CAG TTT CAT TGG 1701
	Asn Val Ile Phe Ala Val Gln Gly Lys Gln Phe His Trp
	340 345 350
35	TAT AAG GAC CTT CTA CCC CTC TTG CCG GGT ACC ATT GCT 1740
	Tyr Lys Asp Leu Leu Pro Leu Leu Pro Gly Thr Ile Ala
	355 360
40	GGT GAA ATA GAA TCA AAG GCT GCA AAC CTC AAT AAT TTG 1779
	Gly Glu Ile Glu Ser Lys Ala Ala Asn Leu Asn Asn Leu
	365 370 375
45	GTA GTA GAA GCC TAT CAG AAA CTC ATT TCA GAA GTG AAA 1818
	Val Val Glu Ala Tyr Gln Lys Leu Ile Ser Glu Val Lys
	380 385
50	GTG CAG GTG GAA AGC AAA GTG CCA GGC GTC TAC TTT AAC 1857
	Val Gln Val Glu Ser Lys Val Pro Gly Val Tyr Phe Asn
	390 395 400
55	GTC ACA GCC ATC TGT CCA GAT GGA GCC AGG AAG CTG GGC 1896
	Val Thr Ala Ile Cys Pro Asp Gly Ala Arg Lys Leu Gly
	405 410 415
60	ATG GAA GGA TGC AGC AAT GTA ACA AGC AGT GAC GAA GTG 1935
	Met Glu Gly Cys Ser Asn Val Thr Ser Ser Asp Glu Val
	420 425
65	CTC TTC AAT GTA ACA GTG ACA ATG GAA AAA TGC AGT GTC 1974
	Leu Phe Asn Val Thr Val Thr Met Glu Lys Cys Ser Val
	430 435 440
70	ACA GGA GGG AAG AAC TAT GCA ATA ATC AAA CCT ATT GGT 2013
	Thr Gly Gly Lys Asn Tyr Ala Ile Ile Lys Pro Ile Gly
	445 450
75	TTC AAT GAA ACC AGT AAA ATC CAT ATA CAC CAA AAC TGT 2052
	Phe Asn Glu Thr Ser Lys Ile His Ile His Gln Asn Cys
	455 460 465
80	GGC TGT GAG TGT GAG GCC AGC AGA GGA GGT GCA GCG AAG 2091
	Gly Cys Glu Cys Glu Ala Ser Arg Gly Gly Ala Ala Lys
	470 475 480

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	TGT	GCC	GAG	GAA	GCA	CCC	CTG	GAT	TCC	ACG	TGT	CCC	CAG	2130
	Cys	Ala	Glu	Glu	Ala	Pro	Leu	Asp	Ser	Thr	Cys	Pro	Gln	
					485					490				
5	TGC	CAG	GAG	AGT	CAG	TGT	CAT	CAA	GAG	GAA	GCA	CAA	TCT	2169
	Cys	Gln	Glu	Ser	Gln	Cys	His	Gln	Glu	Glu	Ala	Gln	Ser	
		495					500					505		
10	CCC	AGT	CAG	GGC	TGC	AAG	GCC	CAC	GAG	GAC	CAA	CCG	GTG	2208
	Pro	Ser	Gln	Gly	Cys	Lys	Ala	His	Glu	Asp	Gln	Pro	Val	
				510					515					
15	TGC	AGT	GGC	CGA	GGG	GTT	TGC	GTT	TGT	GGG	AAA	TGC	CTA	2247
	Cys	Ser	Gly	Arg	Gly	Val	Cys	Val	Cys	Gly	Lys	Cys	Leu	
	520					525					530			
20	TGT	CAC	AAG	ATG	AAG	CTT	GGA	AAA	GTG	TAT	GGA	AAA	TAC	2286
	Cys	His	Lys	Met	Lys	Leu	Gly	Lys	Val	Tyr	Gly	Lys	Tyr	
			535					540					545	
25	TGT	GAA	AAA	GAT	GAC	TTT	TCC	TGT	CCC	TAT	CAT	CAT	GGC	2325
	Cys	Glu	Lys	Asp	Asp	Phe	Ser	Cys	Pro	Tyr	His	His	Gly	
					550					555				
30	AGT	CTG	TGT	GCT	GGG	CAC	GGA	GAG	TGT	GAA	GCG	GGC	AGA	2364
	Ser	Leu	Cys	Ala	Gly	His	Gly	Glu	Cys	Glu	Ala	Gly	Arg	
		560					565					570		
35	TGC	CAA	TGC	TTC	AGT	GGC	TGG	GAA	GGG	GAT	CGG	TGC	CAG	2403
	Cys	Gln	Cys	Phe	Ser	Gly	Trp	Glu	Gly	Asp	Arg	Cys	Gln	
				575					580					
40	TGC	CCG	TCA	GCA	GCA	GCC	CAG	CAC	TGT	GTC	AAT	TCC	AAG	2442
	Cys	Pro	Ser	Ala	Ala	Ala	Gln	His	Cys	Val	Asn	Ser	Lys	
		585				590					595			
45	GGC	CAA	GTG	TGC	AGC	GGA	AGA	GGC	ACG	TGT	GTG	TGT	GGC	2481
	Gly	Gln	Val	Cys	Ser	Gly	Arg	Gly	Thr	Cys	Val	Cys	Gly	
			600					605					610	
50	AGG	TGC	GAG	TGC	AGC	GAT	CCC	AGG	AGC	ATC	GGT	CGC	TTC	2520
	Arg	Cys	Glu	Cys	Ser	Asp	Pro	Arg	Ser	Ile	Gly	Arg	Phe	
					615					620				
55	TGT	GAA	CAC	TGT	CCC	ACA	TGT	CCT	ACA	GCC	TGC	AGT	GAA	2559
	Cys	Glu	His	Cys	Pro	Thr	Cys	Pro	Thr	Ala	Cys	Ser	Glu	
		625					630					635		
60	AAC	TGG	AAT	TGT	GTG	CAA	TGC	CTT	CAC	CCT	CAC	AAT	CTG	2598
	Asn	Trp	Asn	Cys	Val	Gln	Cys	Leu	His	Pro	His	Asn	Leu	
				640					645					
65	TCC	CAG	GCT	ATA	CTT	GAT	CAG	TGT	AGA	ACC	TCA	TGT	GCT	2637
	Ser	Gln	Ala	Ile	Leu	Asp	Gln	Cys	Arg	Thr	Ser	Cys	Ala	
						655					660			
70	TCC	ATG	GAG	CAG	CCT	TAT	GTG	GAG	CAG	GCA	TCA	GAG	TGT	2676
	Ser	Met	Glu	Gln	Pro	Tyr	Val	Glu	Gln	Ala	Ser	Glu	Cys	
			665					670					675	
75	TTC	TCT	AGC	CCA	AGC	TAC	TTG	AGG	ATT	TTT	TTC	ATC	ATA	2715
	Phe	Ser	Ser	Pro	Ser	Tyr	Leu	Arg	Ile	Phe	Phe	Ile	Ile	
					680					685				
80	TTC	ATA	GTC	ACG	TTC	TTG	ATT	GGG	TTG	CTT	AAA	ATC	CTG	2754
	Phe	Ile	Val	Thr	Phe	Leu	Ile	Gly	Leu	Leu	Lys	Ile	Leu	
			690				695					700		

