FULLY HUMAN ANTIBODIES AGAINST HUMAN 4-1BB (CD137)

Field of the Invention:

The invention is directed to fully human antibodies and, more specifically, to fully human antibodies to human 4-1BB (CD137).

BACKGROUND OF THE INVENTION:

An extensive body of evidence has unequivocally demonstrated that some degree of immune response against cancer exists in humans and animals. In cancer patients, cellular components of the immune system are able to recognize antigens expressed by tumor cells, such as differentiation of oncofetal antigens or mutated gene products (S. Rosenberg, Nature, 411:380-4 (2001); P. van der Bruggen et al., Immunological Rev., 188:51-64 (2002)). A number of clinical studies have shown that tumor-infiltrating lymphocytes have favorable prognostic significance (E. Halapi, Med. Oncol., 15(4):203-11 (1998); Y. Naito et al., Cancer Res., 58(16):3491-4 (1998); L. Zhang et al., N.E. J. Med., 348(3):203-13 (2003)). Furthermore, treatment with immunomodulators, such as cytokines or bacterial products, cancer vaccines, or adoptive immunotherapy has led to tumor regression in a number of patients (S. Rosenberg, Cancer J. Sci. Am. 6(S):2 (2000); P. Bassi, Surg. Oncol., 11(1-2):77-83 (2002); S. Antonia et al., Current Opinion in Immunol., 16:130-6 (2004)). Despite these responses, immunity against cancer frequently fails to effectively eliminate tumor cells. The causes for this failure can be grouped into three major categories: (i) impaired tumor recognition by immune cells, either by variable expression of tumor antigens or reduced expression of class I major histocompatibility complex (MHC); (ii) immunosuppressive tumor microenvironment, as a result of secretion of inhibitory cytokines by tumor cells (e.g., TGF-β); and (iii) poor tumor immunogenicity due to the lack of expression of co-stimulatory molecules on tumor cells, which results in the inability of the tumor cells to effectively stimulate T-cells. Advances in our understanding of the requirements for tumor antigen recognition and immune effector function indicate that a potential strategy to enhance an anti-tumor immune response is to provide co-stimulation through an auxiliary molecule. Tumor antigen-specific T-cells require costimulation to initiate and maintain effector

functions. Thus, therapies that target costimulatory molecules can be applied to modulate and enhance the immune response to tumors.

The current model for T-cell activation postulates that naive T-cells require two signals for full activation: (i) a signal provided through the binding of processed antigens presented to the T-cell receptor by major histocompatibility complex (MHC) class I molecules; and (ii) an additional signal provided by the interaction of co-stimulatory molecules on the surface of T-cells and their ligands on antigen presenting cells. Recognition of an antigen by a naive T-cell is insufficient in itself to trigger T-cell activation. Without a co-stimulatory signal, T-cells may be eliminated either by death or by induction of anergy. Signaling through the CD28 costimulatory molecule appears to be key for the initiation of T-cell responses. However, CD137 (4-1BB) signaling has been shown to be primordial for the maintenance and expansion of the immune response to antigens, as well as, for the generation of memory T-cells.

CD137 (4-1BB) is a member of the tumor necrosis receptor (TNF-R) gene family, which includes proteins involved in regulation of cell proliferation, differentiation, and programmed cell death. CD137 is a 30 kDa type I membrane glycoprotein expressed as a 55 kDa homodimer. The receptor was initially described in mice (B. Kwon et al., P.N.A.S. USA, 86:1963-7 (1989)), and later identified in humans (M. Alderson et al., Eur. J. Immunol., 24: 2219-27 (1994); Z. Zhou et al., Immunol. Lett., 45:67 (1995)) (See, also, Published PCT Applications WO95/07984 and WO96/29348, and U.S. Patent No. 6,569,997, hereby incorporated by reference (See, SEQ ID NO:2.)). The human and murine forms of CD137 are 60% identical at the amino acid level. Conserved sequences occur in the cytoplasmic domain, as well as 5 other regions of the molecule, indicating that these residues might be important for function of the CD137 molecule (Z. Zhou et al., Immunol. Lett., 45:67 (1995)). Expression of CD137 has been shown to be predominantly on cells of lymphoid lineage such as activated T-cells, activated Natural Killer (NK) cells, NKT-cells, CD4CD25 regulatory T-cells, and also on activated thymocytes, and intraepithelial lymphocytes. In addition, CD137 has also been shown to be expressed on cells of myeloid origin like dendritic cells, monocytes, neutrophils, and eosinophils. Even though CD137 expression is mainly restricted to immune/inflammatory cells, there

have been reports describing its expression on endothelial cells associated with a small number of tissues from inflammatory sites and tumors.

Functional activities of CD137 on T-cells have been amply characterized. Signaling through CD137 in the presence of suboptimal doses of anti-CD3 has been demonstrated to induce T-cell proliferation and cytokine synthesis (mainly IFN-γ), and to inhibit activated cell death. These effects have been observed with both murine and human T-cells (W. Shuford et al., J. Exp. Med., 186(1):47-55 (1997); D. Vinay et al., Semin. Immunol., 10(6):481-9 (1998); D. Laderach et al., Int. Immunol., 14(10):1155-67 (2002)). In both humans and mice, co-stimulation enhances effector functions, such as IFN-γ production and cytotoxicity, by augmenting the numbers of antigen-specific and effector CD8+ T-cells. In the absence of anti-CD3 signaling, stimulation through CD137 does not alter T-cell function, indicating that CD137 is a co-stimulatory molecule.

The physiological events observed following CD137 stimulation on T-cells are mediated by NF-κB and PI3K/ERK1/2 signals with separate physiological functions. NF-κB signals trigger expression of Bcl-$_{xL}$, an anti-apopotic molecule, thus resulting in increased survival, whereas PI3K and ERK1/2 signals are specifically responsible for CD137-mediated cell cycle progression (H. Lee et al., J. Immunol., 169(9):4882-8 (2002)). The effect of CD137 activation on the inhibition of activation-induced cell death was shown *in vitro* by Hurtado et al. (J. Hurtado et al., J. Immunol., 158(6):2600-9 (1997)), and in an *in vivo* system in which anti-CD137 monoclonal antibodies (mabs) were shown to produce long-term survival of superantigen-activated CD8+ T-cells by preventing clonal deletion (C. Takahashi et al., J. Immunol., 162:5037 (1999)). Later, two reports demonstrated, under different experimental conditions, that the CD137 signal regulated both clonal expansion and survival of CD8+ T-cells (D. Cooper et al., Eur. J. Immunol., 32(2):521-9 (2002); M. Maus et al., Nat. Biotechnol., 20:143 (2002)). Reduced apoptosis observed after co-stimulation correlated with increased levels of Bcl-$_{xL}$ in CD8+ T-cells, while Bcl-2 expression remained unchanged. Up-regulation of the anti-apoptotic genes Bcl-$_{xL}$ and bfl-1 via 4-1BB was shown to be mediated by NF-κB activation, since PDTC, an NF-κB-specific blocker, inhibited 4-1BB-mediated up-regulation of Bcl-$_{xL}$ (H. Lee et al., J. Immunol., 169(9):4882-8 (2002)). On the other hand, clonal expansion of activated

T-cells appears to be mediated by increased expression of cyclins D2, D3, and E, and down-regulation of the $p27^{kip1}$ protein. This effect occurs in both an IL-2 dependent and independent fashion (H. Lee et al., J. Immunol., 169(9):4882-8 (2002)).

Altogether, CD137 stimulation results in enhanced expansion, survival, and effector functions of newly primed CD8+ T-cells, acting, in part, directly on these cells. Both CD4+ and CD8+ T-cells have been shown to respond to CD137 stimulation, however, it appears that enhancement of T-cell function is greater in CD8+ cells (W. Shuford et al., J. Exp. Med., 186(1):47-55 (1997); I. Gramaglia et al., Eur. J. Immunol., 30(2):392-402 (2000); C. Takahashi et al., J. Immunol., 162:5037 (1999)). Based on the critical role of CD137 stimulation in CD8+ T-cell function and survival, manipulation of the CD137/CD137L system provides a plausible approach for the treatment of tumors and viral pathogens.

Recently, the constitutive expression of CD137 on freshly isolated dendritic cells (DCs) was demonstrated in mice (R. Wilcox et al., J. Immunol., 169(8):4230-6 (2002); T. Futagawa et al., Int. Immunol., 14(3):275-86 (2002)) and humans (S. Pauly et al., J. Leukoc. Biol. 72(1):35-42 (2002)). These reports showed that stimulation of CD137 on DCs resulted in secretion of IL-6 and IL-12, and, more importantly, it enhanced DC ability to stimulate T-cell responses to alloantigens. Furthermore, Pan et al. demonstrated that CD137 signaling in DCs resulted in upregulation of MHC Class I and costimulatory molecules, and produced an increased ability of DCs to infiltrate tumors (P. Pan et al., J. Immunol., 172(8):4779-89 (2004)). Therefore, CD137 costimulation on DCs appears to be a novel pathway for proliferation, maturation, and migration of DCs.

Activated Natural Killer (NK) cells express CD137 following stimulation with cytokines (I. Melero et al., Cell Immunol., 190(2):167-72 (1998); R. Wilcox et al., J. Immunol., 169(8):4230-6 (2002)). Several reports demonstrated that NK cells appear to be critical for the modulation of the antitumor immune response induced by agonistic CD137 antibodies ((I. Melero et al., Cell Immunol., 190(2):167-72 (1998); R. Miller et al., J. Immunol., 169(4):1792-800 (2002); R. Wilcox et al., J. Immunol., 169(8):4230-6 (2002)). Depletion of NK cells significantly reduces the antitumor activity of anti-CD137 mabs. Ligation of CD137 on NK cells induces proliferation and IFN-γ secretion, but does not affect their cytolytic activity. Notably, *in vitro*,

CD137-stimulated NK cells presented an immunoregulatory or "helper" activity for CD8+ cytolytic T-cells resulting in expansion of activated T-cells. Therefore, CD137 signaling on NK cells may modulate innate immunity to tumors.

A paradoxical effect has been described for CD137 stimulation in that agonistic CD137 antibodies can induce suppression of the humoral responses to T-cell antigens in primates and mouse models (H. Hong et al., J. Immunother., 23(6):613-21 (2000); R. Mittler et al., J. Exp. Med., 190(10):1535-40 (1999)). Notably, CD137 agonistic antibodies were shown to produce significant amelioration of the symptoms associated with antibody dependent autoimmune diseases such as systemic lupus erythematosus and experimental autoimmune encephalomyelitis (J. Foell et.al., N.Y. Acad. Sci., 987:230-5 (2003); Y. Sun et al., Nat. Med., 8(12):1405-13 (2002)). Recently, Seo et al. demonstrated that, in a mouse model of rheumatoid arthritis, treatment with an agonistic anti-CD137 antibody prevented the development of the disease, and remarkably, blocked disease progression (S. K. Seo et al., Nat. Med. 10;1099-94 (2004)). The mechanism responsible for this effect has not been well defined, but in the model of rheumatoid arthritis it was shown that treatment with a CD137 agonistic antibody resulted in the expansion of IFN-γ-producing CD11C-CD8+ T cells. IFN-γ in turn stimulated dendritic cells to produce indolamine-2,3-dioxygenase (IDO), which exerts immuno-suppressive activities. It has also been postulated that CD137 signaling on antigen-activated CD4 + T-cells results in induction of IFN-γ secretion which activates macrophages. Activated macrophages can in turn produce death signals for B cells. Continuous signaling through CD137 on CD4+ T-cells may subsequently induce activation-induced cell death (AICD) of these CD4+ activated T-cells. Therefore, by eliminating antigen-activated T-cells and B cells, a reduced antibody response is observed and, consequently, a dramatic reduction of Th2-mediated inflammatory diseases is observed (B. Kwon et al., J. Immunol., 168(11):5483-90 (2002)). These studies suggest a role for the use of agonistic CD137 antibodies for the treatment of inflammatory or autoimmune diseases, without inducing a general suppression of the immune system.

The natural ligand for CD137, CD137 ligand (CD137L), a 34kDa glycoprotein member of the TNF superfamily, is detected mainly on activated antigen-presenting cells (APC), such as B cells, macrophages, dendritic cells, and also on murine B-cell

lymphomas, activated T-cells, and human carcinoma lines of epithelial origin (R. Goodwin et al., Eur. J. Immunol., 23(10):2631-41 (1993); Z. Zhou et al., Immunol. Lett., 45:67 (1995); H. Salih et al., J. Immunol., 165(5):2903-10 (2000)). Human CD137L shares 36% homology with its murine counterpart (M. Alderson et al., Eur. J. Immunol., 24: 2219-27 (1994)).

In addition to delivering signals to CD137-expressing cells, binding of CD137 to CD137L initiates a bidirectional signal resulting in functional effects on CD137L-expressing cells. Langstein et al. demonstrated that binding of CD137-Ig fusion protein to CD137L on activated monocytes induced the production of IL-6, IL-8, and TNF-α, upregulated ICAM, and inhibited IL-10, resulting in increased adherence (J. Langstein et al., J. Immunol., 160(5):2488-94 (1998)). In addition, proliferation of monocytes was demonstrated along with a higher rate of apoptosis (J. Langstein et al., J. Leukoc. Biol., 65(6):829-33 (1999)). These observations were confirmed by the studies of Ju et al. (S. Ju et al., Hybrid Hybridomics, 22(5):333-8 (2003)), which showed that a functional anti-CD137L antibody induced a high rate of proliferation of peripheral blood monocytes. Blocking the ligand resulted in inhibition of T-cell activation. In addition, soluble CD137L was found in the serum of patients with rheumatoid arthritis and hematological malignancies (H. Salih et al., J. Immunol., 167(7):4059-66 (2001)). Thus, the interaction of CD137 with CD137L influences and produces functional effects on T-cells and APC.

In another important aspect of T-cell function, it has been demonstrated that agonistic anti-CD137 antibodies rescued T-cell responses to protein antigens in aged mice. It has been well documented that an age-related decline in the immune response to antigens occurs, a process known as immunosenescence (R. Miller, Science, 273:70-4 (1996); R. Miller, Vaccine, 18:1654-60 (2000); F. Hakim et al., Curr. Opinion Immunol., 16:151-156 (2004)). This phenomenon appears to be due to alterations in the equilibrium between the extent of cellular expansion and cellular survival or death. Bansal-Pakala et al. tested the hypothesis that secondary costimulation through CD137 can be used to enhance T-cell responses in situations where T-cells do not receive sufficient stimulation, due to either reduced expression of CD3 or CD28, or reduced quality of signals. Their studies showed that aged mice had a deficient *in vitro* recall response compared to young mice (P. Bansal-Pakala et

al., J. Immunol., 169(9):5005-9 (2002)). However, when aged mice were treated with anti-CD137 mabs, the proliferative and cytokine responses of T-cells were identical to the responses observed in young mice. While the specific mechanism responsible for this effect was not elucidated, it was speculated that enhancing both the expression of anti-apoptotic molecules like Bcl-$_{XL}$, and the promotion of IL-2 secretion *in vivo* may play a role in rescuing defective T-cell responses. These studies demonstrated the potential for agonistic anti-CD137 antibodies to rescue weak T-cell responses in elderly immuno-compromised individuals, and has profound implications for the use of anti-CD137 antibodies in cancer patients.

A role for CD137 targeted therapy in the treatment of cancer was suggested by *in vivo* efficacy studies in mice utilizing agonistic anti-murine CD137 monoclonal antibodies. In a paper by Melero et al., agonistic anti-mouse CD137 antibody produced cures in P815 mastocytoma tumors, and in the low immunogenic tumor model Ag104 (I. Melero et al., Nat. Med., 3(6):682-5 (1997)). The anti-tumor effect required both CD4+ and CD8+ T-cells and NK cells, since selective *in vivo* depletion of each subpopulation resulted in the reduction or complete loss of the anti-tumor effect. It was also demonstrated that a minimal induction of an immune response was necessary for anti-CD137 therapy to be effective. Several investigators have used anti-CD137 antibodies to demonstrate the viability of this approach for cancer therapy (J. Kim et al., Cancer Res., 61(5):2031-7 (2001); O. Martinet et al., Gene Ther., 9(12):786-92 (2002); R. Miller et al., J. Immunol., 169(4):1792-800 (2002); R. Wilcox et al., Cancer Res., 62(15):4413-8 (2002)).

In support of the anti-tumor efficacy data with agonistic CD137 antibodies, signals provided by CD137L have been shown to elicit CTL activity and anti-tumor responses (M. DeBenedette et al., J. Immunol., 163(9):4833-41 (1999); B. Guinn et al., J. Immunol., 162(8):5003-10 (1999)). Several reports demonstrated that gene transfer of CD137 ligand into murine carcinomas resulted in tumor rejection, demonstrating the requirement of costimulation in generating an efficient immune response (S. Mogi et al., Immunology, 101(4):541-7 (2000); I. Melero et al., Cell Immunol., 190(2):167-72 (1998); B. Guinn et al., J. Immunol., 162(8):5003-10 (1999)). Salih et al. reported the expression of CD137L in human carcinomas and human carcinoma cell lines (H. Salih et al., J. Immunol., 165(5):2903-10 (2000)), and

demonstrated that tumors cells expressing the ligand were able to deliver a co-stimulatory signal to T-cells which resulted in the release of IFN-γ and IL-2, and that this effect correlated with the levels of CD137L on tumors. Whether expression of CD137L in human tumors could make these tumors more susceptible to agonistic CD137 antibodies is not known.

CD137L -/- mice have underscored the importance of the CD137/CD137L system in T-cell responses to both viruses and tumors (M. DeBenedette et al., J. Immunol., 163(9):4833-41 (1999); J. Tan et al., J. Immunol., 164(5):2320-5 (2000); B. Kwon et al., J. Immunol., 168(11):5483-90 (2002)). Studies using CD137- and CD137L-deficient mice have demonstrated the importance of CD137 costimulation in graft-vs-host disease, and anti-viral cytolytic T-cell responses. CD137-deficient mice had an enhanced proliferation of T-cells, but a reduction in cytokine production and cytotoxic T-cell activity (B. Kwon et al., J. Immunol., 168(11):5483-90 (2002); D. Vinay et al., Immunol. Cell Biol., 81(3):176-84 (2003)). More recently, it was shown that knockout mice (CD137-/-) had a higher frequency of tumor metastases (4-fold) compared to control mice. These data suggest that restoration of CD137 signaling by the use of agonistic anti-CD137 antibodies is a feasible approach for augmenting cellular immune responses to viral pathogens and cancers.

In addition to the data in mouse *in vivo* models which supports the involvement of CD137 signaling in antitumor immune responses, studies conducted in primary human tumor samples have confirmed the role of CD137 in generating effector T-cells. In patients with Ewing sarcoma, Zhang et al. showed that intratumoral effector T-cells presented the CD3+/CD8+/CD28-/CD137+ phenotype. Unexpectedly, coexistence of progressive tumor growth and anti-tumor immunity (effector T-cells) was observed. *Ex vivo* stimulation studies with patients' cells demonstrated that tumor-induced T-cell proliferation and activation required costimulation with CD137L. Stimulation of PBL with anti-CD3/CD137L, but not anti-CD3/anti-CD28, induced tumor lytic effectors. These studies provided further evidence that CD137 mediated costimulation could result in expansion of tumor reactive CTLs (H. Zhang et al., Cancer Biol. Ther., 2(5):579-86 (2003)). Furthermore, expression of CD137 was demonstrated in tumor infiltrating lymphocytes in hepatocellular carcinomas (HCC) (Y. Wan et al., World J.

Gastroenterol, 10(2):195-9 (2004)). CD137 expression was detected in 19 out of 19 HCC by RT-PCR, and in 13/19 by immunofluorescence staining. Conversely, CD137 was not detected in the peripheral mononuclear cells of the same patients. Analyses conducted in healthy donor liver tissues failed to demonstrate expression of CD137. These studies did not attempt to correlate clinical disease with CD137 expression. Thus, studies conducted in Ewing sarcoma and hepatocellular carcinoma revealed the presence of TIL that express CD137, with concomitant disease progression. In Ewing sarcomas it was demonstrated that CD137+TILs were able to kill tumor cells *ex-vivo* suggesting that the CD137 pathway was intact in these patients, and that perhaps suppressive factors in the tumor microenvironment inhibited their function. Hence, it can be postulated that systemic administration of agonistic CD137 antibodies may provide the signal necessary for expansion of these effector T-cells.

In addition to its role in the development of immunity to cancer, experimental data supports the use of CD137 agonistic antibodies for the treatment of autoimmune and viral diseases (B. Kwon et al., Exp. Mol. Med., 35(1):8-16 (2003); H. Salih et al., J. Immunol., 167(7):4059-66 (2001); E. Kwon et al., P.N.A.S. USA, 96:15074–79 (1999); J. Foell et al., N.Y. Acad. Sci., 987:230-5 (2003); Y. Sun et al., Nat. Med., 8(12):1405-13 (2002) S. K. Seo et al, Nat. Med. 10;1099-94 (2004)).

Consequently, based on the roles of 4-1BB in modulating immune response, it would be desirable to produce anti-human 4-1BB antibodies with agonistic activities that could be used for the treatment or prevention of human diseases such as cancer, infectious diseases, and autoimmune diseases.

BRIEF SUMMARY OF THE INVENTION:

The present invention provides fully human antibodies that bind to human 4-1BB (H4-1BB) and that allow binding of H4-1BB to a human 4-1BB ligand (H4-1BBL). Thus, the invention is directed to antibodies that bind to H4-1BB and that do not block the binding of H4-1BB to H4-1BBL, thereby permitting the binding of both an antibody of the invention and H4-1BBL to H4-1BB. The invention also provides antibodies with agonistic activities in that binding of the antibodies to H4-1BB results in an enhancement and stimulation of H4-1BB mediated immune responses. These antibodies can be used as immuno-enhancers of an anti-tumor or anti-viral immune

response, or as immunomodulators of T cell mediated autoimmune diseases. The antibodies can also be used as diagnostic tools for the detection of H4-1BB in blood or tissues of patients with cancer, autoimmune, or other diseases.

