



(86) Date de dépôt PCT/PCT Filing Date: 1996/06/06
(87) Date publication PCT/PCT Publication Date: 1996/12/19
(45) Date de délivrance/Issue Date: 2010/08/24
(85) Entrée phase nationale/National Entry: 1997/12/03
(86) N° demande PCT/PCT Application No.: US 1996/010053
(87) N° publication PCT/PCT Publication No.: 1996/040878
(30) Priorité/Priority: 1995/06/07 (US08/487,550)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01),
A61K 39/395 (2006.01), *C07K 14/705* (2006.01),
C07K 16/46 (2006.01), *C12N 5/10* (2006.01),
A61K 38/00 (2006.01)

(72) Inventeurs/Inventors:
ANDERSON, DARRELL R., US;
BRAMS, PETER, US;
HANNA, NABIL, US;
SHESTOWSKY, WILLIAM S., US

(73) Propriétaire/Owner:
BIOGEN IDEC INC., US

(74) Agent: SMART & BIGGAR

(54) Titre : ANTICORPS MONOCLONAUX SIMIENS DIRIGES SPECIFIQUEMENT CONTRE LE B7.1 ET/OU B7.2 HUMAINS, LEURS FORMES ADAPTEES AUX PRIMATES, ET COMPOSITIONS PHARMACEUTIQUES
(54) Title: MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS, PHARMACEUTICAL COMPOSITIONS

(57) **Abrégé/Abstract:**

The present invention relates to the identification of macaque antibodies to human B7.1 and B7.2 by screening of phase display libraries or monkey heterohybridomas obtained using B lymphocytes from B7.1 and/or B7.2 immunized monkeys. More specifically, the invention provides four monkey monoclonal antibodies 7B6, 16C10, 7C10 and 20C9 which inhibit the B7:CD28 pathway and thereby function as effective immunosuppressants. The invention further provides the complete DNA and amino acid sequences of the light and heavy chain of three primatized* antibodies derived from those monkey monoclonal antibodies which bind B7.1 and possibly B7.2, primatized* 7C10, primatized* 7B6 and primatized 16C10. These primatized* and monkey antibodies may be used as specific immunosuppressants, e.g., for the treatment of autoimmune diseases and to prevent organ transplant rejection.



53361-1

Abstract

The present invention relates to the identification of macaque antibodies to human B7.1 and B7.2 by screening of phase display libraries or monkey heterohybridomas obtained using B lymphocytes from B7.1 and/or B7.2 immunized monkeys. More specifically, the invention provides four monkey monoclonal antibodies 7B6, 16C10, 7C10 and 20C9 which inhibit the B7:CD28 pathway and thereby function as effective immunosuppressants. The invention further provides the complete DNA and amino acid sequences of the light and heavy chain of three primatized* antibodies derived from those monkey monoclonal antibodies which bind B7.1 and possibly B7.2, primatized* 7C10, primatized* 7B6 and primatized 16C10. These primatized* and monkey antibodies may be used as specific immunosuppressants, e.g., for the treatment of autoimmune diseases and to prevent organ transplant rejection.

*Trade-mark

53361-1

-1-

MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS. PHARMACEUTICAL COMPOSITIONS

5

FIELD OF THE INVENTION

The present invention relates to the manufacture and identification of novel monoclonal antibodies to human B7, i.e., human B7.1 and human B7.2 and primatizedTM forms thereof. More specifically, the present invention
10 relates to the production and identification of macaque antibodies to human B7, i.e., human B7.1 and human B7.2 produced by screening of phage display libraries and monkey heterohybridomas using B lymphocytes obtained from B7 immunized monkeys.

15

The invention further relates to specific primatizedTM antibodies which bind to human B7, i.e., human B7.1 and B7.2 as well as their corresponding amino acid and nucleic acid sequences.

20

Also, the present invention relates to pharmaceutical compositions containing monkey monoclonal or primatizedTM antibodies specific to human B7.1 and/or human B7.2 and their use as immunosuppressants by modulating the B7:CD28 pathway, e.g., for the treatment of autoimmune disorders, and the prevention of organ
25 rejection.

BACKGROUND OF THE INVENTION

30

The clinical interface between immunology, hematology, and oncology has long been appreciated. Many conditions treated by the hematologist or
30 oncologist have either an autoimmune or immunodeficient component to their pathophysiology that has led to the widespread adoption of immunosuppressive medications by hematologists, whereas oncologists have sought

-2-

immunologic adjuvants that might enhance endogenous immunity to tumors. To date, these interventions have generally consisted of nonspecific modes of immunosuppression and immune stimulation. In addition to the limited efficacy of these interventions, toxicities secondary to their nonspecificity have also limited their overall success. Therefore, alternative strategies have been sought.

Elucidation of the functional role of a rapidly increasing number of cell surface molecules has contributed greatly to the integration of immunology with clinical hematology and oncology. Nearly 200 cell surface antigens have been identified on cells of the immune and hematopoietic systems (Schlossman SF. Boumsell L. Gilks JM, Harlan T. Kishimoto, C. Morimoto C, Ritz J. Shaw S, Silverstein RL, Springer TA, Tedder TF, Todd RF: CD antigens (1993), Blood 83:879, 1994). These antigens represent both lineage-restricted and more widely distributed molecules involved in a variety of processes, including cellular recognition, adhesion, induction and maintenance of proliferation, cytokine secretion, effector function, and even cell death. Recognition of the functional attributes of these molecules has fostered novel attempts to manipulate the immune response. Although molecules involved in cellular adhesion and antigen-specific recognition have previously been evaluated as targets of therapeutic immunologic intervention, recent attention has focused on a subgroup of cell surface molecules termed co-stimulatory molecules (Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later." Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, 1993; Geppert T, Davis L. Gur H. Wacholtz

-3-

M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991)).

Co-stimulatory molecules do not initiate but rather enable the generation and amplification of antigen-specific T-cell responses and effector function

(Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later." Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, (1993); Geppert T, Davis L. Gur H. Wacholtz M.

Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991); June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994).

Recently, one specific co-stimulatory pathway termed B7:CD28 has been studied by different research groups because of its significant role in B and T cell activation (June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994); June CH, Ledbetter JA: "The role of the CD28 receptor during T-cell responses to antigen." Annu Rev Immunol 11:191, (1993); Schwartz RH: "Co-stimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy." Cell 71:1065, (1992)). Since this

-4-

ligand:receptor pathway was discovered four years ago, a large body of evidence has accumulated suggesting that B7:CD28 interactions represent one of the critical junctures in determining immune reactivity versus anergy (June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994); June CH, Ledbetter JA: "The role of the CD28 receptor during T-cell responses to antigen." Annu Rev Immunol 11:191, (1993); Schwartz RH: "Co-stimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy." Cell 71:1065, (1992); Cohen J: "Mounting a targeted strike on unwanted immune responses" (news; comment). Science 257:751, (1992); Cohen J: "New protein steals the show as 'co-stimulator' of T cells" (news; comment). Science 262:844, (1993)).

In particular, the role of the human B7 antigens, i.e., human B7.1 and B7.2, has been reported to play a co-stimulatory role in T-cell activation.

1. B7.1 and B7.2 Co-stimulatory Role in T Cell Activation

The elaboration of a successful immune response depends on a series of specific interactions between a T cell and an antigen presenting cell. Although the essential first step in this process depends upon the binding of antigen to the T cell receptor, in the context of the MHC class II molecule (Lane, P.J.L., F.M. McConnell, G.L. Schieven, E.A. Clark, and J.A. Ledbetter, (1990), "The Role of Class II Molecules in Human B Cell Activation." The Journal of Immunology, 144:3684-3692), this interaction alone is not sufficient to induce all the events necessary for a sustained

-5-

response to a given antigen (Schwartz, R.H. (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy." Science, 248:1349; Jenkins, M.K. (1992). "The Role of Cell Division in the Induction of Clonal Anergy."

5 Immunology Today, 13:69; Azuma, M., M. Catabyab, D. Buck, J.H. Phillips, and L.L. Lanier, (1992).

"Involvement of CD28 in MHC-unrestricted Cytotoxicity Mediated by a Human Natural Killer Leukemia Cell Line." The Journal of Immunology, 149:1556-1561; Azuma, M., M.

10 Catabyab, D. Buck, J.H. Phillips, and L.L. Lanier, (1992). "CD28 Interaction with B7 Costimulates Primary Allogeneic Proliferative Responses and Cytotoxicity Mediated by Small Resting T Lymphocytes." J. Exp. Med., 175:353-360).

15 The involvement of certain other co-stimulatory molecules is necessary (Norton, S.D., L. Zuckerman, K.B. Urdahl, R. Shefner, J. Miller, and M.K. Jenkins. (1992), "The CD28 Ligand, B7, Enhances IL-2 Production by Providing A Costimulatory Signal to T Cells." The

20 Journal of Immunology, 149:1556-1561). "The homodimers CD28 and CTLA-4 expressed on T cells" (June, C.H., J.A. Ledbetter, P.S. Linsley, and C.B. Thompson, (1990), "Role of the CD28 Receptor in T-Cell Activation."

Immunology Today, 11:211-216; Linsley, P.S., W. Brady, 25 M. Urnes, L.S. Grosmaire, N.K. Damle, and J.A. Ledbetter, (1991), "CTLA-4 is a Second Receptor for the B Cell Activation Antigen B7." J. Exp. Med., 174:561),

together with B7.1 (CD80) and B7.2 (CD86) expressed on antigen presenting cells, are major pairs of co-

30 stimulatory molecules necessary for a sustained immune response (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes." J. Exp. Med., 177:845-850; Freeman, G.J., A.S. Freedman, J.M. Segil,

-6-

G. Lee, J.F. Whitman, and L.M. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells." The Journal of Immunology, 143:2714-2722; Hathcock, K.S., G. Laslo, H.B. Dickler, J. Bradshaw, P. Linsley, and R.J. Hodes, (1993), "Identification of an Alternative CTLA-4 Ligand Costimulatory for T Cell Activation." Science, 262:905-911; Hart, D.N.J., G.C. Starling, V.L. Calder, and N.S. Fernando, (1993). "B7/BB-1 is a Leucocyte Differentiation Antigen on Human Dendritic Cells Induced by Activation." Immunology, 79:616-620). It can be shown *in vitro* that the absence of these co-stimulatory signals leads to an aborted T cell activation pathway and the development of unresponsiveness to the specific antigen, or anergy. (See, e.g., Harding, F.A., J.G. McArthur, J.A. Gross, D.M. Raulet, and J.P. Allison, (1992). "CD28 Mediated Signalling Co-stimulates Murine T Cells and Prevents Induction of Anergy in T Cell Clones." Nature, 356:607-609; Gimmi, C.D., G.J. Freeman, J.G. Gribben, G. Gray, and L.M. Nadler, (1993). "Human T-Cell Clonal Anergy is Induced by Antigen Presentation in the Absence of B7 Costimulation." Proc. Natl. Acad. Sci., 90:6586-6590; Tan, P., C. Anasefti, J.A. Hansen, J. Melrose, M. Brunvand, J. Bradshaw, J.A. Ledbetter, and P.S. Linsley, (1993), "Induction of Alloantigen-specific Hyporesponsiveness in Human T Lymphocytes by Blocking Interaction of CD28 with Its Natural Ligand B7/BB1." J. Exp. Med., 177:165-173). Achievement of *in vivo* tolerance constitutes a mechanism for immunosuppression and a viable therapy for organ transplant rejection and for the treatment of autoimmune diseases. This has been achieved in experimental models following the administration of CTLA4-Ig (Lenschow, D.J., Y. Zeng, R.J. Thistlethwaite, A. Montag, W. Brady,

-7-

M.G. Gibson, P.S. Linsley, and J.A. Bluestone, (1992), "Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA-4Ig." Science, 257:789-795).

5 The molecules B7.1 and B7.2 can bind to either CD28 or CTLA-4, although B7.1 binds to CD28 with a Kd of 200 Nm and to CTLA-4 with a 20-fold higher affinity (Linsley, P.S., E.A. Clark, and J.A. Ledbetter, (1990), "T-Cell Antigen CD28 Mediates Adhesion with B Cells by Interacting with Activation Antigen B7/BB-1." Proc.
10 Natl. Acad. Sci., 87:5031-5035; Linsley et al, (1993), "The Role of the CD28 receptor during T cell responses to antigen," Annu. Rev. Immunol., 11:191-192; Linesley et al, (1993), "CD28 Engagement by B7/BB-1 Induces Transient Down-Regulation of CD28 Synthesis and
15 Prolonged Unresponsiveness to CD28 Signaling," The Journal of Immunology, 150:3151-3169). B7.2 is expressed on activated B cells and interferon induced monocytes, but not resting B cells (Freeman, G.J., G.S. Gray, C.D. Gimmi, D.B. Lomarrd, L-J. Zhou, M. White,
20 J.D. Fingerioth, J.G. Gribben, and LM. Nadler, (1991). "Structure, Expression and T Cell Costimulatory Activity of the Murine Homologue of the Human B Lymphocyte Activation Antigen B7," J. Exp. Med., 174:625-631). B7.2, on the other hand, is constitutively expressed at
25 very low levels on resting monocytes, dendritic cells and B cells, and its expression is enhanced on activated T cells, NK cells and B lymphocytes (Azuma, M. D. Ito, H. Yagita, K. Okumura, J.H. Phillips, L.L. Lanier, and C. Somoza, "1993", "B70 Antigen is a Second Ligand for
30 CTLA-4 and CD28," Nature, 366:76-79). Although B7.1 and B7.2 can be expressed on the same cell type, their expression on B cells occurs with different kinetics (Lenschow, D.J., G.H. Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone,

-8-

(1993), "Expression and Functional Significance of an Additional Ligand for CTLA-4," Proc. Natl. Acad. Sci., USA, 90:11054-11058; Boussiotis, V.A., G.J. Freeman, J.G. Gribben, J. Daley, G. Gray, and L.M. Nadler, (1993), "Activated Human B Lymphocytes Express Three CTLA-4 Counter-receptors that Co-stimulate T-Cell Activation." Proc. Natl. Acad. Sci., USA, 90:11059-11063). Further analysis at the RNA level has demonstrated that B7.2 mRNA is constitutively expressed, whereas B7.1 MRNA is detected 4 hours after activation and initial low levels of B7.1 protein are not detectable until 24 hours after stimulation (Boussiotis, V.A., G.J. Freeman, J.G. Gribben, J. Daley, G. Gray, and L.M. Nadler, (1993), "Activated Human B Lymphocytes Express Three CTLA-4 Counter-receptors that Co-stimulate T-Cell Activation," Proc. Natl. Acad. Sci., USA, 90:11059-11063). CTLA-4/CD28 counter receptors, therefore, may be expressed at various times after B Cell activation.

The differential temporal expression of B7.1 and B7.2 suggests that the interaction of these two molecules with CTLA-4 and/or CD28 deliver distinct but related signals to the T cell (LaSalle, J.M., P.J. Tolentino, G.J. Freeman, L.M. Nadler, and D.A. Hafler, (1992), "CD28 and T Cell Antigen Receptor Signal Transduction Coordinately Regulate Intedeukin 2 Gene Expression In Response to Superantigen Stimulation," J. Exp. Med., 176:177-186; Vandenberghe, P., G.J. Freeman, L.M. Nadler, M.C. Fletcher, M. Kamoun, L.A. Turka, J.A. Ledbetter, C.B. Thompson, and C.H. June, (1992), "Antibody and B7/BB1-mediated Ligation of the CD28 Receptor Induces Tyrosine Phosphorylation in Human T Cells," The Journal of Experimental Medicine, 175:951-960). The exact signaling functions of CTLA-4

-9-

and CD28 on the T cell are currently unknown (Janeway, C.A., Jr. and K. Bottomly, (1994), "Signals and Signs for Lymphocyte Responses," Cell, 76:275-285). However, it is possible that one set of receptors could provide the initial stimulus for T cell activation and the second, a sustained signal to allow further elaboration of the pathway and clonal expansion to take place (Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A. Ledbetter, C. Anasetti, and N.K. Damle, (1992), "Coexpression and Functional Cooperation of CTLA-4 and CD28 on Activated T Lymphocytes," J. Exp. Med., 176:1595-1604). The current data supports the two-signal hypothesis proposed by Jenkins and Schwartz (Schwartz, R.H., (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy," Science, 248:1349; Jenkins, M.K., (1992), "The Role of Cell Division in the Induction of Clonal Anergy," Immunology Today, 13:69) that both a TCR and co-stimulatory signal are necessary for T cell expansion, lymphokine secretion and the full development of effector function (Greenan, V. and G. Kroemer, (1993), "Multiple Ways to Cellular Immune Tolerance," Immunology Today, 14:573). The failure to deliver the second signal results in the inability of T cells to secrete IL-2 and renders the cell unresponsive to antigen.

Structurally, both B7.1 and B7.2 contain extracellular immunoglobulin superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail (Freeman, G.J., J.G. Gribben, V.A. Boussiotis, J.W. Ng, V. Restivo, Jr., L.A. Lombard, G.S. Gray, and L.M. Nadler, (1993), "Cloning of B7-2: A CTLA-4 Counter-receptor that Co-stimulates Human T Cell Proliferation," Science, 262:909). Both B7.1 and B7.2 are heavily glycosylated. B7.1 is a 44-54kD

-10-

glycoprotein comprised of a 223 amino acid extracellular domain, a 23 amino acid transmembrane domain, and a 61 amino acid cytoplasmic tail. B7.1 contains 3 potential protein kinase phosphorylation sites. (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850). B7.2 is a 306 amino acid membrane glycoprotein. It consists of a 220 amino acid extracellular region, a 23 amino acid hydrophobic transmembrane domain and a 60 amino acid cytoplasmic tail (Freeman, G.J., A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and L.M. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells," The Journal of Immunology, 143:2714-2722). Although both B7.1 and B7.2 genes are localized in the same chromosomal region (Freeman, G.J., D.B. Lombard, C.D. Gimmi, S.A. Brod, L Lee, J.C. Laning, D.A. Hafler, M.E. Dorf, G.S. Gray, H. Reiser, C.H. June, C.B. Thompson, and L.M. Nadler, (1992), "CTLA-4 and CD28 mRNA are Coexpressed in Most T Cells After Activation," The Journal of Immunology, 149:3795-3801; Schwartz, R.H., (1992), "Costimulation of T Lymphocytes: The Role of CD28, CTLA-4, and B7/BB1" in Selvakumar, A., B.K. Mohanraj, R.L. Eddy, T.B. Shows, P.C. White, C. Perrin, and B. Dupont, (1992), "Genomic Organization and Chromosomal Location of the Human Gene Encoding the B-Lymphocyte Activation Antigen B7," Immunogenetics, 36:175-181), these antigens do not share a high level of homology. The overall homology between B7.1 and B7.2 is 26% and between murine B7.1 and human S7 is 27% (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850; Freeman, G.J.,

-11-

A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells," The Journal of Immunology, 143:2714-2722).

5 Although alignment of human B7.1 human B7.2 and murine B.1 sequences shows few stretches of lengthy homology, it is known that all three molecules bind to human CTLA-4 and CD28. Thus, there is most likely a common, or closely homologous region shared by the three molecules
10 that may be either contiguous or conformational. This region may constitute the binding site of the B7.1 and B7.2 molecules to their counter-receptors. Antibodies raised against these epitopes could potentially inhibit the interaction of B7 with its counter-receptor on the T
15 cell. Furthermore, antibodies that cross-reacted with this region on both B7.1 and B7.2 molecules would potentially have practical advantages over antibodies directed against B7.1 or B7.2 separately.

2. Blockade of the B7/CD28 Interaction

20 Blocking of the B7/CD28 interaction offers the possibility of inducing specific immunosuppression, with potential for generating long lasting antigen-specific therapeutic effects. Antibodies to either B7.1 or B7.2 have been shown to block T cell activation, as measured
25 by the inhibition of IL-2 production *in vitro* (DeBoer, M., P. Parren, J. Dove, F. Ossendorp, G. van der Horst, and J. Reeder, (1992), "Functional Characterization of a Novel Anti-B7 Monoclonal Antibody," Eur. Journal of Immunology, 22:3071-3075; Azuma, M., H. Yssel, J.H.
30 Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850). However, different antibodies have been shown to vary in their immunosuppressive

-12-

potency, which may reflect either their affinity or epitope specificity. CTLA-4/Ig fusion protein and anti-CD28 Fabs were shown to have similar effects on the down regulation of IL-2 production.

5 *In vivo* administration of a soluble CTLA-4/Ig fusion protein has been shown to suppress T cell - dependent antibody responses in mice (Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A. Ledbetter, C. Anasetti, and N.K. Damle, (1992), "Coexpression and
10 Functional Cooperation of CTLA-4 and CD28 on Activated T Lymphocytes," J. Exp. Med., 176:1595-1604; Lin, H., S.F. Builing, P.S. Linsley, R.O. Wei, C.D. Thompson, and L.A. Turka, (1993), "Long-term Acceptance of Major
15 Histocompatibility Complex Mismatched Cardiac Allografts Induced by CTLA-4-Ig Plus Donor Specific Transfusion," J. Exp. Med., 178:1801) and, furthermore, larger doses were also able to suppress responses to a second immunization, demonstrating the feasibility of this approach for the treatment of antibody mediated
20 autoimmune disease. In addition, CTLA-4/Ig was able to prevent pancreatic islet cell rejection in mice by directly inhibiting the interaction of T cells and B7.1/B7.2 antigen presenting cells (Lenschow, D.J., G.H. Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone, (1993), "Expression and
25 Functional Significance of an Additional Ligand for CTLA-4," Proc. Natl. Acad. Sci., USA, 90:11054-11058). In this case, long term donor specific tolerance was achieved.