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ATC ATT AGA CAA GTG ATA CTA CAA TGG AAT AGC AGT AAA 2793
 Ile Ile Arg Gln Val Ile Leu Gln Trp Asn Ser Ser Lys
 705 710

5 ATC AAG TCC TCC TCA GAT TAC AGA GTG TCA GCC TCA AAA 2832
 Ile Lys Ser Ser Ser Asp Tyr Arg Val Ser Ala Ser Lys
 715 720 725

10 AAG GAT AAG CTG ATT CTG CAG AGT GTT TGC ACA AGA GCA 2871
 Lys Asp Lys Leu Ile Leu Gln Ser Val Cys Thr Arg Ala
 730 735 740

15 GTG ACC TAC CGA CGT GAG AAA CCT GAA GAG ATA AAA TTG 2910
 Val Thr Tyr Arg Arg Glu Lys Pro Glu Glu Ile Lys Leu
 745 750

20 GAT ATC AGT AAA TTA AAT GCT CAT GAA ACT TTC AGG TGC 2949
 Asp Ile Ser Lys Leu Asn Ala His Glu Thr Phe Arg Cys
 755 760 765

AAC TTC TAAGA AAAAAAAAAA AA 2972
 Asn Phe
 768

25 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3789 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (iv) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40 CCCAGAGCCG CCTCCCCCTG TTGCTGGCAT CCCGAGCTTC CTCCCTTGCC 50

45 AGCCAGGACG CTGCCGACTT GTCTTTGCCC GCTGCTCCGC AGACGGGGCT 100

50 GCAAAGCTGC AACTAATGGT GTTGGCCTCC CTGCCCACCT GTGGAAGCAA 150

55 CTGCGCTGAT TGATGCGCCA CAGACTTTTT TCCCCTCGAC CTCGCCGGCG 200

60 TACCCTCCCA CAGATCCAGC ATCACCAGT GAATGTACAT TAGGGTGGTT 250

65 TCCCCCCCAG CTCGGGGCTT TGTTTGGGTT TGATTGTGTT TGGCTCTTCG 300

70 CTAAGCTGAT TTATGCAGCA GAAGCCCCAC CGGCTGGAGA GAAACAAAAG 350

75 CTCTTTTCTT TGTCCCGGAG CAGGCTGCGG AGCCCTTGCA GAGCCCTCTC 400

80 TCCAGTCGCC GCCGGGCCCT TGGCCGTCGA AGGAGGTGCT TCTCGCGGAG 450

85 ACCGCGGGAC CCGCCGTGCC GAGCCGGGAG GGCCGTAGGG GCCCTGAGAT 500

90 GCCGAGCGGT GCCCGGGCCC GCTTACCTGC ACCGCTTGCT CCGAGCCGCG 550

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GGGTCCGCCT GCTAGGCCTG CGGAAAACGT CCTAGCGACA CTCGCCCCGCG 600

GGCCCCGAGG TCGCCCGGGA GGCCGAGCCC GCGTCCGGAA GGCAGCCAGG 650

5 CGGCGGGCGC GGGGCGGGCT GTTTTGCATT ATG TGC GGC TCG 692
Met Cys Gly Ser
1

10 GCC CTG GCT TTT TTT ACC GCT GCA TTT GTC TGC CTG CAA 731
Ala Leu Ala Phe Phe Thr Ala Ala Phe Val Cys Leu Gln
5 10 15

15 AAC GAC CGG CGA GGT CCC GCC TCG TTC CTC TGG GCA GCC 770
Asn Asp Arg Arg Gly Pro Ala Ser Phe Leu Trp Ala Ala
20 25 30

20 TGG GTG TTT TCA CTT GTT CTT GGA CTG GGC CAA GGT GAA 809
Trp Val Phe Ser Leu Val Leu Gly Leu Gly Gln Gly Glu
35 40

25 GAC AAT AGA TGT GCA TCT TCA AAT GCA GCA TCC TGT GCC 848
Asp Asn Arg Cys Ala Ser Ser Asn Ala Ala Ser Cys Ala
45 50 55

30 AGG TGC CTT GCG CTG GGT CCA GAA TGT GGA TGG TGT GTT 887
Arg Cys Leu Ala Leu Gly Pro Glu Cys Gly Trp Cys Val
60 65

35 CAA GAG GAT TTC ATT TCA GGT GGA TCA AGA AGT GAA CGT 926
Gln Glu Asp Phe Ile Ser Gly Gly Ser Arg Ser Glu Arg
70 75 80

40 TGT GAT ATT GTT TCC AAT TTA ATA AGC AAA GGC TGC TCA 965
Cys Asp Ile Val Ser Asn Leu Ile Ser Lys Gly Cys Ser
85 90 95

45 GTT GAT TCA ATA GAA TAC CCA TCT GTG CAT GTT ATA ATA 1004
Val Asp Ser Ile Glu Tyr Pro Ser Val His Val Ile Ile
100 105

50 CCC ACT GAA AAT GAA ATT AAT ACC CAG GTG ACA CCA GGA 1043
Pro Thr Glu Asn Glu Ile Asn Thr Gln Val Thr Pro Gly
110 115 120

55 GAA GTG TCT ATC CAG CTG CGT CCA GGA GCC GAA GCT AAT 1082
Glu Val Ser Ile Gln Leu Arg Pro Gly Ala Glu Ala Asn
125 130

60 TTT ATG CTG AAA GTT CAT CCT CTG AAG AAA TAT CCT GTG 1121
Phe Met Leu Lys Val His Pro Leu Lys Lys Tyr Pro Val
135 140 145

65 GAT CTT TAT TAT CTT GTT GAT GTC TCA GCA TCA ATG CAC 1160
Asp Leu Tyr Tyr Leu Val Asp Val Ser Ala Ser Met His
150 155 160

AAT AAT ATA GAA AAA TTA AAT TCC GTT GGA AAC GAT TTA 1199
Asn Asn Ile Glu Lys Leu Asn Ser Val Gly Asn Asp Leu
165 170

TCT AGA AAA ATG GCA TTT TTC TCC CGT GAC TTT CGT CTT 1238
Ser Arg Lys Met Ala Phe Phe Ser Arg Asp Phe Arg Leu
175 180 185