In one aspect, the invention provides a monoclonal antibody or antigen-binding portion thereof that specifically binds to H4-1BB, comprising a light chain variable region and a heavy chain variable region, wherein the light chain variable region comprises a CDR1 (complementary determining region 1), a CDR2 (complementary determining region 2), and a CDR3 (complementary determining region 3) as depicted in FIG. 4, and the heavy chain variable region comprises a CDR1 (complementary determining region 1), a CDR2 (complementary determining region 2), and a CDR3 (complementary determining region 3) as depicted in FIG. 3 or FIG. 7. The monoclonal antibody (mab) can be, for example, an IgG4 antibody or IgG1 antibody.

In another aspect, the invention provides a monoclonal antibody or antigen-binding portion thereof, wherein the light chain comprises a variable region as depicted in FIG. 4, and the heavy chain comprises a variable region as depicted in FIG. 3 or FIG. 7.

In another aspect, the invention provides a monoclonal antibody comprising a light chain and a heavy chain, wherein the light chain comprises amino acid residues 21-236 of SEQ ID NO:6 and the heavy chain comprises amino acid residues 20-467 of SEQ ID NO:3. In another aspect, the invention provides a monoclonal antibody comprising a light chain and a heavy chain, wherein the light chain comprises amino acid residues 21-236 of SEQ ID NO:6 and the heavy chain comprises amino acid residues 20-470 of SEQ ID NO:9.

The antibodies of the invention have wide therapeutic applications as immunomodulators of diseases such as cancer, autoimmune diseases, inflammatory diseases, and infectious diseases.

The invention further provides methods for treating cancer in a subject comprising administering a therapeutically effective amount of an antibody of the invention to the subject. In one aspect, this method further comprises administering a vaccine. Suitable vaccines include, for example, a tumor cell vaccine, a DNA vaccine, a GM-CSF-modified tumor cell vaccine, or an antigen-loaded dendritic cell

vaccine. The cancer can be, for example, prostate cancer, melanoma, or epithelial cancer.

In another aspect, the invention provides a method for enhancing the immune response, comprising administration of an antibody of the invention and a SIV gag vaccine. In another aspect, the invention provides a method for enhancing the immune response, comprising administration of an antibody of the invention and a PSA vaccine. In another aspect, the invention provides a method for enhancing the immune response to a SIV gag vaccine, comprising administration of an antibody of the invention. In another aspect, the invention provides a method for enhancing the immune response to a PSA vaccine, comprising administration of an antibody of the invention.

The invention also provides pharmaceutical compositions comprising an antibody of the invention, or an antigen-binding portion thereof, and a pharmaceutically acceptable carrier. The pharmaceutical composition can be administered alone or in combination with an agent, e.g., an agent for treating cancer such as a chemotherapeutic agent or a vaccine or other immunomodulatory agent.

The invention also provides isolated polynucleotides comprising a nucleotide sequence selected from: (a) nucleotides that encode the amino acid sequence of amino acid residues 20-467 of SEQ ID NO:3; (b) nucleotides that encode the amino acid sequence of SEQ ID NO:3; (c) nucleotides that encode the amino acid sequence of amino acid residues 21-236 of SEQ ID NO:6; (d) nucleotides that encode the amino acid sequence of SEQ ID NO:6; (e) nucleotides that encode the amino acid sequence of amino acid residues 20-470 of SEQ ID NO:9; (f) nucleotides that encode the amino acid sequence of SEQ ID NO:9; and (g) nucleotides that encode a fragment of an amino acid sequence of (a) to (f), such as a variable region, constant region, or one or more CDRs. The isolated polynucleotides of the invention further comprise nucleotide sequences encoding at least one CDR of FIG. 3, at least one CDR of FIG. 4, or at least one CDR of FIG. 7. The invention further provides isolated polynucleotides that comprise the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7.

The invention also provides isolated polypeptides comprising an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, and

SEQ ID NO:9. In another aspect, the invention provides isolated polypeptides comprising the amino acid sequence of amino acid residues 20-467 of SEQ ID NO:3, isolated polypeptides comprising the amino acid sequence of amino acid residues 21-236 of SEQ ID NO:6, and isolated polypeptides comprising the amino acid sequence of amino acid residues 20-470 of SEQ ID NO:9. In another aspect, the invention provides isolated polypeptides comprising the amino acid sequence of at least one CDR of FIG. 3, FIG. 4, or FIG. 7, or at least the variable or constant region of FIG. 3, FIG. 4, or FIG. 7.

The invention further includes an immunoglobulin having binding specificity for H4-1BB, said immunoglobulin comprising an antigen binding region. In one aspect, the immunoglobulin is a Fab or $F(ab')_2$ of an antibody of the invention.

The invention also includes a cell line that produces an antibody or antigen-binding portion thereof of the invention, recombinant expression vectors that include the nucleotides of the invention, and methods to make the antibodies of the invention by culturing an antibody-producing cell line.

BRIEF DESCRIPTION OF THE DRAWINGS:

FIG. 1 shows a plasmid map of pD17-20H4.9.h4a.

FIG. 2 shows a plasmid map of pD16gate-20H4.9.LC.

FIG. 3 (FIGS. 3A-3H) shows the nucleotide sequence of the plasmid pD17-20H4.9.h4a, including the coding strand (SEQ ID NO:1), complementary strand (SEQ ID NO:2), and amino acid sequence (leader peptide is amino acid residues 1-19 of SEQ ID NO:3; heavy chain is amino acid residues 20-467 of SEQ ID NO:3) encoded by the coding strand.

FIG. 4 (FIGS. 4A-4F) shows the nucleotide sequence of the plasmid pD16gate-20H4.9.LC, including the coding strand (SEQ ID NO:4), complementary strand (SEQ ID NO:5), and amino acid sequence (leader peptide is amino acid residues 1-20 of SEQ ID NO:6; light chain is amino acid residues 21-236 of SEQ ID NO:6) encoded by the coding strand.

FIG. 5 shows a schematic of the 20H4.9-IgG1 heavy chain sequence construct.

FIG. 6 shows a schematic of the 20H4.9 light chain sequence construct.

FIG. 7 (FIGS. 7A-7D shows the nucleotide and amino acid sequences of the 20H4.9-IgG1 heavy chain construct, including the coding strand (SEQ ID NO:7), complementary strand (SEQ ID NO:8), and amino acid sequence (leader peptide is amino acid residues 1-19 of SEQ ID NO:9; heavy chain is amino acid residues 20-470 of SEQ ID NO:9) encoded by the coding strand.

FIG. 8 (FIGS. 8A-8B) illustrates the results obtained from the binding of mab 20H4.9-IgG1 to human CD137 by ELISA (FIG. 8A) and the effect of mab 20H4.9-IgG1 on CD137-CD137L interaction (FIG. 8B).

FIG. 9 (FIGS. 9A-9B) illustrates the results obtained from the binding of mab 20H4.9-IgG1 to PMA-ionomycin stimulated human or cynomolgus monkey cells. Human CEM (FIG. 9A) or monkey PBMC (FIG. 9B) were incubated with 20H4.9-IgG1 or human CD137L fusion protein.

FIG. 10 (FIGS. 10A-10B) illustrates the results obtained by induction of IFN-γ in co-stimulatory studies with anti-CD137 antibodies, which are expressed as fold increase in pg/ml over controls. Due to the variable background response among donors, data was normalized relative to control treatments (=1). Median IFN-γ baseline level for human T-cells (FIG. 10A) or monkey PBMC (FIG. 10B) stimulated with anti-CD3 alone was 592 pg/ml and 505 pg/ml respectively.

FIG. 11 provides plasmon resonance plots of binding of mab 20H4.9-IgG4 and mab 20H4.9-IgG1 to human CD137.

FIG. 12 illustrates the concentration-dependent binding of 20H4.9-IgG4 to PMA ionomycin stimulated human CEM cells, but no binding to unstimulated CEM cells.

FIG. 13 (FIGS. 13A-B) illustrates the induction of IFN-γ in co-stimulatory studies with anti-CD137 antibodies. The results are expressed as fold increase in pg/ml over controls. Due to the variable background response among donors, data was normalized relative to control treatments (=1). Median IFN-γ baseline level for human T-cells (FIG. 13A) or monkey PBMC (FIG. 13B) stimulated with anti-CD3 alone was 592 pg/ml and 505 pg/ml respectively.

FIG. 14 (FIGS. 14A-14B) illustrates the results obtained of dose-dependent enhancement of IFN-γ synthesis by mab 20H4.9-IgG4 (FIG. 14A), and effect of

antibody crosslinking by addition of crosslinking anti-human IgG antibody (7 μg/ml) (FIG. 14B).

FIG. 15 illustrates the effect of mab 20H4.9-IgG4 on T-cell survival and cell cycle progression. Human T-cells were costimulated with anti-CD3 (1 ug/ml) ± mab 20H4.9-IgG4 at the concentrations listed. Six days after initiation of the assays, cells were collected and stained with Annexin-V and propidium iodide to determine the number of live cells (Annexin V/PI negative), or PE-conjugated cyclin D2 to detect cycling cells. Results represent the mean (±SD) of 4 lots of mab 20H4.9-IgG4 tested in parallel.

FIG. 16 (FIGS. 16A-16D) shows in cynomolgus monkeys the antigen-specific IFN-γ response as measured by ELISPOT after treatment with a DNA vaccine ± anti-human 4-1BB antibodies. Animals were treated with a SIV gag vaccine (day 0, 28, 56; FIG. 16A), SIV gag vaccine (day 0, 28, 56) and mab 20H4.9-IgG4 (day 12, 15 and 19; FIG. 16B), or SIV gag vaccine (day 0, 28, 56) and hu39E3.G4 (day 12, 15 and 19; FIG 16C). A group of animals was left untreated (FIG. 16D). At various times following treatment, blood was collected, and PBMC were separated and evaluated for their ability to secrete IFN-γ in the presence of antigen stimulation.

DETAILED DESCRIPTION OF THE INVENTION:

The invention is directed to the preparation and characterization of antibodies, and antigen binding fragments thereof (including fusion proteins that comprise an antigen binding fragment of an antibody of the invention), for use in the treatment of a disease, such as a cancer, infectious disease, inflammatory disease, or autoimmune disease. The cancer can be, for example, prostate cancer, melanoma, or epithelial cancer.

The antibodies are capable of binding to H4-1BB, and can present high affinity for H4-1BB and effectively enhance T cell responses. In one aspect, the antibody induces IFN-γ production in co-stimulatory assays, but does not affect the binding of H4-1BB to its corresponding ligand, H4-1BBL, and does not fix complement.

The antibodies of the invention may be produced by methods well known in the art. In one aspect, the antibodies can be produced by expression in transfected cells, such as immortalized eukaryotic cells, such as myeloma or hybridoma cells.

The antibodies of the invention may be used alone, or together with other therapeutic agents such as radiotherapy (including radiation), hormonal therapy, cytotoxic agents, vaccines, and other immunomodulatory agents, such us cytokines and biological response modifiers. These agents are particularly useful in treating cancer and immune-proliferative disorders.

In one aspect, the invention provides the monoclonal antibody (mab) 20H4.9-IgG4. FIGS. 1 and 2 provide plasmid maps of pD17-20H4.9.h4a and pD16gate-20H4.9.LC, respectively, that can be used to produce mab 20H4.9-IgG4. FIG. 3 (FIGS. 3A-3H) provides the nucleotide sequence of the plasmid pD17-20H4.9.h4a, including the coding strand (SEQ ID NO:1), complementary strand (SEQ ID NO:2), and amino acid sequence (leader peptide is amino acid residues 1-19 of SEQ ID NO:3; heavy chain is amino acid residues 20-467 of SEQ ID NO:3) encoded by the coding strand. FIG. 4 (FIGS. 4A-4F) shows the nucleotide sequence of the plasmid pD16gate-20H4.9.LC, including the coding strand (SEQ ID NO:4), complementary strand (SEQ ID NO:5), and amino acid sequence (leader peptide is amino acid residues 1-20 of SEQ ID NO:6; light chain is amino acid residues 21-236 of SEQ ID NO:6) encoded by the coding strand.

In another aspect, the invention provides the monoclonal antibody (mab) 20H4.9-IgG1. FIG. 5 schematically shows a heavy chain sequence construct of mab 20H4.9-IgG1. FIG. 6 schematically shows a light chain sequence construct of mab 20H4.9, for both mab 20H4.9-IgG4 and 20 H4.9-IgG1. FIG. 7 provides the nucleotide sequence (coding strand (SEQ ID NO:7) and complementary strand (SEQ ID NO:8)) of the heavy chain sequence construct of FIG. 5, and the amino acid sequence (leader peptide is amino acid residues 1-19 of SEQ ID NO:9; heavy chain is amino acid residues 20-470 of SEQ ID NO:9) encoded by the coding strand. The light chain of mab 20H4.9-IgG1 is the same as the light chain of mab 20H4.9-IgG4.

The invention also encompasses antibodies with conservative amino acid substitutions from the heavy and light chain amino acid sequences depicted in SEQ ID NOS:3, 6, and 9 that have substantially no effect on H4-1BB binding.

Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

The polynucleotides encoding the polypeptides of the invention typically further comprise an expression control sequence operably linked to the polypeptide coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into an appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences and, as desired, the collection and purification of the light chain, heavy chain, light/heavy chain dimers or intact antibody, binding fragments or other immunoglobulin form may follow. (See, S. Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y. (1979)). Single chain antibodies or minibodies (single chain antibodies fused to one or more CH domains) may also be produced by joining nucleic acid sequences encoding the VL and VH regions disclosed herein with DNA encoding a polypeptide linker.

Prokaryotic hosts, such as E. coli, and other microbes, such as yeast, may be used to express an antibody of the invention. In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the antibodies of the invention. Eukaryotic cells may be preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed including, for example, CHO (chinese hamster ovary) cell lines, COS (African green monkey fibroblast cell line) cell lines, HeLa cells, myeloma cell lines, and hybridomas. Expression vectors for these cells can include expression control sequences, such as a promoter or enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences, all well known in the art.

The vectors containing the DNA segments of interest (e.g., the heavy and/or light chain encoding sequences and expression control sequences) can be transferred

into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly used for prokaryotic cells, whereas calcium phosphate treatment, lipofection, or electroporation may be used for other cellular hosts. (See, e.g., T. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1982)).

Once expressed, the antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms, can be purified according to standard procedures in the art, such as ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis, and the like. Substantially pure immunoglobulins of at least 90 to 95% homogeneity are desirable, and 98 to 99% or more homogeneity are more desirable.

The antibodies of the invention are useful for modulating T cell and antibody-mediated immune responses. Typical disease states suitable for treatment include cancers, infectious diseases, inflammatory diseases, and autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myaesthenia gravis.

The invention also provides pharmaceutical compositions comprising at least one antibody of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be sterilized by conventional well known sterilization techniques. The pharmaceutical compositions can also contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stability enhancing agents such as mannitol or tween 80, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, or human albumin.

The antibodies and pharmaceutical compositions of the invention are particularly useful for parenteral administration, including subcutaneous, intramuscular, and intravenous administration. The pharmaceutical compositions for parenteral administration can comprise a solution of the antibody dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, all well known in the art, e.g., water, buffered water, saline, glycine and the like. These solutions are sterile and generally free of particulate matter. It is especially

advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

The pharmaceutical composition can further comprise an additional agent for treatment of a disease. In one aspect, the pharmaceutical composition includes an agent for treatment of a cancer, an infectious disease, inflammatory disease, or autoimmune disease. The antibody of the invention can also be co-administered or separately administered with an additional agent for treatment of a disease.

The antibodies of the invention can be used with other agents to enhance the immune response to cancerous cells in a patient. In one aspect, the antibody is used in combination with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), or cells transfected with genes encoding immune stimulating cytokines and cell surface antigens. In another aspect, the antibody is used in combination with a vaccine such as, for example, a tumor cell vaccine, a DNA vaccine, a gene-modified tumor cell vaccine, such as GM-CSF-modified tumor cell vaccine, a peptide vaccine, or an antigen-loaded dendritic cell vaccine.

Many experimental strategies for vaccination against tumors have been devised. In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al., P.N.A.S., 90:3539-43 (1993); E. Jafee et al., J. Clin. Oncol., 19:145-56 (2001); R. Salgia et al., J. Clin. Oncol., 21:624-30 (2003)).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (S. Rosenberg, Immunity 10:281-7 (1999)). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp 100, MAGE antigens, Trp-2. Many of these antigens can be shown to be the targets of tumor specific T cells found in the host. The antibodies of the invention may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to amplify and direct the immune response to these antigens towards a Th1 response. These proteins

are normally viewed by the immune system as self antigens and are therefore tolerant to them.

In one aspect of the invention, the antibody is combined with an immunodulatory agent comprising the SIV gag antigen (as a model for HIV DNA vaccine) or prostate specific antigen (PSA), or a DNA vaccine comprising a nucleotide sequence that encodes the SIV gag antigen or prostate specific antigen (PSA). PSA vaccines are described in, for example, M. Pavlenko et al., Br. J. Cancer, 91(4):688-94 (2004); J. Wolchok et al., Semin. Oncol., 30(5):659-66 (2003); J. Kim et al., Clin. Cancer Res., 7(3 Suppl.):882s-889s (2001). SIV gag vaccines are described in, for example, B. Makitalo et al., J. Gen. Virol., 85(Pt 8):2407-19 (2004); N. Letvin et al., J. Virol., 78(14):7490-7 (2004); S. Mossman et al., AIDS Res. Hum. Retroviruses., 20(4):425-34 (2004); F. Bertley et al., J. Immunol., 172(6):3745-57 (2004); L. Patterson et al., J. Virol., 78(5):2212-21 (2004); E. O'Neill et al., AIDS Res. Hum. Retroviruses, 19(10):883-90 (2003); Z. Bu et al., Virology, 309(2):272-81 (2003).

The tumor antigen may also include, for example, the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (N. Kim et al., Science, 266, 2011-2013 (1994)). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences, or idiotype from B cell tumors. Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV), and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used with an antibody of the invention is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (R. Suot et al., Science 269: 1585-1588 (1995); Y. Tamura et al., Science 278: 117-120 (1997)).

The antibodies of the invention can also be used to enhance the immune response to vaccines to viral antigens, such as HIV or HCV. The antibodies of the

invention can also be used to enhance the immune response to other immunomodulatory agents, and to elicit a memory immune response. Examples of these agents are cytokines such as GM-CSF, IL-2, IL-15, IL-12, Fl3 ligand, CD40 ligand, adjuvants such as CpG-oligodeoxynucleotide (bacterial DNA), or antibodies to OX-40 or CTLA-4.

The pharmaceutical compositions of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, the pharmaceutical composition is administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest or treat the disease. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease state and the patient (including, for example, the general state of the patient's own immune system), and can be determined by one skilled in the art. In prophylactic applications, the pharmaceutical composition is administered to a patient not already in the disease state, to enhance the patient's resistance to the disease state. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts depend upon the patient's state of health (including, for example, the general state of the patient's own immune system), and can be determined by one skilled in the art. In one aspect, the prophylactic use is for the prevention of tumor recurrence.

Examples:

Example 1: Generation of Antibodies

Materials and Methods

Fully human monoclonal antibodies to the human CD137 (4-1BB) receptor were generated in the HuMAb-Mouse® (Medarex, Inc., Princeton, New Jersey). HuMAb mice were immunized five times intraperitoneally (i.p.) and subcutaneously (s.c.) with 25 μg of the extracellular domain of human CD137 in RIBI adjuvant (Ribi Immunochemical). Prior to fusion, mice were boosted intravenously (i.v.) with the same amount of antigen. Spleen cells from immunized mice with adequate titers of antibodies to huCD137 were fused to mouse myeloma cells following standard procedures.

Anti-human CD3 mab (clone:HIT3a), ELISA kits for human and monkey IFN-γ, cytometric bead array (CBA) kits, and all conjugated antibodies for flow cytometry were purchased from BD Pharmingen (San Diego, California). Human IgG_1 λ and Human IgG_1 κ were purchased from Sigma-Aldrich (St. Louis, Missouri). CEM cells (ATCC-CRL 2265) were purchased from ATCC. Culture media (RPMI), and fetal bovine serum (FBS) were purchased from Mediatech Inc. (Herndon, Virginia). Sheep Red Blood Alsevers was purchased from Colorado Serum Co. (Denver, Colorado).

Hybridoma screening: Detection of binding to huCD137 by ELISA: To identify hybridomas secreting anti-human CD137 antibodies, ELISA plates (Nunc MaxiSorp) were coated with human CD137-mouse IgG_{2b} fusion protein at 1 μg/ml in PBS overnight at 4°C. Plates were then washed 3 times with PBS with 0.01% Tween-80 (PBS-T), and subsequently blocked with PBS-T plus 1% bovine serum albumin (BSA), for 20 min at room temperature. Fifty microliters of supernatants diluted 1:3 in PBS-T were added to the plates and incubated for 1-2 hr at ambient temperature. Afterwards, plates were washed as before, and binding of antibodies was detected by an incubation with alkaline phosphatase-conjugated goat $F(ab')_2$ anti-human IgG antibody (Jackson Laboratories, West Grove, Pennsylvania). Plates were developed with pNPP and read at 405 nm.

Blocking assay: Twenty-six hybridomas secreting antibodies that recognized huCD137 by ELISA were evaluated for their ability to allow CD137-CD137L interactions. These analyses were conducted initially in an ELISA format. Plates were coated with human CD137-muIgG_{2b} at 0.2 μg/ml, 100 μl/well. Serial dilutions of the mab 20H4.9-IgG1, or control antibodies, diluted in PBS-T and 1% bovine serum albumin, were added to the plate. CD137L-CD8 fusion protein was added to the wells at a concentration of 0.2 μg/ml. Binding of antibodies was detected with a biotinylated anti-CD8 antibody (0.2 μg/ml, Ancell Corporation, Bayport, Minnesota). After several washes, streptavidin-alkaline phosphatase (1:2000) and pNPP for the detection of bound antibodies were added, and the plates were read at 405 nm.