-13-

3. Recombinant Phage Display Technology for Antibody Selection

To date, no monoclonal antibodies which crossreact with both B7.1 and B7.2 have been reported. As noted, such antibodies would potentially be highly desirable as immunosuppressants. Phage display technology is beginning to replace traditional methods for isolating antibodies generated during the immune response, because a much greater percentage of the immune repertoire can be assessed than is possible using traditional methods. This is in part due to PEG fusion inefficiency, chromosomal instability, and the large amount of tissue culture and screening associated with heterohybridoma production. Phage display technology, by contrast, relies on molecular techniques for potentially capturing the entire repertoire of immunoglobulin genes associated with the response to a given antigen.

This technique is described by Barber et al, Proc. Natl. Acad. Sci., USA, 88, 7978-7982, (1991). Essentially, immunoglobulin heavy chain genes are PCR amplified and cloned into a vector containing the gene encoding the minor coat protein of the filamentous phage M13 in such a way that a heavy chain fusion protein is created. The heavy chain fusion protein is incorporated into the M13 phage particle together with the light chain genes as it assembles. Each recombinant phage contains, within its genome, the genes for a different antibody Fab molecule which it displays on its surface. Within these libraries, in excess of 10^6 different antibodies can be cloned and displayed. The phage library is panned on antigen coated microliter wells, non-specific phage are washed off, and antigen binding phage are eluted. The genome from the antigen-specific

53361-1

-14-

clones is isolated and the gene III is excised, so that antibody can be expressed in soluble Fab form for further characterization. Once a single Fab is selected as a potential therapeutic candidate, it may easily be converted to a whole antibody. A previously described expression system for converting Fab sequences to whole antibodies is IDEC's mammalian expression vector NEOSPLATM. This vector contains either human gamma 1 or gamma 4 constant region genes. CHO cells are transfected with the NEOSPLATM vectors and after amplification this vector system has been reported to provide very high expression levels (> 30 pg/cell/day) can be achieved.

4. Primatized Antibodies

Another highly efficient means for generating recombinant antibodies is disclosed by Newman, (1992), Biotechnology, 10, 1455-1460. More particularly, this technique results in the generation of primatizedTM antibodies which contain monkey variable domains and human constant sequences. Moreover, this technique is also described in commonly assigned U.S. Application No. 08/379,072, filed on January 25, 1995 (now US Pat. No. 5,658,570), which is a continuation of U.S. Serial No. 07/912,292, filed July 10, 1992, which is a continuation-in-part of U.S. Serial No. 07/856,281, filed March 23, 1992, which is finally a continuation-in-part of U.S. Serial No. 07/735,064, filed July 25, 1991.

This technique modifies antibodies such that they are not antigenically rejected upon administration in humans. This technique relies on immunization of

-15-

cynomolgus monkeys with human antigens or receptors. This technique was developed to create high affinity monoclonal antibodies directed to human cell surface antigens.

5 Antibodies generated in this manner have previously been reported to display human effector function, have reduced immunogenicity, and long serum half-life. The technology relies on the fact that despite the fact that
10 cynomolgus monkeys are phylogenetically similar to humans, they still recognize many human proteins as foreign and therefore mount an immune response. Moreover, because the cynomolgus monkeys are phylogenetically close to humans, the antibodies generated in these monkeys have been discovered to have
15 a high degree of amino acid homology to those produced in humans. Indeed, after sequencing macaque immunoglobulin light and heavy chain variable region genes, it was found that the sequence of each gene family was 85-98% homologous to its human counterpart
20 (Newman et al, (1992), Id.). The first antibody generated in this way, an anti-CD4 antibody, was 91-92% homologous to the consensus sequence of human immunoglobulin framework regions. Newman et al, Biotechnology, 10:1458-1460, (1992).

25 Monoclonal antibodies specific to the human B7 antigen have been previously described in the literature. For example, Weyl et al, Hum. Immunol., 31(4), 271-276, (1991) describe epitope mapping of human monoclonal antibodies against HLA-B-27 using natural and
30 mutated antigenic variants. Also, Toubert et al, Clin. Exp. Immunol., 82(1), 16-20, (1990) describe epitope mapping of an HLA-B27 monoclonal antibody that also reacts with a 35-KD bacterial outer membrane protein. Also, Valle et al, Immunol., 69(4), 531-535, (1990)

-16-

describe a monoclonal antibody of the IgG1 subclass which recognizes the B7 antigen expressed in activated B cells and HTLV-1-transformed T cells. Further, Toubert et al, J. Immunol., 141(7), 2503-9, (1988) describe
5 epitope mapping of HLA-B27 and HLA-B7 antigens using intradomain recombinants constructed by making hybrid genes between these two alleles in *E. coli*.

High expression of B7 antigen has been correlated to autoimmune diseases by some researchers. For
10 example, Ionesco-Tirgoviste et al, Med. Interre, 24(1), 11-17, (1986) report increased B7 antigen expression in type 1 insulin-dependent diabetes. Also, the involvement of B7 antigen expression on dermal dendritic cells obtained from psoriasis patients has been
15 reported. (Nestle et al, J. Clin. Invest., 94(1), 202-209, (1994)).

Further, the inhibition of anti-HLA-B7 alloreactive CTL using affinity-purified soluble HLA-B7 has been reported in the literature. (Zavazava et al,
20 Transplantation, 51(4), 838-42, (1991)). Further, the use of B7 receptor soluble ligand, CTLA-4-Ig to block B7 activity (See, e.g., Lenschow et al, Science, 257, 789, 7955 (1992)) in animal models and a B7-1-Ig fusion protein capable of inhibiting B7 has been reported.

25 SUMMARY AND OBJECTS OF THE INVENTION

An object of the invention is to produce and identify novel macaque antibodies to human B7 antigen, more specifically to human B7.1 antigen and/or human B7.2 antigen.

30 More specifically, it is an object of the present invention to produce and identify novel macaque antibodies to human B7 antigen, i.e., human B7.1 and human B7.2 antigen by screening of phage display

53361-1

-17-

libraries and/or monkey heterohybridomas using B lymphocytes obtained from human B7 antigen, i.e., human B7.1 or B7.2 antigen immunized monkeys.

It is another specific object of the invention to
5 provide anti-B7 monkey monoclonal antibodies and
primatizedTM forms thereof which specifically bind human
B7.1 and/or B7.2 antigen which inhibit the B7/CD86
pathway and B7 stimulation of activated T cells, thereby
inhibiting IL-2 production and T cell proliferation and
10 functioning as effective immunosuppressants.

It is another object of the invention to provide
anti-human B7.1 and anti-human B7.2 monkey monoclonal
antibodies and primatizedTM forms thereof which inhibit
antigen driven responses in donor spleen cell cultures,
15 e.g., antigen specific IgG responses, IL-2 production
and cell proliferation.

It is another specific object of the invention to
identify particular monkey monoclonal antibodies
specific to human B7.1 and human B7.2 antigen and
20 primatizedTM forms thereof having advantageous properties,
i.e., affinity, immunosuppressive activity, which are
useful as therapeutics. More specifically, these monkey
antibodies and primatizedTM forms thereof are to be used,
e.g., as immunosuppressants, i.e., to block antigen
25 driven immune responses, to treat autoimmune diseases
such as psoriasis, rheumatoid arthritis, systemic
erythematosus (SLE), type 1 diabetes mellitus,
idiopathic thrombocytopenia purpura (ITP), and to
prevent organ rejection.

30 It is another object of the invention to provide
pharmaceutical compositions containing one or more
monkey monoclonal antibodies specific to human B7
antigen, i.e., human B7.1 and/or human B7.2 antigen, or
primatizedTM forms thereof, and a pharmaceutically

53361-1

-18-

acceptable carrier or excipient. These compositions will be used, e.g., as immunosuppressants to treat autoimmune diseases, e.g., idiopathic thrombocytopenia purpura (ITP) and systemic lupus erythematosus (SLE), to
5 block antigen driven immune responses, and to prevent organ rejection in transplant recipients.

It is another object of the invention to provide novel methods of therapy by administration of therapeutically effective amounts of one or more monkey
10 or primatizedTM monoclonal antibodies which specifically bind to B7 antigen, i.e., human B7.1 and/or B7.2 antigens. Such therapeutic methods are useful for treatment of diseases treatable by inhibition of the B7:CD28 pathway e.g., autoimmune diseases such as
15 idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, psoriasis, rheumatoid arthritis, multiple sclerosis, aplastic anemia, as well as for preventing rejection in transplantation subjects.

20 It is still another object of the invention to provide transfectants, e.g., CHO cells, which express at least the variable heavy and light domains of monkey monoclonal antibodies specific to the human B7.1 and/or B7.2 antigen.

25 It is another object of the invention to provide nucleic acid sequences which encode the variable heavy and/or light domains of monkey monoclonal antibodies specific to human B7.1 and/or human B7.2 antigen, and expression vectors which provide for the expression of
30 primatizedTM antibodies containing these nucleic acid sequences.

53361-1

-18a-

In one aspect, the invention provides a monkey monoclonal antibody, a primatized form thereof, or a fragment of the monkey monoclonal antibody or primatized form thereof, that binds to human CD80 antigen and comprises light and heavy chain variable region polypeptide sequences selected from the group consisting of: (a) the light and heavy chain variable region polypeptide sequences of antibody 7C10 contained in SEQ ID NO:2 and SEQ ID NO:4, respectively; (b) the light and heavy chain variable region polypeptide sequences of antibody 7B6 contained in SEQ ID NO:6 and SEQ ID NO:8, respectively; and (c) the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

In another aspect, the invention provides a monkey monoclonal antibody, a primatized form thereof, or a fragment of the monkey monoclonal antibody or primatized form thereof, that binds to an epitope of human CD80 antigen that is also bound by an antibody comprising light and heavy chain variable region polypeptide sequences selected from the group consisting of: (a) the light and heavy chain variable region polypeptide sequences of antibody 7C10 contained in SEQ ID NO:2 and SEQ ID NO:4, respectively; (b) the light and heavy chain variable region polypeptide sequences of antibody 7B6 contained in SEQ ID NO:6 and SEQ ID NO:8, respectively; and (c) the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

In another aspect, the invention provides the monoclonal antibody as described above, that comprises a light chain constant region of a human antibody light chain, and a heavy chain constant region of a human antibody heavy chain.

53361-1

-18b-

In another aspect, the invention provides a cultured cell that produces an antibody or fragment thereof as described above.

In another aspect, the invention provides a
5 pharmaceutical composition that contains an antibody or a fragment thereof that binds to human CD80 antigen as described above and a pharmaceutical carrier.

In another aspect, the invention provides use of an antibody or fragment thereof that binds to human CD80
10 antigen as described above in the manufacture of a medicament for the treatment or prevention of a disease or disorder selected from the group consisting of autoimmune disorder, graft versus host disease, rejection of a transplanted organ or tissue, infectious disease,
15 inflammatory disease, and B cell lymphoma.

In another aspect, the invention provides use of an antibody or fragment thereof that binds to human CD80 antigen as described above in the manufacture of a medicament for the treatment or prevention of a B cell
20 lymphoma.

In another aspect, the invention provides use of an antibody or fragment thereof that binds to human CD80 antigen as described above in the treatment or prevention of a disease or disorder selected from the group consisting of
25 autoimmune disorder, graft versus host disease, rejection of a transplanted organ or tissue, infectious disease, inflammatory disease, and B cell lymphoma.

In another aspect, the invention provides use of an antibody or fragment thereof that binds to human CD80
30 antigen as described above in the treatment or prevention of graft versus host disease or rejection of a transplanted

53361-1

-18c-

organ or tissue, wherein the graft or transplanted organ or tissue is selected from the group consisting of kidney, heart, lung, bone marrow, skin, and cornea.

In another aspect, the invention provides use of
5 an antibody or fragment thereof that binds to human CD80 antigen as described above in the treatment or prevention of a B cell lymphoma.

In another aspect, the invention provides an antibody or fragment thereof that binds to human CD80
10 antigen as described above for use in the treatment or prevention of a disease or disorder selected from the group consisting of autoimmune disorder, graft versus host disease, rejection of a transplanted organ or tissue, infectious disease, inflammatory disease, and B cell
15 lymphoma.

In another aspect, the invention provides an antibody or fragment thereof that binds to human CD80 antigen as described above for use in the treatment or prevention of graft versus host disease or rejection of a
20 transplanted organ or tissue, wherein the graft or transplanted organ or tissue is selected from the group consisting of kidney, heart, lung, bone marrow, skin, and cornea.

In another aspect, the invention provides an
25 antibody or fragment thereof that binds to human CD80 antigen as described above for use in the treatment or prevention of a B cell lymphoma.

Definitions

The following terms are defined so that the invention may be more clearly understood.

Depleting antibody - an antibody which kills activated B cells or other antigen presenting cells.

Non-depleting antibody - an antibody which blocks the co-stimulatory action of B7 and T cell activating ligands CD28 and CTLA-4. Thus, it anergizes but does not eliminate the antigen presenting cell.

10 PrimatizedTM antibody - a recombinant antibody which has been engineered to contain the variable heavy and light domains of a monkey antibody, in particular, a cynomolgus monkey antibody, and which contains human constant domain sequences, preferably the human
15 immunoglobulin gamma 1 or gamma 4 constant domain (or PE variant). The preparation of such antibodies is described in Newman et al, (1992), "Primatization of Recombinant Antibodies for Immunotherapy of Human Diseases: A Macaque/Human Chimeric Antibody Against
20 Human CDH, Biotechnology, 10:1458-1460; also in commonly assigned 08/379,072 (US 5,658,570). These antibodies have been reported to exhibit a high degree of homology to human antibodies, i.e., 85-98%, display human effector functions, have reduced immunogenicity, and may
25 exhibit high affinity to human antigens.

B7 antigens - B7 antigens in this application include, e.g., human B7, B7.1 and B7.2 antigens. These antigens bind to CD28 and/or CTLA-4. These antigens have a co-stimulatory role in T cell activation. Also, these B7
30 antigens all contain extracellular immunoglobulin superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail. (See,

53361-1

-20-

Freeman et al, Science, 262:909, (1993)), and are heavily glycosylated.

Anti-B7 antibodies - Antibodies, preferably monkey monoclonal antibodies or primatizedTM forms thereof, which specifically bind human B7 antigens, e.g., human B7.1 and/or B7.2 antigen with a sufficient affinity to block the B7:CD28 interaction and thereby induce immunosuppression.

BRIEF DESCRIPTION OF THE FIGURES

10 Figure 1 depicts the pMS vector used to screen recombinant immunoglobulin libraries produced against B7 displayed on the surface of filamentous phage which contains primers based on macaque immunoglobulin sequences.

15 Figure 2 depicts the NEOSPLATM expression vector used to express the subject primatizedTM antibodies specific to human B7.1 antigen.

20 Figure 3 depicts monkey serum anti-B7.1 titers directed against cell surface B7.1 on transfected CHO cells.

 Figure 4 depicts inhibition of radiolabeled SB7.1 binding by SB7.1 affinity-purified monkey antibodies in the presence of unlabeled SB7 and Mab L307.4 murine anti-B7.1.

25 Figure 5 depicts inhibition of binding of radiolabeled monkey 135 and L3707.4 anti-B7.1 antibodies to B7 positive human SB cells by competition with affinity-purified SB7.1.

30 Figure 6 depicts inhibition of radiolabeled B7-Ig binding to activated human peripheral blood T cells by competing with unlabeled SB7.1 murine anti-B7.1 (L307.4) and monkey 1127 affinity purified serum antibodies.

53361-1

-21-

Figure 7 depicts inhibition of IL-2 protein in mixed lymphocyte cultures by anti-B7.1 affinity-purified monkey serum antibodies.

Figure 8a depicts the amino acid and nucleic acid sequence of a primatized[™] form of the light chain of 7C10 (SEQ ID NOS: 1-2).

Figures 8b-1 and 8b-2 depict the amino acid and nucleic acid sequence of a primatized[™] form of the heavy chain of 7C10 (SEQ ID NOS: 3-4).

Figure 9a depicts the amino acid and nucleic acid sequence of a primatized[™] form of the light chain of 7B6 (SEQ ID NOS: 5-6).

Figures 9b-1 and 9b-2 depict the amino acid and nucleic acid sequence of a primatized[™] form of the heavy chain of 7B6 (SEQ ID NOS: 7-8).

Figure 10a depicts the amino acid and nucleic acid sequence of a primatized[™] light chain 16C10 (SEQ ID NOS: 9-10).

Figures 10b-1 and 10b-2 depict the amino acid and nucleic acid sequence of a primatized[™] heavy chain 16C10 (SEQ ID NOS: 11-12).

DETAILED DESCRIPTION OF THE INVENTION

As described above, the present invention relates to the manufacture of novel monkey monoclonal antibodies which specifically bind human B7.1 and/or human B7.2 antigen, as well as primatized[™] antibodies derived therefrom. These antibodies possess high affinity to human B7.1 and/or B7.2 and therefore may be used as immunosuppressants which inhibit the B7:CD86 pathway.

Preparation of monkey monoclonal antibodies will preferably be effected by screening of phage display libraries or by preparation of monkey heterohybridomas using B lymphocytes obtained from B7 (e.g., human B7.1 and/or B7.2) immunized monkeys.

As noted, the first method for generating anti-B7 antibodies involves recombinant phage display

53361-1

-22-

technology. This technique is generally described *supra*.

Essentially, this will comprise synthesis of recombinant immunoglobulin libraries against B7 antigen displayed on the surface of filamentous phage and selection of phage which secrete antibodies having high affinity to B7.1 and/or B7.2 antigen. As noted *supra*, preferably antibodies will be selected which bind to both human B7.1 and B7.2. To effect such methodology, the present inventors have created a unique library for monkey libraries which reduces the possibility of recombination and improves stability. This vector, pMS, is described in detail *infra*, and is shown in Figure 1.

Essentially, to adopt phage display for use with macaque libraries, this vector contains specific primers for PCR amplifying monkey immunoglobulin genes. These primers are based on macaque sequences obtained while developing the primatizedTM technology and databases containing human sequences.

Suitable primers are disclosed in commonly assigned 08/379,072 (US 5,658,570).

The second method involves the immunization of monkeys, i.e., macaques, against human B7 antigen, preferably against human B7.1 and B7.2 antigen. The inherent advantage of macaques for generation of monoclonal antibodies is discussed *supra*. In particular, such monkeys, i.e., cynomolgus monkeys, may be immunized against human antigens or receptors. Moreover, the resultant antibodies may be used to make primatized antibodies according to the methodology of Newman et al, Biotechnology, 10, 1455-1460, (1992), and Newman et al, commonly assigned U.S. Serial No. 08/379,072, filed January 25, 1995, (US 5,658,570).

-23-

The significant advantage of antibodies obtained from cynomolgus monkeys is that these monkeys recognize many human proteins as foreign and thereby provide for the formation of antibodies, some with high affinity to
5 desired human antigens, e.g., human surface proteins and cell receptors. Moreover, because they are phylogenetically close to humans, the resultant antibodies exhibit a high degree of amino acid homology to those produced in humans. As noted above, after sequencing
10 macaque immunoglobulin light and heavy variable region genes, it was found that the sequence of each gene family was 85-88% homologous to its human counterpart (Newman et al, (1992), Id.).

Essentially, cynomolgus macaque monkeys are
15 administered human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, B cells are isolated therefrom, e.g., lymph node biopsies are taken from the animals, and B lymphocytes are then fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol
20 (PEG). Heterohybridomas secreting antibodies which bind human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, are then identified.

Antibodies which bind to both B7.1 and B7.2 are desirable because such antibodies potentially may be
25 used to inhibit the interaction of B7.1 and B7.2, as well as B7 with their counter-receptors, i.e., human CTLA-4 and CD28. Antibodies against these epitopes may inhibit the interaction of both human B7.1 and human B7.2 with their counter receptors on the T cell. This
30 may potentially provide synergistic effects.

However, antibodies which bind to only one of human B7 antigen, B7.1 antigen or B7.2 antigen, are also highly desirable because of the co-involvement of these molecules in T cell activation, clonal expansion

-24-

lymphokine (IL-2) secretion, and responsiveness to antigen. Given that both human B7.1 and B7.2 bind to human CTLA-4 and CD28, it is probable that there is at least one common or homologous region (perhaps a shared conformational epitope or epitopes) to which macaque antibodies may potentially be raised.

The present inventors elected to immunize macaques against human B7.1 antigen using recombinant soluble B7.1 antigen produced in CHO cells and purified by affinity chromatography using a L307.4-sepharose affinity column. However, the particular source of human B7 antigen, human B7.1 antigen or human B7.2 antigen is not critical, provided that it is of sufficient purity to result in a specific antibody response to the particular administered B7 antigen and potentially to other B7 antigens.

The human B7 antigen, human B7.1 antigen (also called CD80) and human B7.2 antigen (also called CD86) genes have been cloned, and sequenced, and therefore may readily be manufactured by recombinant methods.

Preferably, the administered human B7 antigen, human B7.1 antigen and/or human B7.2 antigen will be administered in soluble form, e.g., by expression of a B7, B7.1 or B7.2 gene which has its transmembrane and cytoplasmic domains removed, thereby leaving only the extracellular portion, i.e., the extracellular superfamily V and C-like domains. (See, e.g., Grumet et al, Hum. Immunol., 40(3), p. 228-234, 1994, which teaches expression of a soluble form of human B7, which is incorporated by reference in its entirety herein).

The macaques will be immunized with the B7, B7.1 and/or B7.2 antigen, preferably a soluble form thereof, under conditions which result in the production of antibodies specific thereto. Preferably, the soluble

53361-1

-25-

human B7, B7.1 or B7.2 antigen will be administered in combination with an adjuvant, e.g., Complete Freund's Adjuvant (CFA), Alum, Saponin, or other known adjuvants, as well as combinations thereof. In general, this will
5 require repeated immunization, e.g., by repeated injection, over several months. For example, administration of soluble B7.1 antigen was effected in adjuvant, with booster immunizations, over a 3 to 4 month period, with resultant production of serum
10 containing antibodies which bound human B7.1 antigen.