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	GGA TTT GGC TCA TAC GTT GAT AAA ACA GTT TCA CCA TAC 1277
	Gly Phe Gly Ser Tyr Val Asp Lys Thr Val Ser Pro Tyr
	190 195
5	ATT AGC ATC CAC CCC GAA AGG ATT CAT AAT CAA TGC AGT 1316
	Ile Ser Ile His Pro Glu Arg Ile His Asn Gln Cys Ser
	200 205 210
10	GAC TAC AAT TTA GAC TGC ATG CCT CCC CAT GGA TAC ATC 1355
	Asp Tyr Asn Leu Asp Cys Met Pro Pro His Gly Tyr Ile
	215 220 225
15	CAT GTG CTG TCT TTG ACA GAG AAC ATC ACT GAG TTT GAG 1394
	His Val Leu Ser Leu Thr Glu Asn Ile Thr Glu Phe Glu
	230 235
20	AAA GCA GTT CAT AGA CAG AAG ATC TCT GGA AAC ATA GAT 1433
	Lys Ala Val His Arg Gln Lys Ile Ser Gly Asn Ile Asp
	240 245 250
25	ACA CCA GAA GGA GGT TTT GAC GCC ATG CTT CAG GCA GCT 1472
	Thr Pro Glu Gly Gly Phe Asp Ala Met Leu Gln Ala Ala
	255 260
30	GTC TGT GAA AGT CAT ATC GGA TGG CGA AAA GAG GCT AAA 1511
	Val Cys Glu Ser His Ile Gly Trp Arg Lys Glu Ala Lys
	265 270 275
35	AGA TTG CTG CTG GTG ATG ACA GAT CAG ACG TCT CAT CTC 1550
	Arg Leu Leu Leu Val Met Thr Asp Gln Thr Ser His Leu
	280 285 290
40	GCT CTT GAT AGC AAA TTG GCA GGC ATA GTG GTG CCC AAT 1589
	Ala Leu Asp Ser Lys Leu Ala Gly Ile Val Val Pro Asn
	295 300
45	GAC GGA AAC TGT CAT CTG AAA AAC AAC GTC TAC GTC AAA 1628
	Asp Gly Asn Cys His Leu Lys Asn Asn Val Tyr Val Lys
	305 310 315
50	TCG ACA ACC ATG GAA CAC CCC TCA CTA GGC CAA CTT TCA 1667
	Ser Thr Thr Met Glu His Pro Ser Leu Gly Gln Leu Ser
	320 325
55	GAG AAA TTA ATA GAC AAC AAC ATT AAT GTC ATC TTT GCA 1706
	Glu Lys Leu Ile Asp Asn Asn Ile Asn Val Ile Phe Ala
	330 335 340
60	GTT CAA GGA AAA CAA TTT CAT TGG TAT AAG GAT CTT CTA 1745
	Val Gln Gly Lys Gln Phe His Trp Tyr Lys Asp Leu Leu
	345 350 355
65	CCC CTC TTG CCA GGC ACC ATT GCT GGT GAA ATA GAA TCA 1784
	Pro Leu Leu Pro Gly Thr Ile Ala Gly Glu Ile Glu Ser
	360 365
70	AAG GCT GCA AAC CTC AAT AAT TTG GTA GTG GAA GCC TAT 1823
	Lys Ala Ala Asn Leu Asn Asn Leu Val Val Glu Ala Tyr
	370 375 380
75	CAG AAG CTC ATT TCA GAA GTG AAA GTT CAG GTG GAA AAC 1862
	Gln Lys Leu Ile Ser Glu Val Lys Val Gln Val Glu Asn
	385 390
80	CAG GTA CAA GGC ATC TAT TTT AAC ATT ACC GCC ATC TGT 1901
	Gln Val Gln Gly Ile Tyr Phe Asn Ile Thr Ala Ile Cys
	395 400 405

	CCA	GAT	GGG	TCC	AGA	AAG	CCA	GGC	ATG	GAA	GGA	TGC	AGA	1940
	Pro	Asp	Gly	Ser	Arg	Lys	Pro	Gly	Met	Glu	Gly	Cys	Arg	
			410					415					420	
5	AAC	GTG	ACG	AGC	AAT	GAT	GAA	GTT	CTT	TTC	AAT	GTA	ACA	1979
	Asn	Val	Thr	Ser	Asn	Asp	Glu	Val	Leu	Phe	Asn	Val	Thr	
					425					430				
10	GTT	ACA	ATG	AAA	AAA	TGT	GAT	GTC	ACA	GGA	GGA	AAA	AAC	2018
	Val	Thr	Met	Lys	Lys	Cys	Asp	Val	Thr	Gly	Gly	Lys	Asn	
			435				440						445	
15	TAT	GCA	ATA	ATC	AAA	CCT	ATT	GGT	TTT	AAT	GAA	ACC	GCT	2057
	Tyr	Ala	Ile	Ile	Lys	Pro	Ile	Gly	Phe	Asn	Glu	Thr	Ala	
				450					455					
20	AAA	ATT	CAT	ATA	CAC	AGA	AAC	TGC	AGC	TGT	CAG	TGT	GAG	2096
	Lys	Ile	His	Ile	His	Arg	Asn	Cys	Ser	Cys	Gln	Cys	Glu	
						465					470			
25	GAC	AAC	AGA	GGA	CCT	AAA	GGA	AAG	TGT	GTA	GAT	GAA	ACT	2135
	Asp	Asn	Arg	Gly	Pro	Lys	Gly	Lys	Cys	Val	Asp	Glu	Thr	
			475					480					485	
30	TTT	CTA	GAT	TCC	AAG	TGT	TTC	CAG	TGT	GAT	GAG	AAT	AAA	2174
	Phe	Leu	Asp	Ser	Lys	Cys	Phe	Gln	Cys	Asp	Glu	Asn	Lys	
					490					495				
35	TGT	CAT	TTT	GAT	GAA	GAT	CAG	TTT	TCT	TCT	GAG	AGT	TGC	2213
	Cys	His	Phe	Asp	Glu	Asp	Gln	Phe	Ser	Ser	Glu	Ser	Cys	
			500				505					510		
40	AAG	TCA	CAC	AAG	GAT	CAG	CCT	GTT	TGC	AGT	GGT	CGA	GGA	2252
	Lys	Ser	His	Lys	Asp	Gln	Pro	Val	Cys	Ser	Gly	Arg	Gly	
				515					520					
45	GTT	TGT	GTT	TGT	GGG	AAA	TGT	TCA	TGT	CAC	AAA	ATT	AAG	2291
	Val	Cys	Val	Cys	Gly	Lys	Cys	Ser	Cys	His	Lys	Ile	Lys	
						530					535			
50	CTT	GGA	AAA	GTG	TAT	GGA	AAA	TAC	TGT	GAA	AAG	GAT	GAC	2330
	Leu	Gly	Lys	Val	Tyr	Gly	Lys	Tyr	Cys	Glu	Lys	Asp	Asp	
			540					545					550	
55	TTT	TCT	TGT	CCA	TAT	CAC	CAT	GGA	AAT	CTG	TGT	GCT	GGG	2369
	Phe	Ser	Cys	Pro	Tyr	His	His	Gly	Asn	Leu	Cys	Ala	Gly	
					555					560				
60	CAT	GGA	GAG	TGT	GAA	GCA	GGC	AGA	TGC	CAA	TGC	TTC	AGT	2408
	His	Gly	Glu	Cys	Glu	Ala	Gly	Arg	Cys	Gln	Cys	Phe	Ser	
			565				570					575		
65	GGC	TGG	GAA	GGT	GAT	CGA	TGC	CAG	TGC	CCT	TCA	GCA	GCA	2447
	Gly	Trp	Glu	Gly	Asp	Arg	Cys	Gln	Cys	Pro	Ser	Ala	Ala	
				580					585					
70	GCC	CAG	CAC	TGT	GTC	AAT	TCA	AAG	GGC	CAA	GTG	TGC	AGT	2486
	Ala	Gln	His	Cys	Val	Asn	Ser	Lys	Gly	Gln	Val	Cys	Ser	
						595					600			
75</														

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ACC TGT TAT ACA GCC TGC AAG GAA AAC TGG AAT TGT ATG 2603
 Thr Cys Tyr Thr Ala Cys Lys Glu Asn Trp Asn Cys Met
 630 635 640

5 CAA TGC CTT CAC CCT CAC AAT TTG TCT CAG GCT ATA CTT 2642
 Gln Cys Leu His Pro His Asn Leu Ser Gln Ala Ile Leu
 645 650

10 GAT CAG TGC AAA ACC TCA TGT GCT CTC ATG GAA CAA CAG 2681
 Asp Gln Cys Lys Thr Ser Cys Ala Leu Met Glu Gln Gln
 655 660 665