To confirm that the selected antibodies did not alter CD137-CD137L binding, purified antibodies were further characterized by BIAcore analyses. All experiments were carried out on a BIAcore 3000 instrument (BIAcore Inc., Piscataway, New

Jersey). Human CD137 was immobilized covalently at a high density on a carboxy-methylated dextran surface of a BIAcore sensorchip (BIAcore Inc., Piscataway, New Jersey). Injections were conducted at 2 μg/mL in 10 mM acetate buffer, pH 5.0. Unoccupied active esters were subsequently blocked by injection of an excess of ethanolamine. Regeneration of the surface was done with 10 mM glycine, pH 2.0.

Purified samples of anti-CD137 antibodies were diluted to concentrations between 200 and 1000 nM using HEPES buffered saline, pH 7.4, supplemented with 0.15 M NaCl and 0.005% surfactant P20 (HBS-EP). Human CD137L-CD8 fusion proteins (huCD137L) were used as source of CD137 ligand. Experiments were conducted in which huCD137L was injected prior to anti-CD137 antibodies, or vice versa. Injections were performed at a flow rate of 5 μL/min. Bound ligand and antibodies were removed by regeneration with 10 mM glycine buffer, pH 2.0.

Human T-cell purification: T-cells or PBMCs were obtained from healthy human donors. Blood was collected in EDTA, suspended in elutriation buffer (RPMI containing 2.5 mM EDTA, 10 μg/ml polymyxin B), underlayed with Lymphocyte Separation Medium (LSM, Mediatech Inc., Herndon, Virginia), and centrifuged at 1800 rpm for 25 minutes. Cellular interfaces were collected, and centrifuged at 1500 rpm for 10 minutes. Afterwards, cell pellets were resuspended in elutriation buffer and washed Sheep Red Blood Cells (SRBC, 1:10 dilution), and incubated on ice for 1 hour. Cells were then underlayed with LSM and centrifuged at 2500 rpm for 25 minutes. Interfaces were removed and SRBC were lysed with SRBC Lysis Buffer. Isolated T-cells were washed and resuspended in 10% FBS/RPMI.

Flow Cytometric analyses: Binding of anti-human CD137 antibodies to CD137 expressed on cells was determined by flow cytometry. A human T-cell leukemia cell line (CEM) or cynomolgus monkey peripheral blood monocytic cells (PBMC) were used for these studies. These cells do not express CD137 constitutively, but the receptor can be induced by stimulation with phorbol myristate (PMA, 10 ng/ml) and ionomycin (1 μM) for 18 hr. Cells were then washed and incubated with various concentrations of the antibodies in staining buffer (phosphate buffer saline, PBS, plus 1 % FCS, and 0.01 % sodium azide). Binding of the antibodies to stimulated or non-stimulated cells was detected by a fluorescein (FITC) or phycoerithrin (PE) conjugated goat anti-human IgG (Jackson Immunoresearch,

West Grove, Pennsylvania). To confirm expression of CD137, a fusion protein consisting of the extracellular domain of CD137 ligand and mouse CD8 was used (Ancell Corporation, Bayport, Minnesota), followed by incubation with PE-conjugated anti-mouse CD8 (BD Pharmingen, San Diego, California). Samples were fixed in 1% formalin, kept at 4 °C, and read by flow cytometry.

Functional assays: Primary human T-cells or monkey PBMC obtained from healthy donors were stimulated with immobilized anti-CD3 antibody to provide the first signal for T-cell activation, and co-stimulated with human anti-human CD137 antibodies. As a non-specific control, a humanized anti-carcinoma antibody (BR96) was used at the same antibody concentration. Plates were coated with anti-CD3 antibody (0.5-1 μg/ml) at 4 °C overnight. The next day T-cells or PBMC were plated at 1-1.5 x10^5 /well concentrations. Synthesis of IFN-γ was measured after 72 hours of culture at 37 °C either by cytometric bead array (CBA) or by ELISA.

Cytokine assays

ELISA: After stimulation of T-cells at various times, plates were centrifuged and media was removed. Cytokine levels were detected by an ELISA in accordance with the manufacturer's instructions (BD Pharmingen, San Diego, California). In brief, test samples and standards were added to anti-cytokine-coated 96-well plates. After incubation for 2 hr at ambient temperature, plates were washed 3 times in PBS-T and then incubated first with a working detector antibody, followed by the addition of substrate. Absorbance was read at 405 nm, and concentrations were calculated based on the standard curve.

Cytometric Bead Array: Another method used to determine cytokine production *in vitro* was flow cytometry using the Cytometric Bead array (CBA) developed by BD Pharmingen. Levels of IFN-γ, IL-2, IL-5, IL-4, IL-10, and TNF-α were measured in culture supernatants following manufacturers' instructions. Results were analyzed by flow cytometry with the CBA analysis software.

Results

Hybridomas secreting antibodies that showed binding to human CD137 were further expanded, and subcloned. Secreted antibodies were purified and tested for their ability to bind to huCD137 and to allow the interaction of CD137-CD137L. Of the panel of anti-human CD137 antibodies evaluated, mab 20H4.9-IgG1 was selected

for further evaluation based on its binding profile and non-blocking properties. The 20H4.9-IgG1 antibody is IgG1 kappa as determined by ELISA using alkaline phosphatase anti-human IgG1, 2, 3, 4, and anti-kappa and lambda reagents (Southern Biotech, Birmingham, Alabama). FIG. 8 (FIG. 8A - binding to human CD137 by ELISA; FIG. 8B - effect of mab 20H4.9-IgG1 on CD137-CD137L interaction) provides the initial characterization of mab 20H4.9-IgG1. Serial dilutions of mab 20H4.9-IgG1, 26G6 (a blocking anti-CD137 antibody), or tetanus toxoid (TT, negative control) were evaluated for their ability to alter binding of CD137 to CD137L. Mab 20H4.9-IgG1 at concentrations up to 10 μg/ml did not block CD137L binding, whereas mab 26G6 inhibited binding at concentrations > 0.37 μg/ml.

Mab 20H4.9-IgG1 was also tested for reactivity towards CD137 expressed on human T-cells (CEM) and in cynomolgus monkey peripheral blood monocytic cells (PBMC) stimulated with PMA and ionomycin. Previous studies determined that CD137 is upregulated on T-cells following activation with PMA and ionomycin. Control molecules consisted of an irrelevant human IgG antibody (negative control) or CD137L-CD8 fusion protein (positive control, BD Pharmingen, San Diego, California). Results from these studies indicated that mab 20H4.9-IgG1 bound to activated human CEM and PBMCs from cynomolgus monkeys, with minimum binding to unstimulated cells. Similar percentages of positive cells were detected with either mab 20H4.9-IgG1 or CD137L. FIG. 9 provides the results obtained demonstrating the binding of mab 20H4.9-IgG1 to PMA-ionomycin stimulated human or cynomolgus monkey cells. Human CEM (FIG. 9A) or monkey PBMC (FIG. 9B) were incubated with 20H4.9-IgG1 or human CD137L fusion protein. Secondary antibodies were added and samples were read by flow cytometry.

Next, it was determined whether mab 20H4.9-IgG1 could induce enhancement of IFN-γ in costimulatory assays in the presence of anti-CD3 stimulation, the key functional effect desired for an agonistic CD137 antibody. Mab 20H4.9-IgG1 was evaluated for its co-stimulatory activity in functional studies in human and monkey lymphocytes. Based on initial data, a concentration of 20 ug/ml anti-CD137 antibody (excess antibody) was used in these studies. Levels of anti-CD3 antibody between 0.2-1 μg/ml were tested which resulted in 10-20% CD137-positive lymphocytes. Levels of IFN-γ in supernatants were measured after 72 h of culture. As shown in

FIG. 10, mab 20H4.9-IgG1 enhanced IFN-γ synthesis in both human and monkey costimulatory assays to levels significantly higher than controls. Results of studies conducted with T-cells isolated from 8 healthy human donors showed that in six of them, mab 20H4.9-IgG1enhanced IFN-γ synthesis between 2.2 - 4.3-fold compared to controls. One of the other two donors showed a 1.6-fold increase. The level of enhancement was superior to that observed with hu39E3.G4, a humanized anti-CD137 antibody provided in published PCT Application WO04/010947 (herein incorporated by reference) which showed augmentation of IFN-γ in 5 out of 8 donors and at levels lower than mab 20H4.9-IgG1 (1.5 - 2-fold increase) (FIG. 10A). In monkey costimulatory studies, mab 20H4.9-IgG1 also demonstrated enhanced functional activity resulting in significant augmentation of IFN-γ over controls (FIG. 10B). As in the human studies, enhancement of IFN-γ was consistently higher than with hu39E3.G4.

Induction of TNF-α synthesis above control levels was also observed in human cultures, albeit at much lower levels than IFN-γ. TNF-α levels induced by anti-CD3 antibody alone (baseline) were about 20-50 fold lower than baseline levels for IFN-γ. Mab 20H4.9-IgG1 induced an increase of ~2 to 4.7-fold in 3 out of 8 donors. Again, hu39E3.G4 (tested in parallel) induced ~2-fold increase in the same donors but at lower levels. Other cytokines tested, IL-2, IL-5, IL-10, and IL-4 did not change significantly with either treatment.

Together these studies demonstrated that mab 20H4.9-IgG1 presented the functional activity desired in both humans and monkeys by inducing a Th1-type of response. Significantly, since *in vivo* anti-tumor activity is associated with the ability of anti-CD137 antibodies to induce IFN-γ synthesis, these results supported the selection of mab 20H4.9-IgG1 for isotype switching.

Example 2: *In vitro* characterization of mab 20H4.9-IgG4

Based on its binding kinetics, inability to block CD137-CD137L interaction, and functional effects on human T-cells, mab 20H4.9-IgG1 was selected for switching to an IgG4 form. The IgG4 form of mab 20H4.9-IgG1 is 20H4.9-IgG4 (depicted in FIGS. 3 and 4).

The second phase of these studies involved the comparison of the *in vitro* properties of mab 20H4.9-IgG4 and mab 20H4.9-IgG1. In this section, the binding

kinetic properties, and functional effects of both antibodies in human and monkey lymphocytes are described.

Binding kinetics

Kinetic properties of anti-human CD137 antibodies were evaluated by surface plasmon resonance using a BIAcore 3000 instrument. The antigen, human CD137-murine IgG_{2a}, was immobilized covalently at a low density on the surface of a CM5 sensorchip. Mab 20H4.9-IgG4 and mab 20H4.9-IgG1 were injected at concentrations between 25 and 200 nM. FIG. 11 depicts injections at 100 nM for both mab 20H4.9-IgG1 and mab 20H4.9-IgG4. Data calculated using BIAevaluation software (bivalent model, global curve fit analysis) resulted in kinetic parameters that were similar for both antibodies (see Table 1). Dissociation constants K_D for mab 20H4.9-IgG1 and mab 20H4.9-IgG4 were determined as 11.2 and 16.6 nM, respectively. Under similar experimental conditions, mab 20H4.9-IgG4 did not bind to murine 4-1BB.

Table 1 - Comparison of the binding kinetics of mab 20H4.9-IgG4 and mab 20H4.9-IgG1

antibody	k_{a1} (1/Ms)	k_{d1} (1/s)	ka2 (1/RUs)	kd2 (1/s)	Rmax (RU)	K_A1	K_D1 (nM)
20H4.9-IgG1	3.43E+04	3.85E-04	2.30E-05	1.51E-03	262	8.91E+07	11.22
20H4.9-IgG4	3.92E+04	6.51E-04	0.0755	0.105	409	6.02E+07	16.61

Flow cytometric analyses

Biotinylated mab 20H4.9-IgG4 at concentrations ranging from 0.32 ng/ml to 5 µg/ml was tested for binding to CEM cells ± PMA-ionomycin. Mab 20H4.9-IgG4 bound to PMA-ionomycin stimulated CEM cells in a concentration-dependent manner. Saturation was achieved at 0.2 µg/ml. On the other hand, as shown for its parental molecule, mab 20H4.9-IgG1, mab 20H4.9-IgG4 did not bind to CEM cells that were not stimulated with PMA-ionomycin (FIG. 12). Concentration-dependent binding of mab 20H4-.9-IgG4 was demonstrated in PMA-ionomycin stimulated CEM cells (FIG. 12). Samples were read by flow cytometry.

Cellular/Functional Assays

To confirm that the process of switching the isotype of mab 20H4.9-IgG1 did not alter the activity of the antibody, *in vitro* studies were conducted to compare the activity of mab 20H4.9-IgG4 to the parent mab 20H4.9-IgG1 in monkey PBMC and human T-cells. The functional effects of mab 20H4.9-IgG4 on human and monkey T-cells or PBMC were determined and compared to its parental molecule, mab 20H4.9-IgG1. Primary human T-cells or monkey PBMC obtained from healthy donors were stimulated with anti-CD3 antibody (0.5 μg/ml-1 μg/ml) +/- anti-human CD137 antibodies. Synthesis of IFN-γ was measured after 72 h of culture at 37 °C by cytometric bead array (CBA) for human samples or by ELISA for monkey samples. Antibodies were tested in costimulatory assays in the presence of suboptimal concentrations of anti-CD3 antibody (1 μg/ml) or Concavalin A (1 μg/ml) (donors M5170 and 81 only). Results are expressed as fold increase in pg/ml over controls. Due to the variable background response among donors, data was normalized relative to control treatments (=1). FIG. 13A provides the human T-cell results and FIG. 13B provides the monkey PBMC results. As shown in FIGS. 13A-13B, mab 20H4.9-IgG4 demonstrated costimulatory properties yielding higher levels of IFN-γ in human and monkey cells compared to controls. The level of enhancement of IFN-γ synthesis was comparable to its parental molecule in human and monkey samples.

Subsequently, the effect of antibody cross-linking on the functional effect of mab 20H4.9-IgG4 was evaluated. It has been shown that cross-linking of antibodies may result in potentiation of their signaling ability. Thus, a study was conducted to determine the functional activity of several batches of mab 20H4.9-IgG4 ± an anti-human IgG antibody. As shown in FIG. 14A, significant enhancement of IFN-γ synthesis was observed for all lots tested in the absence of cross-linking antibodies, with a plateau at concentrations of 400 ng/ml. The augmentation of IFNγ synthesis by mab 20H4.9-IgG4 was further enhanced by the addition of anti-human IgG cross-linking antibody as shown in FIG. 14B. Different batches of mab 20H4.9-IgG4 had comparable cellular activities.

Thus, cross-linking of mab 20H4.9-IgG4 resulted in an enhancement of the ability of the antibody to induce IFN-γ synthesis. Antibody cross-linking *in vivo* may

occur by cellular receptors for the Fc portion of immunoglobulins or by antibody dimerization. Mab 20H4.9-IgG4 is of the IgG4 isotype, which, compared to other IgG isotypes, has low affinity for Fc receptors. However, IgG4 can bind to FcγRI (CD64) expressed on monocytes and neutrophils.

Two other approaches were used to further characterize mab 20H4.9-IgG4: (i) effect on T-cell survival and (ii) effect on cyclin D2 expression. To determine whether mab 20H4.9-IgG4 could elicit signaling through CD137 on human T-cells and provide co-stimulatory signals to T-cells leading to cell survival and expansion, human T-cells stimulated with anti-CD3 antibodies +/- mab 20H4.9-IgG4 at concentrations known to induce IFN-γ synthesis were stained with annexin-V and propidium iodide to determine the number of live cells (Annexin V/Propidium iodide negative), and with Cyclin D2 to determine its effect on cell progression. FIG. 15 shows the average results of 4 different lots of mab 20H4.9-IgG4 on cyclin D2 expression and survival of T-cells. Concentrations of mab 20H4.9-IgG4 of 0.4-10 μg/ml resulted in an increase in the number of live cells by approximately 1.8 - 2 fold, and yielded a significant increase in the number of cyclin D2-expressing T-cells (2.5 - 3 fold).

Example 3: In vivo evaluation of 4-1BB antibodies in a pharmacodynamic model in cynomolgus monkeys.

This example illustrates the ability of mab 20H4.9-IgG4 and mab hu39E3.G4 to enhance the antigen specific immune response elicited by DNA vaccines.

Materials and Methods

Experimental animal groups: Female and male cynomolgus monkeys (2.5 to 5.0 kg) were purchased from Charles River BRF (Houston, Texas) for this study and were housed in pairs. Each experimental group consisted of 4 males and 2 females which were randomized into groups by body weight. Experimental groups were as follows:

Group 1 - SIV gag and PSA DNA vaccine (2 mg each), day 0, 28, 56, i.m., plus saline control, i.v., on days 12, 15 and 19;

Group 2 - SIV gag and PSA DNA vaccine (2 mg each), day 0, 28, 56, i.m., plus mab hu39E3.G4, i.v., on days 12, 15 and 19;

Group 3 - SIV gag and PSA DNA vaccine (2 mg each), day 0, 28, 56, i.m., plus mab 20H4.9-IgG4, i.v., on days 12, 15 and 19;

Group 4 - untreated control group.

Immunizations and antibody treatments: PSA and SIV gag DNA vaccines were obtained from David B. Weiner, Department of Pathology and Laboratory of Medicine, University of Pennsylvania. (See, Kim et al., Oncogene 20, 4497-4506 (2001); Muthumani et al., Vaccine 21, 629-637 (2003).)

Monkeys were immunized by the intramuscular route with both PSA and SIV gag DNA constructs (2 mg/construct/immunization) simultaneously, followed by two boosts 4 weeks apart (days 0, 28, and 56). Twelve days after the initial immunization, treatment with mab 20H4.9-IgG4 or mab hu39E3.G4 was initiated. Antibodies were administered i.v, at 50 mg/kg, on days 12, 15, and 19 after the first immunization. This schedule was chosen because it was shown to suppress the antibody response to mab hu39E3.G4.

Clinical and Clinical pathology

Throughout the course of the study, physical examinations were conducted on all monkeys by the attending veterinarians. Blood samples for hematology and serum chemistry analyses were collected prior to vaccinations and then 12, 42, 70, 97, 134, and 168 days after immunizations.

Immunological assays

To determine the effect on the immune responses induced by these therapeutic regimens, an enzyme-linked immune spot assay (ELISPOT) was used for the detection of IFN-γ production by antigen-specific stimulated lymphocytes. Blood samples for ELISPOT analyses were collected prior to vaccinations and then 12, 42, 70, 97, 134, and 168 days after immunizations. Synthetic peptides corresponding to the complete sequences of SIV gag and the PSA antigen were used for ex-vivo stimulation of PBMC.

Results

Antigen-specific IFN-γ secreting cells in response to PSA or SIV gag peptides were quantitated by ELISPOT. FIG. 16 (FIGS. 16A-16D) illustrates the results obtained from Groups 1-4, respectively. The level of response to PSA was very low in all groups, indicating that the vaccine by itself did not induce a measurable and

consistent immune response when compared to non-vaccinated animals. On the other hand, SIV gag vaccination alone resulted in significant number of antigen-specific IFN-γ secreting cells that augmented over time (FIG. 16A). Untreated animals (not vaccinated) showed 100-1,000 spots/10^6 PBMC throughout the course of the study (FIG. 16D). These results established the threshold response to the vaccine; animals that presented < 1,000 spots/10^6 PBMC were considered non-responders. In the group of animals that received vaccine, 5 out of 6 monkeys showed an increased response overtime, with a mean number of spots after the third immunization (day 70) of 1,727 spots/10^6 PBMC (SD=242, range=1,403-1,968 spots/10^6 PBMC). One monkey was considered a non-responder (620 spots/million PBMC). Since in these studies MHC typing was not done, it is likely that the lack of T cell responses to the vaccine by some monkeys may be due to MHC-mismatch. Remarkably, on day 70, 4 out of 6 animals treated with SIV gag·plus mab 20H4.9-IgG4 presented a significant higher number of IFN-γ spots (FIG. 16C) compared to control animals (FIG. 16D) and to macaques that were immunized with DNA vaccine alone (FIG. 16A). The mean number of spots after the third immunization for the mab 20H4.9-IgG4-treated group was of 3,465 spots/10^6 PBMC (SD=1,236, range=2,070-4,780 spots/10^6 PBMC). Two monkeys in that group did not respond to the vaccine (< 800 spots/million PBMC). Following the third immunization (day 70), treatment with mab hu39E3.G4 plus DNA vaccine resulted in 6 out of 6 animals considered as responders with a mean number of spots/10^6 PBMC of 2,348 (SD=588, range=1,738-3,283) (FIG. 16B). For this group, the range of the number of spots was lower compared to those macaques treated with mab 20H4.9-IgG4.

Treatment with both mab 20H4.9-IgG4 and mab hu9E3.G4 was well tolerated and did not result in any significant changes in clinical signs, clinical chemistry, or hematological parameters relative to control monkeys.

These data show that mab 20H4.9-IgG4 treatment in combination with a DNA vaccine elicited an *in vivo* enhancement of the magnitude of the specific cellular response to the test antigen relative to controls or to treatment with mab hu39E3.G4, as measured by antigen specific IFN-γ-secreting cells. Since only one dose level of the antibodies and one dosing regimen were used in these preliminary studies, it is unlikely that maximal responses were induced, and further work to optimize

conditions is required. Clearly, however, even with this non-optimized protocol, an enhancement of the cellular response to test antigens was achieved with mab 20H4.9-IgG4, suggesting that modulation of CD137 function may be an attractive approach for augmenting the effectiveness of DNA vaccines.

5

Although the invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

CLAIMS:

What is claimed is:

1. A monoclonal antibody or antigen-binding portion thereof that specifically binds to 4-1BB, comprising a light chain variable region and a heavy chain variable region, wherein:

said light chain variable region comprises a CDR1 having amino acids 44-54 of SEQ ID NO:6, a CDR2 having amino acids 70-76 of SEQ ID NO:6, and a CDR3 having amino acids 109-119 of SEQ ID NO:6; and

said heavy chain variable region comprises a CDR1 having amino acids 50-54 of SEQ ID NO:3, a CDR2 having amino acids 69-84 of SEQ ID NO:3, and a CDR3 having amino acids 117-129 of SEQ ID NO:3.

2. The monoclonal antibody or antigen-binding portion thereof of claim 1, wherein:

said light chain comprises a variable region having amino acids 21-129 of SEQ ID NO:6; and

said heavy chain comprises a variable region having amino acids 20-140 of SEQ ID NO:3.