After immunization B cells are collected, e.g., by lymph node biopsies taken from the immunized animals and B lymphocytes fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol. Methods
15 for preparation of such heteromyelomas are known and may be found in U.S. Serial No. 08/379,072 by Newman et al, filed on January 25, 1995.

Heterohybridomas which secrete antibodies which
20 bind human B7, B7.1 and/or B7.2 are then identified. This may be effected by known techniques. For example, this may be determined by ELISA or radioimmunoassay using enzyme or radionuclide labelled human B7, B7.1 and/or B7.2 antigen.

25 Cell lines which secrete antibodies having the desired specificity to human B7, B7.1 and/or B7.2 antigen are then subcloned to monoclonality.

In the present invention, the inventors screened purified antibodies for their ability to bind to soluble
30 B7.1 antigen coated plates in an ELISA assay, antigen positive B cells, and CHO transfectomas which express human B7.1 antigen on their cell surface. In addition, the antibodies were screened for their ability to block B cell/T cell interactions as measured by IL-2

53361-1

-26-

production and tritiated thymidine uptake in a mixed lymphocyte reaction (MLR), with B7 binding being detected using ^{125}I -radiolabeled soluble B7.1 (SB7.1).

Also, affinity purified antibodies from macaques
5 were tested for their reactivity against CHO
transfectants which expressed B7.1/Ig fusion proteins,
and against CHO cells which produced human B7.2 antigen.
These results indicated that the B7.1 immune sera bound
to the B7.2 transfectomas. Binding of antibodies to
10 B7.2 antigen may be confirmed using soluble B7.2-Ig
reagents. As discussed in the examples, this may be
effected by producing and purifying B7.2-Ig from CHO
transfectomas in sufficient quantities to prepare a
B7.2-Ig-sepharoseTM affinity column. Those antibodies
15 which cross-react with B7.2 will bind the B7.2-Ig-
sepharose column.

Cell lines which express antibodies which
specifically bind to human B7 antigen, B7.1 antigen
and/or B7.2 antigen are then used to clone variable
20 domain sequences for the manufacture of primatizedTM
antibodies essentially as described in Newman et al,
(1992), Id. and Newman et al, U.S. Serial No. 379,072,
filed January 25, 1995, (US 5,658,570). Essentially, this
entails extraction of RNA therefrom, conversion to cDNA, and
25 amplification thereof by PCR using Ig specific primers.
Suitable primers are described in Newman et al, 1992,
Id. and in U.S. Serial No. 379,072 (US 5,658,570). (See, in
particular, Figure 1 of U.S. Serial No. 379,072 (US 5,658,570)).

The cloned monkey variable genes are then inserted
30 into an expression vector which contains human heavy and
light chain constant region genes. Preferably, this is
effected using a proprietary expression vector of IDEC,
Inc., referred to as NEOSPLATM. This vector is shown in

53361-1

-27-

Figure 2 and contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation sequence, neomycin
5 phosphotransferase exon 1 and exon 2, human immunoglobulin kappa or lambda constant region, the dihydrofolate reductase gene, the human immunoglobulin gamma 1 or gamma 4 PE constant region and leader
10 sequence. This vector has been found to result in very high level expression of primatizedTM antibodies upon incorporation of monkey variable region genes, transfection in CHO cells, followed by selection in G418 containing medium and methotrexate amplification.

For example, this expression system has been
15 previously disclosed to result in primatizedTM antibodies having high avidity ($K_d \leq 10^{-10}$ M) against CD4 and other human cell surface receptors. Moreover, the antibodies have been found to exhibit the same affinity, specificity and functional activity as the original
20 monkey antibody. This vector system is substantially disclosed in commonly assigned U.S. Serial No. 379,072, (US 5,658,570) as well as U.S. Serial No. 08/149,099 (US 5,736,136), filed on November 3, 1993. This system
25 provides for high expression levels, i.e., > 30 pg/cell/day.

As discussed *infra*, the subject inventors have selected four lead candidate monkey monoclonal antibodies which specifically bind the B7.1 antigen, and which may also bind the B7.2 antigen. These monkey monoclonal antibodies are referred to herein as 7B6,
30 16C10, 7C10 and 20C9.

As discussed in greater detail *infra*, these antibodies were evaluated for their ability to block B

-28-

cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in a mixed lymphocyte reaction for T cell binding experiments for T cell binding, human body coat peripheral blood lymphocytes were cultured for 3-6 days in the presence of PHA stimulator. B7 binding was radioassayed using ¹²⁵I-radiolabeled soluble B7.1. The observed results indicate that all of these antibodies bind B7.1 antigen with high affinity and effectively block B cell/T cell interactions as evidenced by reduced IL-2 production and reduced proliferation of mixed lymphocyte cultures.

The properties of these particular monkey monoclonal antibodies are summarized below:

1. To demonstrate the monkey antibodies' ability to block the physical interaction between CTLA4-Ig, varying concentrations of the monkey anti-B7.1 antibodies and unlabeled CTLA4-IG were incubated with radiolabeled CTLA4-Ig¹²⁵. The results of the inhibition assay showed that the IC50 (the concentration of inhibitor which results in 50% inhibition) for the monkey antibodies are:

a:	7C10:	0.39 µg/Ml
b:	16C10:	1.60 µg/Ml
c:	20C9:	3.90 µg/Ml
d:	7B6:	39.0 µg/Ml

2. Scatchard analysis showed that the apparent affinity constants (Kd) for the monkey antibodies binding to B7-Ig coated plates were approximated to be:

a:	7C10:	6.2 x 10 ⁻⁹ M
b:	16C10:	8.1 x 10 ⁻⁹ M
c:	7B6:	10.7 x 10 ⁻⁹ M

-29-

d: 20C9: $16.8 \times 10^{-9}M$

3. The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR showed that all 4 anti-B7.1 antibodies inhibit IL-2 production to different extents as shown by the following Ibgo values:

a: 7B6: 5.0 $\mu g/M$
b: 16C10: <0.1 $\mu g/M$
c: 20C9: 2.0 $\mu g/M$
d: 7C10: 5.0 $\mu g/M$

4. The monkey anti-B7.1 antibodies were tested for their ability to bind B7 on human peripheral blood lymphocytes (PBL). FACS analysis showed that all 4 monkey antibodies tested positive.

5. Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were tested for C1q binding by FACS analysis. Results showed 7C10 monkey Ig had strong human C1q binding after incubating with B7.1 CHO-transfected cells. 16C10 was positive, while 20C9 and 7B6 monkey antibodies were negative.

6. To select an animal model for path-tox studies, the monkey antibodies were tested with animal blood from different species. It was determined that the monkey anti-B7.1 antibodies cross-reacted with human, chimpanzee, and possibly baboon.

Based on these properties, it would appear that three monkey monoclonal antibodies possess the most advantageous properties, 16C10, 7C10 and 20C9, with 16C10 and 7C10 being somewhat better than 20C9.

53361-1

-30-

Using the techniques described *supra*, and in commonly assigned U.S. Serial No. 08/379,072 (US 5,658,570), the present inventors have cloned the variable domains of 7C10, 7B6 and 16C10, and provide the amino acid and nucleic acid sequences of primatizedTM forms of the 7C10 light chain, 7C10 heavy chain, 7B6 light chain, 7B6 heavy chain, 16C10 light chain and 16C10 heavy chain. These amino acid and nucleic acid sequences may be found in Figures 8a and 8b, 9a and 9b, and 10a and 10b. The DNA and amino acid sequence for the human gamma 1 constant domain may be found in 08/379,072.

As discussed *supra*, these primatized antibodies are preferably expressed using the NEOSPLATM expression vector shown in Figure 2 which is substantially described in commonly assigned 08/379,072 (US 5,658,570) and 08/149,099 (US 5,736,137).

As previously noted, the subject primatizedTM antibodies will preferably contain either the human immunoglobulin gamma 1 or gamma 4 constant region, with gamma 4 preferably mutated at two positions to create gamma 4 PE. The gamma 4 PE mutant contains two mutations, a glutamic acid in the CH2 region introduced to eliminate residual FCR binding, and a proline substitution in the hinge region, intended to enhance the stability of the heavy chain disulfide bond interaction. (See, Alegre et al, J. Immunol., 148, 3461-3468, (1992); and Angel et al, Mol. Immunol., 30, 105-158; (1993)).

Whether the subject primatizedTM antibodies contain the gamma 1, gamma 4 or gamma 4 PE constant region largely depends on the particular disease target. Preferably, depleting and non-depleting primatizedTM IgG1

53361-1

-31-

and IgG4 antibodies are created and tested against specific disease targets.

Given the described binding and functional properties of the subject monkey monoclonal antibodies, these anti-B7.1 monoclonal antibodies and primatizedTM forms thereof should be well suited as therapeutic agents for blocking the B7:CD28 interaction thereby providing for immunosuppression. In particular, given their high affinity to B7.1 antigen and ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in mixed lymphocyte culture as well as their ability to effectively inhibit antigen driven responses in donor spleen cell cultures as shown by reduced antigen specific IgG responses, IL-2 production and cell proliferation, these monkey monoclonal antibodies and primatizedTM forms thereof should function as effective immunosuppressants which modulate the B7:CD28 pathway. This is significant for the treatment of many diseases wherein immunosuppression is therapeutically desirable, e.g., autoimmune diseases, to inhibit undesirable antigen specific IgG responses, and also for prevention of organ rejection and graft-versus-host disease. Essentially, the subject antibodies will be useful in treating any disease wherein suppression of the B7:CD28 pathway is therapeutically desirable.

Key therapeutic indications for the subject anti-B7.1 antibodies include, by way of example, autoimmune diseases such as idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, multiple sclerosis, aplastic anemia, psoriasis and rheumatoid arthritis.

Another significant therapeutic indication of the subject anti-B7.1 antibodies is for prevention of graft-

53361-1

-32-

versus-host-disease (GVHD) during organ transplant and bone marrow transplant (BMT). The subject antibodies may be used to induce host tolerance to donor-specific alloantigens and thereby facilitate engraftment and
5 reduce the incidence of graft rejection. It has been shown in a murine model of allogeneic cardiac transplantation that intravenous administration of CTLA4-Ig can result in immunosuppression or even induction of tolerance to alloantigen. (Lin et al, J. Exp. Med. 178:1801, 1993; Torka et al, Proc. Natl. Acad. Sci., USA, 89:11102, 1992). It is expected that the
10 subject primatized anti-B7.1 antibodies will exhibit similar or greater activity.

Antibodies produced in the manner described above,
15 or by equivalent techniques, can be purified by a combination of affinity and size exclusion chromatography for characterization in functional biological assays. These assays include determination of specificity and binding affinity as well as effector
20 function associated with the expressed isotype, e.g., ADCC, or complement fixation. Such antibodies may be used as passive or active therapeutic agents against a number of human diseases, including B cell lymphoma, infectious diseases including AIDS, autoimmune and
25 inflammatory diseases, and transplantation. The antibodies can be used either in their native form, or as part of an antibody/chelate, antibody/drug or antibody/toxin complex. Additionally, whole antibodies or antibody fragments (Fab₂, Fab, Fv) may be used as
30 imaging reagents or as potential vaccines or immunogens in active immunotherapy for the generation of anti-idiotypic responses.

The amount of antibody useful to produce a therapeutic effect can be determined by standard

-33-

techniques well known to those of ordinary skill in the art. The antibodies will generally be provided by standard technique within a pharmaceutically acceptable buffer, and may be administered by any desired route.

5 Because of the efficacy of the presently claimed antibodies and their tolerance by humans it is possible to administer these antibodies repetitively in order to combat various diseases or disease states within a human.

10 The anti-B7.1 antibodies (or fragments thereof) of this invention are useful for inducing immunosuppression, i.e., inducing a suppression of a human's or animal's immune system. This invention therefore relates to a method of prophylactically or
15 therapeutically inducing immunosuppression in a human or other animal in need thereof by administering an effective, non-toxic amount of such an antibody of this invention to such human or other animal.

The ability of the compounds of this invention to
20 induce immunosuppression has been demonstrated in standard tests used for this purpose, for example, a mixed lymphocyte reaction test or a test measuring inhibition of T-cell proliferation measured by thymidine uptake.

25 The fact that the antibodies of this invention have utility in inducing immunosuppression indicates that they should be useful in the treatment or prevention of resistance to or rejection of transplanted organs or tissues (e.g., kidney, heart, lung, bone marrow, skin,
30 cornea, etc.); the treatment or prevention of autoimmune, inflammatory, proliferative and hyperproliferative diseases, and of cutaneous manifestations of immunologically mediated diseases (e.g., rheumatoid arthritis, lupus erythematosus,

-34-

systemic lupus erythematosus, Hashimotos thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, nephrotic syndrome, psoriasis, atypical dermatitis, contact dermatitis and further eczematous dermatitides, seborrheic dermatitis, Lichen planus, Pemphigus, bullous pemphigus, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythema, cutaneous eosinophilias, Alopecia areata, etc.); the treatment of reversible obstructive airways disease, intestinal inflammations and allergies (e.g., Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis) and food-related allergies (e.g., migraine, rhinitis and eczema).

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of antibody would be for the purpose of inducing immunosuppression. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The antibodies (or fragments thereof) of this invention should also be useful for treating tumors in a mammal. More specifically, they should be useful for reducing tumor size, inhibiting tumor growth and/or prolonging the survival time of tumor-bearing animals. Accordingly, this invention also relates to a method of treating tumors in a human or other animal by administering to such human or animal an effective, non-toxic amount of an antibody. One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of anti-B7 antibody would be for the purpose of treating carcinogenic tumors. Generally, however, an effective dosage is

-35-

expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

5 The antibodies of the invention may be administered to a human or other animal in accordance with the
10 aforementioned methods of treatment in an amount sufficient to produce such effect to a therapeutic or prophylactic degree. Such antibodies of the invention can be administered to such human or other animal in a conventional dosage form prepared by combining the
15 antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is
dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

The route of administration of the antibody (or fragment thereof) of the invention may be oral,
20 parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. The subcutaneous and intramuscular forms of parenteral administration are
25 generally preferred.

The daily parenteral and oral dosage regimens for employing compounds of the invention to prophylactically or therapeutically induce immunosuppression, or to
30 therapeutically treat carcinogenic tumors will generally be in the range of about 0.05 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

The antibodies of the invention may also be administered by inhalation. By "inhalation" is meant

-36-

intranasal and oral inhalation administration.

Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The preferred dosage amount of a compound of the invention to be employed is generally within the range of about 10 to 100 milligrams.

The antibodies of the invention may also be administered topically. By topical administration is meant non-systemic administration and includes the application of an antibody (or fragment thereof) compound of the invention externally to the epidermis, to the buccal cavity and instillation of such an antibody into the ear, eye and nose, and where it does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of an antibody required for therapeutic or prophylactic effect will, of course, vary with the antibody chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician. A suitable topical dose of an antibody of the invention will generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

Formulations

While it is possible for an antibody or fragment thereof to be administered alone, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g., from 1% to 2% by weight of the formulation, although it may comprise as much as 10%

-37-

w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

5 The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredients(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

10 Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for
15 administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a
20 bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by
25 autoclaving or maintaining at 90°-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops
30 are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

-38-

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared
5 by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor
10 oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided
15 or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap;
20 a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any
25 suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous
30 silicas, and other ingredients such as lanolin, may also be included.

The subject anti-B7.1 antibodies or fragments thereof may also be administered in combination with other moieties which modulate the B7:CD28 pathway. Such

-39-

moieties include, by way of example, cytokines such as IL-7 and IL-10, CTLA4-Ig, soluble CTLA-4 and anti-CD28 antibodies and fragments thereof.

5 It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an antibody or fragment thereof of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular animal being treated,
10 and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of
15 days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Without further elaboration, it is believed that one skilled in the art can, using the preceding
20 description, utilize the present invention to its fullest extent. The following formulations are, therefore, to be construed as merely illustrative embodiments and not a limitation of the scope of the present invention in any way.

25 Capsule Composition

A pharmaceutical composition of this invention in the form of a capsule is prepared by filling a standard two-piece hard gelatin capsule with 50 mg. of an antibody or fragment thereof of the invention, in
30 powdered form, 100 mg. of lactose, 32 mg. of talc and 8 mg. of magnesium stearate.

53361-1

-40-

Injectable Parenteral Composition

A pharmaceutical composition of this invention in a form suitable for administration by injection is prepared by stirring 1.5% by weight of an antibody or
5 fragment thereof of the invention in 10% by volume propylene glycol and water. The solution is sterilized by filtration.

Ointment Composition

Antibody or fragment thereof of the invention
10 1.0 g.

White soft paraffin to 100.0 g.

The antibody or fragment thereof of the invention is dispersed in a small volume of the vehicle to produce a smooth, homogeneous product. Collapsible metal tubes
15 are then filled with the dispersion.

Topical Cream Composition

Antibody or fragment thereof of the invention
1.0 g.

PolawaxTM GP 200 20.0 g.

20 Lanolin Anhydrous 2.0 g.

White Beeswax 2.5 g.

Methyl hydroxybenzoate 0.1 g.

Distilled Water to 100.0 g.

The polawax, beeswax and lanolin are heated
25 together at 60°C. A solution of methyl hydroxybenzoate is added and homogenization is achieved using high speed stirring. The temperature is then allowed to fall to 50°C. The antibody or fragment thereof of the invention is then added and dispersed throughout, and the
30 composition is allowed to cool with slow speed stirring.

-41-

Topical Lotion Composition

Antibody or fragment thereof of the invention
1.0 g.

Sorbitan Monolaurate 0.6 g.

5 Polysorbate 20 0.6 g.

Cetostearyl Alcohol 1.2 g.

Glycerin 6.0 g.

Methyl Hydroxybenzoate 0.2 g.

10 Purified Water B.P. to 100-00 ml. (B.P. = British
Pharmacopeia)

15 The methyl hydroxybenzoate and glycerin are
dissolved in 70 ml. of the water at 75°C. The sorbitan
monolaurate, polysorbate 20 and cetostearyl alcohol are
melted together at 75°C and added to the aqueous
solution. The resulting emulsion is homogenized,
allowed to cool with continuous stirring and the
antibody or fragment thereof of the invention is added
as a suspension in the remaining water. The whole
suspension is stirred until homogenized.

20 Eye Drop Composition

Antibody or fragment thereof of the invention
0.5 g.

Methyl Hydroxybenzoate 0.01 g.

Propyl Hydroxybenzoate 0.04 g.

25 Purified Water B.P. to 100-00 ml.

30 The methyl and propyl hydroxybenzoates are
dissolved in 70 ml. purified water at 75°C and the
resulting solution is allowed to cool. The antibody or
fragment thereof of the invention is then added, and the
solution is sterilized by filtration through a membrane
filter (0.022 μ m pore size), and packed aseptically into
suitable sterile containers.

-42-

Composition for Administration by Inhalation

For an aerosol container with a capacity of 15-20 ml: mix 10 mg. of an antibody or fragment thereof of the invention with 0.2-0.5% of a lubricating agent, such as polysorbate 85 or oleic acid, and disperse such mixture in a propellant, such as freon, preferably in a combination of (1,2 dichlorotetrafluoroethane) and difluorochloro-methane and put into an appropriate aerosol container adapted for either intranasal or oral inhalation administration.

Composition for Administration by Inhalation

For an aerosol container with a capacity of 15-20 ml: dissolve 10 mg. of an antibody or fragment thereof of the invention in ethanol (6-8 ml.), add 0.1-0.2% of a lubricating agent, such as polysorbate 85 or oleic acid; and disperse such in a propellant, such as freon, preferably in combination of (1.2 dichlorotetrafluoroethane) and difluorochloromethane, and put into an appropriate aerosol container adapted for either intranasal or oral inhalation administration.

The antibodies and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of an antibody or fragment thereof of the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., water, buffered water, 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well-known sterilization techniques. The

53361-1

-43-

compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as Ph adjusting and buffering agents, etc. The concentration of the
5 antibody or fragment thereof of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight, and will be selected primarily based on fluid volumes, viscosities, etc.,
10 according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 Ml sterile buffered water, and 50 mg. of an antibody
15 or fragment thereof of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml. of sterile Ringer's solution, and 150 mg. of an antibody or fragment thereof of the invention. Actual methods
20 for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

25

The antibodies (or fragments thereof) of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with
30 conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed.

Depending on the intended result, the pharmaceutical composition of the invention can be

-44-

administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least
5 partially arrest the disease and its complications. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance.

10 Single or multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical composition of the invention should provide a quantity of the
15 altered antibodies (or fragments thereof) of the invention sufficient to effectively treat the patient.

It should also be noted that the antibodies of this invention may be used for the design and synthesis of either peptide or non-peptide compounds (mimetics) which
20 would be useful in the same therapy as the antibody. See, e.g., Saragovi et al., Science, 253, 792-795 (1991).

To further illustrate the invention, the following examples are provided. These examples are not intended,
25 nor are they to be construed, as further limiting the invention.