15 CAT TAT GTC GAC CAA ACT TCA GAA TGT TTC TCC AGC CCA 2720
 His Tyr Val Asp Gln Thr Ser Glu Cys Phe Ser Ser Pro
 670 675 680

AGC TAC TTG AGA ATA TTT TTC ATC ATT TTC ATA GTT ACA 2759
 Ser Tyr Leu Arg Ile Phe Phe Ile Ile Phe Ile Val Thr
 685 690

20 TTC TTG ATT GGG TTG CTT AAA GTC CTG ATC ATT AGA CAG 2798
 Phe Leu Ile Gly Leu Leu Lys Val Leu Ile Ile Arg Gln
 695 700 705

25 GTG ATA CTA CAA TGG AAT AGT AAT AAA ATT AAG TCC TCA 2837
 Val Ile Leu Gln Trp Asn Ser Asn Lys Ile Lys Ser Ser
 710 715

30 TCA GAT TAC AGA GTG TCA GCC TCA AAA AAG GAT AAG TTG 2876
 Ser Asp Tyr Arg Val Ser Ala Ser Lys Lys Asp Lys Leu
 720 725 730

35 ATT CTG CAA AGT GTT TGC ACA AGA GCA GTC ACC TAC CGA 2915
 Ile Leu Gln Ser Val Cys Thr Arg Ala Val Thr Tyr Arg
 735 740 745

CGT GAG AAG CCT GAA GAA ATA AAA ATG GAT ATC AGC AAA 2954
 Arg Glu Lys Pro Glu Glu Ile Lys Met Asp Ile Ser Lys
 750 755

40 TTA AAT GCT CAT GAA ACT TTC AGG TGC AAC TTC TAA 2990
 Leu Asn Ala His Glu Thr Phe Arg Cys Asn Phe
 760 765 769

45 AAAAAAGATTT TTAAACACTT AATGGGAAAC TGGAATTGTT AATAATTGCT 3040

CCTAAAGATT ATAATTTTAA AAGTCACAGG AGGAGACAAA TTGCTCACGG 3090

50 TCATGCCAGT TGCTGGTTGT ACACTCGAAC GAAGACTGAC AAGTATCCTC 3140

55 ATCATGATGT GACTCACATA GCTGCTGACT TTTTCAGAGA AAAATGTGTC 3190

TTACTACTGT TTGAGACTAG TGTCGTTGTA GCACTTTACT GTAATATATA 3240

60 ACTTATTTAG ATCAGCATAG AATGTAGATC CTCTGAAGAG CACTGATTAC 3290

ACTTTACAGG TACCTGTTAT CCCTACGCTT CCCAGAGAGA ACAATGCTGT 3340

65 GAGAGAGTTT AGCATTGTGT CACTACAAGG GTACAGTAAT CCCTGCACTG 3390

-55-

5 GACATGTGAG GAAAAAATA ATCTGGCAAG TATATTCTAA GGTGCCCCAA 3440

CACTTCAACA GTTGGTGGTT GAATAGACAA GAACAGCTAG ATGAATAAAT 3490

10 GATTCGTGTT TCACTCTTTC AAGAGGTGAA CAGATACAAC CTTAATCTTA 3540

AAAGATTATT GCTTTTTTAA GTGTGTAGTT TTATGCATGT GTGTTTATGG 3590

15 TTTGCTTATT TTTGCAAGAT GGATACTAAT TCCAGCATTC TCTCCTCTTT 3640

GCCTTTATGT TTTGTTTTCT TTTTACAGG ATAAGTTTAT GTATGTCACA 3690

20 GATGACTGGA TTAATTAAGT GCTAAGTTAC TACTGCCATA AAAAATAAT 3740

AATACAATGT CACTTTATCA GAATACTAGT TTTAAAAGCT GAATGTTAA 3789

25

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- 30 (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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

35 Glu Glu Ile Lys Met Asp Ile Ser Lys Leu Asn Ala His Glu Thr

1 5 10 15

Phe Arg Cys Asn Phe

20

40

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- 45 (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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

TTTCATCATTT TCATAGTTAC ATTC 24

55

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- 60 (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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

CATTAAGTGT TTAAAAATCT TTTT 24

WE CLAIM:

1. An isolated beta-8 integrin subunit polypeptide.
2. The beta-8 integrin subunit polypeptide of claim 1 that is human.
3. A composition comprising the beta-8 integrin subunit polypeptide of claim 1,
5 which composition is free of contaminating polypeptides of the animal species that is the source of the polypeptide.
4. An antibody that is capable of binding the beta-8 integrin subunit polypeptide of claim 1 that is free of antibody capable of binding beta integrin subunits 1-7.
5. An antibody that is capable of binding to a complex comprising an isolated beta-
10 8 integrin subunit polypeptide, which polypeptide is associated with an alpha integrin subunit polypeptide, which antibody is free of antibody capable of binding beta integrin subunits 1-7.
6. An isolated nucleic acid molecule encoding the beta-8 integrin subunit.
7. The nucleic acid molecule of claim 6 which is DNA and has the translated DNA
sequence shown in Figure 2A-H.
- 15 8. The nucleic acid molecule of claim 6 which is DNA and contains greater than about 45 bases.
9. The nucleic acid molecule of claim 6 further comprising a promoter operably linked to the nucleic acid sequence.
10. An expression vector comprising the nucleic acid sequence of claim 6 operably
20 linked to control sequences recognized by a host cell transformed with the vector.
11. A host cell transformed with the vector of claim 10.
12. The host cell of claim 11 further transformed with an expression vector comprising nucleic acid encoding the alpha-v integrin subunit.
13. A method of using a nucleic acid molecule encoding the beta-8 integrin subunit,
25 comprising expressing the nucleic acid molecule in a cultured host cell transformed with a vector comprising the nucleic acid molecule encoding the beta-8 integrin subunit operably linked to control sequences recognized by the host cell transformed with the vector, and recovering beta-8 integrin subunit from the host cell.
14. The method of claim 13 wherein the beta-8 integrin subunit is recovered from
30 the host cell culture medium.
15. A method for producing a beta-8 integrin subunit comprising inserting into the DNA of a cell containing nucleic acid encoding the beta-8 integrin subunit a transcription modulatory element in sufficient proximity and orientation to the nucleic acid molecule to influence transcription thereof.
- 35 16. A method for obtaining cells having increased or decreased transcription of nucleic acid encoding a beta-8 integrin subunit comprising:
 - (a) providing cells containing the nucleic acid;
 - (b) introducing into the cells a transcription modulating element;

(c) screening the cells for a cell in which the transcription of the nucleic acid molecule is increased or decreased.

- 5 17. A method of determining the presence of beta-8 integrin subunit, comprising hybridizing DNA encoding the beta-8 integrin subunit to a test sample nucleic acid and determining the presence of beta-8 integrin subunit DNA.

18. A method of amplifying a nucleic acid test sample comprising priming a nucleic acid polymerase reaction with nucleic acid encoding a beta-8 integrin subunit.

19. A composition comprising the beta-8 integrin subunit of claim 1 and a pharmaceutically acceptable carrier.

- 10 20. An isolated beta-8 integrin subunit polypeptide associated with an alpha integrin subunit polypeptide.