3. A monoclonal antibody that specifically binds to 4-1BB comprising a light chain and a heavy chain, wherein said light chain comprises amino acid residues 21-236 of SEQ ID NO:6 and said heavy chain comprises amino acid residues 20-467 of SEQ ID NO:3.

4. A pharmaceutical composition comprising:

the monoclonal antibody or antigen-binding portion thereof of claim 1; and

a pharmaceutically acceptable carrier.

5. A pharmaceutical composition comprising:
the monoclonal antibody of claim 3; and
a pharmaceutically acceptable carrier.

6. The monoclonal antibody or antigen-binding portion thereof of claim 1 that is a monoclonal antibody.

7. The monoclonal antibody or antigen-binding portion thereof of claim 2 that is a monoclonal antibody.

SEQUENCE LISTING

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<213> Artificial

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Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser
100 105 110

Asn Trp Pro Pro Ala Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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<213> Artificial

<220>
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Val Leu Ser Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys
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Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe
35 40 45

Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Ser Pro Glu Lys Gly Leu
50 55 60

Glu Trp Ile Gly Glu Ile Asn His Gly Gly Tyr Val Thr Tyr Asn Pro
65 70 75 80

Ser Leu Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
85 90 95

Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
100 105 110

Tyr Cys Ala Arg Asp Tyr Gly Pro Gly Asn Tyr Asp Trp Tyr Phe Asp
115 120 125

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
210 215 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
225 230 235 240

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
245 250 255

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
260 265 270

```
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                 280                 285

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                 295                 300

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                 310                 315                 320

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                 330                 335

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                 345                 350

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
    370                 375                 380

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
                405                 410                 415

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
            420                 425                 430

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
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Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
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Ser Leu Ser Pro Gly Lys
465                 470
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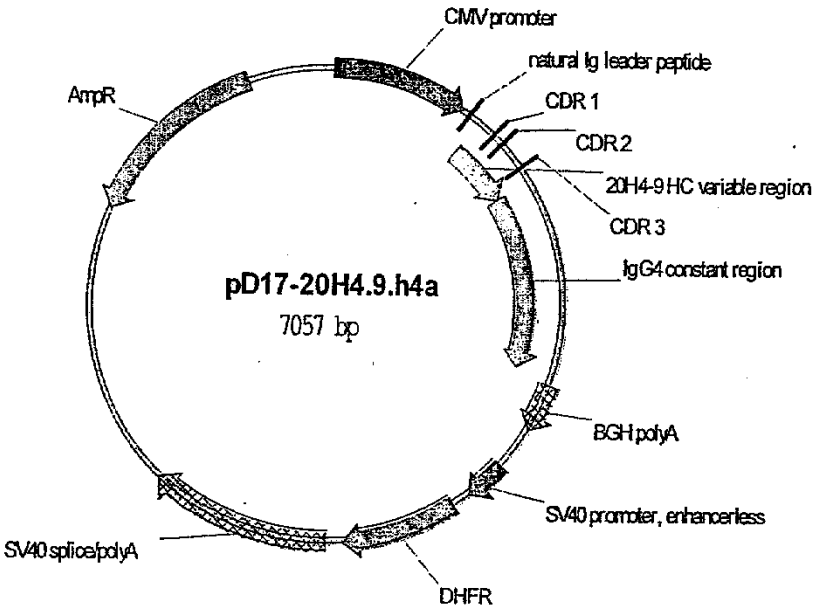

CMV promoter
natural Ig leader peptide
CDR 1
CDR 2
20H4-9 HC variable region
CDR 3
IgG4 constant region
pD17-20H4.9.h4a
7057 bp
BGH polyA
SV40 promoter, enhancerless
DHFR
SV40 splice/polyA
AmpR

FIG. 1

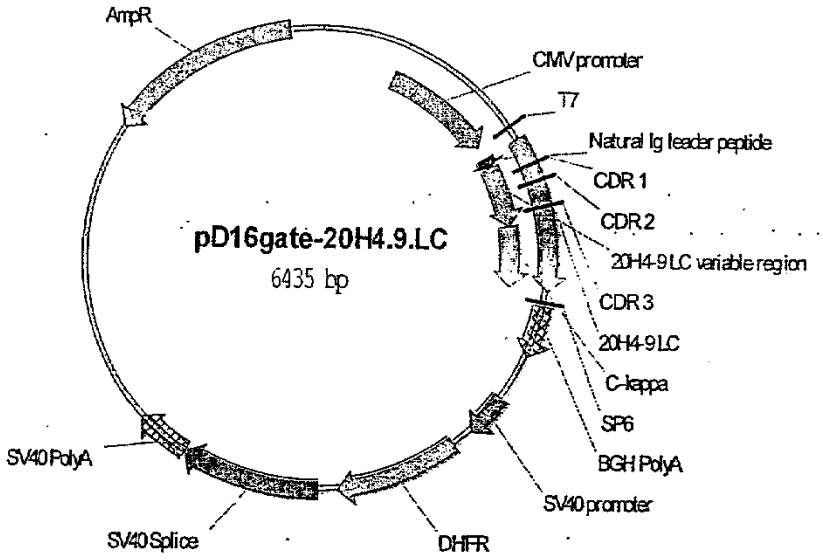

AmpR
CMV promoter
T7
Natural Ig leader peptide
CDR 1
CDR 2
20H4-9 LC variable region
CDR 3
20H4-9 LC
C-kappa
SP6
BGH PolyA
SV40 promoter
DHFR
SV40 Splice
SV40 PolyA
pD16gate-20H4.9.LC
6435 bp

FIG. 2

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       GCTACATGCC CGGTCTATAT GCGCAACTGT AACTAATAAC TGATCAATAA
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       TTATCATTAG TTAATGCCCC AGTAATCAAG TATCGGGTAT ATACCTCAAG
  101  CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA
       GCGCAATGTA TTGAATGCCA TTTACCGGGC GGACCGACTG GCGGGTTGCT
  151  CCCCCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCATA GTAACGCCAA
       GGGGGCGGGT AACTGCAGTT ATTACTGCAT ACAAGGGTAT CATTGCGGTT
  201  TAGGGACTTT CCATTGACGT CAATGGGTGG ACTATTTACG GTAAACTGCC
       ATCCCTGAAA GGTAACTGCA GTTACCCACC TGATAAATGC CATTTGACGG
  251  CACTTGGCAG TACATCAAGT GTATCATATG CCAAGTACGC CCCCTATTGA
       GTGAACCGTC ATGTAGTTCA CATAGTATAC GGTTCATGCG GGGGATAACT
  301  CGTCAATGAC GGTAAATGGC CCGCCTGGCA TTATGCCCAG TACATGACCT
       GCAGTTACTG CCATTTACCG GGCGGACCGT AATACGGGTC ATGTACTGGA
  351  TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT
       ATACCCTGAA AGGATGAACC GTCATGTAGA TGCATAATCA GTAGCGATAA
  401  ACCATGGTGA TGCGGTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT
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  451  TGACTCACGG GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT
       ACTGAGTGCC CCTAAAGGTT CAGAGGTGGG GTAACTGCAG TTACCCTCAA
  501  TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCG TAACAACTCC
       ACAAAACCGT GGTTTTAGTT GCCCTGAAAG GTTTTACAGC ATTGTTGAGG
  551  GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
       CGGGGTAACT GCGTTTACCC GCCATCCGCA CATGCCACCC TCCAGATATA
  601  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG
       TTCGTCTCGA GAGACCGATT GATCTCTTGG GTGACGAATG ACCGAATAGC
                                                    ~~~~~
                                                  M  K  ·
  651  AAATTAATAC GACTCACTAT AGGGAGACCC AAGCTTGGTA CCGCCATGAA
       TTTAATTATG CTGAGTGATA TCCCTCTGGG TTCGAACCAT GGCGGTACTT
                  natural Ig leader peptide
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·  H  L  W   F  F  L  L   L  V  A   A  P  R   W  V  L  S  ·
  701  ACACCTGTGG TTCTTCCTCC TCCTGGTGGC AGCTCCCAGA TGGGTCCTGT
       TGTGGACACC AAGAAGGAGG AGGACCACCG TCGAGGGTCT ACCCAGGACA
       natural Ig leader sequence
       ~~
                    20H4-9 HC variable region
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·  Q  V  Q   L  Q  Q   W  G  A  G   L  L  K   P  S  E
  751  CCCAGGTGCA ACTACAGCAG TGGGGCGCAG GACTGTTGAA GCCTTCGGAG
       GGGTCCACGT TGATGTCGTC ACCCCGCGTC CTGACAACTT CGGAAGCCTC
                                                    CDR 1
                                                   ~~~~~~~~
                    20H4-9 HC variable region
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        T  L  S  L   T  C  A   V  Y  G   G  S  F  S   G  Y  Y  ·
  801  ACCCTGTCCC TCACCTGCGC TGTCTATGGT GGGTCCTTCA GTGGTTACTA
       TGGGACAGGG AGTGGACGCG ACAGATACCA CCCAGGAAGT CACCAATGAT
        CDR 1                                              CDR 2
       ~~~~~~~                                             ~
                    20H4-9 HC variable region
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·  W  S  W   I  R  Q  S   P  E  K   G  L  E   W  I  G  E  ·
  851  CTGGAGCTGG ATACGCCAGT CCCCAGAGAA GGGGCTGGAG TGGATTGGGG
       GACCTCGACC TATGCGGTCA GGGGTCTCTT CCCCGACCTC ACCTAACCCC
```

FIG. 3A

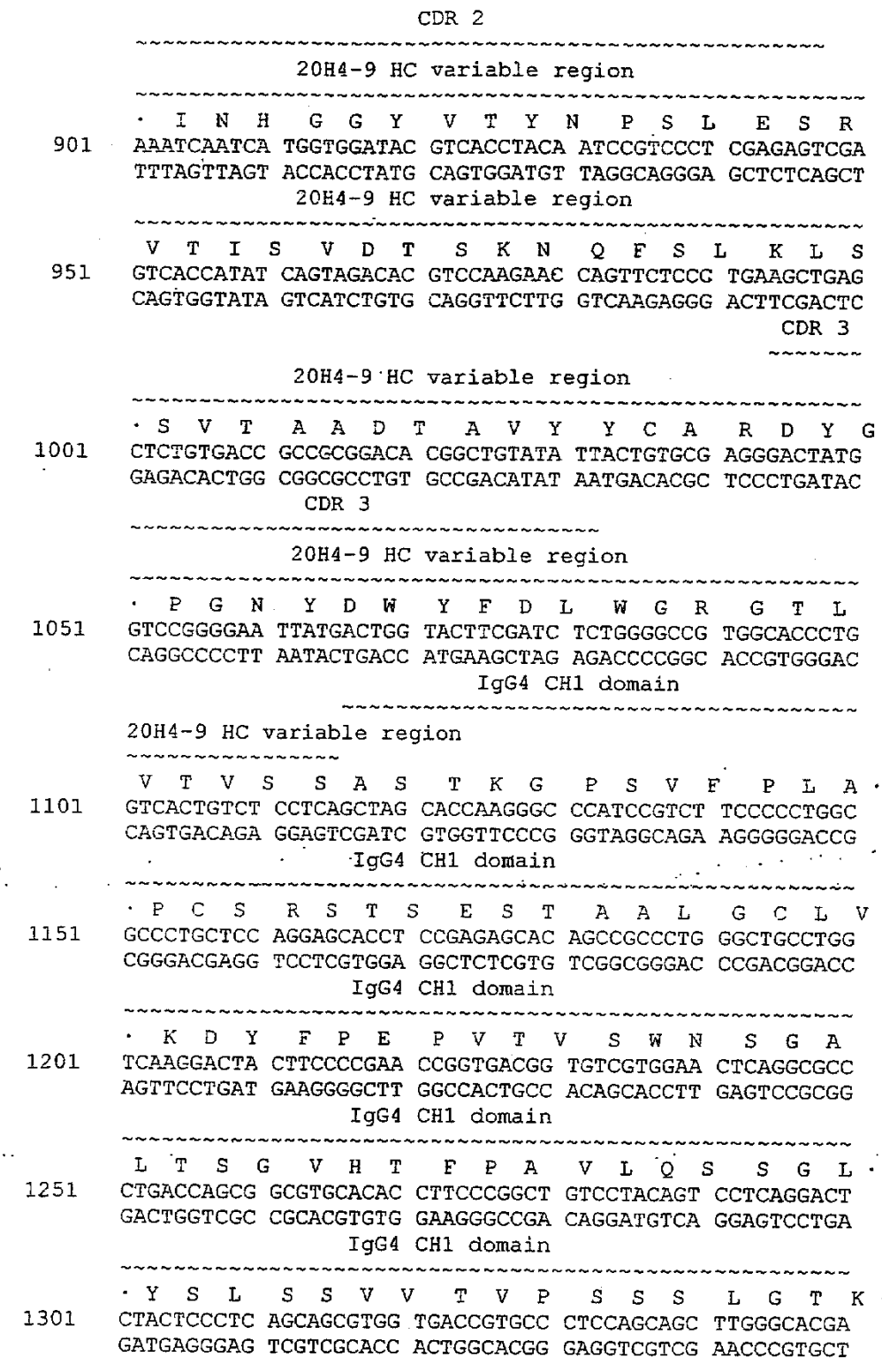

CDR 2
20H4-9 HC variable region
· I N H G G Y V T Y N P S L E S R
901 AAATCAATCA TGGTGGATAC GTCACCTACA ATCCGTCCCT CGAGAGTCGA
TTTAGTTAGT ACCACCTATG CAGTGGATGT TAGGCAGGGA GCTCTCAGCT
20H4-9 HC variable region
V T I S V D T S K N Q F S L K L S ·
951 GTCACCATAT CAGTAGACAC GTCCAAGAAC CAGTTCTCCC TGAAGCTGAG
CAGTGGTATA GTCATCTGTG CAGGTTCTTG GTCAAGAGGG ACTTCGACTC
CDR 3
20H4-9 HC variable region
· S V T A A D T A V Y Y C A R D Y G ·
1001 CTCTGTGACC GCCGCGGACA CGGCTGTATA TTACTGTGCG AGGGACTATG
GAGACACTGG CGGCGCCTGT GCCGACATAT AATGACACGC TCCCTGATAC
CDR 3
20H4-9 HC variable region
· P G N Y D W Y F D L W G R G T L
1051 GTCCGGGGAA TTATGACTGG TACTTCGATC TCTGGGGCCG TGGCACCCTG
CAGGCCCCTT AATACTGACC ATGAAGCTAG AGACCCCGGC ACCGTGGGAC
IgG4 CH1 domain
20H4-9 HC variable region
V T V S S A S T K G P S V F P L A ·
1101 GTCACTGTCT CCTCAGCTAG CACCAAGGGC CCATCCGTCT TCCCCCTGGC
CAGTGACAGA GGAGTCGATC GTGGTTCCCG GGTAGGCAGA AGGGGGACCG
IgG4 CH1 domain
· P C S R S T S E S T A A L G C L V ·
1151 GCCCTGCTCC AGGAGCACCT CCGAGAGCAC AGCCGCCCTG GGCTGCCTGG
CGGGACGAGG TCCTCGTGGA GGCTCTCGTG TCGGCGGGAC CCGACGGACC
IgG4 CH1 domain
· K D Y F P E P V T V S W N S G A
1201 TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
IgG4 CH1 domain
L T S G V H T F P A V L Q S S G L ·
1251 CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT
GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA
IgG4 CH1 domain
· Y S L S S V V T V P S S S L G T K ·
1301 CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACGA
GATGAGGGAG TCGTCGCACC ACTGGCACGG GAGGTCGTCG AACCCGTGCT

FIG. 3B

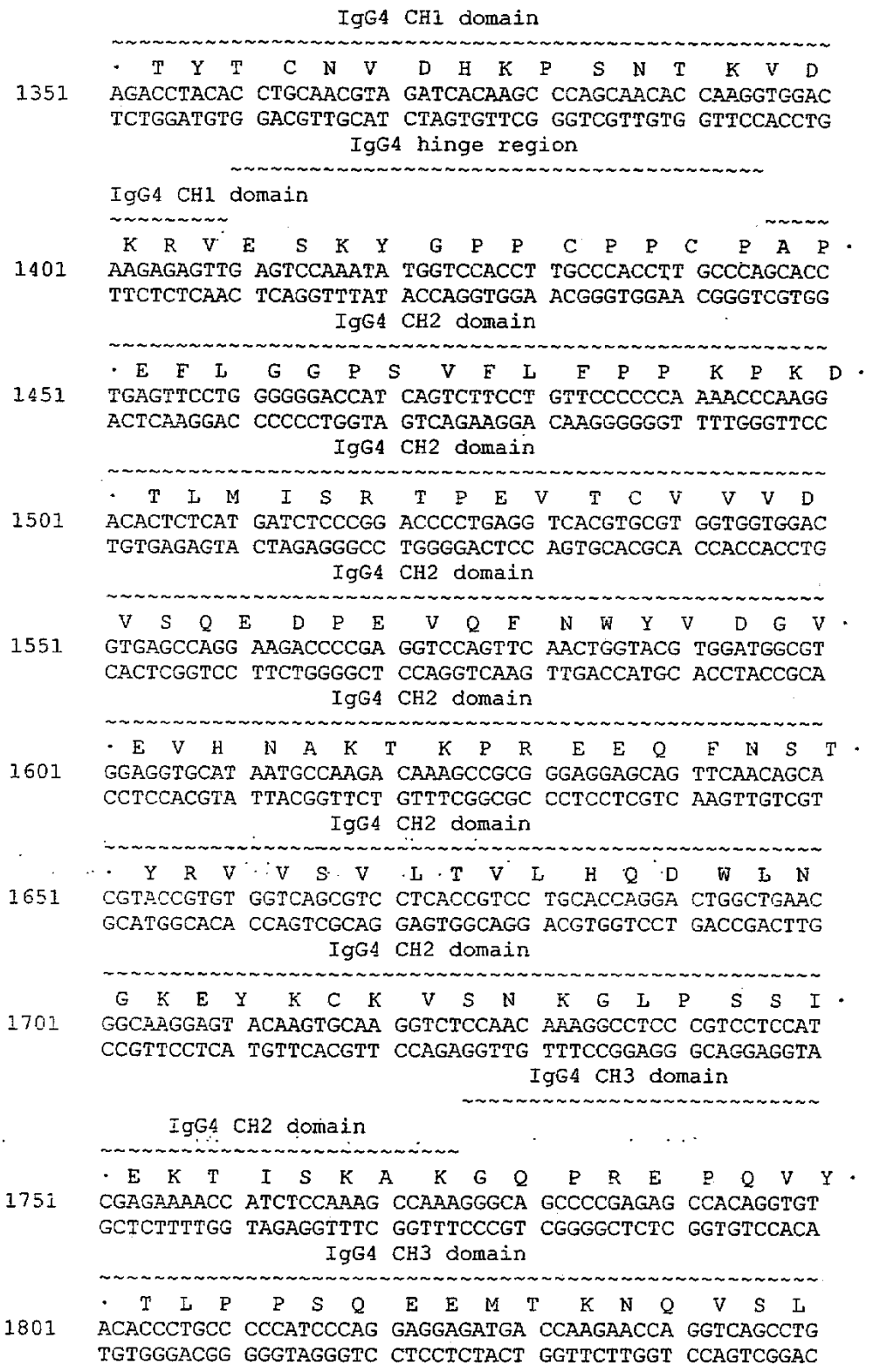

IgG4 CH1 domain
· T Y T C N V D H K P S N T K V D
1351 AGACCTACAC CTGCAACGTA GATCACAAGC CCAGCAACAC CAAGGTGGAC
TCTGGATGTG GACGTTGCAT CTAGTGTTCG GGTCGTTGTG GTTCCACCTG
IgG4 hinge region
IgG4 CH1 domain
K R V E S K Y G P P C P P C P A P ·
1401 AAGAGAGTTG AGTCCAAATA TGGTCCACCT TGCCCACCTT GCCCAGCACC
TTCTCTCAAC TCAGGTTTAT ACCAGGTGGA ACGGGTGGAA CGGGTCGTGG
IgG4 CH2 domain
· E F L G G P S V F L F P P K P K D ·
1451 TGAGTTCCTG GGGGGACCAT CAGTCTTCCT GTTCCCCCCA AAACCCAAGG
ACTCAAGGAC CCCCCTGGTA GTCAGAAGGA CAAGGGGGGT TTTGGGTTCC
IgG4 CH2 domain
· T L M I S R T P E V T C V V V D
1501 ACACTCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
TGTGAGAGTA CTAGAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG
IgG4 CH2 domain
V S Q E D P E V Q F N W Y V D G V ·
1551 GTGAGCCAGG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGATGGCGT
CACTCGGTCC TTCTGGGGCT CCAGGTCAAG TTGACCATGC ACCTACCGCA
IgG4 CH2 domain
· E V H N A K T K P R E E Q F N S T ·
1601 GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA
CCTCCACGTA TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT
IgG4 CH2 domain
· Y R V V S V L T V L H Q D W L N
1651 CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC
GCATGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG
IgG4 CH2 domain
G K E Y K C K V S N K G L P S S I ·
1701 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CGTCCTCCAT
CCGTTCCTCA TGTTCACGTT CCAGAGGTTG TTTCCGGAGG GCAGGAGGTA
IgG4 CH3 domain
IgG4 CH2 domain
· E K T I S K A K G Q P R E P Q V Y ·
1751 CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAG CCACAGGTGT
GCTCTTTTGG TAGAGGTTTC GGTTTCCCGT CGGGGCTCTC GGTGTCCACA
IgG4 CH3 domain
· T L P P S Q E E M T K N Q V S L
1801 ACACCCTGCC CCCATCCCAG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
TGTGGGACGG GGGTAGGGTC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC

FIG. 3C

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                    IgG4 CH3 domain
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E
1851  ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
      TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT
                    IgG4 CH3 domain
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D
1901  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
      CTCGTTACCC GTCGGCCTCT TGTTGATGTT CTGGTGCGGA GGGCACGACC
                    IgG4 CH3 domain
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           S  D  G  S  F  F  L  Y  S  R  L  T  V  D  K  S
1951  ACTCCGACGG CTCCTTCTTC CTCTACAGCA GGCTAACCGT GGACAAGAGC
      TGAGGCTGCC GAGGAAGAAG GAGATGTCGT CCGATTGGCA CCTGTTCTCG
                    IgG4 CH3 domain
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           R  W  Q  E  G  N  V  F  S  C  S  V  M  H  E  A  L
2001  AGGTGGCAGG AGGGGAATGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
      TCCACCGTCC TCCCCTTACA GAAGAGTACG AGGCACTACG TACTCCGAGA
                    IgG4 CH3 domain
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           H  N  H  Y  T  Q  K  S  L  S  L  S  L  G  K
2051  GCACAACCAC TACACACAGA AGAGCCTCTC CCTGTCTCTG GGTAAATGAT
      CGTGTTGGTG ATGTGTGTCT TCTCGGAGAG GGACAGAGAC CCATTTACTA
2101  CTAGAGGGCC CTATTCTATA GTGTCACCTA AATGCTAGAG CTCGCTGATC
      GATCTCCCGG GATAAGATAT CACAGTGGAT TTACGATCTC GAGCGACTAG
2151  AGCCTCGACT GTGCCTTCTA GTTGCCAGCC ATCTGTTGTT TGCCCCTCCC
      TCGGAGCTGA CACGGAAGAT CAACGGTCGG TAGACAACAA ACGGGGAGGG
2201  CCGTGCCTTC CTTGACCCTG GAAGGTGCCA CTCCCACTGT CCTTTCCTAA
      GGCACGGAAG GAACTGGGAC CTTCCACGGT GAGGGTGACA GGAAAGGATT
2251  TAAAATGAGG AAATTGCATC GCATTGTCTG AGTAGGTGTC ATTCTATTCT
      ATTTTACTCC TTTAACGTAG CGTAACAGAC TCATCCACAG TAAGATAAGA
2301  GGGGGGTGGG GTGGGGCAGG ACAGCAAGGG GGAGGATTGG GAAGACAATA
      CCCCCCACCC CACCCCGTCC TGTCGTTCCC CCTCCTAACC CTTCTGTTAT
2351  GCAGGCATGC TGGGGATGCG GTGGGCTCTA TGGCTTCTGA GGCGGAAAGA
      CGTCCGTACG ACCCCTACGC CACCCGAGAT ACCGAAGACT CCGCCTTTCT
2401  ACCAGCTGGG GCTCTAGGGG GTATCCCCAC GCGCCCTGTA GCGGCGCATT
      TGGTCGACCC CGAGATCCCC CATAGGGGTG CGCGGGACAT CGCCGCGTAA
2451  AAGCGCGGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT ACACTTGCCA
      TTCGCGCCGC CCACACCACC AATGCGCGTC GCACTGGCGA TGTGAACGGT
2501  GCGCCCTAGC GCCCGCTCCT TTCGCTTTCT TCCCTTCCTT TCTCGCCACG
      CGCGGGATCG CGGGCGAGGA AAGCGAAAGA AGGGAAGGAA AGAGCGGTGC
2551  TTCGCCGGGC CTCTCAAAAA AGGGAAAAAA AGCATGCATC TCAATTAGTC
      AAGCGGCCCG GAGAGTTTTT TCCCTTTTTT TCGTACGTAG AGTTAATCAG
2601  AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
      TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
2651  CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT
      GGTCAAGGCG GGTAAGAGGC GGGGTACCGA CTGATTAAAA AAAATAAATA
2701  GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG
      CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC
2751  AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTTGGA CAGCTCAGGG
      TCCGAAAAAA CCTCCGGATC CGAAAACGTT TTTCGAACCT GTCGAGTCCC
2801  CTGCGATTTC GCGCCAAACT TGACGGCAAT CCTAGCGTGA AGGCTGGTAG
      GACGCTAAAG CGCGGTTTGA ACTGCCGTTA GGATCGCACT TCCGACCATC
```

FIG. 3D

```
2851  GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT GCATCGTCGC
      CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA CGTAGCAGCG
2901  CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGGCCTC
      GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACCGGAG
2951  CGCTCAGGAA CGAGTTCAAG TACTTCCAAA GAATGACCAC AACCTCTTCA
      GCGAGTCCTT GCTCAAGTTC ATGAAGGTTT CTTACTGGTG TTGGAGAAGT
3001  GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC
      CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG
3051  CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT ATAGTTCTCA
      GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA TATCAAGAGT
3101  GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT
      CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA
3151  TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG CAAGTAAAGT
      AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC GTTCATTTCA
3201  AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA
      TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT
3251  ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT GCAGGAATTT
      TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA CGTCCTTAAA
3301  GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACTTCT
      CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGAAGA
3351  CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA AAAGGCATCA
      GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT TTTCCGTAGT
3401  AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC
      TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG
3451  AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA GACCATGGGA
      TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT CTGGTACCCT
3501  CTTTTGCTGG CTTTAGATCT CTTTGTGAAG GAACCTTACT TCTGTGGTGT
      GAAAACGACC GAAATCTAGA GAAACACTTC CTTGGAATGA AGACACCACA
3551  GACATAATTG GACAAACTAC CTACAGAGAT TTAAAGCTCT AAGGTAAATA
      CTGTATTAAC CTGTTTGATG GATGTCTCTA AATTTCGAGA TTCCATTTAT
3601  TAAAATTTTT AAGTGTATAA TGTGTTAAAC TACTGATTCT AATTGTTTGT
      ATTTTAAAAA TTCACATATT ACACAATTTG ATGACTAAGA TTAACAAACA
3651  GTATTTTAGA TTCCAACCTA TGGAACTGAT GAATGGGAGC AGTGGTGGAA
      CATAAAATCT AAGGTTGGAT ACCTTGACTA CTTACCCTCG TCACCACCTT
3701  TGCCTTTAAT GAGGAAAACC TGTTTTGCTC AGAAGAAATG CCATCTAGTG
      ACGGAAATTA CTCCTTTTGG ACAAAACGAG TCTTCTTTAC GGTAGATCAC
3751  ATGATGAGGC TACTGCTGAC TCTCAACATT CTACTCCTCC AAAAAAGAAG
      TACTACTCCG ATGACGACTG AGAGTTGTAA GATGAGGAGG TTTTTTCTTC
3801  AGAAAGGTAG AAGACCCCAA GGACTTTCCT TCAGAATTGC TAAGTTTTTT
      TCTTTCCATC TTCTGGGGTT CCTGAAAGGA AGTCTTAACG ATTCAAAAAA
3851  GAGTCATGCT GTGTTTAGTA ATAGAACTCT TGCTTGCTTT GCTATTTACA
      CTCAGTACGA CACAAATCAT TATCTTGAGA ACGAACGAAA CGATAAATGT
3901  CCACAAAGGA AAAAGCTGCA CTGCTATACA AGAAAATTAT GGAAAAATAT
      GGTGTTTCCT TTTTCGACGT GACGATATGT TCTTTTAATA CCTTTTTATA
3951  TCTGTAACCT TTATAAGTAG GCATAACAGT TATAATCATA ACATACTGTT
      AGACATTGGA AATATTCATC CGTATTGTCA ATATTAGTAT TGTATGACAA
4001  TTTTCTTACT CCACACAGGC ATAGAGTGTC TGCTATTAAT AACTATGCTC
      AAAAGAATGA GGTGTGTCCG TATCTCACAG ACGATAATTA TTGATACGAG
4051  AAAAATTGTG TACCTTTAGC TTTTTAATTT GTAAAGGGGT TAATAAGGAA
      TTTTTAACAC ATGGAAATCG AAAAATTAAA CATTTCCCCA ATTATTCCTT
4101  TATTTGATGT ATAGTGCCTT GACTAGAGAT CATAATCAGC CATACCACAT
      ATAAACTACA TATCACGGAA CTGATCTCTA GTATTAGTCG GTATGGTGTA
4151  TTGTAGAGGT TTTACTTGCT TTAAAAAACC TCCCACACCT CCCCCTGAAC
      AACATCTCCA AAATGAACGA AATTTTTTGG AGGGTGTGGA GGGGGACTTG
4201  CTGAAACATA AAATGAATGC AATTGTTGTT GTTAACTTGT TTATTGCAGC
```

FIG. 3E

```
       GACTTTGTAT TTTACTTACG TTAACAACAA CAATTGAACA AATAACGTCG
4251   TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTC ACAAATAAAG
       AATATTACCA ATGTTTATTT CGTTATCGTA GTGTTTAAAG TGTTTATTTC
4301   CATTTTTTTC ACTGCATTCT AGTTGTGGTT TGTCCAAACT CATCAATGTA
       GTAAAAAAAG TGACGTAAGA TCAACACCAA ACAGGTTTGA GTAGTTACAT
4351   TCTTATCATG TCTGGATCGG CTGGATGATC CTCCAGCGCG GGGATCTCAT
       AGAATAGTAC AGACCTAGCC GACCTACTAG GAGGTCGCGC CCCTAGAGTA
4401   GCTGGAGTTC TTCGCCCACC CCAACTTGTT TATTGCAGCT TATAATGGTT
       CGACCTCAAG AAGCGGGTGG GGTTGAACAA ATAACGTCGA ATATTACCAA
4451   ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA
       TGTTTATTTC GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT
4501   CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT
       GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA
4551   CTGTATACCG TCGACCTCTA GCTAGAGCTT GGCGTAATCA TGGTCATAGC
       GACATATGGC AGCTGGAGAT CGATCTCGAA CCGCATTAGT ACCAGTATCG
4601   TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA
       ACAAAGGACA CACTTTAACA ATAGGCGAGT GTTAAGGTGT GTTGTATGCT
4651   GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG TGAGCTAACT
       CGGCCTTCGT ATTTCACATT TCGGACCCCA CGGATTACTC ACTCGATTGA
4701   CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG GGAAACCTGT
       GTGTAATTAA CGCAACGCGA GTGACGGGCG AAAGGTCAGC CCTTTGGACA
4751   CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG
       GCACGGTCGA CGTAATTACT TAGCCGGTTG CGCGCCCCTC TCCGCCAAAC
4801   CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT
       GCATAACCCG CGAGAAGGCG AAGGAGCGAG TGACTGAGCG ACGCGAGCCA
4851   CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT
       GCAAGCCGAC GCCGCTCGCC ATAGTCGAGT GAGTTTCCGC CATTATGCCA
4901   TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC
       ATAGGTGTCT TAGTCCCCTA TTGCGTCCTT TCTTGTACAC TCGTTTTCCG
4951   CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA
       GTCGTTTTCC GGTCCTTGGC ATTTTTCCGG CGCAACGACC GCAAAAAGGT
5001   TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA
       ATCCGAGGCG GGGGGACTGC TCGTAGTGTT TTTAGCTGCG AGTTCAGTCT
5051   GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA
       CCACCGCTTT GGGCTGTCCT GATATTTCTA TGGTCCGCAA AGGGGGACCT
5101   AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT
       TCGAGGGAGC ACGCGAGAGG ACAAGGCTGG GACGGCGAAT GGCCTATGGA
5151   GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAA TGCTCACGCT
       CAGGCGGAAA GAGGGAAGCC CTTCGCACCG CGAAAGAGTT ACGAGTGCGA
5201   GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG
       CATCCATAGA GTCAAGCCAC ATCCAGCAAG CGAGGTTCGA CCCGACACAC
5251   CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG
       GTGCTTGGGG GGCAAGTCGG GCTGGCGACG CGGAATAGGC CATTGATAGC
5301   TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG GCAGCAGCCA
       AGAACTCAGG TTGGGCCATT CTGTGCTGAA TAGCGGTGAC CGTCGTCGGT
5351   CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC
       GACCATTGTC CTAATCGTCT CGCTCCATAC ATCCGCCACG ATGTCTCAAG
5401   TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT
       AACTTCACCA CCGGATTGAT GCCGATGTGA TCTTCCTGTC ATAAACCATA
5451   CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT
       GACGCGAGAC GACTTCGGTC AATGGAAGCC TTTTTCTCAA CCATCGAGAA
5501   GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG
       CTAGGCCGTT TGTTTGGTGG CGACCATCGC CACCAAAAAA ACAAACGTTC
5551   CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT
       GTCGTCTAAT GCGCGTCTTT TTTTCCTAGA GTTCTTCTAG GAAACTAGAA
```

FIG. 3F

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5601  TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT
      AAGATGCCCC AGACTGCGAG TCACCTTGCT TTTGAGTGCA ATTCCCTAAA
5651  TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA
      ACCAGTACTC TAATAGTTTT TCCTAGAAGT GGATCTAGGA AAATTTAATT
5701  AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA
      TTTACTTCAA AATTTAGTTA GATTTCATAT ATACTCATTT GAACCAGACT
5751  CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT
      GTCAATGGTT ACGAATTAGT CACTCCGTGG ATAGAGTCGC TAGACAGATA
5801  TTCGTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA
      AAGCAAGTAG GTATCAACGG ACTGAGGGGC AGCACATCTA TTGATGCTAT
5851  CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC
      GCCCTCCCGA ATGGTAGACC GGGGTCACGA CGTTACTATG GCGCTCTGGG
5901  ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG
      TGCGAGTGGC CGAGGTCTAA ATAGTCGTTA TTTGGTCGGT CGGCCTTCCC
5951  CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT
      GGCTCGCGTC TTCACCAGGA CGTTGAAATA GGCGGAGGTA GGTCAGATAA
6001  AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG
      TTAACAACGG CCCTTCGATC TCATTCATCA AGCGGTCAAT TATCAAACGC
6051  CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG
      GTTGCAACAA CGGTAACGAT GTCCGTAGCA CCACAGTGCG AGCAGCAAAC
6101  GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA
      CATACCGAAG TAAGTCGAGG CCAAGGGTTG CTAGTTCCGC TCAATGTACT
6151  TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT
      AGGGGGTACA ACACGTTTTT TCGCCAATCG AGGAAGCCAG GAGGCTAGCA
6201  TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC
      ACAGTCTTCA TTCAACCGGC GTCACAATAG TGAGTACCAA TACCGTCGTG
6251  TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT
      ACGTATTAAG AGAATGACAG TACGGTAGGC ATTCTACGAA AAGACACTGA
6301  GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG
      CCACTCATGA GTTGGTTCAG TAAGACTCTT ATCACATACG CCGCTGGCTC
6351  TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA
      AACGAGAACG GGCCGCAGTT ATGCCCTATT ATGGCGCGGT GTATCGTCTT
6401  CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA
      GAAATTTTCA CGAGTAGTAA CCTTTTGCAA GAAGCCCCGC TTTTGAGAGT
6451  AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC
      TCCTAGAATG GCGACAACTC TAGGTCAAGC TACATTGGGT GAGCACGTGG
6501  CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT GGGTGAGCAA
      GTTGACTAGA AGTCGTAGAA AATGAAAGTG GTCGCAAAGA CCCACTCGTT
6551  AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA
      TTTGTCCTTC CGTTTTACGG CGTTTTTTCC CTTATTCCCG CTGTGCCTTT
6601  TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA
      ACAACTTATG AGTATGAGAA GGAAAAAGTT ATAATAACTT CGTAAATAGT
6651  GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA
      CCCAATAACA GAGTACTCGC CTATGTATAA ACTTACATAA ATCTTTTTAT
6701  AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
      TTGTTTATCC CCAAGGCGCG TGTAAAGGGG CTTTTCACGG TGGACTGCAG
6751  GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA
      CTGCCTAGCC CTCTAGACGA TCCACTGGAC TCCGCGCGGC CGAAGCTTAT
6801  GCCAGAGTAA CCTTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG
      CGGTCTCATT GGAAAAAAAA ATTAAAATAA AATAAAATAA AAACTCTACC
6851  AGTTTGGCGC CGATCTCCCG ATCCCCTATG GTCGACTCTC AGTACAATCT
      TCAAACCGCG GCTAGAGGGC TAGGGGATAC CAGCTGAGAG TCATGTTAGA
6901  GCTCTGATGC CGCATAGTTA AGCCAGTATC TGCTCCCTGC TTGTGTGTTG
      CGAGACTACG GCGTATCAAT TCGGTCATAG ACGAGGGACG AACACACAAC
6951  GAGGTCGCTG AGTAGTGCGC GAGCAAAATT TAAGCTACAA CAAGGCAAGG
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FIG. 3G

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        CTCCAGCGAC TCATCACGCG CTCGTTTTAA ATTCGATGTT GTTCCGTTCC
7001    CTTGACCGAC AATTGCATGA AGAATCTGCT TAGGGTTAGG CGTTTTGCGC
        GAACTGGCTG TTAACGTACT TCTTAGACGA ATCCCAATCC GCAAAACGCG
7051    TGCTTCG
        ACGAAGC
```