Example 1

Recombinant immunoglobulin libraries displayed on the surface of filamentous phage were first described by
30 McCafferty et al, Nature, 348:552-554, 1990 and Barbas et al, Proc. Natl. Acad. Sci., USA 88:7978-7982, 1991. Using this technology, high affinity antibodies have been isolated from immune human recombinant libraries

53361-1

-45-

(Barbas et al, Proc. Natl. Acad. Sci., USA 589:10164-10168, 1992). Although the phage display concept used is substantially similar to that described by Barbas, 1991, Id. the technique has been modified by the

5 substitution of a unique vector for monkey libraries to reduce the possibility of recombination and improve stability. This vector, pMS, Figure 1 contains a single lac promoter/operator for efficient transcription and translation of polycistronic heavy and light chain

10 monkey DNA. This vector contains two different leader sequences, the omp A (Movva et al, J. Biol. Chem., 255: 27-29, (1980), for the light chain and the pel B (Lei, J. Bact., 4379-109:4383 (1987) for the heavy chain Fd. Both leader sequences are translated into hydrophobic

15 signal peptides that direct the secretion of the heavy and light chain cloned products into the periplasmic space. In the oxidative environment of the periplasm, the two chains fold and disulfide bonds form to create stable Fab fragments. We derived the backbone of the

20 vector from the phagemid bluescriptTM. (Stratagene, La Jolla, CA). It contains the gene for the enzyme beta-lactamase that confers ampicillin (carbenicillin) resistance to bacteria that harbor pMS DNA. We also derived, from bluescriptTM, the origin of replication of

25 the multicopy plasmid ColEI and the origin of replication of the filamentous bacteriophage f1. The origin of replication of phage f1 (the so-called intragenic region), signals the initiation of synthesis of single stranded pMS DNA, the initiation of capsid

30 formation and the termination of RNA synthesis by viral enzymes. The replication and assembly of pMS DNA strands into phage particles requires viral proteins that must be provided by a helper phage. We have used helper phage VCSM13 which is particularly suited for

-46-

this, since it also contains a gene coding for kanamycin resistance. Bacteria infected with VCSM13 and pMS can be selected by adding both kanamycin and carbenicillin to the growth medium. The bacteria will ultimately
5 produce filamentous phage particles containing either pMS or VCSM13 genomes. Packaging of the helper phage is less efficient than that of pMS, resulting in a mixed phage population that contains predominately recombinant pMS phages. The ends of the phage pick up minor coat
10 proteins specific to each end. Of particular interest here is the gene III product which is present in three to five copies at one end of the phage. The gene III product is 406 amino acid residues and is required for phage infection of *E. coli* via the F pili. The first
15 two domains of the heavy chain, the variable and the CH1 domain, are fused to the carboxy-terminal half of the gene III protein. This recombinant pili protein, directed by the pel B leader, is secreted to the periplasm where it accumulates and forms disulfide bonds
20 with the light chain before it is incorporated in the coat of the phage. Also, another vector contains a FLAG sequence engineered downstream of the gene III. The FLAG is an 8 amino acid peptide expressed at the carboxy terminal of the Fd protein. We are using commercially
25 available monoclonal anti-FLAG M2 for both purification and detection of phage Fab by ELISA (Brizzard, Bio Techniques, 16(4):730-731, (1994)).

After constructing the vector pMS, we tested its ability to produce phage bound Fab using control
30 antibody genes. We cloned an anti-tetanus toxoid antibody, (obtained from Dr. Carlos Barbas), into pMS and transformed XLI-blue. We co-infected our cells with VCSM13 and generated phage displaying the anti-tetanus toxoid antibody. We performed efficiency experiments

53361-1

-47-

where anti-tetanus toxoid phage were combined with phage beading an irrelevant antibody at 1:100,000. We performed three rounds of panning by applying 50 μ l of the mixed phage to antigen (tetanus toxoid) coated polystyrene wells. Non-adherent phage were washed off and the adherent phage were eluted with acid. The eluted phage were used to infect a fresh aliquot of XL1-Blue bacteria and helper phage was added. After overnight amplification, phage were prepared and again panned on antigen coated plates. After three rounds of panning, we were able to show that we had successfully enriched for the anti-tetanus toxoid phage. The success of this technology also depends on the ability to prepare soluble Fabs for characterization of the final panned product. This was achieved by excising gene III from the pMS DNA using the restriction enzyme Nhe I followed by re-ligation. After the gene III was excised, the Fab was no longer displayed on the phage surface but accumulated in the periplasmic space. Lysates were prepared from bacteria expressing soluble Fab and tested for antigen specificity using an ELISA. High levels of soluble Fab were detected.

In order to adapt phage display technology for use with macaque libraries, we developed specific primers for PCR amplifying monkey immunoglobulin genes. These were based on macaque sequences we obtained while developing the PRIMATIZED™ antibody technology (See, 08/379,072 (US 5,658,570) and databases containing human sequences. (Kabat et al, (1991), "Sequences of Proteins of Immunological Interest," U.S. Dept. of Health and Human Services, National Institute of Health).

We developed three sets of primers to cover amplification of the macaque repertoire. Our first set

53361-1

-48-

of primers was designed for amplification of the heavy chain VH and CH1 (Fd) domains. It consisted of a 3' CH1 domain primer and six 5' VH family specific primers that bind in the framework 1 region. Our second set of
5 primers, for amplifying the whole lambda chain, covers the many lambda chain subgroups. It consists of a 3' primer and three 5' degenerate primers that bind in the VL framework 1 region. Our third set of primers was designed for amplification of the kappa chain subgroups.
10 It consists of one 3' primer and five VK framework 1 primers. Using each of these sets, PCR parameters were optimized to obtain strong enough signals from each primer pair so that ample material was available for cloning of the library. We recently created macaque
15 combinatorial libraries in our pMS vector using these optimized PCR conditions. Bone marrow biopsies were taken from CD4 immune monkeys as the source of immunoglobulin RNA. The libraries contained approximately 10^6 members and are currently being panned
20 for specific binders on antigen coated wells.

Example 2

Development of B7/CTLA-4 Reagents

We have generated a number of reagents for the purpose of immunizing monkeys, developing binding and
25 functional assays *in vitro*, screening heterohybridomas and panning phage libraries. Table 1 lists each reagent and its intended purpose. In the case of B7.1, RNA was extracted from SB cells and converted to cDNA using reverse transcriptase. The first strand cDNA was PCR
30 amplified using B7.1 specific primers and cloned into IDEC's NEOSPLATM mammalian expression vectors. CHO cells were transfected with B7.1 NEOSPLATM DNA and clones expressing membrane associated B7.1 were identified.

53361-1

-49-

The B7.1 fusion protein was generated similarly, except that the PCR amplified B7.1 gene was cloned into a NEOSPLATM cassette vector containing the human CH2 and CH3 immunoglobulin genes. CHO cells were transformed with the B7.1/Ig NEOSPLATM DNA and stable clones secreting B7.1/Ig fusion protein were amplified. In general, the B7.2 and CTLA4 reagents were generated in the same manner, except that for B7.2 the RNA was isolated from human spleen cells that had been stimulated 24 hours with anti-Ig and IL-4, and for the CTLA4 constructs the gene source was PHA activated human T cells.

Table 1

Reagent	Purpose	CHO Expression
Soluble B7.1	Immunization, immunoassays	Yes
B7.1 Transfectant	Screening, ELISA	Yes
B7.1/Ig Fusion Protein	Inhibition studies, panning	Yes
B7.2 Transfectant	Screening, ELISA	Yes
B7.2/Ig Fusion Protein	Inhibition studies, panning	To be completed
CTLA4 Transfectant	Inhibition studies	To be completed
CTLA4/Ig	Inhibition studies	To be completed.

The availability of these reagents, together with monoclonal antibodies to B7.1 (L3074) (Becton Dickinson, 1994) and B7.2 (Fun-1 (Engel et al, Blood, 84, 1402-1407, (1994) and purified goat and rabbit antisera, specifically developed to detect monkey Fab fragments, facilitates identification of antibodies having the desired properties.

53361-1

-50-

Example 3**Investigation of the Immune Response in Cynomolgus Monkeys to Soluble and Cell Associated Human B7.1**

To evaluate the feasibility of producing monkey
5 antibodies to human B7.1 antigen, we first purified
recombinant SB7.1 from CHO cell media by affinity
chromatography using a L307.4-sepharoseTM affinity column.
SB7.1 was then injected, with adjuvant, into five mature
cynomolgus macaques. After a 3 to 4 month period of
10 booster immunizations, sera from the monkeys immunized
with SB7.1 or human SB cells were tested for antigen
binding.

Serum samples from the five monkeys immunized with
SB7.1. and three additional animals immunized with B7.1
15 positive human SB cells, were tested for antibody titers
against membrane associated B7.1 expressed in
transfected CHO cells. The results summarized in Figure
3 showed that four out of five monkeys immunized with
affinity-purified SB7.1 produced antibody titers in
20 excess of 1:5000. The three animals immunized with SB
cells containing cell associated B7.1 expressed lower
titers of antibodies ranging from 1:1400 to 1:2800.

Example 4

We purified antibodies from sera of all eight
25 immunized monkeys using SB7.1-sepharoseTM and then tested
their ability to bind to 1) SB7.1 coated plates in
ELISA; 2) antigen positive B cells and 3) B7.1 CHO
transfectomas. In addition, they were evaluated for
their ability to block B cell interactions as measured
30 by IL-2 production and tritiated thymidine uptake in a
mixed lymphocyte reaction (MLR). For T cell binding
experiments, human buffy coat peripheral blood

53361-1

-51-

lymphocytes were cultured for 3-6 days in the presence of PHA stimulator. B7 binding was detected by radio assay using ^{125}I -radiolabeled soluble B7.1 (SB7.1).

Example 5

5 Direct binding of monkey antibodies to radiolabeled SB7. ^{125}I radiolabeled SB7.1 was tested for binding to anti-B7.1 antibodies at 4, 1 and 0.25 $\mu\text{g/ml}$ in solution. The results shown in Table 2 suggest that most of the antibodies produced by monkeys immunized with SB7.1 were
10 capable of binding the affinity-purified ^{125}I -SB7.1 in a concentration dependent manner. To evaluate the specificity of binding to labeled SB7.1, unlabelled SB7.1 competition experiments were done with antibodies from two animals. Affinity-purified antibodies from
15 monkeys 1133 and 1144 were coated onto microwell plates at 400 ng/well. Affinity-purified unlabeled SB7.1 (500 and 100 ng/well) was used as competitor. The results shown in Figure 4 demonstrated that SB7.1 preparations are effective in inhibiting the ^{125}I -SB7.1 from binding to
20 the antibodies.

Table 2

Binding of SB7- I^{125} to Monkey Antibodies Affinity
Purified on a SB7-Sepharose Affinity ColumnTM

Antibody ($\mu\text{g/ml}$)	Monkey Numbers							
	769	908	1133	1135	1137	1139	1144	1146
4	175	213	9,056	12,771	4,318	226	5,781	108
1	106	142	6,569	7,940	3,401	110	3,901	80
0.25	95	104	1,803	2,673	1,219	100	1,186	94

-52-

Data are mean values of duplicate assays and represent cpm SB7-I¹²⁵ bound.

Example 6

5 Direct binding of radiolabeled affinity-purified monkey antibodies to B7⁺ cells and inhibition by SB7.1.

Affinity-purified radiolabeled monkey anti-B7.1 antibodies from monkey PRI135 were compared with radiolabeled L307.4 MAb for direct binding to B7 positive human SB cells. As a specificity control, 10 unlabeled SB7.1 (0.002 - 20 µg/ml) was added to compete with both radiolabeled antibodies. We demonstrated that monkey antibodies can bind cell associated B7.1 and are inhibited with SB7.1, as shown in Figure 5. Inhibition as high as 90% was observed with SB7.1.

15

Example 7

Direct binding of radiolabeled B7-Ig fusion protein to activated T cells and Inhibition by affinity-purified monkey antibodies.

Human peripheral blood T lymphocytes were activated 20 for 3-6 days and tested for direct binding of ¹²⁵I-B7.1-Ig. Because of Fc receptor upregulation on activated human T cells, it was necessary to pre-incubate the cells with heat-aggregated pre-immune immunoglobulin to block Fc binding sites prior to 25 addition of B7.1-Ig to the cells. A background control using SP2/0 murine myeloma cells was included to allow correction of the background binding. Figure 6 shows that inhibition of ¹²⁵I-B7.1-Ig fusion protein binding to

-53-

activated T cells was achieved with affinity-purified monkey antibodies at concentrations from 200 to 8 $\mu\text{g/ml}$. Unlabeled SB7.1 and L307.4 MAb used as controls were also effective in inhibiting B7.1-Ig fusion protein cell binding.

Example 8

Inhibition of IL-2 production in mixed lymphocyte reactions by monkey anti-B7 antibodies.

The blocking of CD28/B7 interaction leads to inhibition of IL-2 production by T lymphocytes. In the experiment shown in Figure 7, affinity-purified monkey antibodies from two monkeys immunized with SB7.1 (monkeys 1137 and 1135) and one immunized with B7 positive SB cells (monkey 1146) were evaluated for their abilities to inhibit human T cell activation in mixed lymphocyte reaction (MLR), as measured by inhibition of IL-2 production. The results of this experiment show that affinity-purified anti-B7.1 antibodies from monkeys 1146 and 1137 inhibited IL-2 production when added at concentrations of 50 $\mu\text{g/ml}$. Monkey 1135 antibodies could not be evaluated at the two highest concentrations due to lack of material, yet gave significant inhibition at lower concentrations. The murine MAb L307.4 was inhibitory at concentrations of 10 $\mu\text{g/ml}$. Other monkey sera tested at these concentrations were negative (data not shown). These results demonstrate that at least three of the monkeys immunized with both soluble and membrane associated forms of the B7 antigen are producing B7-blocking antibodies with immunosuppressive potential.

53361-1

-54-

Example 9**Investigation of Cross-reactivity in B7.1 Immunized Monkey Serum to B7.2 Antigen.**

Antibodies raised against B7.1 are to be tested for cross-reactivity to B7.2. Preliminary results using B7.1 affinity-purified antibodies from B7.1 immune sera provided suggestive evidence of binding to B7.2 transfected CHO cells (not shown). These data should be confirmed by using soluble B7.2Ig reagents. We will first purify additional monkey antibodies from B7.1 immunized animals by affinity chromatography on B7.1Ig--sepharoseTM. We will then produce and purify B7.2Ig from CHO cells in sufficient quantities to prepare a B7.2Ig-sepharoseTM affinity column. We will select from the B7.1 specific antibody population those antibodies which cross-react with B7.2 by binding to the B7.2Ig-sepharoseTM column. Any cross-reactive antibodies identified will be further characterized by direct binding to both B7.1 and B7.2 transfected CHO cells and inhibition of binding to B7.2 transfected cells by B7.1Ig.

Example 10**Generation of a Phage Display Library**

Recombinant phage display libraries are generated from B7.1 and B7.2 immune monkeys. Lymph node and bone marrow biopsies are performed 7-12 days after immunization to harvest RNA rich B cells and plasma cells. RNA is isolated from the lymphocytes using the method described by Chomczynski Anal. Biochem., 162(1), 156-159, (1987). RNA is converted to cDNA using an oligo dT primer and reverse transcriptase. The first

53361-1

-55-

strand cDNA is divided into aliquots and PCR amplified using the sets of kappa, lambda, and heavy chain Fd region primers described earlier and either Pfu polymerase (Stratagene, San Diego) or Taq polymerase (Promega, Madison). The heavy chain PCR amplified products are pooled, cut with Xho VSpe I restriction enzymes and cloned into the vector pMS. Subsequently, the light chain PCR products are pooled, cut with Sac I/Xba I restriction enzymes, and cloned to create the recombinant library. XLI-Blue *E. coli* is transformed with the library DNA and super-infected with VCSM13 to produce the phage displaying antibodies. The library is panned four rounds on polystyrene wells coated with B7.1 or B7.2 antigen. Individual phage clones from each round of panning are analyzed. The pMS vector DNA is isolated and the gene III excised. Soluble Fab fragments are generated and tested in ELISA for binding to B7.1 and B7.2.

Example 11

20 **Characterization of Phage Fab Fragments**

The monkey phage Fab fragments are characterized for their specificity and the ability to block B7.1-Ig and B7.2-Ig binding to CTLA-4-Ig or CTLA-4 transfected cells. Phage fragments are also characterized for cross-reactivity after first panning for 4 rounds on the B7 species used for immunization in order to select for high affinity fragments. Fab fragments identified from four rounds of panning either on B7.1 or B7.2 antigen coated surfaces are scaled up by infection and grown in 24 hour fermentation cultures of *E coli*. Fragments are purified by KodakTM FLAG binding to a anti-FLAG affinity column. Purified phage Fabs are tested for affinity by an ELISA based direct binding modified Scatchard

-56-

analysis (Kato et al, J. Chem. BioEng., 76:451-454, (1993)) using Goat anti-monkey Fab antibodies or anti-FLAG MAb conjugated with horseradish peroxidase. The anti-monkey Fab reagents will be absorbed against human heavy chain constant region Ig to remove any cross-reactivity to B7-Ig. Kd values are calculated for each fragment after measurements of direct binding to B7.1-Ig or B7.2-Ig coated plates.

Example 12

10 Phage Fab Fragment Blocking of CTLA-4/B7 Binding

Fab fragments most effectively blocking the binding of B7-Ig at the lowest concentrations are selected as lead candidates. Selections are made by competing off ¹²⁵I-B7-Ig binding to CTLA-4-Ig or CTLA-4 transfected cells. Additional selection criteria include, blocking of mixed lymphocyte reaction (MLR), as measured by inhibiting 3H-thymidine uptake in responder cells (Azuma et al, J. Exp. Med., 177:845-850,; Azuma et al, Nature, 301:76-79, (1993)) and direct analysis of IL-2 production using IL-2 assay kits. The three or four candidates which are most effective in inhibiting of MLR and CTLA-4 binding assays are chosen for cloning into the above-described mammalian expression vector for transfection into CHO cells and expression of chimeric monkey/human antibodies.

Example 13

Generation of Monkey Heterohybridomas

Monkey heterohybridomas secreting monoclonal antibodies are generated from existing immunized animals whose sera tested positive for B7.1 and/or B7.2. Lymph node biopsies are taken from animals positive to either, or both, antigens. The method of hybridoma production

-57-

is similar to the established method used for the generation of monkey anti-CD4 antibodies (Newman, 1992(Id.)). Monkeys with high serum titers will have sections of inguinal lymph nodes removed under
5 anesthesia. Lymphocytes are washed from the tissue and fused with KH6/B5 heteromyeloma cells (Carrol et al, J. Immunol. Meth., 89:61-72, (1986)) using polyethylene glycol (PEG). Hybridomas are selected on H.A.T. media and stabilized by repeated subcloning in 96 well plates.

10 Monkey monoclonal antibodies specific for B7.1 antigen are screened for cross-reactivity to B7.2. Monkey anti-B7 antibodies will be characterized for blocking of B7/CTLA-4 binding using the ¹²⁵I-B7-Ig binding
15 assay. Inhibition of MLR by 3H-Thymidine uptake and direct measurement of IL-2 production is used to select three candidates. Two candidates will be brought forward in Phase II studies and expressed in CHO cells while repeating all functional studies. For the
20 purposes of developing an animal model for *in vivo* pharmacology, anti-B7 antibodies will be tested on cells of several animal species. The establishment of an animal model will allow preclinical studies to be carried out for the selected clinical indication.

Example 14

25 As discussed *supra*, using the above heterohybridoma methods, 4 lead monkey anti-B7.1 antibodies have been identified: 16C10, 7B6, 7C10 and 20C9. These antibodies were characterized as follows:

30 To demonstrate the monkey antibodies' ability to block the physical interaction between CTLA4-Ig, varying concentrations of the monkey anti-B7.1 antibodies and unlabeled CTLA4-Ig were incubated

-58-

with radiolabeled CTLA4-Ig¹¹²⁵. The results of the inhibition assay showed that the IC50 (the concentration of inhibitor which results in 50% inhibition) for the monkey antibodies are:

5 a: 7C10: 0.39 µg/Ml
 b: 16C10: 1.60 µg/Ml
 c: 20C9: 3.90 µg/Ml
 d: 7B6: 39.0 µg/Ml

10 Scatchard analysis showed that the apparent affinity constants (Kd) for the monkey antibodies binding to B7-Ig coated plates were approximated to be:

 a: 7C10: 6.2 x 10⁻⁹M
 b: 16C10: 8.1 x 10⁻⁹M
15 c: 7B6: 10.7 x 10⁻⁹M
 d: 20C9: 16.8 x 10⁻⁹M

20 The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR showed that all 4 anti-B7.1 antibodies inhibit IL-2 production to different extents:

 a: 7B6: 5.0 µg/Ml
 b: 16C10: 0.1 µg/Ml
 c: 20C9: 2.0 µg/Ml
 d: 7C10: 5.0 µg/Ml

25 The monkey anti-B7.1 antibodies were tested for their ability to bind B7 on human peripheral blood lymphocytes (PBL). FACS analysis showed that all 4 monkey antibodies tested positive.

30 Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were tested for C1q binding by FACS analysis. Results

53361-1

-59-

showed 7C10 monkey Ig had strong human C1q binding after incubating with B7.1 CHO-transfected cells. 16C10 was negative, as were the 20C9 and 7B6 monkey antibodies.

5

Example 15

Using the primatizedTM antibody methodology incorporated by reference to commonly assigned U.S. Serial No. 08/379,072 (now US 5,658,570), and using the NEOSPLATM vector system shown in Figure 2, the heavy and light variable domains of 7C10, 7B6 and 16C10 were cloned and primatized forms thereof have been synthesized in CHO cells using the NEOSPLATM vector system. The amino acid and nucleic acid sequences for the primatizedTM 7C10 light and heavy chain, 7B6 light and heavy chain, and 16C10 light and heavy chain are respectively shown in Figures 8a (SEQ ID NOS: 1-2), 8b-1 and 8b-2 (SEQ ID NOS: 3-4), 9a (SEQ ID NOS: 5-6), 9b-1 and 9b-2 (SEQ ID NOS: 7-8), 10a (SEQ ID NOS: 9-10) and 10b-1 and 10b-2 (SEQ ID NOS: 11-12).