21. An isolated polypeptide comprising the beta-8 integrin subunit fused to a heterologous polypeptide.

GCGATGGCT CGCGGGGAGC CTGCGGGGCGT CGCAGGGCG TGCTAGGGCG CTCCCGGAGC CGCCTCCCCG GGCTGCTGGC
 GCCCAGAGCT TCCTCCCTGG CCCGCCGGGC GCGGGGCTGC AAGCCGGGGG ACGTGGCCTC TCCTGCCCCAC CTGTGGAAGG
 AGCTCGCGCC GATGGCTGCG CCCTCCGGCC TCGCAGGCGT CCCCTCCAC TAAGGCAGCA TCACCCAGCG AATGTACTTT
 AGGGTGGTTT CCCCCTCCCC AGCTTCGGGC TTGTGTTGGG TTGTATTGTG TTTGGCTCTC CGCTAAGCTG ATTTATGCAG
 CACAAGCCCC ATCGGCTGGC GAGAAACAAA AGCTCTTTTC TTTGTCCCGG GAGCGGGCTT GCGGAGCCCC GGCTCGCGTC
 GCGACCGGG CCATCGGCCG TCGCGGGAGG CGCTGCTGGC CGAGGCCGCG CCGAGCTGGG AGGCCCGCGG GGGCCCTGGG
 ATGCCGAGCG GCGCGGGGC CCGCGTACCT GCACCGCCTG CCGGGGAGCG CCTGCCAGGC CTGCTGGAGA CGTCTAGCG
 GGCTCGGCCC GGGCCCCGAG GTCGCCCGGG AGGCCGAGCG CGCGTCCCCA GAGCGGCCAG GCGCGGGCG CGGGCGGGG

CGCTGTGCAG 650

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T ATG TGC GGC TCG GCC CTG GGT CTC CCT CCC GCC GCA TTC GTC CGC CTG CGC AGC TGC CGG CCA
 MET CYS GLY SER ALA LEU GLY LEU PRO PRO ALA ALA PHE VAL ARG LEU ARG SER CYS ARG PRO
 1 5 10 15 20

GGT CCT GCG GCG TTC CTC GCG GCG GCC TGG GTG CTC TCA CTT GTT CTC GGA CTG GGT CGA AGC GAA
 GLY PRO ALA ALA PHE LEU ARG ALA ALA TRP VAL LEU SER LEU VAL LEU GLY LEU GLY ARG SER GLU
 25 30 35 40

AAC AGT AGA TGC GCA TCC TCC CAT GCT GTG TCG TGT TCC GAG TGC CTT GCG CTG GGT CCA GAC TGT
 ASN SER ARG CYS ALA SER SER HIS ALA VAL SER CYS SER GLU CYS LEU ALA LEU GLY PRO ASP CYS
 45 50 55 60 65

GGA TGG TGT GTT CAC GAG GAT TTC ATC TCA GGT GGA CCA AGG AGT GAG CGC TGT GAT ATT GTT TCC
 GLY TRP CYS VAL HIS GLU ASP PHE ILE SER GLY GLY PRO ARG SER GLU ARG CYS ASP ILE VAL SER
 70 75 80 85

FIG. 1A

AAT TTA ATA AGC AAA GGC TGC CCG GTG GAT TCA ATA GAA TAC CCA TCT GTG CAT GTG ACA ATT CCA
 ASN LEU ILE SER LYS GLY CYS PRO 95 VAL ASP SER ILE GLU TYR PRO SER VAL HIS 105 VAL THR ILE PRO
 90

AGT GAA AAT GAA GTT AAT ACC CAG GTG ACA CCA GGA GAA GTG TCG ATT CAG CTG CGA CCA GGA GCT
 SER GLU ASN GLU VAL 115 ASN THR GLN VAL THR PRO GLY GLU VAL SER ILE GLN LEU ARG PRO GLY ALA
 110

GCA GCT AAT TTT ATG CTG AAA ATT CAT CCG CTG AAG AAA TAT CCT GTG GAT CTT TAT TAT CTG GTA
 ALA ALA ASN PHE MET 135 LEU MET LEU LYS ILE HIS CAT 140 PRO VAL ASP LEU TYR 150 TYR LEU VAL
 135

GAT GTC TCA GCA TCA ATG CAC AAC AAT ATA GAA AAA TTA AAT TCT GTT GGA AAT GAC TTA TCT AGA
 ASP VAL SER ALA SER MET 155 HIS ASN ASN ILE GLU LYS LEU ASN SER VAL GLY ASN ASP LEU SER ARG
 155

AAA ATG GCA TTT TTC TCC CGT GAC TTC CGC CTT GGG TTC GGC TCC TAT GTT GAT AAA ACA GTC TCA
 LYS MET ALA PHE 180 PHE SER ARG ASP PHE PHE GLY GLY SER TYR VAL ASP LYS THR VAL SER
 180

CCA TAC ATC AGT ATC CAC CCA GAA AGG ATT CAC AAC CAA TGC AGT GAC TAC AAC AAC TTA GAC TGC ATG
 PRO TYR ILE SER ILE HIS PRO GLU ARG 205 GLU ARG ILE HIS ASN GLN CYS SER ASP TYR ASN LEU ASP CYS MET
 200

CCC CCC CAC GGA TAC ATC CAT GTG CTG TCC CTG ACG GAG AAC ATC ACG GAG TTC GAG CGA GCT GTG
 PRO PRO HIS GLY TYR 225 ILE HIS VAL LEU SER 230 LEU THR GLU PHE 235 GLU ARG 240 ALA VAL
 220

CAC AGA CAG AAA ATC TCT GGC AAC ATC GAC ACA CCC GAA GGA GGC TTT GAT GCC ATG CTG CAG GCT
 HIS ARG GLN LYS ILE SER GLY ASN ILE ASP THR PRO GLU GLY GLY GGC TTT PHE ASP ALA MET 260
 245

FIG. 1B

GCC GTC TGC GAG AGT CAC ATC GGA TGG CGA AAA GAA GCT AAA AGA TTG CTG CTG GTG ATG ACG GAT
 ALA VAL CYS GLU SER HIS ILE GLY TRP ARG LYS GLU ALA LYS ARG LEU LEU MET THR ASP 285
 265 270 275

CAG ACA TCT CAT CTG GCC GTC TAC GTC AAG TCC ACA ACC ATG GAA CAT CCC TCA CTA GGC CAA CTT TCA
 GLN THR SER HIS LEU ASP 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395

CAT CTG AGA AAC AAC GTC TAC GTC AAG TCC ACA ACC ATG GAA CAT CCC TCA CTA GGC CAA CTT TCA
 HIS LEU ARG ASN ASN VAL TYR VAL 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395

GAG AAA TTA ATA GAC AAC AAC ATC AAT GTC ATC TTT GCA GTT CAA GGA AAA CAG TTT CAT TGG TAT
 GLU LYS LEU ILE ASP 330 335 340 345 350 355 360 365 370 375 380 385 390 395

AAG GAC CTT CTA CCC CTC TTG CCG GGT ACC ATT GCT GGT GAA ATA GAA TCA AAG GCT GCA AAC CTC
 LYS ASP LEU LEU PRO LEU LEU PRO GLY THR ILE ALA GGT GAA GLU ILE GLU SER LYS LYS ALA ASN LEU

AAT AAT TTG GTA GAA GCC TAT CAG AAA CTC ATT TCA GAA GTG AAA GTG CAG GTG GAA AGC AAA
 ASN ASN LEU VAL VAL GLU ALA TYR GLN LYS LEU ILE SER GLU VAL LYS VAL GLN VAL GLU SER LYS 395