FIG. 3H

```
   1  GACGGATCGG GAGATCTGCT AGCCCGGGTG ACCTGAGGCG CGCCGGCTTC
      CTGCCTAGCC CTCTAGACGA TCGGGCCCAC TGGACTCCGC GCGGCCGAAG
  51  GAATAGCCAG AGTAACCTTT TTTTTTAATT TTATTTTATT TTATTTTTGA
      CTTATCGGTC TCATTGGAAA AAAAAATTAA AATAAAATAA AATAAAAACT
 101  GATGGAGTTT GGCGCCGATC TCCCGATCCC CTATGGTCGA CTCTCAGTAC
      CTACCTCAAA CCGCGGCTAG AGGGCTAGGG GATACCAGCT GAGAGTCATG
 151  AATCTGCTCT GATGCCGCAT AGTTAAGCCA GTATCTGCTC CCTGCTTGTG
      TTAGACGAGA CTACGGCGTA TCAATTCGGT CATAGACGAG GGACGAACAC
 201  TGTTGGAGGT CGCTGAGTAG TGCGCGAGCA AAATTTAAGC TACAACAAGG
      ACAACCTCCA GCGACTCATC ACGCGCTCGT TTTAAATTCG ATGTTGTTCC
 251  CAAGGCTTGA CCGACAATTG CATGAAGAAT CTGCTTAGGG TTAGGCGTTT
      GTTCCGAACT GGCTGTTAAC GTACTTCTTA GACGAATCCC AATCCGCAAA
 301  TGCGCTGCTT CGCGATGTAC GGGCCAGATA TACGCGTTGA CATTGATTAT
      ACGCGACGAA GCGCTACATG CCCGGTCTAT ATGCGCAACT GTAACTAATA
 351  TGACTAGTTA TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA
      ACTGATCAAT AATTATCATT AGTTAATGCC CCAGTAATCA AGTATCGGGT
 401  TATATGGAGT TCCGCGTTAC ATAACTTACG GTAAATGGCC CGCCTGGCTG
      ATATACCTCA AGGCGCAATG TATTGAATGC CATTTACCGG GCGGACCGAC
 451  ACCGCCCAAC GACCCCCGCC CATTGACGTC AATAATGACG TATGTTCCCA
      TGGCGGGTTG CTGGGGGCGG GTAACTGCAG TTATTACTGC ATACAAGGGT
 501  TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT GGAGTATTTA
      ATCATTGCGG TTATCCCTGA AAGGTAACTG CAGTTACCCA CCTCATAAAT
 551  CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC
      GCCATTTGAC GGGTGAACCG TCATGTAGTT CACATAGTAT ACGGTTCATG
 601  GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC
      CGGGGGATAA CTGCAGTTAC TGCCATTTAC CGGGCGGACC GTAATACGGG
 651  AGTACATGAC CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA
      TCATGTACTG GAATACCCTG AAAGGATGAA CCGTCATGTA GATGCATAAT
 701  GTCATCGCTA TTACCATGGT GATGCGGTTT TGGCAGTACA TCAATGGGCG
      CAGTAGCGAT AATGGTACCA CTACGCCAAA ACCGTCATGT AGTTACCCGC
 751  TGGATAGCGG TTTGACTCAC GGGGATTTCC AAGTCTCCAC CCCATTGACG
      ACCTATCGCC AAACTGAGTG CCCCTAAAGG TTCAGAGGTG GGGTAACTGC
 801  TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGACTT TCCAAAATGT
      AGTTACCCTC AAACAAAACC GTGGTTTTAG TTGCCCTGAA AGGTTTTACA
 851  CGTAACAACT CCGCCCCATT GACGCAAATG GGCGGTAGGC GTGTACGGTG
      GCATTGTTGA GGCGGGGTAA CTGCGTTTAC CCGCCATCCG CACATGCCAC
 901  GGAGGTCTAT ATAAGCAGAG CTCTCTGGCT AACTAGAGAA CCCACTGCTT
      CCTCCAGATA TATTCGTCTC GAGAGACCGA TTGATCTCTT GGGTGACGAA
 951  ACTGGCTTAT CGAAATTAAT ACGACTCACT ATAGGGAGAC CCAAGCTTAT
      TGACCGAATA GCTTTAATTA TGCTGAGTGA TATCCCTCTG GGTTCGAATA
                                        Natural Ig leader
                                        ~~~~~~~~~~~~~~~~~~
                                         M  E  A  P  A  Q
1001  CAACAAGTTT GTACAAAAAA GCAGGCTGGT ACCATGGAAG CCCCAGCTCA
      GTTGTTCAAA CATGTTTTTT CGTCCGACCA TGGTACCTTC GGGGTCGAGT
                                                  ~~~~~~~
              Natural Ig leader peptide
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      L  L  F  L  L  L  L  W  L  P  D  T  T  G  E  I  V
1051  GCTTCTCTTC CTCCTGCTAC TCTGGCTCCC AGATACCACC GGAGAAATTG
      CGAAGAGAAG GAGGACGATG AGACCGAGGG TCTATGGTGG CCTCTTTAAC
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FIG. 4A

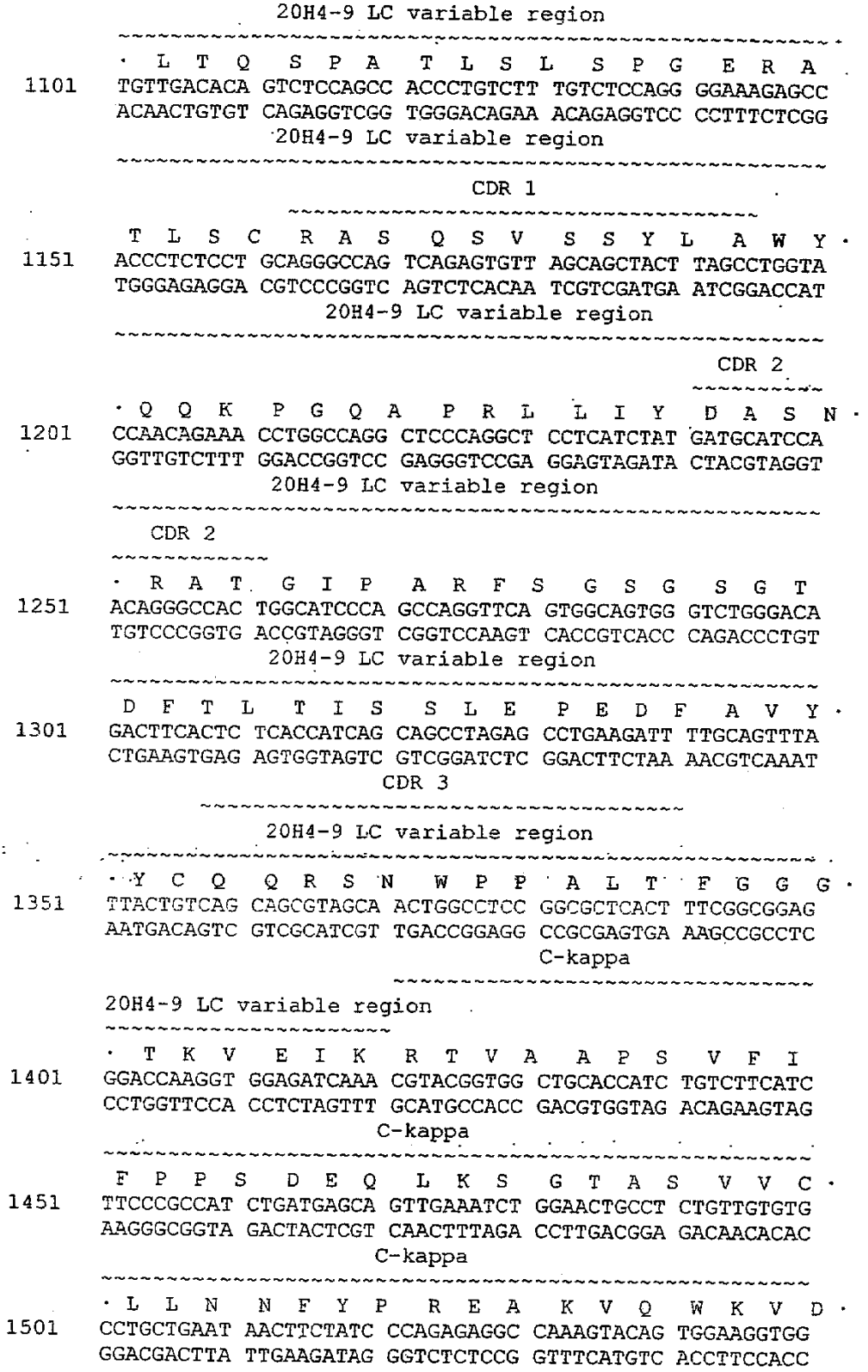

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ACAACTGTGT CAGAGGTCGG TGGGACAGAA ACAGAGGTCC CCTTTCTCGG
20H4-9 LC variable region
CDR 1
T L S C R A S Q S V S S Y L A W Y ·
1151 ACCCTCTCCT GCAGGGCCAG TCAGAGTGTT AGCAGCTACT TAGCCTGGTA
TGGGAGAGGA CGTCCCGGTC AGTCTCACAA TCGTCGATGA ATCGGACCAT
20H4-9 LC variable region
CDR 2
· Q Q K P G Q A P R L L I Y D A S N ·
1201 CCAACAGAAA CCTGGCCAGG CTCCCAGGCT CCTCATCTAT GATGCATCCA
GGTTGTCTTT GGACCGGTCC GAGGGTCCGA GGAGTAGATA CTACGTAGGT
20H4-9 LC variable region
CDR 2
· R A T G I P A R F S G S G S G T
1251 ACAGGGCCAC TGGCATCCCA GCCAGGTTCA GTGGCAGTGG GTCTGGGACA
TGTCCCGGTG ACCGTAGGGT CGGTCCAAGT CACCGTCACC CAGACCCTGT
20H4-9 LC variable region
D F T L T I S S L E P E D F A V Y ·
1301 GACTTCACTC TCACCATCAG CAGCCTAGAG CCTGAAGATT TTGCAGTTTA
CTGAAGTGAG AGTGGTAGTC GTCGGATCTC GGACTTCTAA AACGTCAAAT
CDR 3
20H4-9 LC variable region
· Y C Q Q R S N W P P A L T F G G G ·
1351 TTACTGTCAG CAGCGTAGCA ACTGGCCTCC GGCGCTCACT TTCGGCGGAG
AATGACAGTC GTCGCATCGT TGACCGGAGG CCGCGAGTGA AAGCCGCCTC
C-kappa
20H4-9 LC variable region
· T K V E I K R T V A A P S V F I
1401 GGACCAAGGT GGAGATCAAA CGTACGGTGG CTGCACCATC TGTCTTCATC
CCTGGTTCCA CCTCTAGTTT GCATGCCACC GACGTGGTAG ACAGAAGTAG
C-kappa
F P P S D E Q L K S G T A S V V C ·
1451 TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAACTGCCT CTGTTGTGTG
AAGGGCGGTA GACTACTCGT CAACTTTAGA CCTTGACGGA GACAACACAC
C-kappa
· L L N N F Y P R E A K V Q W K V D ·
1501 CCTGCTGAAT AACTTCTATC CCAGAGAGGC CAAAGTACAG TGGAAGGTGG
GGACGACTTA TTGAAGATAG GGTCTCTCCG GTTTCATGTC ACCTTCCACC

FIG. 4B

C-kappa

```
                                                                    ~~~~~~~~~~
                                                                    · N A L
1551  ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTCAC AGAGCAGGAC
      TATTGCGGGA GGTTAGCCCA TTGAGGGTCC TCTCACAGTG TCTCGTCCTG
                                                                    C-kappa
                                                                    ~~~~~~~~~~
                                                                    Q S G N S
1601  AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC TGAGCAAAGC
      TCGTTCCTGT CGTGGATGTC GGAGTCGTCG TGGGACTGCG ACTCGTTTCG
                                                                    C-kappa
                                                                    ~~~~~~~~~~
                                                                    Q E S V T
1651  AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC CATCAGGGCC
      TCTGATGCTC TTTGTGTTTC AGATGCGGAC GCTTCAGTGG GTAGTCCCGG
                                                                    C-kappa
                                                                    ~~~~~~~~~~
                                                                    E Q D
1701  TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG TTAGACCCAG
      ACTCGAGCGG GCAGTGTTTC TCGAAGTTGT CCCCTCTCAC AATCTGGGTC
1751  CTTTCTTGTA CAAAGTGGTT GATCTAGAGG GCCCTATTCT ATAGTGTCAC
      GAAAGAACAT GTTTCACCAA CTAGATCTCC CGGGATAAGA TATCACAGTG
1801  CTAAATGCTA GAGCTCGCTG ATCAGCCTCG ACTGTGCCTT CTAGTTGCCA
      GATTTACGAT CTCGAGCGAC TAGTCGGAGC TGACACGGAA GATCAACGGT
1851  GCCATCTGTT GTTTGCCCCT CCCCCGTGCC TTCCTTGACC CTGGAAGGTG
      CGGTAGACAA CAAACGGGGA GGGGGCACGG AAGGAACTGG GACCTTCCAC
1901  CCACTCCCAC TGTCCTTTCC TAATAAAATG AGGAAATTGC ATCGCATTGT
      GGTGAGGGTG ACAGGAAAGG ATTATTTTAC TCCTTTAACG TAGCGTAACA
1951  CTGAGTAGGT GTCATTCTAT TCTGGGGGGT GGGGTGGGGC AGGACAGCAA
      GACTCATCCA CAGTAAGATA AGACCCCCCA CCCCACCCCG TCCTGTCGTT
2001  GGGGGAGGAT TGGGAAGACA ATAGCAGGCA TGCTGGGGAT GCGGTGGGCT
      CCCCCTCCTA ACCCTTCTGT TATCGTCCGT ACGACCCCTA CGCCACCCGA
2051  CTATGGCTTC TGAGGCGGAA AGAACCAGCT GGGGCTCTAG GGGGTATCCC
      GATACCGAAG ACTCCGCCTT TCTTGGTCGA CCCCGAGATC CCCCATAGGG
2101  CACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG
      GTGCGCGGGA CATCGCCGCG TAATTCGCGC CGCCCACACC ACCAATGCGC
2151  CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT
      GTCGCACTGG CGATGTGAAC GGTCGCGGGA TCGCGGGCGA GGAAAGCGAA
2201  TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG GGCCTCTCAA AAAAGGGAAA
      AGAAGGGAAG GAAAGAGCGG TGCAAGCGGC CCGGAGAGTT TTTTCCCTTT
2251  AAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC
      TTTTCGTACG TAGAGTTAAT CAGTCGTTGG TATCAGGGCG GGGATTGAGG
2301  GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG
      CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC
2351  GCTGACTAAT TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT
      CGACTGATTA AAAAAAATAA ATACGTCTCC GGCTCCGGCG GAGCCGGAGA
2401  GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG
      CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC
2451  CAAAAAGCTT GGGGGGACAG CTCAGGGCTG CGATTTCGCG CCAAACTTGA
      GTTTTTCGAA CCCCCCTGTC GAGTCCCGAC GCTAAAGCGC GGTTTGAACT
2501  CGGCAATCCT AGCGTGAAGG CTGGTAGGAT TTTATCCCCG CTGCCATCAT
      GCCGTTAGGA TCGCACTTCC GACCATCCTA AAATAGGGGC GACGGTAGTA
2551  GGTTCGACCA TTGAACTGCA TCGTCGCCGT GTCCCAAAAT ATGGGGATTG
      CCAAGCTGGT AACTTGACGT AGCAGCGGCA CAGGGTTTTA TACCCCTAAC
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FIG. 4C

```
2601  GCAAGAACGG AGACCTACCC TGGCCTCCGC TCAGGAACGA GTTCAAGTAC
      CGTTCTTGCC TCTGGATGGG ACCGGAGGCG AGTCCTTGCT CAAGTTCATG
2651  TTCCAAAGAA TGACCACAAC CTCTTCAGTG GAAGGTAAAC AGAATCTGGT
      AAGGTTTCTT ACTGGTGTTG GAGAAGTCAC CTTCCATTTG TCTTAGACCA
2701  GATTATGGGT AGGAAAACCT GGTTCTCCAT TCCTGAGAAG AATCGACCTT
      CTAATACCCA TCCTTTTGGA CCAAGAGGTA AGGACTCTTC TTAGCTGGAA
2751  TAAAGGACAG AATTAATATA GTTCTCAGTA GAGAACTCAA AGAACCACCA
      ATTTCCTGTC TTAATTATAT CAAGAGTCAT CTCTTGAGTT TCTTGGTGGT
2801  CGAGGAGCTC ATTTTCTTGC CAAAAGTTTG GATGATGCCT TAAGACTTAT
      GCTCCTCGAG TAAAAGAACG GTTTTCAAAC CTACTACGGA ATTCTGAATA
2851  TGAACAACCG GAATTGGCAA GTAAAGTAGA CATGGTTTGG ATAGTCGGAG
      ACTTGTTGGC CTTAACCGTT CATTTCATCT GTACCAAACC TATCAGCCTC
2901  GCAGTTCTGT TTACCAGGAA GCCATGAATC AACCAGGCCA CCTCAGACTC
      CGTCAAGACA AATGGTCCTT CGGTACTTAG TTGGTCCGGT GGAGTCTGAG
2951  TTTGTGACAA GGATCATGCA GGAATTTGAA AGTGACACGT TTTTCCCAGA
      AAACACTGTT CCTAGTACGT CCTTAAACTT TCACTGTGCA AAAAGGGTCT
3001  AATTGATTTG GGGAAATATA AACTTCTCCC AGAATACCCA GGCGTCCTCT
      TTAACTAAAC CCCTTTATAT TTGAAGAGGG TCTTATGGGT CCGCAGGAGA
3051  CTGAGGTCCA GGAGGAAAAA GGCATCAAGT ATAAGTTTGA AGTCTACGAG
      GACTCCAGGT CCTCCTTTTT CCGTAGTTCA TATTCAAACT TCAGATGCTC
3101  AAGAAAGACT AACAGGAAGA TGCTTTCAAG TTCTCTGCTC CCCTCCTAAA
      TTCTTTCTGA TTGTCCTTCT ACGAAAGTTC AAGAGACGAG GGGAGGATTT
3151  GCTATGCATT TTTATAAGAC CATGGGACTT TTGCTGGCTT TAGATCTGAT
      CGATACGTAA AAATATTCTG GTACCCTGAA AACGACCGAA ATCTAGACTA
3201  CTTTGTGAAG GAACCTTACT TCTGTGGTGT GACATAATTG GACAAACTAC
      GAAACACTTC CTTGGAATGA AGACACCACA CTGTATTAAC CTGTTTGATG
3251  CTACAGAGAT TTAAAGCTCT AAGGTAAATA TAAAATTTTT AAGTGTATAA
      GATGTCTCTA AATTTCGAGA TTCCATTTAT ATTTTAAAAA TTCACATATT
3301  TGTGTTAAAC TACTGATTCT AATTGTTTGT GTATTTTAGA TTCCAACCTA
      ACACAATTTG ATGACTAAGA TTAACAAACA CATAAAATCT AAGGTTGGAT
3351  TGGAACTGAT GAATGGGAGC AGTGGTGGAA TGCCTTTAAT GAGGAAAACC
      ACCTTGACTA CTTACCCTCG TCACCACCTT ACGGAAATTA CTCCTTTTGG
3401  TGTTTTGCTC AGAAGAAATG CCATCTAGTG ATGATGAGGC TACTGCTGAC
      ACAAAACGAG TCTTCTTTAC GGTAGATCAC TACTACTCCG ATGACGACTG
3451  TCTCAACATT CTACTCCTCC AAAAAAGAAG AGAAAGGTAG AAGACCCCAA
      AGAGTTGTAA GATGAGGAGG TTTTTTCTTC TCTTTCCATC TTCTGGGGTT
3501  GGACTTTCCT TCAGAATTGC TAAGTTTTTT GAGTCATGCT GTGTTTAGTA
      CCTGAAAGGA AGTCTTAACG ATTCAAAAAA CTCAGTACGA CACAAATCAT
3551  ATAGAACTCT TGCTTGCTTT GCTATTTACA CCACAAAGGA AAAAGCTGCA
      TATCTTGAGA ACGAACGAAA CGATAAATGT GGTGTTTCCT TTTTCGACGT
3601  CTGCTATACA AGAAAATTAT GGAAAAATAT TCTGTAACCT TTATAAGTAG
      GACGATATGT TCTTTTAATA CCTTTTTATA AGACATTGGA AATATTCATC
3651  GCATAACAGT TATAATCATA ACATACTGTT TTTTCTTACT CCACACAGGC
      CGTATTGTCA ATATTAGTAT TGTATGACAA AAAAGAATGA GGTGTGTCCG
3701  ATAGAGTGTC TGCTATTAAT AACTATGCTC AAAAATTGTG TACCTTTAGC
      TATCTCACAG ACGATAATTA TTGATACGAG TTTTTAACAC ATGGAAATCG
3751  TTTTTAATTT GTAAAGGGGT TAATAAGGAA TATTTGATGT ATAGTGCCTT
      AAAAATTAAA CATTTCCCCA ATTATTCCTT ATAAACTACA TATCACGGAA
3801  GACTAGAGAT CGATCATAAT CAGCCATACC ACATTTGTAG AGGTTTTACT
      CTGATCTCTA GCTAGTATTA GTCGGTATGG TGTAAACATC TCCAAAATGA
3851  TGCTTTAAAA AACCTCCCAC ACCTCCCCCT GAACCTGAAA CATAAAATGA
      ACGAAATTTT TTGGAGGGTG TGGAGGGGGA CTTGGACTTT GTATTTTACT
3901  ATGCAATTGT TGTTGTTAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA
      TACGTTAACA ACAACAATTG AACAAATAAC GTCGAATATT ACCAATGTTT
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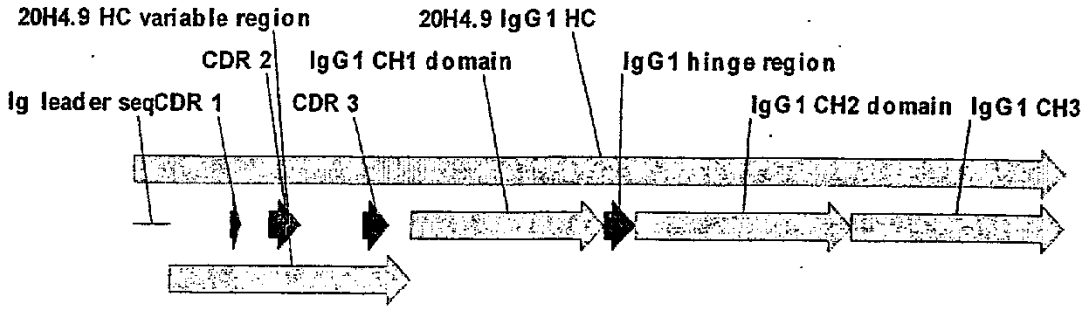
FIG. 4D

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3951  TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
      ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT
4001  TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA
      AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT
4051  TCGGCTGGAT GATCCTCCAG CGCGGGGATC TCATGCTGGA GTTCTTCGCC
      AGCCGACCTA CTAGGAGGTC GCGCCCCTAG AGTACGACCT CAAGAAGCGG
4101  CACCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG
      GTGGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC
4151  CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG
      GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC
4201  GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGTAT ACCGTCGACC
      CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACATA TGGCAGCTGG
4251  TCTAGCTAGA GCTTGGCGTA ATCATGGTCA TAGCTGTTTC CTGTGTGAAA
      AGATCGATCT CGAACCGCAT TAGTACCAGT ATCGACAAAG GACACACTTT
4301  TTGTTATCCG CTCACAATTC CACACAACAT ACGAGCCGGA AGCATAAAGT
      AACAATAGGC GAGTGTTAAG GTGTGTTGTA TGCTCGGCCT TCGTATTTCA
4351  GTAAAGCCTG GGGTGCCTAA TGAGTGAGCT AACTCACATT AATTGCGTTG
      CATTTCGGAC CCCACGGATT ACTCACTCGA TTGAGTGTAA TTAACGCAAC
4401  CGCTCACTGC CCGCTTTCCA GTCGGGAAAC CTGTCGTGCC AGCTGCATTA
      GCGAGTGACG GGCGAAAGGT CAGCCCTTTG GACAGCACGG TCGACGTAAT
4451  ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT GGGCGCTCTT
      TACTTAGCCG GTTGCGCGCC CCTCTCCGCC AAACGCATAA CCCGCGAGAA
4501  CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA
      GGCGAAGGAG CGAGTGACTG AGCGACGCGA GCCAGCAAGC CGACGCCGCT
4551  GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG
      CGCCATAGTC GAGTGAGTTT CCGCCATTAT GCCAATAGGT GTCTTAGTCC
4601  GGATAACGCA GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA
      CCTATTGCGT CCTTTCTTGT ACACTCGTTT TCCGGTCGTT TTCCGGTCCT
4651  ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT CCGCCCCCCT
      TGGCATTTTT CCGGCGCAAC GACCGCAAAA AGGTATCCGA GGCGGGGGGA
4701  GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC
      CTGCTCGTAG TGTTTTTAGC TGCGAGTTCA GTCTCCACCG CTTTGGGCTG
4751  AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT
      TCCTGATATT TCTATGGTCC GCAAAGGGGG ACCTTCGAGG GAGCACGCGA
4801  CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT
      GAGGACAAGG CTGGGACGGC GAATGGCCTA TGGACAGGCG GAAAGAGGGA
4851  TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC
      AGCCCTTCGC ACCGCGAAAG AGTATCGAGT GCGACATCCA TAGAGTCAAG
4901  GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTTC
      CCACATCCAG CAAGCGAGGT TCGACCCGAC ACACGTGCTT GGGGGGCAAG
4951  AGCCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA GTCCAACCCG
      TCGGGCTGGC GACGCGGAAT AGGCCATTGA TAGCAGAACT CAGGTTGGGC
5001  GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG
      CATTCTGTGC TGAATAGCGG TGACCGTCGT CGGTGACCAT TGTCCTAATC
5051  CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA
      GTCTCGCTCC ATACATCCGC CACGATGTCT CAAGAACTTC ACCACCGGAT
5101  ACTACGGCTA CACTAGAAGG AACAGTATTT GGTATCTGCG CTCTGCTGAA
      TGATGCCGAT GTGATCTTCC TTGTCATAAA CCATAGACGC GAGACGACTT
5151  GCCAGTTACC TTCGGAAAAA GAGTTGGTAG CTCTTGATCC GGCAAACAAA
      CGGTCAATGG AAGCCTTTTT CTCAACCATC GAGAACTAGG CCGTTTGTTT
5201  CCACCGCTGG TAGCGGTGGT TTTTTTGTTT GCAAGCAGCA GATTACGCGC
      GGTGGCGACC ATCGCCACCA AAAAAACAAA CGTTCGTCGT CTAATGCGCG
5251  AGAAAAAAAG GATCTCAAGA AGATCCTTTG ATCTTTTCTA CGGGGTCTGA
      TCTTTTTTTC CTAGAGTTCT TCTAGGAAAC TAGAAAAGAT GCCCCAGACT
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FIG. 4E

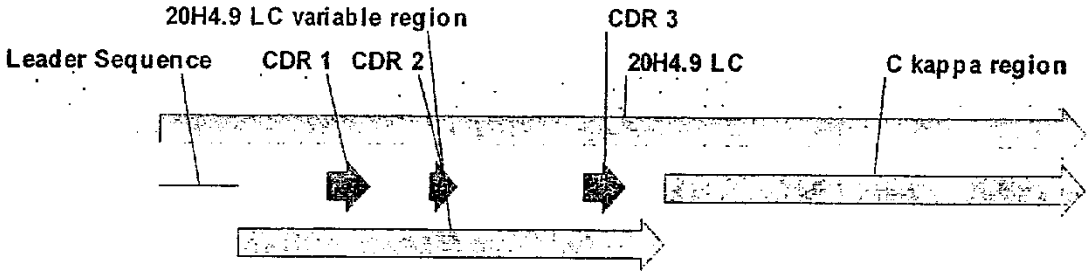
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5301   CGCTCAGTGG AACGAAAACT CACGTTAAGG GATTTTGGTC ATGAGATTAT
       GCGAGTCACC TTGCTTTTGA GTGCAATTCC CTAAAACCAG TACTCTAATA
5351   CAAAAAGGAT CTTCACCTAG ATCCTTTTAA ATTAAAAATG AAGTTTTAAA
       GTTTTTCCTA GAAGTGGATC TAGGAAAATT TAATTTTTAC TTCAAAATTT
5401   TCAATCTAAA GTATATATGA GTAAACTTGG TCTGACAGTT ACCAATGCTT
       AGTTAGATTT CATATATACT CATTTGAACC AGACTGTCAA TGGTTACGAA
5451   AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTCGT TCATCCATAG
       TTAGTCACTC CGTGGATAGA GTCGCTAGAC AGATAAAGCA AGTAGGTATC
5501   TTGCCTGACT CCCCGTCGTG TAGATAACTA CGATACGGGA GGGCTTACCA
       AACGGACTGA GGGGCAGCAC ATCTATTGAT GCTATGCCCT CCCGAATGGT
5551   TCTGGCCCCA GTGCTGCAAT GATACCGCGA GACCCACGCT CACCGGCTCC
       AGACCGGGGT CACGACGTTA CTATGGCGCT CTGGGTGCGA GTGGCCGAGG
5601   AGATTTATCA GCAATAAACC AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG
       TCTAAATAGT CGTTATTTGG TCGGTCGGCC TTCCCGGCTC GCGTCTTCAC
5651   GTCCTGCAAC TTTATCCGCC TCCATCCAGT CTATTAATTG TTGCCGGGAA
       CAGGACGTTG AAATAGGCGG AGGTAGGTCA GATAATTAAC AACGGCCCTT
5701   GCTAGAGTAA GTAGTTCGCC AGTTAATAGT TTGCGCAACG TTGTTGCCAT
       CGATCTCATT CATCAAGCGG TCAATTATCA AACGCGTTGC AACAACGGTA
5751   TGCTACAGGC ATCGTGGTGT CACGCTCGTC GTTTGGTATG GCTTCATTCA
       ACGATGTCCG TAGCACCACA GTGCGAGCAG CAAACCATAC CGAAGTAAGT
5801   GCTCCGGTTC CCAACGATCA AGGCGAGTTA CATGATCCCC CATGTTGTGC
       CGAGGCCAAG GGTTGCTAGT TCCGCTCAAT GTACTAGGGG GTACAACACG
5851   AAAAAAGCGG TTAGCTCCTT CGGTCCTCCG ATCGTTGTCA GAAGTAAGTT
       TTTTTTCGCC AATCGAGGAA GCCAGGAGGC TAGCAACAGT CTTCATTCAA
5901   GGCCGCAGTG TTATCACTCA TGGTTATGGC AGCACTGCAT AATTCTCTTA
       CCGGCGTCAC AATAGTGAGT ACCAATACCG TCGTGACGTA TTAAGAGAAT
5951   CTGTCATGCC ATCCGTAAGA TGCTTTTCTG TGACTGGTGA GTACTCAACC
       GACAGTACGG TAGGCATTCT ACGAAAAGAC ACTGACCACT CATGAGTTGG
6001   AAGTCATTCT GAGAATAGTG TATGCGGCGA CCGAGTTGCT CTTGCCCGGC
       TTCAGTAAGA CTCTTATCAC ATACGCCGCT GGCTCAACGA GAACGGGCCG
6051   GTCAATACGG GATAATACCG CGCCACATAG CAGAACTTTA AAAGTGCTCA
       CAGTTATGCC CTATTATGGC GCGGTGTATC GTCTTGAAAT TTTCACGAGT
6101   TCATTGGAAA ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG
       AGTAACCTTT TGCAAGAAGC CCCGCTTTTG AGAGTTCCTA GAATGGCGAC
6151   TTGAGATCCA GTTCGATGTA ACCCACTCGT GCACCCAACT GATCTTCAGC
       AACTCTAGGT CAAGCTACAT TGGGTGAGCA CGTGGGTTGA CTAGAAGTCG
6201   ATCTTTTACT TTCACCAGCG TTTCTGGGTG AGCAAAAACA GGAAGGCAAA
       TAGAAAATGA AAGTGGTCGC AAAGACCCAC TCGTTTTTGT CCTTCCGTTT
6251   ATGCCGCAAA AAAGGGAATA AGGGCGACAC GGAAATGTTG AATACTCATA
       TACGGCGTTT TTTCCCTTAT TCCCGCTGTG CCTTTACAAC TTATGAGTAT
6301   CTCTTCCTTT TTCAATATTA TTGAAGCATT TATCAGGGTT ATTGTCTCAT
       GAGAAGGAAA AAGTTATAAT AACTTCGTAA ATAGTCCCAA TAACAGAGTA
6351   GAGCGGATAC ATATTTGAAT GTATTTAGAA AAATAAACAA ATAGGGGTTC
       CTCGCCTATG TATAAACTTA CATAAATCTT TTTATTTGTT TATCCCCAAG
6401   CGCGCACATT TCCCCGAAAA GTGCCACCTG ACGTC
       GCGCGTGTAA AGGGGCTTTT CACGGTGGAC TGCAG
```