It is expected that these primatizedTM antibodies, given their probable low antigenicity and human effector function, will be well suited as therapeutics. In fact, it has recently been shown that primatizedTM 16C10 exhibits human C1, binding, whereas 16C10 does not.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be embraced by the following claims.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 53361-1 Seq 09-10-08 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> Biogen Idec Inc.

<120> MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2
PRIMATIZED
FORMS, PHARMACEUTICAL COMPOSITIONS

<130> 53361-1

<140> 2,223,532

<141> 1996-06-06

<150> PCT/US96/10053

<151> 1996-06-06

<150> 08/487,550

<151> 1995-06-07

<160> 12

<170> PatentIn Ver. 2.1

<210> 1

<211> 705

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(702)

<400> 1

atg	agg	gtc	ccc	gct	cag	ctc	ctg	ggg	ctc	ctg	ctg	ctc	tgg	ctc	cca	48
Met	Arg	Val	Pro	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Pro	
1				5				10					15			
ggt	gca	cga	tgt	gcc	tat	gaa	ctg	act	cag	cca	ccc	tcg	gtg	tca	gtg	96
Gly	Ala	Arg	Cys	Ala	Tyr	Glu	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Val	
			20					25					30			
tcc	cca	gga	cag	acg	gcc	agg	atc	acc	tgt	ggg	gga	gac	aac	agt	aga	144
Ser	Pro	Gly	Gln	Thr	Ala	Arg	Ile	Thr	Cys	Gly	Gly	Asp	Asn	Ser	Arg	
			35					40					45			

60a

```

aat gaa tat gtc cac tgg tac cag cag aag cca gcg cgg gcc cct ata 192
Asn Glu Tyr Val His Trp Tyr Gln Gln Lys Pro Ala Arg Ala Pro Ile
    50                      55                      60

ctg gtc atc tat gat gat agt gac cgg ccc tca ggg atc cct gag cga 240
Leu Val Ile Tyr Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg
    65                      70                      75                      80

ttc tct ggc tcc aaa tca ggg aac acc gcc acc ctg acc atc aac ggg 288
Phe Ser Gly Ser Lys Ser Gly Asn Thr Ala Thr Leu Thr Ile Asn Gly
                      85                      90                      95

gtc gag gcc ggg gat gag gct gac tat tac tgt cag gtg tgg gac agg 336
Val Glu Ala Gly Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Arg
                      100                      105                      110

gct agt gat cat ccg gtc ttc gga gga ggg acc cgg gtg acc gtc cta 384
Ala Ser Asp His Pro Val Phe Gly Gly Gly Thr Arg Val Thr Val Leu
                      115                      120                      125

ggg cag ccc aag gct gcc ccc tcg gtc act ctg ttc ccg ccc tcc tct 432
Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
    130                      135                      140

gag gag ctt caa gcc aac aag gcc aca ctg gtg tgt ctc ata agt gac 480
Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
    145                      150                      155                      160

ttc tac ccg gga gcc gtg aca gtg gcc tgg aag gca gat agc agc ccc 528
Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
                      165                      170                      175

gtc aag gcg gga gtg gag acc acc aca ccc tcc aaa caa agc aac aac 576
Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
                      180                      185                      190

aag tac gcg gcc agc agc tac ctg agc ctg acg cct gag cag tgg aag 624
Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
                      195                      200                      205

tcc cac aga agc tac agc tgc cag gtc acg cat gaa ggg agc acc gtg 672
Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
    210                      215                      220

gag aag aca gtg gcc cct aca gaa tgt tca tga 705
Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
    225                      230

```

<210> 2
 <211> 234
 <212> PRT
 <213> Homo sapiens

<400> 2
 Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro
 1 5 10 15
 Gly Ala Arg Cys Ala Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val
 20 25 30

60b

```

Ser Pro Gly Gln Thr Ala Arg Ile Thr Cys Gly Gly Asp Asn Ser Arg
      35      40      45
Asn Glu Tyr Val His Trp Tyr Gln Gln Lys Pro Ala Arg Ala Pro Ile
      50      55      60
Leu Val Ile Tyr Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg
      65      70      75      80
Phe Ser Gly Ser Lys Ser Gly Asn Thr Ala Thr Leu Thr Ile Asn Gly
      85      90      95
Val Glu Ala Gly Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Arg
      100      105      110
Ala Ser Asp His Pro Val Phe Gly Gly Gly Thr Arg Val Thr Val Leu
      115      120      125
Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
      130      135      140
Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
      145      150      155      160
Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
      165      170      175
Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
      180      185      190
Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
      195      200      205
Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
      210      215      220
Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
      225      230

```

<210> 3

<211> 1431

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1428)

<400> 3

```

atg aaa cac ctg tgg ttc ttc ctc ctc ctg gtg gca gct ccc aga tgg      48
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
      1      5      10      15

gtc ctg tcc cag gtg aag ctg cag cag tgg ggc gaa gga ctt ctg cag      96
Val Leu Ser Gln Val Lys Leu Gln Gln Trp Gly Glu Gly Leu Leu Gln
      20      25      30

cct tcg gag acc ctg tcc cgc acc tgc gtt gtc tct ggt ggc tcc atc      144
Pro Ser Glu Thr Leu Ser Arg Thr Cys Val Val Ser Gly Gly Ser Ile
      35      40      45

agc ggt tac tac tac tgg acc tgg atc cgc cag acc cca ggg agg gga      192
Ser Gly Tyr Tyr Tyr Trp Thr Trp Ile Arg Gln Thr Pro Gly Arg Gly
      50      55      60

ctg gag tgg att ggc cat att tat ggt aat ggt gcg acc acc aac tac      240
Leu Glu Trp Ile Gly His Ile Tyr Gly Asn Gly Ala Thr Thr Asn Tyr
      65      70      75      80

```

60c

aat	ccc	tcc	ctc	aag	agt	cga	gtc	acc	att	tca	aaa	gac	acg	tcc	aag	288
Asn	Pro	Ser	Leu	Lys	Ser	Arg	Val	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Lys	
				85					90					95		
aac	cag	ttc	ttc	ctg	aac	ttg	aat	tct	gtg	acc	gac	gcg	gac	acg	gcc	336
Asn	Gln	Phe	Phe	Leu	Asn	Leu	Asn	Ser	Val	Thr	Asp	Ala	Asp	Thr	Ala	
			100					105					110			
gtc	tat	tac	tgt	gcg	aga	ggc	cct	cgc	cct	gat	tgc	aca	acc	att	tgt	384
Val	Tyr	Tyr	Cys	Ala	Arg	Gly	Pro	Arg	Pro	Asp	Cys	Thr	Thr	Ile	Cys	
			115				120					125				
tat	ggc	ggc	tgg	gtc	gat	gtc	tgg	ggc	ccg	gga	gac	ctg	gtc	acc	gtc	432
Tyr	Gly	Gly	Trp	Val	Asp	Val	Trp	Gly	Pro	Gly	Asp	Leu	Val	Thr	Val	
	130					135					140					
tcc	tca	gct	agc	acc	aag	ggc	cca	tcg	gtc	ttc	ccc	ctg	gca	ccc	tcc	480
Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	
145					150					155					160	
tcc	aag	agc	acc	tct	ggg	ggc	aca	gcg	gcc	ctg	ggc	tgc	ctg	gtc	aag	528
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	
				165				170						175		
gac	tac	ttc	ccc	gaa	ccg	gtg	acg	gtg	tcg	tgg	aac	tca	ggc	gcc	ctg	576
Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	
			180					185					190			
acc	agc	ggc	gtg	cac	acc	ttc	ccg	gct	gtc	cta	cag	tcc	tca	gga	ctc	624
Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	
		195					200					205				
tac	tcc	ctc	agc	agc	gtg	gtg	acc	gtg	ccc	tcc	agc	agc	ttg	ggc	acc	672
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
	210					215					220					
cag	acc	tac	atc	tgc	aac	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	720
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
225					230					235					240	
gac	aag	aaa	gca	gag	ccc	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	768
Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	
				245				250						255		
ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	816
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	
			260					265					270			
ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	864
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	
		275					280					285				
aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	912
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
	290					295					300					
aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	960
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	
305					310					315					320	

60d

```

cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc 1008
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
          325                      330                      335

gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc 1056
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
          340                      345                      350

tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc 1104
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
          355                      360                      365

aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg 1152
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
          370                      375                      380

gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc 1200
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
385                      390                      395                      400

ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg 1248
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
          405                      410                      415

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc 1296
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
          420                      425                      430

ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag 1344
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
          435                      440                      445

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac 1392
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
          450                      455                      460

tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga 1431
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465                      470                      475

```

<210> 4

<211> 476

<212> PRT

<213> Homo sapiens

<400> 4

```

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

```

60e

Asn	Gln	Phe	Phe	Leu	Asn	Leu	Asn	Ser	Val	Thr	Asp	Ala	Asp	Thr	Ala		
			100					105					110				
Val	Tyr	Tyr	Cys	Ala	Arg	Gly	Pro	Arg	Pro	Asp	Cys	Thr	Thr	Ile	Cys		
		115					120					125					
Tyr	Gly	Gly	Trp	Val	Asp	Val	Trp	Gly	Pro	Gly	Asp	Leu	Val	Thr	Val		
	130					135					140						
Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser		
145					150					155					160		
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys		
			165					170						175			
Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu		
		180						185					190				
Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu		
		195					200					205					
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr		
	210					215					220						
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val		
225					230					235					240		
Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro		
			245						250					255			
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe		
			260					265					270				
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val		
		275					280					285					
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe		
	290					295					300						
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro		
305					310					315					320		
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr		
			325						330					335			
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val		
			340					345					350				
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala		
		355					360					365					
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg		
	370					375					380						
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly		
385					390					395					400		
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro		
			405					410						415			
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser		
			420					425					430				
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln		
		435					440					445					
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His		
	450					455					460						
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys						
465					470					475							

<210> 5

<211> 720

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(717)

60f

<400> 5

atg agc ctc cct gct cag ctc ctc ggg ctg cta ttg ctc tgc gtc ccc	48
Met Ser Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Cys Val Pro	
1 5 10 15	
ggg tcc agt ggg gaa gtt gtg atg act cag tct cca ctg tcc ctt ccc	96
Gly Ser Ser Gly Glu Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro	
20 25 30	
atc aca cct gga gag ccg gcc tcc atc tcc tgt agg tct agt caa agc	144
Ile Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser	
35 40 45	
ctt aaa cac agt aat gga gac acc ttc ctg agt tgg tat cag cag aag	192
Leu Lys His Ser Asn Gly Asp Thr Phe Leu Ser Trp Tyr Gln Gln Lys	
50 55 60	
cca ggc caa cct cca agg ctc ctg att tat aag gtt tct aac cgg gac	240
Pro Gly Gln Pro Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Asp	
65 70 75 80	
tct ggg gtc cca gac aga ttc agc ggc agt ggg gca ggg aca gat ttc	288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe	
85 90 95	
aca ctg aaa atc agc gca gtg gag gct gaa gat gtt ggg gtt tat ttc	336
Thr Leu Lys Ile Ser Ala Val Glu Ala Glu Asp Val Gly Val Tyr Phe	
100 105 110	
tgc ggg caa ggt aca agg act cct ccc act ttc ggc gga ggg acc aag	384
Cys Gly Gln Gly Thr Arg Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys	
115 120 125	
gtg gaa atc aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg	432
Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
130 135 140	
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
145 150 155 160	
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
165 170 175	
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
180 185 190	
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
195 200 205	
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag	672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln	
210 215 220	
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tga	720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	
225 230 235	

60g

<210> 6
 <211> 239
 <212> PRT
 <213> Homo sapiens

<400> 6
 Met Ser Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Cys Val Pro
 1 5 10 15
 Gly Ser Ser Gly Glu Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30
 Ile Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45
 Leu Lys His Ser Asn Gly Asp Thr Phe Leu Ser Trp Tyr Gln Gln Lys
 50 55 60
 Pro Gly Gln Pro Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Asp
 65 70 75 80
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe
 85 90 95
 Thr Leu Lys Ile Ser Ala Val Glu Ala Glu Asp Val Gly Val Tyr Phe
 100 105 110
 Cys Gly Gln Gly Thr Arg Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys
 115 120 125
 Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 130 135 140
 Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 145 150 155 160
 Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 165 170 175
 Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 180 185 190
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 195 200 205
 Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 210 215 220
 Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

<210> 7
 <211> 1437
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(1434)

<400> 7
 atg ggt tgg agc ctc atc ttg ctc ttc ctt gtc gct gtt gct acg cgt 48
 Met Gly Trp Ser Leu Ile Leu Leu Phe Leu Val Ala Val Ala Thr Arg
 1 5 10 15

 gtc cag tgt gag gtg caa ctg gtg gag tct ggg gga ggc ttg gtc cag 96
 Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
 20 25 30

 cct ggc ggg tcc ctg aga gtc tcc tgt gca gtc tct gga ttc acc ttc 144
 Pro Gly Gly Ser Leu Arg Val Ser Cys Ala Val Ser Gly Phe Thr Phe
 35 40 45

60h

agt gac cac tac atg tat tgg ttc cgc cag gct cca ggg aag ggg ccg	192
Ser Asp His Tyr Met Tyr Trp Phe Arg Gln Ala Pro Gly Lys Gly Pro	
50 55 60	
gaa tgg gta ggt ttc att aga aac aaa ccg aac ggt ggg aca aca gaa	240
Glu Trp Val Gly Phe Ile Arg Asn Lys Pro Asn Gly Gly Thr Thr Glu	
65 70 75 80	
tac gcc gcg tct gtg aaa gac aga ttc acc atc tcc aga gat gat tcc	288
Tyr Ala Ala Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser	
85 90 95	
aaa agc atc gcc tat ctg caa atg agc agc ctg aaa atc gag gac acg	336
Lys Ser Ile Ala Tyr Leu Gln Met Ser Ser Leu Lys Ile Glu Asp Thr	
100 105 110	
gcc gtc tat tac tgt act aca tcc tac att tca cat tgt cgg ggt ggt	384
Ala Val Tyr Tyr Cys Thr Thr Ser Tyr Ile Ser His Cys Arg Gly Gly	
115 120 125	
gtc tgc tat gga ggt tac ttc gaa ttc tgg ggc cag ggc gcc ctg gtc	432
Val Cys Tyr Gly Gly Tyr Phe Glu Phe Trp Gly Gln Gly Ala Leu Val	
130 135 140	
acc gtc tcc tca gct agc acc aag ggc cca tcg gtc ttc ccc ctg gca	480
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala	
145 150 155 160	
ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg	528
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu	
165 170 175	
gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc	576
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly	
180 185 190	
gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca	624
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser	
195 200 205	
gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg	672
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu	
210 215 220	
ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc	720
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr	
225 230 235 240	
aag gtg gac aag aaa gca gag ccc aaa tct tgt gac aaa act cac aca	768
Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr	
245 250 255	
tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc	816
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe	
260 265 270	
ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct	864
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro	
275 280 285	

60i

gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc	912
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val	
290 295 300	
aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca	960
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr	
305 310 315 320	
aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc	1008
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val	
325 330 335	
ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc	1056
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys	
340 345 350	
aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc	1104
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser	
355 360 365	
aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca	1152
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro	
370 375 380	
tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc	1200
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val	
385 390 395 400	
aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg	1248
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly	
405 410 415	
cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac	1296
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp	
420 425 430	
ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg	1344
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp	
435 440 445	
cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac	1392
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His	
450 455 460	
aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga	1437
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
465 470 475	

<210> 8

<211> 478

<212> PRT

<213> Homo sapiens

<400> 8

Met Gly Trp Ser Leu Ile Leu Leu Phe Leu Val Ala Val Ala Thr Arg	
1 5 10 15	
Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln	
20 25 30	

60j

Pro	Gly	Gly	Ser	Leu	Arg	Val	Ser	Cys	Ala	Val	Ser	Gly	Phe	Thr	Phe
		35					40					45			
Ser	Asp	His	Tyr	Met	Tyr	Trp	Phe	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Pro
	50					55					60				
Glu	Trp	Val	Gly	Phe	Ile	Arg	Asn	Lys	Pro	Asn	Gly	Gly	Thr	Thr	Glu
	65				70					75					80
Tyr	Ala	Ala	Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser
				85					90					95	
Lys	Ser	Ile	Ala	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Lys	Ile	Glu	Asp	Thr
			100					105					110		
Ala	Val	Tyr	Tyr	Cys	Thr	Thr	Ser	Tyr	Ile	Ser	His	Cys	Arg	Gly	Gly
		115					120					125			
Val	Cys	Tyr	Gly	Gly	Tyr	Phe	Glu	Phe	Trp	Gly	Gln	Gly	Ala	Leu	Val
	130					135					140				
Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala
	145				150					155					160
Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu
			165						170					175	
Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly
		180						185					190		
Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser
		195					200					205			
Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu
	210					215					220				
Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr
	225				230					235					240
Lys	Val	Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr
			245						250					255	
Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe
			260					265					270		
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro
		275					280					285			
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val
	290					295					300				
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr
	305				310					315					320
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val
			325						330					335	
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys
			340					345					350		
Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser
		355					360					365			
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro
	370					375					380				
Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val
	385				390					395					400
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly
			405						410					415	
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp
			420					425					430		
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp
		435					440					445			
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His
	450					455					460				
Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys		
	465				470					475					

<210> 9

<211> 711

60k

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(708)

<400> 9

atg agg gtc ccc gct cag ctc ctg ggg ctc ctg ctg ctc tgg ctc cca	48
Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro	
1 5 10 15	
ggt gca cga tgt gag tct gtc ctg aca cag ccg ccc tca gtg tct ggg	96
Gly Ala Arg Cys Glu Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly	
20 25 30	
gcc cca ggg cag aag gtc acc atc tcg tgc act ggg agc acc tcc aac	144
Ala Pro Gly Gln Lys Val Thr Ile Ser Cys Thr Gly Ser Thr Ser Asn	
35 40 45	
att gga ggt tat gat cta cat tgg tac cag cag ctc cca gga acg gcc	192
Ile Gly Gly Tyr Asp Leu His Trp Tyr Gln Gln Leu Pro Gly Thr Ala	
50 55 60	
ccc aaa ctc ctc atc tat gac att aac aag cga ccc tca gga att tct	240
Pro Lys Leu Leu Ile Tyr Asp Ile Asn Lys Arg Pro Ser Gly Ile Ser	
65 70 75 80	
gac cga ttc tct ggc tcc aag tct ggt acc gcg gcc tcc ctg gcc atc	288
Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ala Ala Ser Leu Ala Ile	
85 90 95	
act ggg ctc cag act gag gat gag gct gat tat tac tgc cag tcc tat	336
Thr Gly Leu Gln Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr	
100 105 110	
gac agc agc ctg aat gct cag gta ttc gga gga ggg acc cgg ctg acc	384
Asp Ser Ser Leu Asn Ala Gln Val Phe Gly Gly Gly Thr Arg Leu Thr	
115 120 125	
gtc cta ggt cag ccc aag gct gcc ccc tcg gtc act ctg ttc ccg ccc	432
Val Leu Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro	
130 135 140	
tcc tct gag gag ctt caa gcc aac aag gcc aca ctg gtg tgt ctc ata	480
Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile	
145 150 155 160	
agt gac ttc tac ccg gga gcc gtg aca gtg gcc tgg aag gca gat agc	528
Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser	
165 170 175	
agc ccc gtc aag gcg gga gtg gag acc acc aca ccc tcc aaa caa agc	576
Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser	
180 185 190	
aac aac aag tac gcg gcc agc agc tac ctg agc ctg acg cct gag cag	624
Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln	
195 200 205	

60m

gtc	ctg	tcc	cag	gtg	cag	ctg	cag	gag	tcg	ggc	cca	gga	ctg	gtg	aag	96
Val	Leu	Ser	Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	
			20					25					30			
cct	tcg	gag	acc	ctg	tcc	ctc	acc	tgc	gct	gtc	tct	ggg	ggc	tcc	atc	144
Pro	Ser	Glu	Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Ser	Gly	Gly	Ser	Ile	
		35					40					45				
agc	ggg	ggg	tat	ggc	tgg	ggc	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggg	192
Ser	Gly	Gly	Tyr	Gly	Trp	Gly	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Gly	
	50					55					60					
ctg	gag	tgg	att	ggg	agt	ttc	tat	agt	agt	agt	ggg	aac	acc	tac	tac	240
Leu	Glu	Trp	Ile	Gly	Ser	Phe	Tyr	Ser	Ser	Ser	Gly	Asn	Thr	Tyr	Tyr	
65					70					75					80	
aac	ccc	tcc	ctc	aag	agt	caa	gtc	acc	att	tca	aca	gac	acg	tcc	aag	288
Asn	Pro	Ser	Leu	Lys	Ser	Gln	Val	Thr	Ile	Ser	Thr	Asp	Thr	Ser	Lys	
				85					90					95		
aac	cag	ttc	tcc	ctg	aag	ctg	aac	tct	atg	acc	gcc	gcg	gac	acg	gcc	336
Asn	Gln	Phe	Ser	Leu	Lys	Leu	Asn	Ser	Met	Thr	Ala	Ala	Asp	Thr	Ala	
			100					105					110			
gtg	tat	tac	tgt	gtg	aga	gat	cgt	ctt	ttt	tca	gtt	gtt	gga	atg	gtt	384
Val	Tyr	Tyr	Cys	Val	Arg	Asp	Arg	Leu	Phe	Ser	Val	Val	Gly	Met	Val	
		115					120					125				
tac	aac	aac	tgg	ttc	gat	gtc	tgg	ggc	ccg	gga	gtc	ctg	gtc	acc	gtc	432
Tyr	Asn	Asn	Trp	Phe	Asp	Val	Trp	Gly	Pro	Gly	Val	Leu	Val	Thr	Val	
	130					135					140					
tcc	tca	gct	agc	acc	aag	ggc	cca	tcg	gtc	ttc	ccc	ctg	gca	ccc	tcc	480
Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	
145					150					155					160	
tcc	aag	agc	acc	tct	ggg	ggc	aca	gcg	gcc	ctg	ggc	tgc	ctg	gtc	aag	528
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	
				165				170						175		
gac	tac	ttc	ccc	gaa	ccg	gtg	acg	gtg	tcg	tgg	aac	tca	ggc	gcc	ctg	576
Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	
			180					185					190			
acc	agc	ggc	gtg	cac	acc	ttc	ccg	gct	gtc	cta	cag	tcc	tca	gga	ctc	624
Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	
		195					200					205				
tac	tcc	ctc	agc	agc	gtg	gtg	acc	gtg	ccc	tcc	agc	agc	ttg	ggc	acc	672
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
	210					215					220					
cag	acc	tac	atc	tgc	aac	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	720
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
225					230					235					240	
gac	aag	aaa	gca	gag	ccc	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	768
Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	
				245					250					255		