GTG CCA GGC GTC TAC TTT AAC GTC ACA GCC ATC TGT CCA GAT GGA GCC AGG AAG CTG GGC ATG GAA
 VAL PRO GLY VAL TYR PHE ASN VAL THR 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495

GGA TGC AGC AAT GTA ACA AGC AGT GAC GAA GTG CTC TTC AAT GTA ACA GTG ACA ATG GAA AAA TGC
 GLY CYS SER ASN VAL THR SER 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495

FIG. 1C

AGT GTC ACA GGA GGG AAG AAC TAT GCA ATA ATC AAA CCT ATT GGT TTC AAT GAA ACC AGT AAA ATC
 SER VAL THR GLY GLY LYS ASN TYR ALA ILE ILE ILE LYS PRO ILE GLY PHE 455
 440 445 450 460
 CAT ATA CAC CAA AAC TGT GGC TGT GAG TGT GAG GCC AGC AGA GGA GGT GCA GCG AAG TGT GCC GAG
 HIS ILE HIS GLN ASN CYS GLY CYS GLU CYS GLU ALA SER ARG GLY GLY ALA 475
 465 470 480
 GAA GCA CCC CTG GAT TCC ACG TGT CCC CAG TGC TGC CAG GAG AGT CAG TGT CAT CAA GAG GAA GCA CAA
 GLU ALA PRO LEU ASP SER THR CYS PRO GLN CYS GLN GLU SER GLN CYS HIS 500
 485 490 495 505
 TCT CCC AGT CAG GGC TGC AAG GCC CAC GAG GAC CAA CCG GTG TGC AGT GGC CGA GGG GTT TGC GTT
 SER PRO SER GLN GLY CYS LYS ALA HIS GLU ASP 515
 510 520 525
 TGT GGG AAA TGC CTA TGT CAC AAG ATG AAG CTT GGA AAA GGA AAA TAC TGT GAA AAA GAT
 CYS GLY LYS CYS LEU CYS HIS LYS MET LYS VAL LYS TYR GLY LYS 540
 530 535 545
 GAC TTT TCC TGT CCC TAT CAT CAT GGC AGT CTG TGT GCT GGG CAC GGA GAG TGT GAA GCG GGC AGA
 ASP PHE SER CYS PRO TYR HIS HIS GLY SER LEU CYS ALA GLY HIS GLY GLU CYS GLU ALA 565
 550 555 560 570
 TGC CAA TGC TCC TTC AGT GGC TGG GAA GGG GAT CGG TGC CAG TGC CCG TCA GCA GCA GCC CAG CAC TGT
 CYS GLN CYS PHE SER GLY TRP GLU GLY ASP ARG CYS GLN CYS PRO SER ALA ALA 585
 575 580 590
 GTC AAT TCC AAG GGC CAA GTG TGC AGC GGA AGA GGC ACG TGT TGT GGT TGT GGC AGG TGC GAG TGC AGC
 VAL ASN SER LYS GLY GLN VAL CYS SER GLY ARG 605
 595 600 610 615

FIG. 1D

GAT CCC AGG AGC ATC GGT CGC TTC TGT GAA CAC TGT CCT ACA GCC TGC AGT GAA AAC
 ASP PRO ARG SER ILE GLY ARG PHE CYS TGT CYS PRO THR ALA CYS SER GLU ASN
 620 625 630 635

TGG AAT TGT GTG CAA TGC CTT CAC CCT CAC AAT CTG TCC CAG GCT ATA CTT GAT CAG TGT AGA ACC
 TRP ASN CYS VAL GLN CYS LEU HIS CAC HIS ASN LEU SER GLN ALA ILE LEU ASP GLN CYS ARG THR
 640 645 650 655

TCA TGT GCT TCC ATG GAG CAG CCT TAT GTG GAG CAG TCA GAG TGT TTC TCT AGC CCA AGC TAC
 SER CYS ALA SER MET GLU GLN PRO TYR VAL GLU GLN ALA SER GLU CYS PHE SER PRO SER TYR
 660 665 670 675 680

TTG AGG ATT TTT TTC ATC ATA TTC ATA GTC ACG TTC TTG ATT AAA ATC CTG ATC ATT
 LEU ARG ILE PHE PHE ILE ILE PHE ILE VAL THR PHE LEU ILE GLY LEU LYS ILE LEU ILE ILE
 685 690 695 700

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AGA CAA GTG ATA CTA CAA TGG AAT AGC AGT AAA ATC AAG TCC TCC TCA GAT TAC AGA GTG TCA GCC
 ARG GLN VAL ILE LEU GLN TRP ASN SER SER LYS ILE LYS SER SER SER ASP TYR ARG VAL SER ALA
 705 710 715 720 725

TCA AAA AAG GAT AAG CTG ATT CTG CAG AGT GTT TGC ACA AGA GCA GTG ACC TAC CGA CGT GAG AAA
 SER LYS LYS ASP LYS LEU ILE ILE LEU GLN SER VAL CYS THR ARG ALA VAL THR TYR ARG ARG GLU LYS
 730 735 740 745

CCT GAA GAG ATA AAA TTG GAT ATC AGT AAA TTA AAT GCT CAT GAA ACT TTC AGG TGC AAC TTC
 PRO GLU GLU ILE LYS LEU ASP ILE SER LYS LEU ASN ALA HIS GLU THR PHE ARG CYS ASN PHE
 750 755 760 765 768

TAAGA AAAAAAAAAA AA 2972

FIG. 1E

CCCAGAGCCG CCTCCCCCTG TTGCTGGCAT CCGAGCTTC CTCCCTTGCC AGCAGGACG CTGCCGACTT GTCCTTGCCC
 GCTGCTCCGC AGACGGGGCT GCAAAGCTGC AACTAATGGT GTTGGCCTCC CTGCCCCACCT GTGGAAGCAA CTGCGCTGAT
 TGATGCGCCA CAGACTTTT TCCCTCGAC CTCGCCGGCG TACCCTCCCA CAGATCCAGC ATCACCCAGT GAATGTACAT
 TAGGGTGGTT TCCCCCCCAG CTTCCGGCTT TGTGTTGGTT TGATTGTGTT TGGCTCTTCG CTAAGCTGAT TTATGCAGCA
 GAAGCCCCAC CGGCTGGAGA GAAACAAAAG CTCCTTTCTT TGTCCCGGAG CAGGCTGCGG AGCCCTTGCA GAGCCCTCTC
 TCCAGTCGCC GCCGGGCCCT TGGCCGTCCA AGGAGGTGCT TCTCGCGGAG ACCGGGGAC CCGCCGTGCC GAGCCGGGAG
 GGCCGTAGGG GCCCTGAGAT GCGAGCGGT GCCCGGGCCC GCTTACCTGC ACCGCTTGCT CCGAGCCGCG GGTCCGCCCT
 GCTAGGCCCTG CGGAAACGT CCTAGCGACA CTCGCCCGCG GGCCCCGAGG TCGCCCCGGA GGCCGAGCCC GCGTCCGGAA
 GGCAGCCAGG CGCGGGGCGC GGGCGGGCT GTTTTGCAAT
 ATG TGC GGC TCG GCC CTG GCT TTT ACC GCT GCA TTT GTC TGC CTG CAA AAC GAC CGG CGA
 MET CYS GLY SER ALA LEU ALA PHE PHE THR ALA ALA PHE VAL CYS LEU GLN ASN ASP ARG ARG
 1 5 10 15 20
 GGT CCC GCC TCG TTC CTC TGG GCA GCC TGG GTG TTT TCA CTT GTT CTT GGA CTG GGC CAA GGT
 GLY PRO ALA SER PHE LEU TRP ALA ALA TRP VAL PHE SER LEU VAL LEU GLY LEU GLY GLN GLY
 25 30 35 40
 GAA GAC AAT AGA TGT GCA TCT TCA AAT GCA GCA TCC TGT GCC AGG TGC CTT GCG CTG GGT CCA
 GLU ASP ASN ARG CYS ALA SER SER ASN ALA ALA SER CYS ALA ARG CYS LEU ALA LEU GLY PRO
 45 50 55 60
 GAA TGT GGA TGG TGT GTT CAA GAG GAT TTC ATT TCA GGT GGA TCA AGA AGT GAA CGT TGT GAT
 GLU CYS GLY TRP CYS VAL GLN GLU ASP PHE ILE SER GLY GLY SER ARG SER GLU ARG CYS ASP
 65 70 75 80