FIG. 4F



20H4.9-IgG1 Heavy Chain

FIG. 5



20H4.9 Light chain

FIG. 6

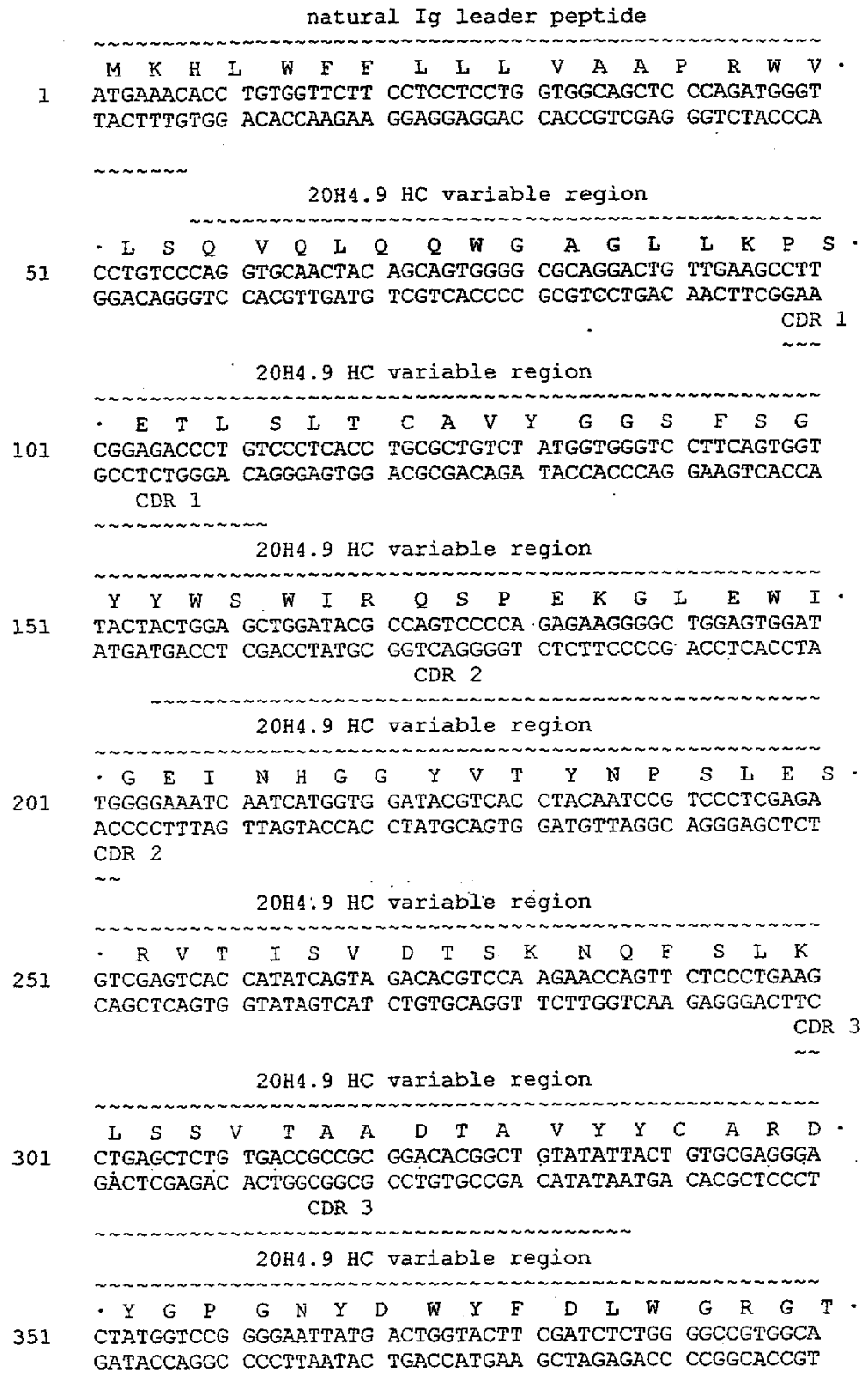

natural Ig leader peptide
M K H L W F F L L L V A A P R W V ·
1 ATGAAACACC TGTGGTTCTT CCTCCTCCTG GTGGCAGCTC CCAGATGGGT
TACTTTGTGG ACACCAAGAA GGAGGAGGAC CACCGTCGAG GGTCTACCCA
20H4.9 HC variable region
· L S Q V Q L Q Q W G A G L L K P S ·
51 CCTGTCCCAG GTGCAACTAC AGCAGTGGGG CGCAGGACTG TTGAAGCCTT
GGACAGGGTC CACGTTGATG TCGTCACCCC GCGTCCTGAC AACTTCGGAA
CDR 1
20H4.9 HC variable region
· E T L S L T C A V Y G G S F S G
101 CGGAGACCCT GTCCCTCACC TGCGCTGTCT ATGGTGGGTC CTTCAGTGGT
GCCTCTGGGA CAGGGAGTGG ACGCGACAGA TACCACCCAG GAAGTCACCA
CDR 1
20H4.9 HC variable region
Y Y W S W I R Q S P E K G L E W I ·
151 TACTACTGGA GCTGGATACG CCAGTCCCCA GAGAAGGGGC TGGAGTGGAT
ATGATGACCT CGACCTATGC GGTCAGGGGT CTCTTCCCCG ACCTCACCTA
CDR 2
20H4.9 HC variable region
· G E I N H G G Y V T Y N P S L E S ·
201 TGGGGAAATC AATCATGGTG GATACGTCAC CTACAATCCG TCCCTCGAGA
ACCCCTTTAG TTAGTACCAC CTATGCAGTG GATGTTAGGC AGGGAGCTCT
CDR 2
20H4.9 HC variable region
· R V T I S V D T S K N Q F S L K
251 GTCGAGTCAC CATATCAGTA GACACGTCCA AGAACCAGTT CTCCCTGAAG
CAGCTCAGTG GTATAGTCAT CTGTGCAGGT TCTTGGTCAA GAGGGACTTC
CDR 3
20H4.9 HC variable region
L S S V T A A D T A V Y Y C A R D ·
301 CTGAGCTCTG TGACCGCCGC GGACACGGCT GTATATTACT GTGCGAGGGA
GACTCGAGAC ACTGGCGGCG CCTGTGCCGA CATATAATGA CACGCTCCCT
CDR 3
20H4.9 HC variable region
· Y G P G N Y D W Y F D L W G R G T ·
351 CTATGGTCCG GGGAATTATG ACTGGTACTT CGATCTCTGG GGCCGTGGCA
GATACCAGGC CCCTTAATAC TGACCATGAA GCTAGAGACC CCGGCACCGT

FIG. 7A

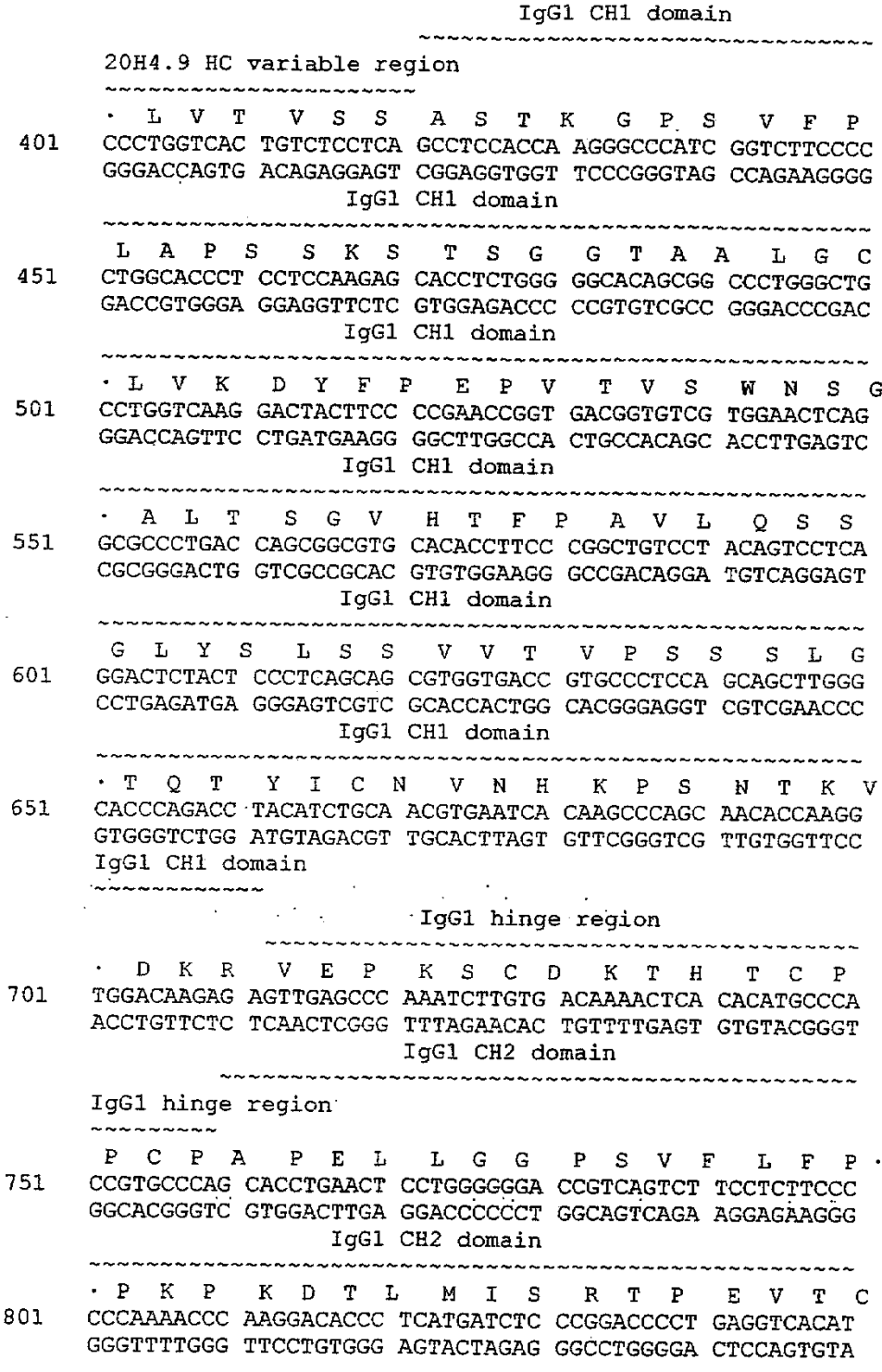

IgG1 CH1 domain
20H4.9 HC variable region
· L V T V S S A S T K G P S V F P
401 CCCTGGTCAC TGTCTCCTCA GCCTCCACCA AGGGCCCATC GGTCTTCCCC
GGGACCAGTG ACAGAGGAGT CGGAGGTGGT TCCCGGGTAG CCAGAAGGGG
IgG1 CH1 domain
L A P S S K S T S G G T A A L G C ·
451 CTGGCACCCT CCTCCAAGAG CACCTCTGGG GGCACAGCGG CCCTGGGCTG
GACCGTGGGA GGAGGTTCTC GTGGAGACCC CCGTGTCGCC GGGACCCGAC
IgG1 CH1 domain
· L V K D Y F P E P V T V S W N S G ·
501 CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG TGGAACTCAG
GGACCAGTTC CTGATGAAGG GGCTTGGCCA CTGCCACAGC ACCTTGAGTC
IgG1 CH1 domain
· A L T S G V H T F P A V L Q S S
551 GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
CGCGGGACTG GTCGCCGCAC GTGTGGAAGG GCCGACAGGA TGTCAGGAGT
IgG1 CH1 domain
G L Y S L S S V V T V P S S S L G ·
601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG
CCTGAGATGA GGGAGTCGTC GCACCACTGG CACGGGAGGT CGTCGAACCC
IgG1 CH1 domain
· T Q T Y I C N V N H K P S N T K V ·
651 CACCCAGACC TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG
GTGGGTCTGG ATGTAGACGT TGCACTTAGT GTTCGGGTCG TTGTGGTTCC
IgG1 CH1 domain
IgG1 hinge region
· D K R V E P K S C D K T H T C P
701 TGGACAAGAG AGTTGAGCCC AAATCTTGTG ACAAAACTCA CACATGCCCA
ACCTGTTCTC TCAACTCGGG TTTAGAACAC TGTTTTGAGT GTGTACGGGT
IgG1 CH2 domain
IgG1 hinge region
P C P A P E L L G G P S V F L F P ·
751 CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT TCCTCTTCCC
GGCACGGGTC GTGGACTTGA GGACCCCCCT GGCAGTCAGA AGGAGAAGGG
IgG1 CH2 domain
· P K P K D T L M I S R T P E V T C ·
801 CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT
GGGTTTTGGG TTCCTGTGGG AGTACTAGAG GGCCTGGGGA CTCCAGTGTA

FIG. 7B

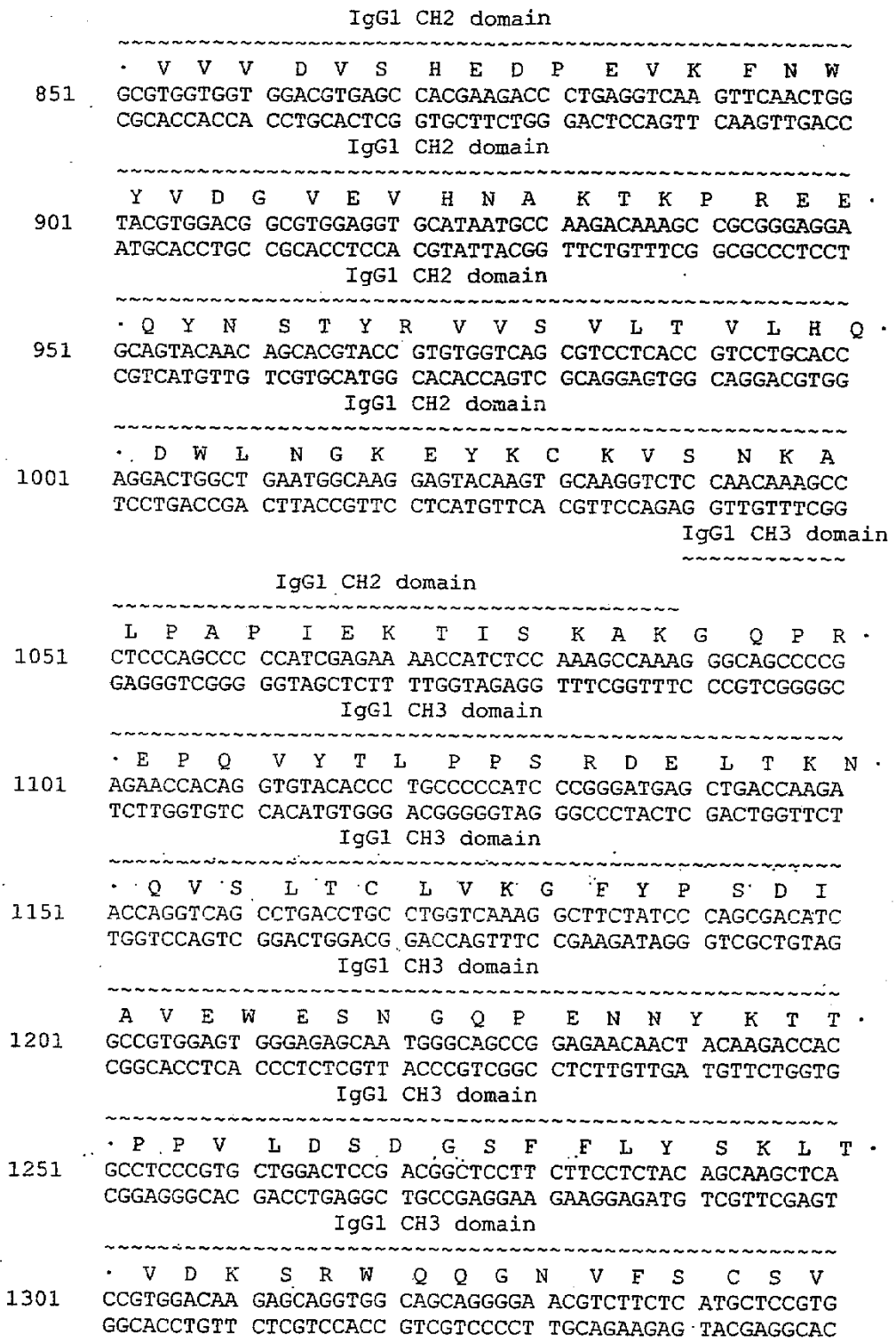

IgG1 CH2 domain
· V V V D V S H E D P E V K F N W
851 GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
CGCACCACCA CCTGCACTCG GTGCTTCTGG GACTCCAGTT CAAGTTGACC
IgG1 CH2 domain
Y V D G V E V H N A K T K P R E E ·
901 TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA
ATGCACCTGC CGCACCTCCA CGTATTACGG TTCTGTTTCG GCGCCCTCCT
IgG1 CH2 domain
· Q Y N S T Y R V V S V L T V L H Q ·
951 GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC
CGTCATGTTG TCGTGCATGG CACACCAGTC GCAGGAGTGG CAGGACGTGG
IgG1 CH2 domain
· D W L N G K E Y K C K V S N K A
1001 AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
TCCTGACCGA CTTACCGTTC CTCATGTTCA CGTTCCAGAG GTTGTTTCGG
IgG1 CH3 domain
IgG1 CH2 domain
L P A P I E K T I S K A K G Q P R ·
1051 CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG
GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG TTTCGGTTTC CCGTCGGGGC
IgG1 CH3 domain
· E P Q V Y T L P P S R D E L T K N ·
1101 AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGATGAG CTGACCAAGA
TCTTGGTGTC CACATGTGGG ACGGGGGTAG GGCCCTACTC GACTGGTTCT
IgG1 CH3 domain
· Q V S L T C L V K G F Y P S D I
1151 ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
TGGTCCAGTC GGACTGGACG GACCAGTTTC CGAAGATAGG GTCGCTGTAG
IgG1 CH3 domain
A V E W E S N G Q P E N N Y K T T ·
1201 GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC
CGGCACCTCA CCCTCTCGTT ACCCGTCGGC CTCTTGTTGA TGTTCTGGTG
IgG1 CH3 domain
· P P V L D S D G S F F L Y S K L T ·
1251 GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA
CGGAGGGCAC GACCTGAGGC TGCCGAGGAA GAAGGAGATG TCGTTCGAGT
IgG1 CH3 domain
· V D K S R W Q Q G N V F S C S V
1301 CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG
GGCACCTGTT CTCGTCCACC GTCGTCCCCT TGCAGAAGAG TACGAGGCAC