60n

ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc	816
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe	
260 265 270	
ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc	864
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val	
275 280 285	
aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc	912
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe	
290 295 300	
aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg	960
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro	
305 310 315 320	
cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc	1008
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr	
325 330 335	
gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc	1056
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val	
340 345 350	
tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc	1104
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala	
355 360 365	
aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc ccg	1152
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg	
370 375 380	
gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc	1200
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly	
385 390 395 400	
ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg	1248
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro	
405 410 415	
gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc	1296
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser	
420 425 430	
ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag	1344
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln	
435 440 445	
ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac	1392
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His	
450 455 460	
tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga	1431
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
465 470 475	

<210> 12

<211> 476

600

<212> PRT

<213> Homo sapiens

<400> 12

Met	Lys	His	Leu	Trp	Phe	Phe	Leu	Leu	Leu	Val	Ala	Ala	Pro	Arg	Trp
1				5				10						15	
Val	Leu	Ser	Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys
			20					25					30		
Pro	Ser	Glu	Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Ser	Gly	Gly	Ser	Ile
		35					40					45			
Ser	Gly	Gly	Tyr	Gly	Trp	Gly	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Gly
	50					55				60					
Leu	Glu	Trp	Ile	Gly	Ser	Phe	Tyr	Ser	Ser	Ser	Gly	Asn	Thr	Tyr	Tyr
	65				70				75						80
Asn	Pro	Ser	Leu	Lys	Ser	Gln	Val	Thr	Ile	Ser	Thr	Asp	Thr	Ser	Lys
			85					90						95	
Asn	Gln	Phe	Ser	Leu	Lys	Leu	Asn	Ser	Met	Thr	Ala	Ala	Asp	Thr	Ala
			100				105						110		
Val	Tyr	Tyr	Cys	Val	Arg	Asp	Arg	Leu	Phe	Ser	Val	Val	Gly	Met	Val
	115					120						125			
Tyr	Asn	Asn	Trp	Phe	Asp	Val	Trp	Gly	Pro	Gly	Val	Leu	Val	Thr	Val
	130					135					140				
Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser
	145				150				155					160	
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys
			165					170					175		
Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu
			180					185					190		
Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu
	195					200						205			
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr
	210					215					220				
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val
	225				230					235					240
Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro
			245					250						255	
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe
			260					265					270		
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val
		275					280					285			
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe
	290				295						300				
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro
	305				310					315					320
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr
			325					330						335	
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val
			340					345					350		
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala
	355						360					365			
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg
	370					375					380				
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly
	385				390					395					400
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro
			405					410						415	
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser
			420				425						430		
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln
		435					440						445		

60p

Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His
	450					455					460				
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys				
465					470					475					

53361-1

-61-

CLAIMS:

1. A monkey monoclonal antibody, a primatized form thereof, or a fragment of the monkey monoclonal antibody or primatized form thereof, that binds to human CD80 antigen and comprises light and heavy chain variable region polypeptide sequences selected from the group consisting of:

(a) the light and heavy chain variable region polypeptide sequences of antibody 7C10 contained in SEQ ID NO:2 and SEQ ID NO:4, respectively;

(b) the light and heavy chain variable region polypeptide sequences of antibody 7B6 contained in SEQ ID NO:6 and SEQ ID NO:8, respectively; and

(c) the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

2. A monkey monoclonal antibody, a primatized form thereof, or a fragment of the monkey monoclonal antibody or primatized form thereof, that binds to an epitope of human CD80 antigen that is also bound by an antibody comprising light and heavy chain variable region polypeptide sequences selected from the group consisting of:

(a) the light and heavy chain variable region polypeptide sequences of antibody 7C10 contained in SEQ ID NO:2 and SEQ ID NO:4, respectively;

(b) the light and heavy chain variable region polypeptide sequences of antibody 7B6 contained in SEQ ID NO:6 and SEQ ID NO:8, respectively; and

53361-1

-62-

(c) the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

3. The monoclonal antibody according to claim 1 or claim 2, that comprises a light chain constant region of a human antibody light chain, and a heavy chain constant region of a human antibody heavy chain.

4. The monoclonal antibody according to claim 3, which comprises a heavy chain constant region selected from human gamma 1 constant region, human gamma 4 constant region, and human gamma 4 PE constant region.

5. The monoclonal antibody according to any one of claims 1 to 4, which is a depleting antibody.

6. The monoclonal antibody according to any one of claims 1 to 4, which is a non-depleting antibody.

7. An antibody fragment according to claim 1 or claim 2, which is a $F(ab')_2$, Fab, or Fv fragment.

8. The antibody according to claim 2, wherein said antibody is a primatized form of a monkey monoclonal antibody which binds to an epitope of human CD80 antigen that is also bound by an antibody comprising the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO: 10 and SEQ ID NO: 12, respectively.

9. An isolated nucleic acid that comprises nucleotide sequences which encode the light and heavy chain variable region polypeptide sequences of an antibody or fragment thereof according to claim 1 or claim 2.

53361-1

-63-

10. The isolated nucleic acid according to claim 9, that comprises nucleotide sequences selected from the group consisting of:

a) the nucleotide sequences encoding the light and heavy chain variable region polypeptide sequences of antibody 7C10 contained in SEQ ID NO:1 and SEQ ID NO:3;

b) the nucleotide sequences encoding the light and heavy chain variable region polypeptide sequences of antibody 7B6 contained in SEQ ID NO:5 and SEQ ID NO:7; and

c) the nucleotide sequences encoding the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:9 and SEQ ID NO:11.

11. A cultured cell that produces an antibody or fragment thereof according to any one of claims 3 to 7.

12. The cultured cell of claim 11, which is a CHO transfectoma.

13. The cultured cell of claim 11, that produces a chimeric antibody that binds to human CD80 antigen and comprises a light chain constant region of a human antibody light chain and a heavy constant region of a human antibody heavy chain, and further comprises variable region polypeptide sequences selected from the group consisting of:

(a) the light and heavy chain variable region polypeptide sequences of antibody 7C10 contained in SEQ ID NO:2 and SEQ ID NO:4, respectively;

(b) the light and heavy chain variable region polypeptide sequences of antibody 7B6 contained in SEQ ID NO:6 and SEQ ID NO:8, respectively; and

53361-1

-64-

(c) the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

14. A pharmaceutical composition that contains an antibody or a fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 and a pharmaceutical carrier.

15. The pharmaceutical composition of claim 14 for use as an immunosuppressant.

16. The pharmaceutical composition according to claim 14, wherein said pharmaceutical composition contains an antibody or a fragment according to claim 2, and said antibody or fragment binds to an epitope of human CD80 antigen that is also bound by an antibody comprising the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

17. The pharmaceutical composition according to claim 14, wherein said pharmaceutical composition contains an antibody according to claim 2, and said antibody is a primatized form of a monkey monoclonal antibody which binds to an epitope of human CD80 antigen that is also bound by an antibody comprising the light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

18. Use of an antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 in the manufacture of a medicament for the treatment or prevention of a disease or disorder selected from the group consisting of autoimmune disorder, graft versus host disease,

53361-1

-65-

rejection of a transplanted organ or tissue, infectious disease, inflammatory disease, and B cell lymphoma.

19. Use according to claim 18, in the manufacture of a medicament for the treatment or prevention of graft versus host disease or rejection of a transplanted organ or tissue, wherein the graft or transplanted organ or tissue is selected from the group consisting of kidney, heart, lung, bone marrow, skin, and cornea.

20. Use according to claim 18, wherein the disease or disorder is selected from the group consisting of AIDS, Hashimoto's thyroiditis, myasthenia gravis, uveitis, nephrotic syndrome, atopic dermatitis, contact dermatitis, seborrheic dermatitis, eczematous dermatitides, lichen planus, pemphigus, bullous pemphigus, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythema, cutaneous eosinophilias, alopecia areata, reversible obstructive airways disease, migraine, eczema, rhinitis, other allergic conditions, proliferative disease, hyperproliferative disease, and diseases associated with intestinal inflammation, including coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis.

21. Use according to claim 18, wherein the autoimmune disorder is selected from idiopathic thrombocytopenia purpura, lupus erythematosus, systemic lupus erythematosus, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis, multiple sclerosis, and aplastic anemia.

22. Use of an antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 in the manufacture of a medicament for the treatment or prevention of a B cell lymphoma.

53361-1

-66-

23. The use according to claim 22, wherein the antibody or fragment thereof comprises an antibody or fragment according to claim 2, and said antibody or fragment binds to an epitope of human CD80 antigen that is also bound by an antibody comprising the light and heavy chain variable region polypeptide sequences of antibody 16C10 as set forth in SEQ ID NO:10 and SEQ ID NO:12, respectively.

24. The use according to claim 22, wherein the antibody or fragment thereof comprises an antibody according to claim 2, and said antibody is a primatized form of a monkey monoclonal antibody which binds to an epitope of human CD80 antigen that is also bound by an antibody comprising light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

25. Use of an antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 in the treatment or prevention of a disease or disorder selected from the group consisting of autoimmune disorder, graft versus host disease, rejection of a transplanted organ or tissue, infectious disease, inflammatory disease, and B cell lymphoma.

26. Use of an antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 in the treatment or prevention of graft versus host disease or rejection of a transplanted organ or tissue, wherein the graft or transplanted organ or tissue is selected from the group consisting of kidney, heart, lung, bone marrow, skin, and cornea.

27. Use according to claim 25, wherein the disease or disorder is selected from the group consisting of AIDS, Hashimoto's thyroiditis, myasthenia gravis, uveitis, nephrotic

53361-1

-67-

syndrome, atypical dermatitis, contact dermatitis, seborrheic dermatitis, eczematous dermatitides, lichen planus, pemphigus, bullous pemphigus, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythema, cutaneous eosinophilias, alopecia areata, reversible obstructive airways disease, migraine, eczema, rhinitis, other allergic conditions, proliferative disease, hyperproliferative disease, and diseases associated with intestinal inflammation, including coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis.

28. Use according to claim 25, wherein the autoimmune disorder is selected from idiopathic thrombocytopenia purpura, lupus erythematosus, systemic lupus erythematosus, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis, multiple sclerosis, and aplastic anemia.

29. Use of an antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 in the treatment or prevention of a B cell lymphoma.

30. The use according to claim 29, wherein the antibody or antibody fragment comprises an antibody or fragment according to claim 2, and said antibody or fragment binds to an epitope of human CD80 antigen that is also bound by an antibody comprising the light and heavy chain variable region polypeptide sequences of antibody 16C10 as set forth in SEQ ID NO:10 and SEQ ID NO:12, respectively.

31. The use according to claim 29, wherein the antibody or antibody fragment comprises an antibody according to claim 2, and said antibody is a primatized form of a monkey monoclonal antibody which binds to an epitope of human CD80 antigen that is also bound by an antibody comprising light and heavy chain variable region polypeptide sequences of

53361-1

-68-

antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

32. An antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 for use in the treatment or prevention of a disease or disorder selected from the group consisting of autoimmune disorder, graft versus host disease, rejection of a transplanted organ or tissue, infectious disease, inflammatory disease, and B cell lymphoma.

33. An antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 for use in the treatment or prevention of graft versus host disease or rejection of a transplanted organ or tissue, wherein the graft or transplanted organ or tissue is selected from the group consisting of kidney, heart, lung, bone marrow, skin, and cornea.

34. The antibody or fragment thereof according to claim 32, wherein the disease or disorder is selected from the group consisting of AIDS, Hashimoto's thyroiditis, myasthenia gravis, uveitis, nephrotic syndrome, atypical dermatitis, contact dermatitis, seborrheic dermatitis, eczematous dermatitides, lichen planus, pemphigus, bullous pemphigus, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythema, cutaneous eosinophilias, alopecia areata, reversible obstructive airways disease, migraine, eczema, rhinitis, other allergic conditions, proliferative disease, hyperproliferative disease, and diseases associated with intestinal inflammation, including coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis.

35. The antibody or fragment thereof according to claim 32, wherein the autoimmune disorder is selected from

53361-1

-69-

idiopathic thrombocytopenia purpura, lupus erythematosus, systemic lupus erythematosus, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis, multiple sclerosis, and aplastic anemia.

36. An antibody or fragment thereof that binds to human CD80 antigen according to any one of claims 1 to 7 for use in the treatment or prevention of a B cell lymphoma.

37. The antibody or fragment thereof according to claim 36, wherein the antibody or antibody fragment comprises an antibody or fragment according to claim 2, and said antibody or fragment binds to an epitope of human CD80 antigen that is also bound by an antibody comprising the light and heavy chain variable region polypeptide sequences of antibody 16C10 as set forth in SEQ ID NO:10 and SEQ ID NO:12, respectively.

38. The antibody or fragment thereof according to claim 36, wherein the antibody or antibody fragment comprises an antibody according to claim 2, and said antibody is a primatized form of a monkey monoclonal antibody which binds to an epitope of human CD80 antigen that is also bound by an antibody comprising light and heavy chain variable region polypeptide sequences of antibody 16C10 contained in SEQ ID NO:10 and SEQ ID NO:12, respectively.

SMART & BIGGAR

OTTAWA, CANADA

PATENT AGENTS

1 / 15

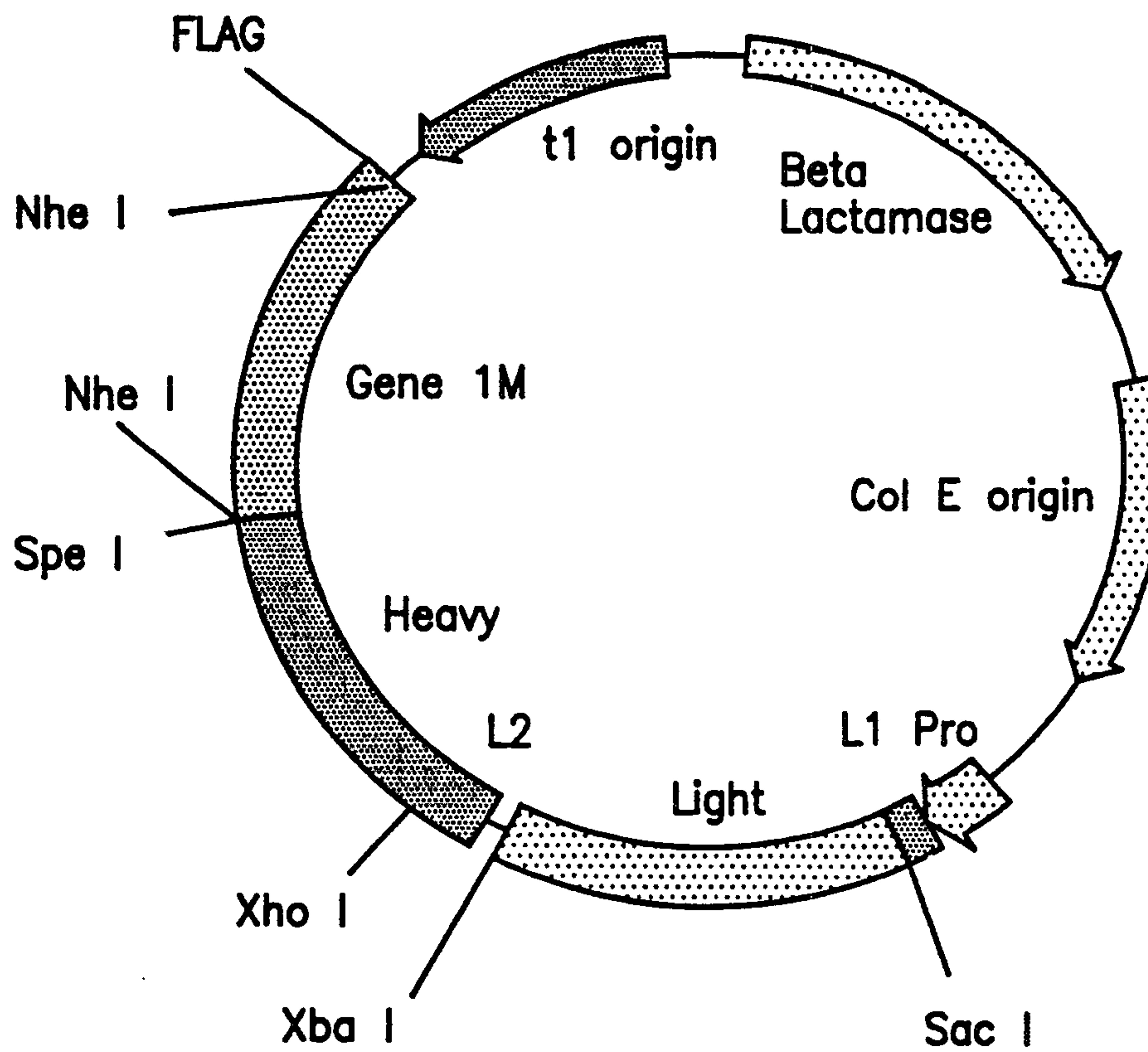


FIG. 1

SUBSTITUTE SHEET (RULE 26)

2 / 15

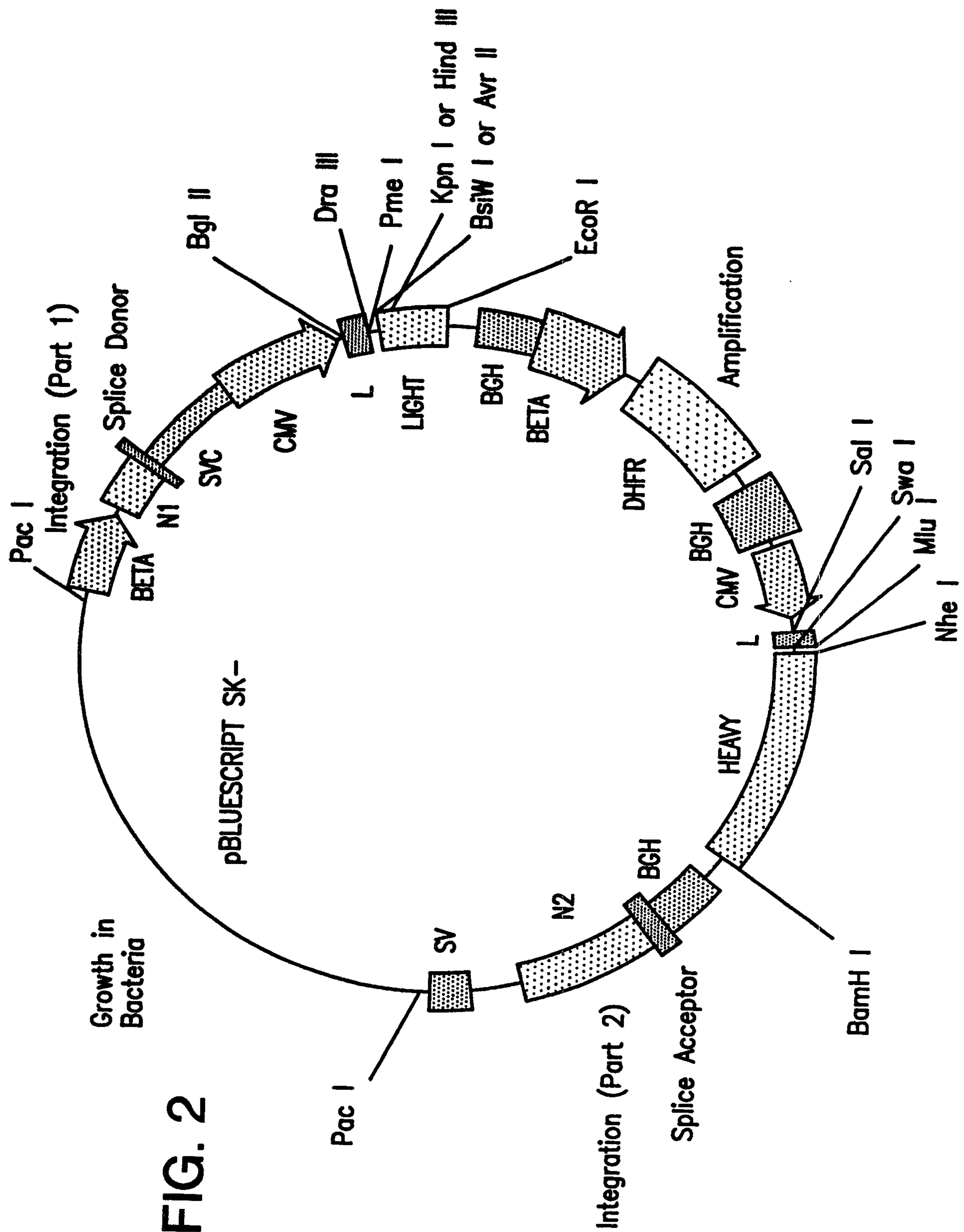


FIG. 2

SUBSTITUTE SHEET (RULE 26)