FIG. 2A

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ATT GTT TCC AAT TTA ATA AGC AAA GGC TGC TCA GTT GAT TCA ATA GAA TAC CCA TCT GTG CAT
ILE VAL SER ASN LEU ILE SER LYS GLY CYS SER SER VAL ASP SER ILE GLU TYR PRO SER VAL HIS
85      90      95
GTT ATA ATA CCC ACT GAA AAT GAA ATT AAT ACC CAG GTG ACA CCA GGA GAA GTG TCT ATC CAG
VAL ILE ILE PRO THR GLU THR GLN ILE ASN THR THR VAL THR PRO GLY GLU VAL SER ILE GLN
110      115
CTG CGT CCA GGA GCC GAA GCT AAT TTT ATG CTG AAA GTT CAT CCT CTG AAG AAA TAT CCT GTG
LEU ARG PRO GLY ALA GLU ALA ASN PHE MET LEU LYS VAL HIS PRO LEU LYS LYS TYR PRO VAL
130      135
GAT CTT TAT TAT CTT GTT GAT GTC TCA GCA TCA ATG CAC AAT AAT ATA GAA AAA TTA AAT TCC
ASP LEU TYR TYR LEU VAL ASP VAL SER ALA SER MET HIS ASN ASN ILE GLU LYS LEU ASN SER
150      155
GTT GGA AAC GAT TTA TCT AGA AAA ATG GCA TTT TTC TCC CGT GAC TTT CGT CTT GGA TTT GGC
VAL GLY ASN ASP LEU SER ARG LYS MET ALA PHE PHE SER ARG ASP PHE ARG LEU GLY PHE GLY
170      175      180      185

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FIG. 2B

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TCA TAC GTT GAT AAA ACA GTT TCA CCA TAC ATT AGC ATC CAC CCC GAA AGG ATT CAT AAT CAA
SER TYR VAL ASP LYS THR 195 TAC TYR PRO PRO TYR ILE SER ILE HIS CAT HIS ASN GLN 210
190

TGC AGT GAC TAC AAT TTA GAC TGC ATG CCT CCC CAT GGA TAC ATC CAT GTG CTG TCT TTG ACA
CYS SER ASP TYR ASN LEU 215 LEU ASP CYS MET PRO PRO PRO HIS VAL LEU SER LEU THR 230
215

GAG AAC ATC ACT GAG TTT GAG AAA GCA GTT CAT AGA CAG AAG ATC TCT GGA AAC ATA GAT ACA
GLU ASN ILE THR 235 GLU PHE PHE GLU LYS ALA VAL HIS ARG GLN LYS ILE SER GLY ASN ILE ASP THR 250
240

CCA GAA GGA GGT TTT GAC GCC ATG CTT CAG GCA GCT GTC TGT GAA AGT CAT ATC GGA TGG CGA
PRO GLU GLY 255 MET LEU GLN ALA ALA VAL 265 CYS GLU SER HIS 270 TRP ARG

AAA GAG GCT AAA AGA CTG CTG GTG ATG ACA GAT CAG ACG TCT CAT CTC GCT CTT GAT AGC AAA
LYS GLU ALA LYS ARG LEU 280 THR ASP GLN THR SER HIS LEU ALA LEU ASP SER LYS 295
275

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FIG. 2C

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TTG GCA GGC ATA GTG GTG CCC AAT GAC GGA AAC TGT CAT CTG AAA AAC AAC GTC TAC GTC AAA
LEU ALA GLY ILE VAL VAL 300 VAL VAL ASP 305 GLY ASN CYS HIS LEU LYS ASN ASN VAL TYR VAL 315
310

TCG ACA ACC ATG GAA CAC CAC CCC TCA CTA GGC CAA CTT TCA GAG AAA TTA ATA GAC AAC AAC ATT
SER THR THR MET GLU HIS CAC HIS PRO PRO SER LEU LEU GLN GLN LEU SER GLU LYS LEU ILE ASP ASN ASN ILE
320 330 335

AAT GTC ATC TTT GCA GTT CAA GGA AAA CAA TTT CAT TGG TAT AAG GAT CTT CTA CCC CTC TTG
ASN VAL ILE PHE ALA VAL VAL GLN GLN GLY LYS LYS PHE HIS TRP TYR LYS ASP LEU LEU PRO LEU LEU
340 345 350 355

CCA GGC ACC ATT GCT GGT GAA ATA GAA TCA AAG GCT GCA AAC CTC AAT AAT TTT GTA GTG GAA
PRO GLY THR ILE ALA GLY GGT GAA GLU ILE GLU SER LYS ALA ALA ASN LEU ASN 375 VAL VAL GLU
360 370 375

GCC TAT CAG AAG CTC ATT TCA GAA GTG AAA GTT CAG GTG GAA AAC AAC CAG GTA GGC ATC TAT
ALA TYR GLN LYS ILE ILE SER SER LYS VAL GLN VAL GLU ASN GLN VAL 395 GLN GLY ILE TYR
380 385 390 395 400

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FIG. 2D

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TTT AAC ATT ACC GCC ATC TGT CCA GAT GGG TCC AGA AAG CCA GGC ATG GAA GGA TGC AGA AAC
PHE ASN ILE THR ALA ILE CYS PRO ASP GLY SER ARG LYS PRO GLY MET GLU GLY CYS ARG ASN
405
410
415
420
GTG ACG AGC AAT GAT GAA GTT CTT TTC AAT GTA ACA GTT ACA ATG AAA AAA TGT GAT GTC ACA
VAL THR SER ASN ASP GLU VAL LEU PHE ASN VAL THR VAL THR MET LYS LYS CYS ASP VAL THR
425
430
435
440
GGA GGA AAA AAC TAT GCA ATA ATC AAA CCT ATT GGT TTT AAT GAA ACC GCT AAA ATT CAT ATA
GLY GLY LYS ASN TYR ALA ILE ILE LYS PRO ILE GLY PHE ASN GLU THR ALA LYS ILE HIS ILE
445
450
455
460
CAC AGA AAC TGC AGC TGT CAG TGT GAG GAC AAC AGA GGA CCT AAA GGA AAG TGT GTA GAT GAA
HIS ARG ASN CYS SER CYS GLN CYS GLU ASP ASN ARG GLY PRO LYS GLY LYS CYS VAL ASP GLU
465
470
475
480
ACT TTT CTA GAT TCC AAG TGT TTC CAG TGT GAT GAG AAT AAA TGT CAT TTT GAT GAA GAT CAG
THR PHE LEU ASP SER LYS CYS PHE GLN CYS ASP CYS TGT CYS HIS PHE ASP GLU ASP GLN
485
490
495
500
505