FIG. 7C

```
                    IgG1 CH3 domain
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S ·
 1351      ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC
           TACGTACTCC GAGACGTGTT GGTGATGTGC GTCTTCTCGG AGAGGGACAG
           IgG1 CH3 domain
           ~~~~~~~~~~~~
           · P  G  K
 1401      CCCGGGTAAA TGA
           GGGCCCATTT ACT
```

FIG. 7D

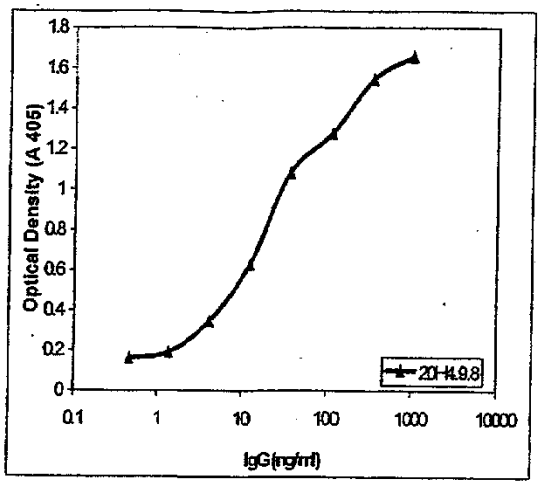

Optical Density (A 405)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.1
1
10
100
1000
10000
IgG(ng/ml)
20H4.9.8

FIG. 8A

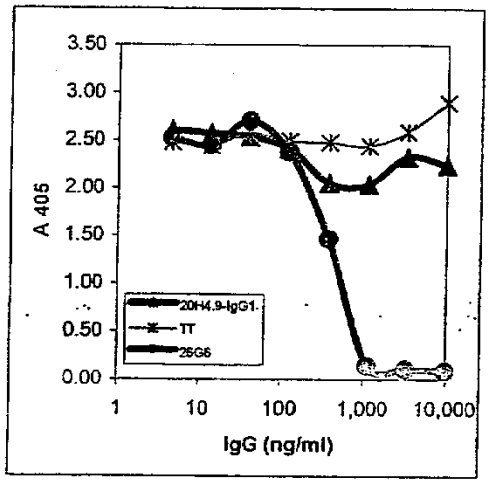

A 405
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1
10
100
1,000
10,000
IgG (ng/ml)
20H4.9-IgG1
TT
25G6

FIG. 8B

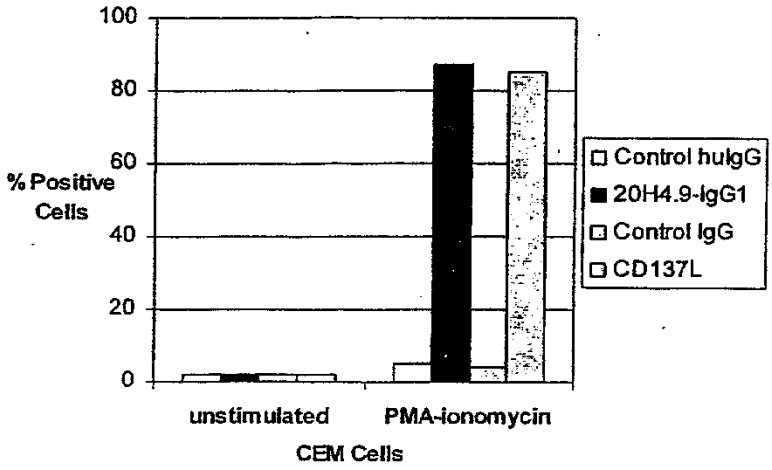

100
80
60
40
20
0
% Positive Cells
Control huIgG
20H4.9-IgG1
Control IgG
CD137L
unstimulated
PMA-ionomycin
CEM Cells

FIG. 9A

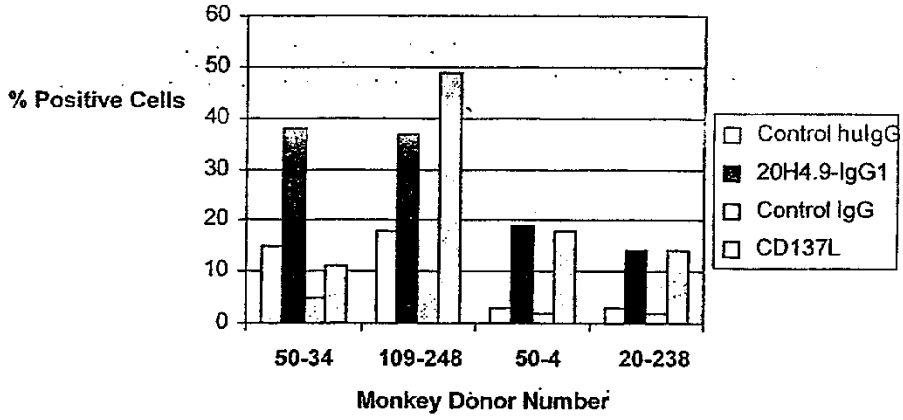

60
50
40
30
20
10
0
% Positive Cells
Control huIgG
20H4.9-IgG1
Control IgG
CD137L
50-34
109-248
50-4
20-238
Monkey Donor Number

FIG. 9B

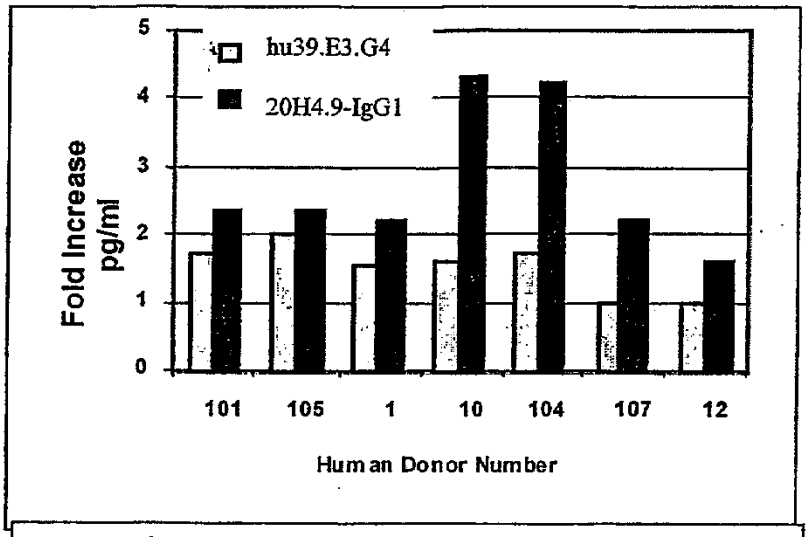


FIG. 10A

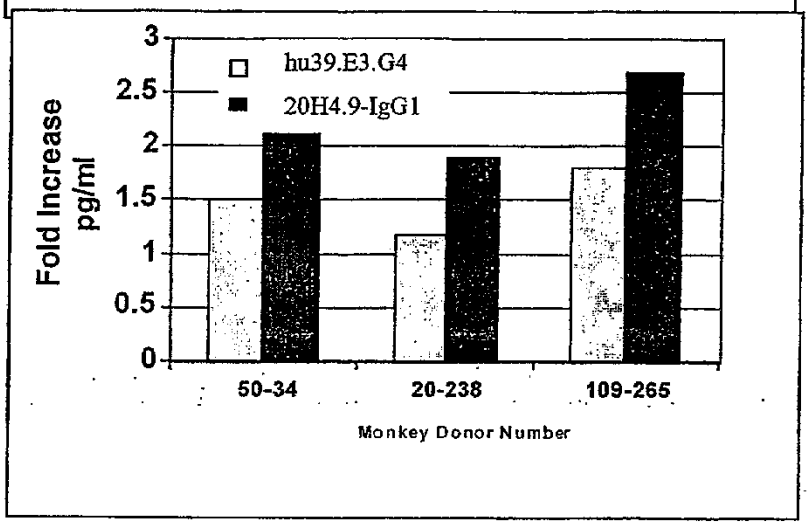


FIG. 10B

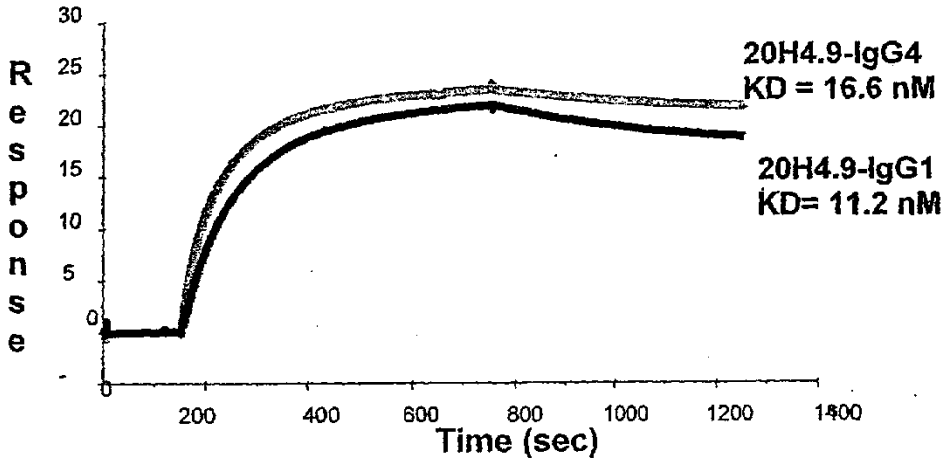

Response
30
25
20
15
10
5
0
0
200
400
600
800
1000
1200
1400
Time (sec)
20H4.9-IgG4
KD = 16.6 nM
20H4.9-IgG1
KD= 11.2 nM

FIG. 11

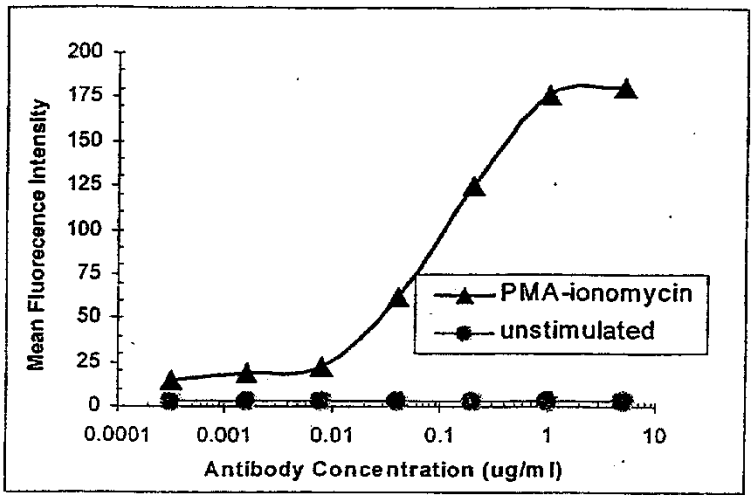

Mean Fluorecence Intensity
200
175
150
125
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
Antibody Concentration (ug/ml)
PMA-ionomycin
unstimulated

FIG. 12

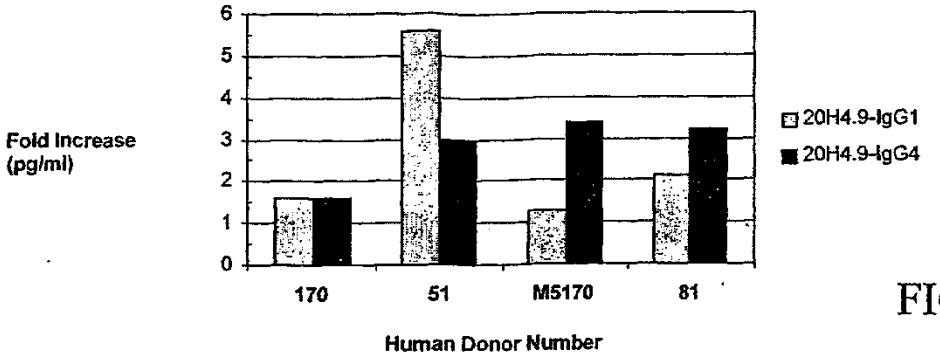


FIG. 13A

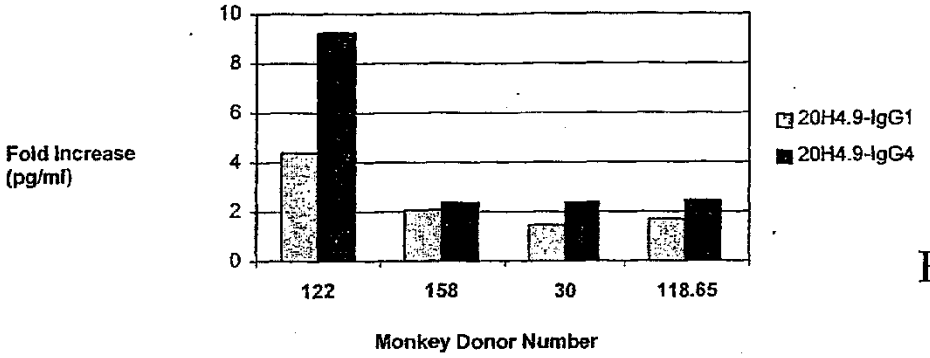


FIG. 13B

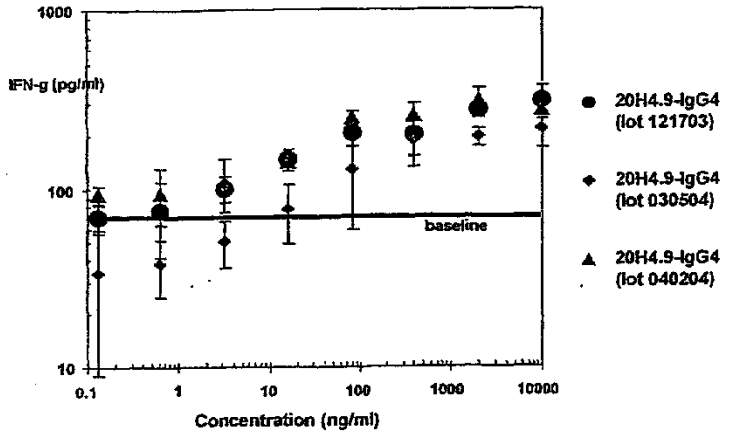


FIG. 14A

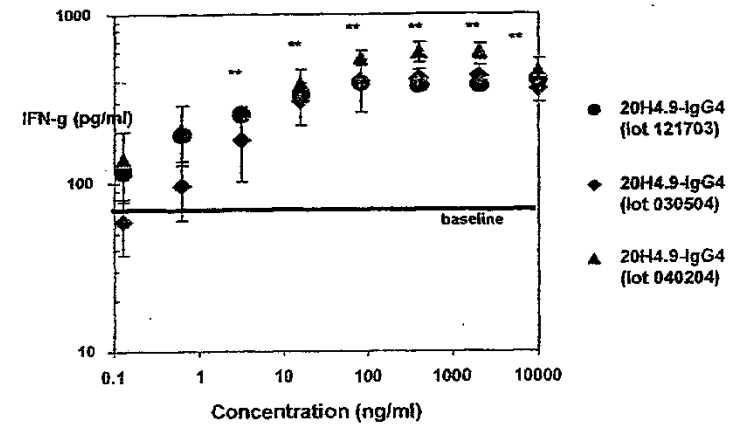


FIG. 14B

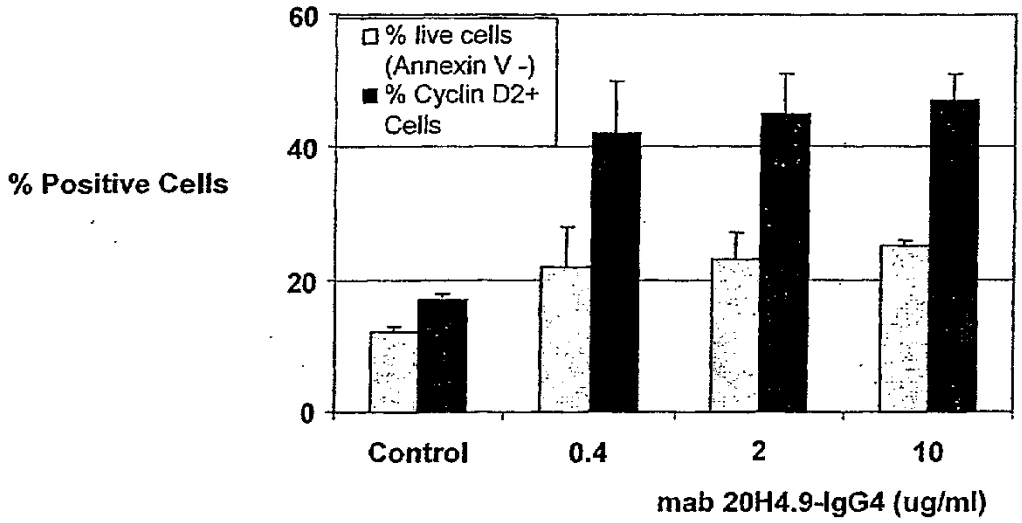

% Positive Cells
60
40
20
0
% live cells (Annexin V -)
% Cyclin D2+ Cells
Control
0.4
2
10
mab 20H4.9-IgG4 (ug/ml)

FIG. 15

FIG. 16A

FIG. 16B

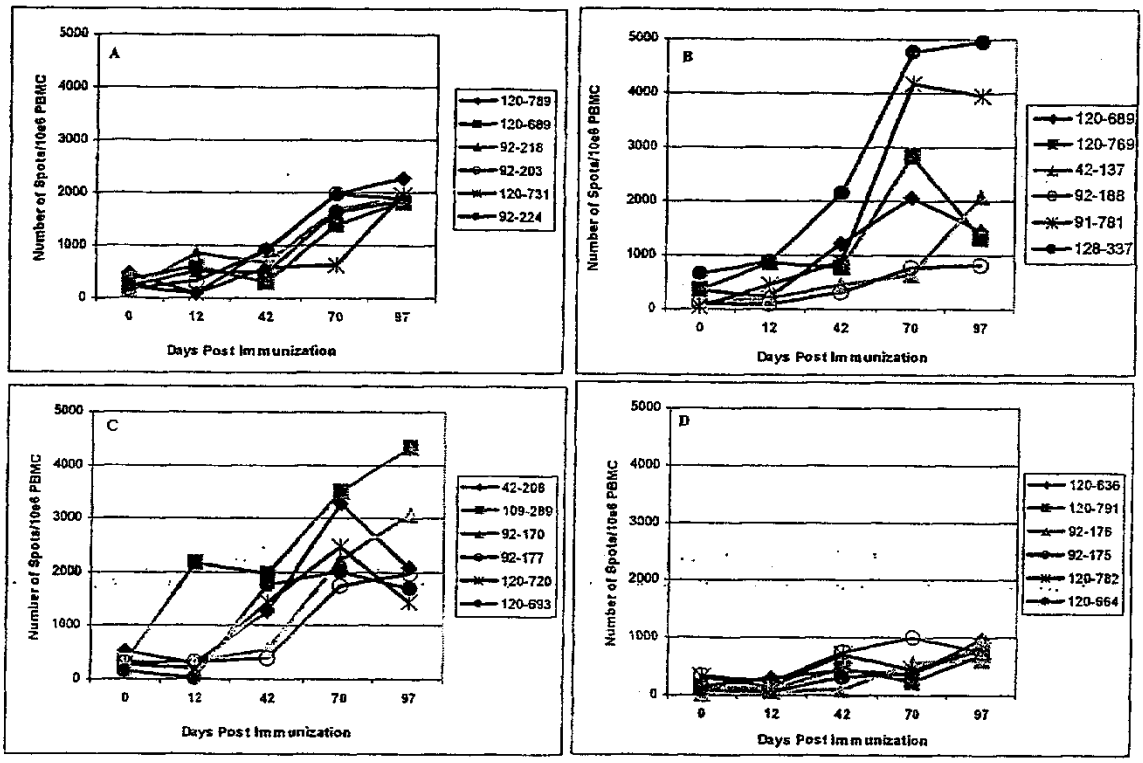


FIG. 16C

FIG. 16D