3 / 15

FIG. 3

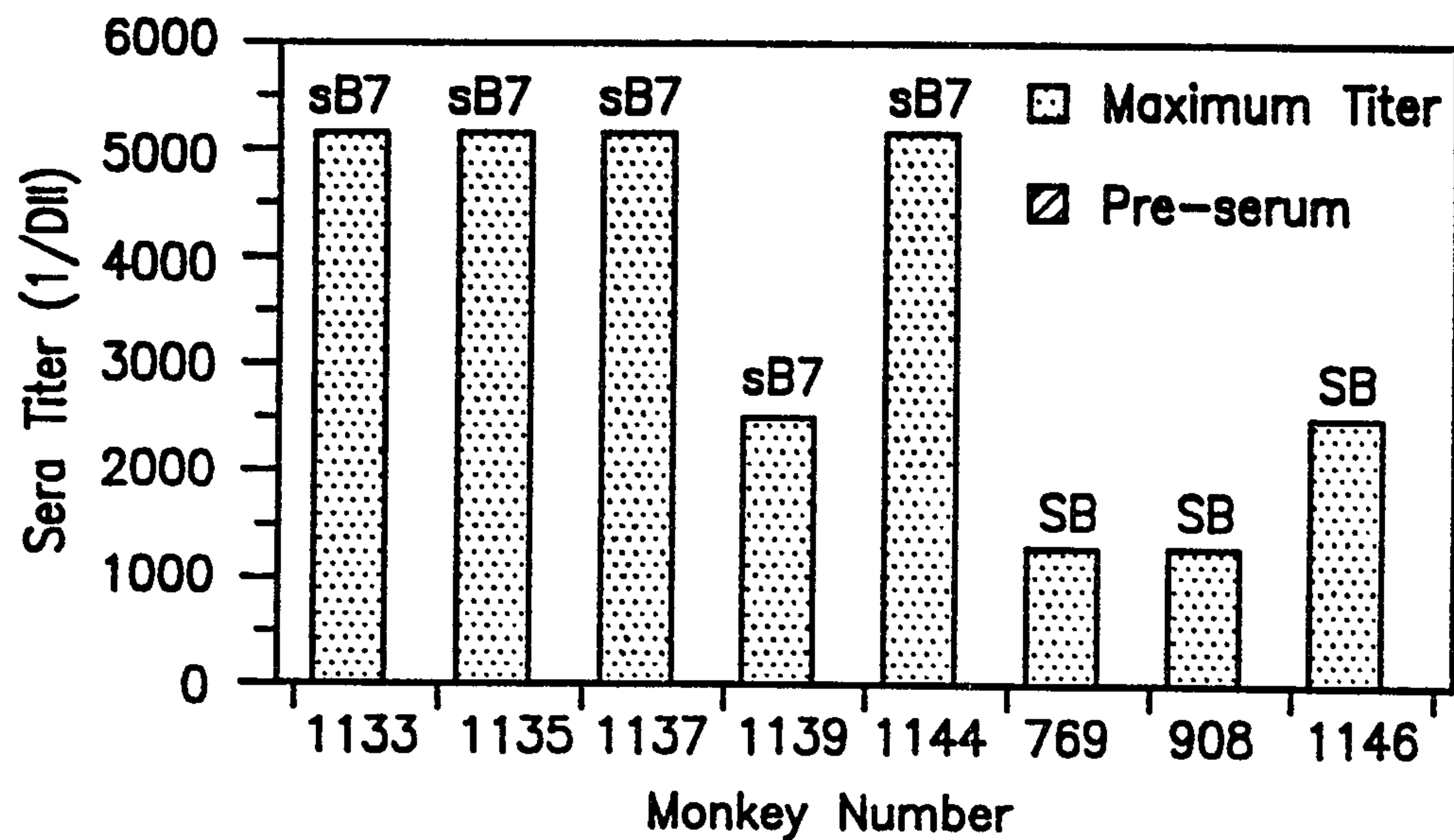
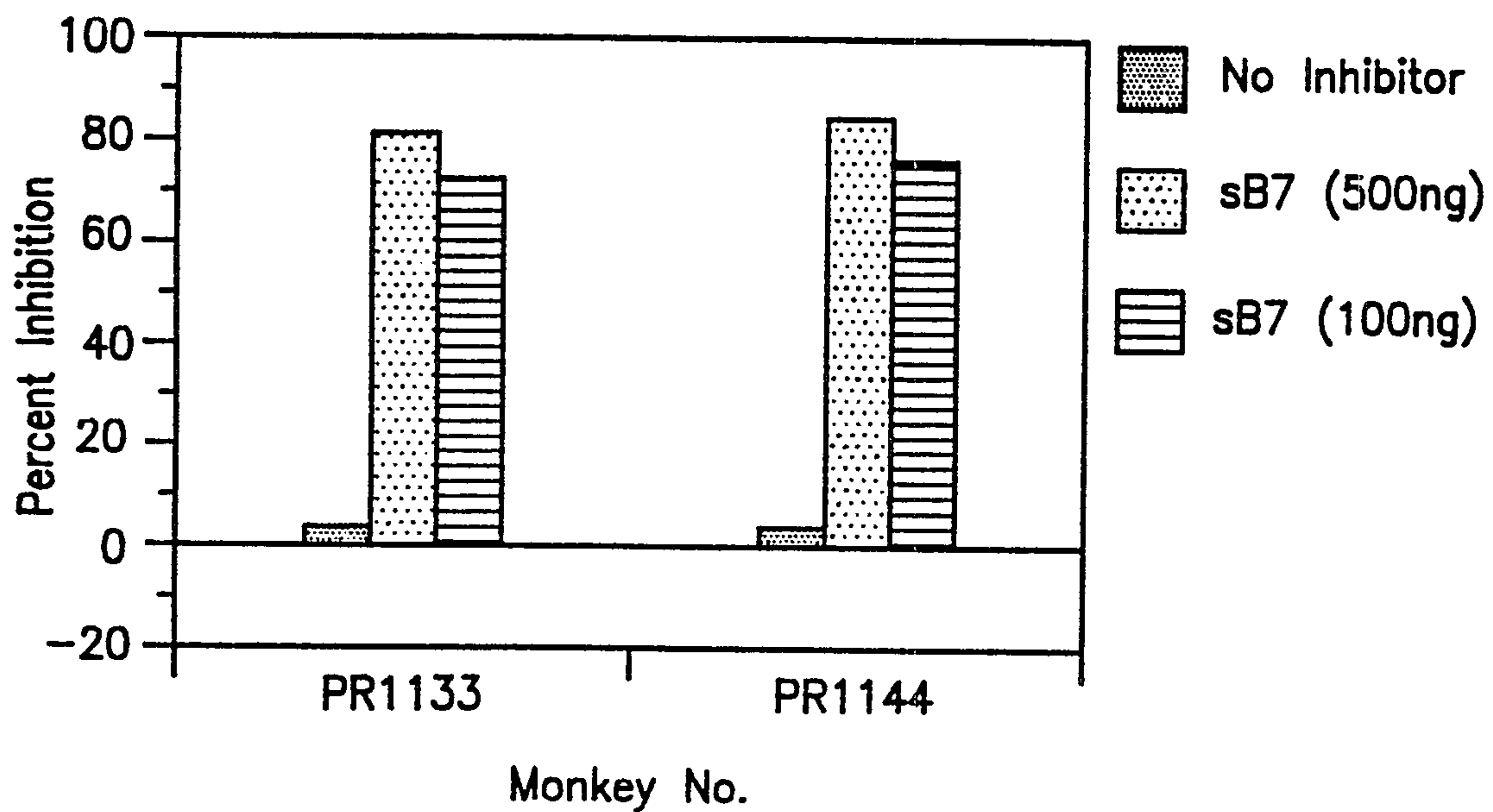
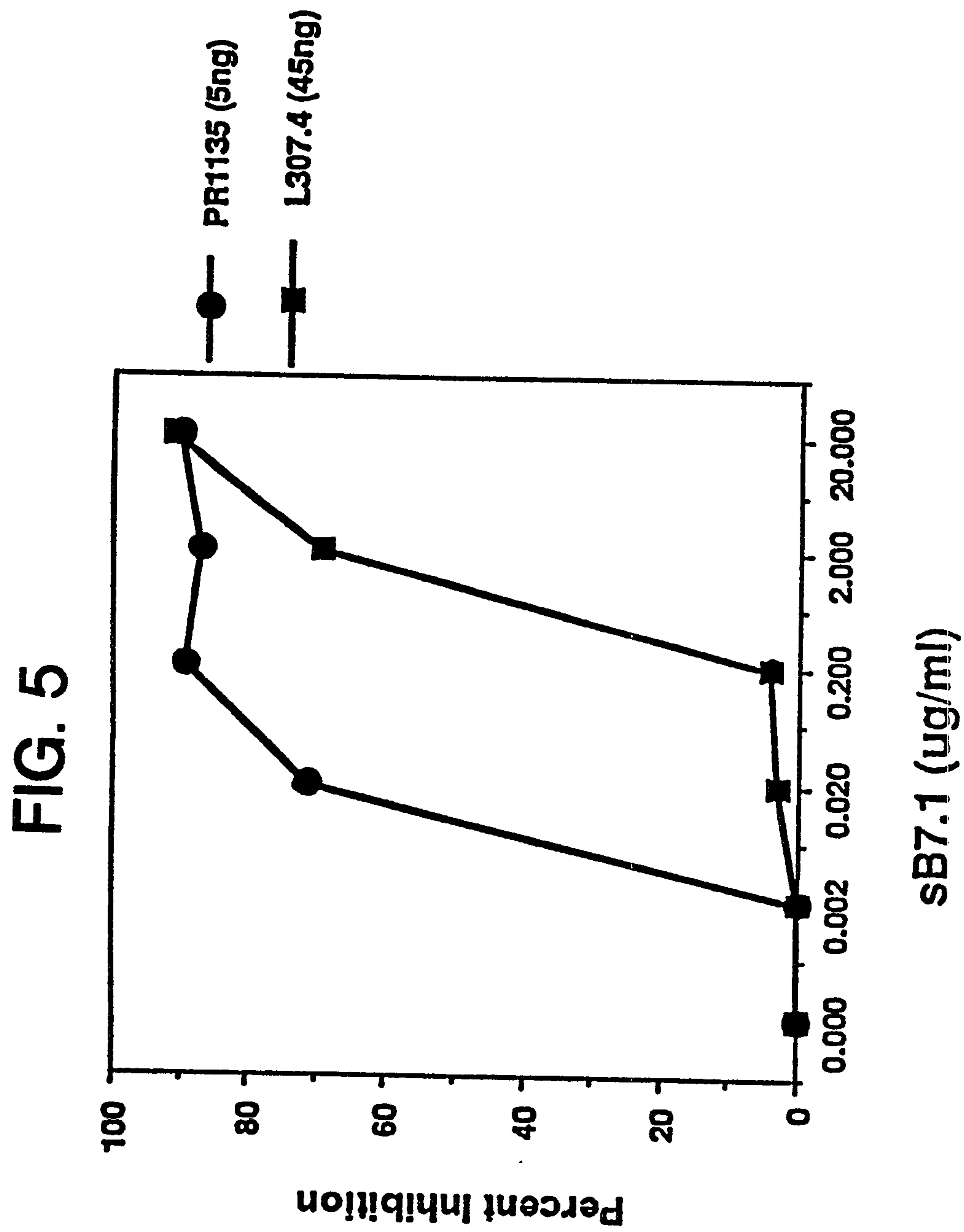


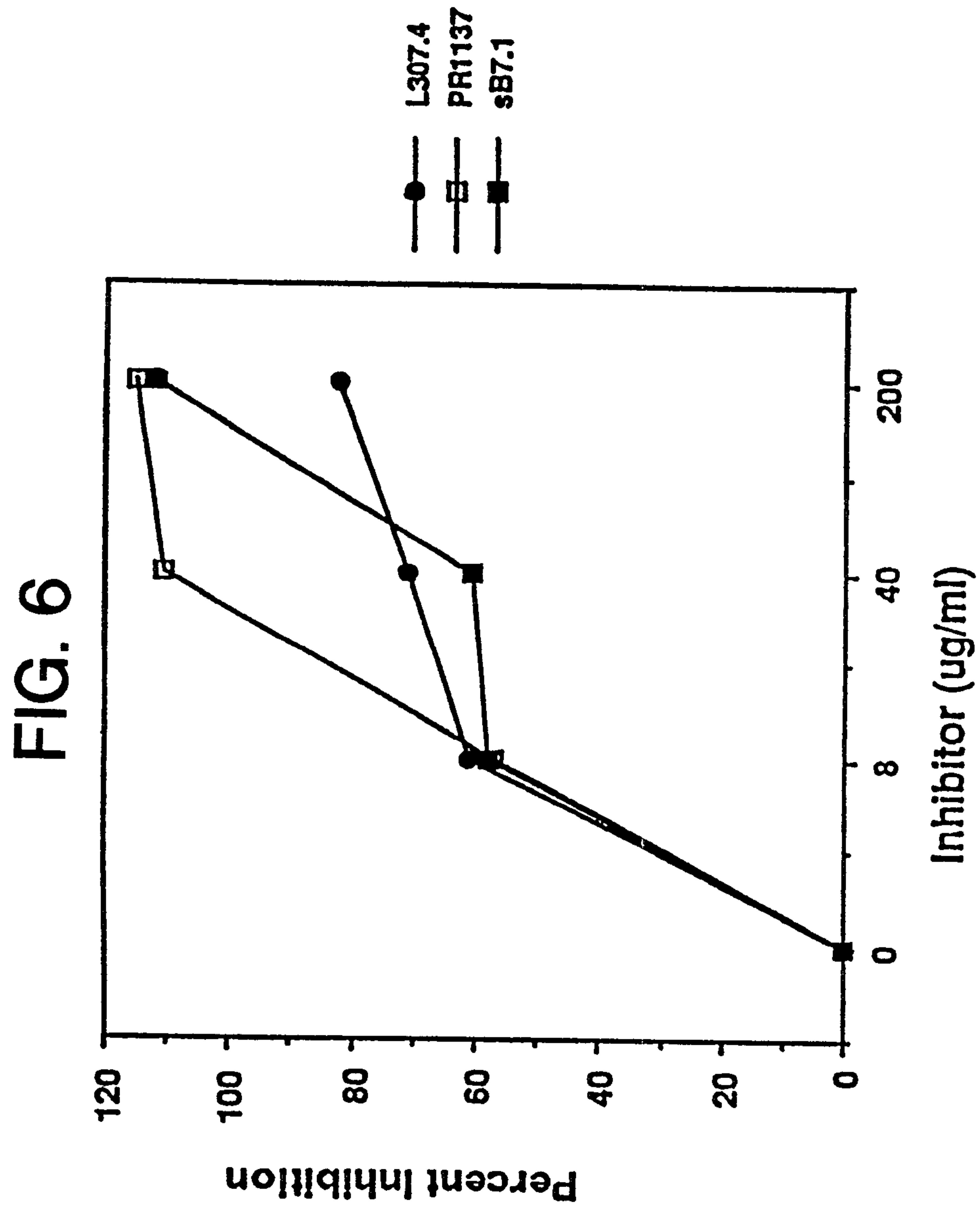
FIG. 4



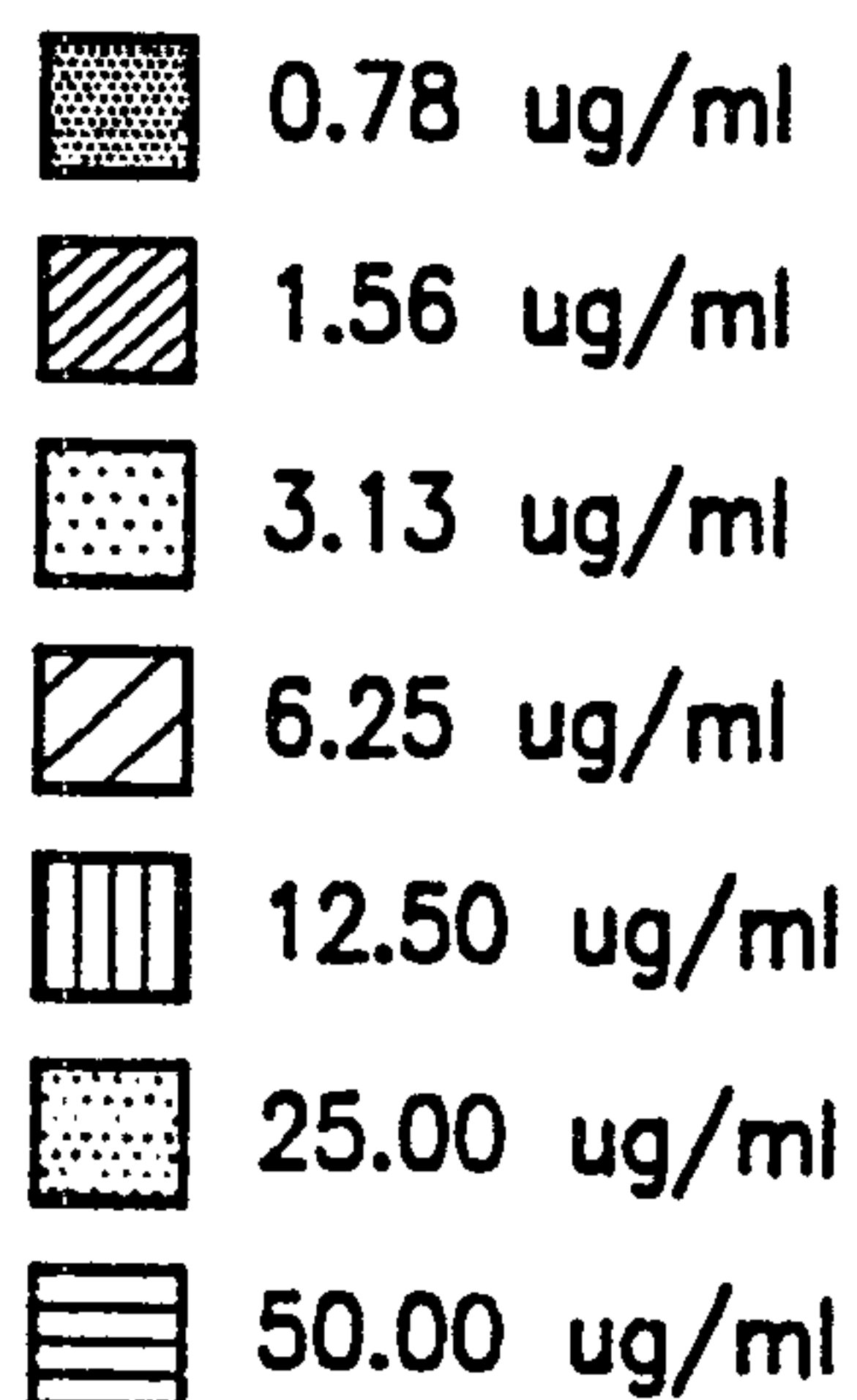
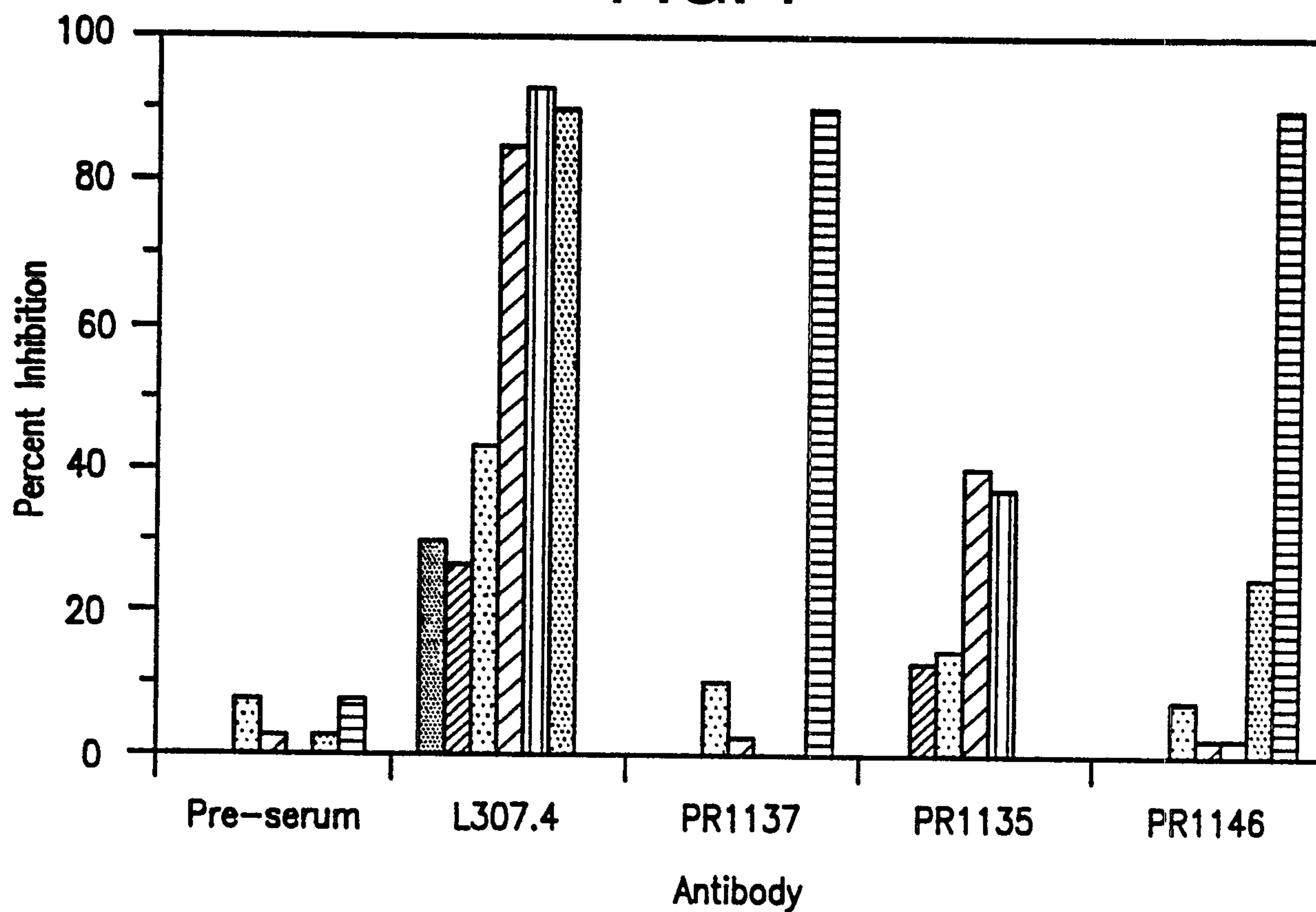
4 / 15