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FIG. 2E

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TTT TCT TCT GAG AGT TGC AAG TCA CAC AAG GAT CAG CCT GTT TGC AGT GGT CGA GGA GTT TGT
PHE SER SER SER GLU SER 510 CYS LYS SER HIS LYS ASP GLN PRO VAL CYS SER GLY ARG GLY VAL CYS
515
GTT TGT GGG AAA TGT TCA TGT CAC AAA ATT AAG CTT GGA AAA GTG TAT GGA AAA TAC TGT GAA
VAL CYS GLY LYS 530 CYS SER CYS HIS 535 TGT CYS HIS LYS ILE LYS LEU GLY LYS VAL TYR GLY LYS TYR CYS GLU
540
AAG GAT GAC TTT TCT TGT TGT CCA TAT CAC CAT GGA AAT CTG TGT GCT GGG CAT GGA GAG TGT GAA
LYS ASP ASP PHE SER CYS PRO TYR HIS 550 CYS HIS GLY ASN LEU CYS ALA GLY HIS GLY GLU CYS GLU
560
GCA GGC AGA TGC CAA TGC TTC AGT GGC TGG GAA GGT GAT CGA TGC CAG TGC CYS GLN 585
ALA GLY ARG CYS GLN CYS PHE SER 575 TGC GLY ASP ARG CYS GLN CYS PRO SER ALA ALA
570
GCC CAG CAC TGT GTC AAT TCA AAG GGC CAA GTG TGC AGT GGA AGA GGC ACG TGT GTG TGT GGA
ALA GLN HIS CYS VAL 595 ASN VAL CYS SER GLY ARG GLY THR CYS VAL CYS GLY
590 600 605 610

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FIG. 2F

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AGG TGT GAG TGC ACC GAT CCC AGG AGC ATC GGC CGC TTC TGT GAA CAC TGC CCC ACC TGT TAT
ARG CYS GLU CYS THR ASP 615 620 625 630
        TGT CYS GLU HIS CYS PRO THR CYS TYR

ACA GCC TGC AAG GAA AAC AAT TGG AAT TGT ATG CAA TGC CTT CAC CCT CAC AAT TTG TCT CAG GCT
THR ALA CYS LYS GLU ASN TRP ASN CYS MET GLN CYS LEU HIS PRO HIS ASN LEU SER GLN ALA
        635 640 645 650

ATA CTT GAT CAG TGC AAA ACC TCA TGT GCT CTC ATG GAA CAA CAG CAT TAT TAT GTC GAC CAA ACT
ILE LEU ASP GLN CYS LYS THR SER CYS ALA LEU MET GLU GLN GLN HIS TYR VAL ASP GLN THR
        655 660 665 670

TCA GAA TGT TTC TCC AGC CCA AGC TAC TTG AGA ATA TTT TTC ATC ATC ATT TTC ATA GTT ACA TTC
SER GLU CYS PHE SER SER PRO SER TYR LEU ARG ILE ILE PHE PHE ILE PHE ILE VAL THR PHE
        675 680 685 690

TTG ATT GGG TTG CTT AAA GTC CTG ATC ATT AGA CAG GTG ATA CTA CAA TGG AAT AGT AAT AAA
LEU ILE GLY LEU LEU LYS VAL LEU ILE ILE ARG GLN VAL ILE LEU GLN TRP ASN SER ASN LYS
        695 700 705 710 715

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FIG. 2G

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ATT AAG TCC TCA TCA GAT TAC AGA GTG TCA GCC TCA AAA AAG GAT AAG TTG ATT CTG CAA AGT
ILE LYS SER SER 720 SER ASP TYR ARG VAL SER ALA SER LYS LYS ASP LYS LEU ILE LEU GLN SER
725 730 735

GTT TGC ACA AGA GCA GTC ACC TAC CGA CGT GAG AAG CCT GAA GAA ATA AAA ATG GAT ATC AGC
VAL CYS THR ARG ALA VAL THR TYR ARG ARG GLU LYS PRO GLU GLU ILE LYS MET ASP ILE SER
740 745 750 755

AAA TTA AAT GCT CAT GAA ACT TTC AGG TGC AAC TTC TAA 2990
LYS LEU ASN ALA HIS GLU THR PHE ARG CYS ASN PHE
760 765 769

AAAAAGATTT TTAACACACTT AATGGGAAAC TGAATTGTT AATAATTGCT CCTAAAGATT ATAATTTTAA AAGTCACAGG
AGGAGACAAA TTGCTCACGG TCATGCCAGT TGCTGGTTGT ACACCTCGAAC GAAGACTGAC AAGTATCCTC ATCATGATGTC
GACTCACATA GCTGCTGACT TTTTCAGAGA AAAATGTGTC TTACTACTGT TTGAGACTAG TGTCGTTGTA GCACTTTACTC
GTAATATATA ACTTATTAG ATCAGCATAG AATGTAGATC CTCTGAAGAG CACTGATTAC ACTTTACAGG TACCTGTTAT
CCCTACGCTT CCCAGAGAGA ACAAATGCTGT GAGAGAGTTT AGCATTGTGT CACTACAAGG GTACAGTAAT CCCTGCACTG
GACATGTGAG GAAAAAATA ATCTGGCAAG TATATTCTAA GGTTGCCAAA CACTTCAACA GTTGGTGGTT GAATAGACAA
GAACAGCTAG ATGAATAAAT GATTTCGTGTT TCACTCTTTC AAGAGGTGAA CAGATACAAC CTTAATCTTA AAAGATTATT
GCTTTTAAA GTGTGTAGTT TTATGCATGT GTGTTTATGG TTTGCTTATT TTTGCAAGAT GGATACTAAT TCCAGCATTC
TCTCCTCTTT GCCTTTATGT TTTGTTTCT TTTTACAGG ATAAGTTTAT GTATGTCACA GATGACTGGA TTAATTAAAGT
GCTAAGTTAC TACTGCCATA AAAAATAAT AATACAATGT CACTTTATCA GAATACTAGT TTTAAAAGCT GAATGTTAA

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FIG. 2H

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/01504

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12N15/62; C07K13/00; C07K15/00
 A61K37/02; G01N33/53; C12Q1/68

II. FIELDS SEARCHEDMinimum Documentation Searched⁷

Classification System

Classification Symbols

Int.Cl. 5

C07K ; C12N

Documentation Searched other than Minimum Documentation
 to the Extent that such Documents are Included in the Fields Searched⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
P,X	J. BIOL. CHEM. vol. 266, no. 29, 15 October 1991, AM. SOC. MOL. BIOL. , INC., US pages 11650 - 11658; M. MOYLE ET AL.: 'Cloning and expression of a divergent integrin subunit beta-8'	1-11,13, 14
T	CELL vol. 69, no. 1, 3 April 1992, CELL PRESS, CAMBRIDGE, NA.; pages 11 - 25; R.O.HYNES: 'Integrins: Versatility, modulation, and signalling in cell adhesion'	

¹⁰ Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 JULY 1992	05.08.92
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer HORNIG H. 