5 / 15



6 / 15

FIG. 7

7 / 15

Frame 1 M R V P A Q L L G L L L L W L P G A R
ATG AGG GTC CCC GCT CAG CTC CTG GGG CTC CTG CTG CTC TGG CTC CCA GGT GCA CGA
9 18 27 36 45 54

C A Y E L T Q P P S V S V S P G Q T A R I
TGT GCC TAT GAA CTG ACT CAG CCA CCC TCG GTG TCA GTG TCC CCA GGA CAG ACG GCC AGG ATC
63 72 81 90 99 108 117

T C G G D N S R N E Y V H W Y Q Q K P A R
ACC TGT GGG GGA GAC AAC AGT AGA AAT GAA TAT GTC CAC TGG TAC CAG CAG AAG CCA GCG CGG
126 135 144 153 162 171 180

A P I L V I Y D D S D R P S G I P E R F S
GCC CCT ATA CTG GTC ATC TAT GAT GAT AGT GAC CGG CCC TCA GGG ATC CCT GAG CGA TTC TCT
189 198 207 216 225 234 243

G S K S G N T A T L T I N G V E A G D E A
GGC TCC AAA TCA GGG AAC ACC GCC ACC CTG ACC ATC AAC GGG GTC GAG GCC GGG GAT GAG GCT
252 261 270 279 288 297 306

D Y Y C Q V W D R A S D H P V F G G G T R
GAC TAT TAC TGT CAG GTG TGG GAC AGG GCT AGT GAT CAT CCG GTC TTC GGA GGA GGG ACC CGG
315 324 333 342 351 360 369

V T V L G Q P K A A P S V T L F P P S S E
GTG ACC GTC CTA GGT CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC TCT GAG
378 387 396 405 414 423 432

E L Q A N K A T L V C L I S D F Y P G A V
GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC TTC TAC CCG GGA GCC GTG
441 450 459 468 477 486 495

T V A W K A D S S P V K A G V E T T T P S
ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC TCC
504 513 522 531 540 549 558

K Q S N N K Y A A S S Y L S L T P E Q W K
AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAC CTG AGC CTG ACG CCT GAG CAG TGG AAG
567 576 585 594 603 612 621

S H R S Y S C Q V T H E G S T V E K T V A
TCC CAC AGA AGC TAC AGC TGC CAG GTG ACG CAT GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC
630 639 648 657 666 675 684

P T E C S
CCT ACA GAA TGT TCA TGA
693 702

FIG. 8a

SUBSTITUTE SHEET (RULE 26)

8 / 15

Frame 1	M	K	H	L	W	F	F	L	L	L	V	A	A	P	R	W	V	L	S	
	ATG	AAA	CAC	CTG	TGG	TTC	TTC	CTC	CTC	CTG	GTG	GCA	GCT	CCC	AGA	TGG	GTC	CTG	TCC	
			9			18			27			36			45			54		
Q	V	K	L	Q	Q	W	G	E	G	L	L	Q	P	S	E	T	L	S	R	T
CAG	GTG	AAG	CTG	CAG	CAG	TGG	GGC	GAA	GGA	CTT	CTG	CAG	CCT	TCG	GAG	ACC	CTG	TCC	CGC	ACC
	63			72			81			90			99			108			117	
C	V	V	S	G	G	S	I	S	G	Y	Y	Y	W	T	W	I	R	Q	T	P
TGC	GTT	GTC	TCT	GGT	GGC	TCC	ATC	AGC	GGT	TAC	TAC	TAC	TGG	ACC	TGG	ATC	CGC	CAG	ACC	CCA
	126			135			144			153			162			171			180	
G	R	G	L	E	W	I	G	H	I	Y	G	N	G	A	T	T	N	Y	N	P
GGG	AGG	GGA	CTG	GAG	TGG	ATT	GGC	CAT	ATT	TAT	GGT	AAT	GGT	GCG	ACC	ACC	AAC	TAC	AAT	CCC
	189			198			207			216			225			234			243	
S	L	K	S	R	V	T	I	S	K	D	T	S	K	N	Q	F	F	L	N	L
TCC	CTC	AAG	AGT	CGA	GTC	ACC	ATT	TCA	AAA	GAC	ACG	TCC	AAG	AAC	CAG	TTC	TTC	CTG	AAC	TTG
	252			261			270			279			288			297			306	
N	S	V	T	D	A	D	T	A	V	Y	Y	C	A	R	G	P	R	P	D	C
AAT	TCT	GTG	ACC	GAC	GCG	GAC	ACG	GCC	GTC	TAT	TAC	TGT	GCG	AGA	GCG	CCT	CGC	CCT	GAT	TGC
	315			324			333			342			351			360			369	
T	T	I	C	Y	G	G	W	V	D	V	W	G	P	G	D	L	V	T	V	S
ACA	ACC	ATT	TGT	TAT	GGC	GGC	TGG	GTC	GAT	GTC	TGG	GGC	CCG	GGA	GAC	CTG	GTC	ACC	GTC	TCC
	378			387			396			405			414			423			432	
S	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G
TCA	GCT	AGC	ACC	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG
	441			450			459			468			477			486			495	
G	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W
GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG
	504			513			522			531			540			549			558	
N	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L
AAC	TCA	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	CTC
	567			576			585			594			603			612			621	
Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C
TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	ACC	TAC	ATC	TGC
	630			639			648			657			666			675			684	
N	V	N	H	K	P	S	N	T	K	V	D	K	K	A	E	P	K	S	C	D
AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA	GCA	GAG	CCC	AAA	TCT	TGT	GAC
	693			702			711			720			729			738			747	
K	T	H	T	C	P	P	C	P	A	P	E	L	L	G	G	P	S	V	F	L
AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT	GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC
	756			765			774			783			792			801			810	
F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
TTC	CCC	CCA	AAA	CCC	AAG	GAC	ACC	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACA	TGC	GTG	GTG
	819			828			837			846			855			864			873	
V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V
GTG	GAC	GTG	AGC	CAC	GAA	GAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG	GAG	GTG
	882			891			900			909			918			927			936	

FIG. 8b-1

SUBSTITUTE SHEET (RULE 26)

9 / 15

H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V
CAT	AAT	GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC	GTC
945				954			963			972			981			990			999	
L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S	N	K
CTC	ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	GTC	TCC	AAC	AAA
1008			1017			1026		1035		1044		1053					1062			
A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q
GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA	GGG	CAG	CCC	CGA	GAA	CCA	CAG
1071			1080			1089		1098		1107			1116				1125			
V	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T	C	L
GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG	ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG
1134			1143			1152		1161		1170			1179				1188			
V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N
GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC
1197			1206			1215		1224		1233			1242				1251			
N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L
AAC	TAC	AAG	ACC	ACG	CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAC	AGC	AAG	CTC
1260			1269			1278		1287		1296			1305				1314			
T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A
ACC	GTG	GAC	AAG	AGC	AGG	TGG	CAG	CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT
1323			1332			1341		1350		1359			1368				1377			
L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K					
CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	TGA				
1386			1395			1404		1413		1422			1431							

FIG. 8b-2

SUBSTITUTE SHEET (RULE 26)

10 / 15

Frame 1	M	S	L	P	A	Q	L	L	G	L	L	L	L	C	V	P	G	S	S	
	ATG	AGC	CTC	CCT	GCT	CAG	CTC	CTC	GGG	CTG	CTA	TTG	CTC	TGC	GTC	CCC	GGG	TCC	AGT	
			9			18			27			36			45			54		
G	E	V	V	M	T	Q	S	P	L	S	L	P	I	T	P	G	E	P	A	S
GGG	GAA	GTT	GTG	ATG	ACT	CAG	TCT	CCA	CTG	TCC	CTT	CCC	ATC	ACA	CCT	GGA	GAG	CCG	GCC	TCC
	63			72		81			90			99			108			117		
I	S	C	R	S	S	Q	S	L	K	H	S	N	G	D	T	F	L	S	W	Y
ATC	TCC	TGT	AGG	TCT	AGT	CAA	AGC	CTT	AAA	CAC	AGT	AAT	GGA	GAC	ACC	TTC	CTG	AGT	TGG	TAT
	126			135		144			153			162			171			180		
Q	Q	K	P	G	Q	P	P	R	L	L	I	Y	K	V	S	N	R	D	S	G
CAG	CAG	AAG	CCA	GGC	CAA	CCT	CCA	AGG	CTC	CTG	ATT	TAT	AAG	GTT	TCT	AAC	CGG	GAC	TCT	GGG
	189			198		207			216			225			234			243		
V	P	D	R	F	S	G	S	G	A	G	T	D	F	T	L	K	I	S	A	V
GTC	CCA	GAC	AGA	TTC	AGC	GGC	AGT	GGG	GCA	GGG	ACA	GAT	TTC	ACA	CTG	AAA	ATC	AGC	GCA	GTG
	252			261		270			279			288			297			306		
E	A	E	D	V	G	V	Y	F	C	G	Q	G	T	R	T	P	P	T	F	G
GAG	GCT	GAA	GAT	GTT	GGG	GTT	TAT	TTC	TGC	GGG	CAA	GGT	ACA	AGG	ACT	CCT	CCC	ACT	TTC	GGC
	315			324		333			342			351			360			369		
G	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	I	F	P	P
GGA	GGG	ACC	AAG	GTG	GAA	ATC	AAA	CGT	ACG	GTG	GCT	GCA	CCA	TCT	GTC	TTC	ATC	TTC	CCG	CCA
	378			387		396			405			414			423			432		
S	D	E	Q	L	K	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P
TCT	GAT	GAG	CAG	TTG	AAA	TCT	GGA	ACT	GCC	TCT	GTT	GTG	TGC	CTG	CTG	AAT	AAC	TTC	TAT	CCC
	441			450		459			468			477			486			495		
R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E	S
AGA	GAG	GCC	AAA	GTA	CAG	TGG	AAG	GTG	GAT	AAC	GCC	CTC	CAA	TCG	GGT	AAC	TCC	CAG	GAG	AGT
	504			513		522			531			540			549			558		
V	T	E	Q	D	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K
GTC	ACA	GAG	CAG	GAC	AGC	AAG	GAC	AGC	ACC	TAC	AGC	CTC	AGC	AGC	ACC	CTG	ACG	CTG	AGC	AAA
	567			576		585			594			603			612			621		
A	D	Y	E	K	H	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P
GCA	GAC	TAC	GAG	AAA	CAC	AAA	GTC	TAC	GCC	TGC	GAA	GTC	ACC	CAT	CAG	GGC	CTG	AGC	TCG	CCC
	630			639		648			657			666			675			684		
V	T	K	S	F	N	R	G	E	C	.										
GTC	ACA	AAG	AGC	TTC	AAC	AGG	GGA	GAG	TGT	TGA										
	693			702		711			720											

FIG. 9a

11 / 15

Frame 1 M G W S L I L L F L V A V A T R V Q C
ATG GGT TGG AGC CTC ATC TTG CTC TTC CTT GTC GCT GTT GCT ACG CGT GTC CAG TGT
9 18 27 36 45 54

E V Q L V E S G G G L V Q P G G S L R V S
GAG GTG CAA CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGC GGG TCC CTG AGA GTC TCC
63 72 81 90 99 108 117

C A V S G F T F S D H Y M Y W F R Q A P G
TGT GCA GTC TCT GGA TTC ACC TTC AGT GAC CAC TAC ATG TAT TGG TTC CGC CAG GCT CCA GGG
126 135 144 153 162 171 180

K G P E W V G F I R N K P N G G T T E Y A
AAG GGG CCG GAA TGG GTA GGT TTC ATT AGA AAC AAA CCG AAC GGT GGG ACA ACA GAA TAC GCC
189 198 207 216 225 234 243

A S V K D R F T I S R D D S K S I A Y L Q
GCG TCT GTG AAA GAC AGA TTC ACC ATC TCC AGA GAT GAT TCC AAA AGC ATC GCC TAT CTG CAA
252 261 270 279 288 297 306

M S S L K I E D T A V Y Y C T T S Y I S H
ATG AGC AGC CTG AAA ATC GAG GAC ACG GCC GTC TAT TAC TGT ACT ACA TCC TAC ATT TCA CAT
315 324 333 342 351 360 369

C R G G V C Y G G Y F E F W G Q G A L V T
TGT CGG GGT GGT GTC TGC TAT GGA GGT TAC TTC GAA TTC TGG GGC CAG GGC GCC CTG GTC ACC
378 387 396 405 414 423 432

V S S A S T K G P S V F P L A P S S K S T
GTC TCC TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC
441 450 459 468 477 486 495

S G G T A A L G C L V K D Y F P E P V T V
TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG
504 513 522 531 540 549 558

S W N S G A L T S G V H T F P A V L Q S S
TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA
567 576 585 594 603 612 621

G L Y S L S S V V T V P S S S L G T Q T Y
GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC
630 639 648 657 666 675 684

I C N V N H K P S N T K V D K K A E P K S
ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GCA GAG CCC AAA TCT
693 702 711 720 729 738 747

C D K T H T C P P C P A P E L L G G P S V
TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC
756 765 774 783 792 801 810

F L F P P K P K D T L M I S R T P E V T C
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC
819 828 837 846 855 864 873

V V V D V S H E D P E V K F N W Y V D G V
GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG
882 891 900 909 918 927 936

FIG. 9b-1

SUBSTITUTE SHEET (RULE 26)

12 / 15

E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V
GAG	GTG	CAT	AAT	GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTG
945				954			963			972			981			990			999	
S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
AGC	GTG	CTC	ACC	GTG	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	GTG	TCC
1008				1017			1026			1035			1044			1053			1062	
N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E
AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA	GGG	CAG	CCC	CGA	GAA
1071				1080			1089			1098			1107			1116			1125	
P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T
CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG	ACC	AAG	AAC	CAG	GTG	AGC	CTG	ACC
1134				1143			1152			1161			1170			1179			1188	
C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P
TGC	CTG	GTG	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG
1197				1206			1215			1224			1233			1242			1251	
E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S
GAG	AAC	AAC	TAC	AAG	ACC	ACG	CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAC	ACC
1260				1269			1278			1287			1296			1305			1314	
K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H
AAG	CTC	ACC	GTG	GAC	AAG	AGC	AGG	TGG	CAG	CAG	GGG	AAC	GTG	TTC	TCA	TGC	TCC	GTG	ATG	CAT
1323				1332			1341			1350			1359			1368			1377	
E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K			
GAG	GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	TGA		
1386				1395			1404			1413			1422			1431				

FIG. 9b-2

13 / 15

Frame 1 M R V P A Q L L G L L L L W L P G A R
ATG AGG GTC CCC GCT CAG CTC CTG GGG CTC CTG CTC TGG CTC CCA GGT GCA CGA
9 18 27 36 45 54

C E S V L T Q P P S V S G A P G Q K V T I
TGT GAG TCT GTC CTG ACA CAG CCG CCC TCA GTG TCT GGG GCC CCA GGG CAG AAG GTC ACC ATC
63 72 81 90 99 108 117

S C T G S T S N I G G Y D L H W Y Q Q L P
TCG TGC ACT GGG AGC ACC TCC AAC ATT GGA GGT TAT GAT CTA CAT TGG TAC CAG CAG CTC CCA
126 135 144 153 162 171 180

G T A P K L L I Y D I N K R P S G I S D R
GGA ACG GCC CCC AAA CTC CTC ATC TAT GAC ATT AAC AAG CGA CCC TCA GGA ATT TCT GAC CGA
189 198 207 216 225 234 243

F S G S K S G T A A S L A I T G L Q T E D
TTC TCT GGC TCC AAG TCT GGT ACC GCG GCC TCC CTG GCC ATC ACT GGG CTC CAG ACT GAG GAT
252 261 270 279 288 297 306

E A D Y Y C Q S Y D S S L N A Q V F G G G
GAG GCT GAT TAT TAC TGC CAG TCC TAT GAC AGC AGC CTG AAT GCT CAG GTA TTC GGA GGA GGG
315 324 333 342 351 360 369

T R L T V L G Q P K A A P S V T L F P P S
ACC CGG CTG ACC GTC CTA GGT CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC
378 387 396 405 414 423 432

S E E L Q A N K A T L V C L I S D F Y P G
TCT GAG GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC TTC TAC CCG GGA
441 450 459 468 477 486 495

A V T V A W K A D S S P V K A G V E T T T
GCC GTG ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA
504 513 522 531 540 549 558

P S K Q S N N K Y A A S S Y L S L T P E Q
CCC TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAC CTG AGC CTG ACG CCT GAG CAG
567 576 585 594 603 612 621

W K S H R S Y S C Q V T H E G S T V E K T
TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG CAT GAA GGG AGC ACC GTG GAG AAG ACA
630 639 648 657 666 675 684

V A P T E C S
GTG GCC CCT ACA GAA TGT TCA TGA
693 702 711

FIG. 10a

14 / 15

Frame 1 M K H L W F F L L L V A A P R W V L S
ATG AAA CAC CTG TGG TTC TTC CTC CTC CTG GTG GCA GCT CCC AGA TGG GTC CTG TCC
9 18 27 36 45 54

Q V Q L Q E S G P G L V K P S E T L S L T
CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG TCC CTC ACC
63 72 81 90 99 108 117

C A V S G G S I S G G Y G W G W I R Q P P
TGC GCT GTC TCT GGT GGC TCC ATC AGC GGT GGT TAT GGC TGG GGC TGG ATC CGC CAG CCC CCA
126 135 144 153 162 171 180

G K G L E W I G S F Y S S S G N T Y Y N P
GGG AAG GGG CTG GAG TGG ATT GGG AGT TTC TAT AGT AGT AGT GGG AAC ACC TAC TAC AAC CCC
189 198 207 216 225 234 243

S L K S Q V T I S T D T S K N Q F S L K L
TCC CTC AAG AGT CAA GTC ACC ATT TCA ACA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG
252 261 270 279 288 297 306

N S M T A A D T A V Y Y C V R D R L F S V
AAC TCT ATG ACC GCC GCG GAC ACG GCC GTG TAT TAC TGT GTG AGA GAT CGT CTT TTT TCA GTT
315 324 333 342 351 360 369

V G M V Y N N W F D V W G P G V L V T V S
GTT GGA ATG GTT TAC AAC AAC TGG TTC GAT GTC TGG GGC CCG GGA GTC CTG GTC ACC GTC TCC
378 387 396 405 414 423 432

S A S T K G P S V F P L A P S S K S T S G
TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG
441 450 459 468 477 486 495

G T A A L G C L V K D Y F P E P V T V S W
GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG
504 513 522 531 540 549 558

N S G A L T S G V H T F P A V L Q S S G L
AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC
567 576 585 594 603 612 621

Y S L S S V V T V P S S S L G T Q T Y I C
TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC
630 639 648 657 666 675 684

N V N H K P S N T K V D K K A E P K S C D
AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GCA GAG CCC AAA TCT TGT GAC
693 702 711 720 729 738 747

K T H T C P P C P A P E L L G G P S V F L
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC
756 765 774 783 792 801 810

F P P K P K D T L M I S R T P E V T C V V
TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG
819 828 837 846 855 864 873

V D V S H E D P E V K F N W Y V D G V E V
GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG

FIG. 10b-1

SUBSTITUTE SHEET (RULE 26)

15 / 15

882	891	900	909	918	927	936
H N A K T K P R E E Q Y N S T Y R V V S V						
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC						
945	954	963	972	981	990	999
L T V L H Q D W L N G K E Y K C K V S N K						
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA						
1008	1017	1026	1035	1044	1053	1062
A L P A P I E K T I S K A K G Q P R E P Q						
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GGC AAA GGG CAG CCC CGA GAA CCA CAG						
1071	1080	1089	1098	1107	1116	1125
V Y T L P P S R D E L T K N Q V S L T C L						
GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG						
1134	1143	1152	1161	1170	1179	1188
V K G F Y P S D I A V E W E S N G Q P E N						
GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC						
1197	1206	1215	1224	1233	1242	1251
N Y K T T P P V L D S D G S F F L Y S K L						
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC						
1260	1269	1278	1287	1296	1305	1314
T V D K S R W Q Q G N V F S C S V M H E A						
ACC GTG GAC AAG AGC AGG TGG CAG CAG GGC AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT						
1323	1332	1341	1350	1359	1368	1377
L H N H Y T Q K S L S L S P G K .						
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA						
1386	1395	1404	1413	1422	1431	

FIG. 10b-2