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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01N 37/18	A1	(11) International Publication Number: **WO 99/57981**
		(43) International Publication Date: 18 November 1999 (18.11.99)

(21) International Application Number: PCT/US99/10065

(22) International Filing Date: 7 May 1999 (07.05.99)

(30) Priority Data:
60/084,870 8 May 1998 (08.05.98) US

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(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS AND METHODS FOR ACTIVE VACCINATION

(57) Abstract

Non-Hodgkin's lymphoma (NHL) is treated, not by administration of an anti-CD20 monoclonal antibody, but by the administration of CD20 itself, or an immunogenic fragment of the extracellular portion thereof, coupled to or administered with an antigenic carrier moiety such as keyhole limpet hemocyanin (KLH). This results in the stimulation of the production of polyclonal antibodies against CD20 (or an immunogenic fragment thereof) which has the effect of reducing the number of B-cells, including malignant B-cells, and thus provides an active vaccine. The same approach can be used for therapeutics for other diseases and conditions in which target cells possess a transmembrane protein, and is particularly applicable to those diseases and conditions for which administration of antibodies to transmembrane proteins or peptides (i.e., passive therapy) have been shown to provide therapeutic benefits, and especially in the situations where the target is also capable of transducing or receiving a signal important for cell growth or function. This would include, for example, Her2/neu, VEGF receptor, epidermal growth factor receptor, the CD19 molecule, interleukin-2-receptor, interleukin-4-receptor, and the P-glycoprotein, also known as the multidrug-resistance protein.

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COMPOSITIONS AND METHODS FOR ACTIVE VACCINATION

BACKGROUND OF THE INVENTION

This application relates to an active vaccine approach to the treatment of cancer and other diseases. The approach is applicable to a number of cancers and diseases, although a preferred embodiment provides an active vaccine for treatment of B cell Non-Hodgkin's Lymphoma (NHL).

NHL is characterized by a clonal proliferation of malignant B cells. The treatment of NHL across a broad spectrum of patients remains a challenge, although numerous therapeutic approaches have been proposed and tried.

The most common therapeutic approach being used today is chemotherapy. While chemotherapy is effective for some period of time in most patients, a significant percentage of patients are not cured and experience a relapse.

Treatments have been proposed based on anti-idiotype therapy. In anti-idiotype therapy, a cell surface molecule which is expressed by malignant cells but not by normal cells is used to create patient-specific antibodies which are then administered to the patient. See, Miller, et al., *New Engl. J. Med.* 306: 517-522 (1982). Autologous patient-derived idiotype proteins have also been conjugated with keyhole limpet hemocyanin to produce a vaccine which has demonstrated efficacy and can elicit B and T cell immune responses. Kwak et al., *New Engl. J. Med.* 327: 1209-1215 (1992). Hybridoma-derived idiotype was co-cultured with patient-derived dendritic cells which acted as antigen presenters upon re-infusion into the patient and showed clinical efficacy. Hsu et al., *Nature Medicine* 2: 52-58 (1996). Idiotypic vaccines made in lipid-based carriers are disclosed in International Patent Publication WO98/14170.

Treatments have also been proposed using antibodies directed to CD20, a transmembrane protein that is expressed by both normal and malignant B-cells during parts of the B cell development cycle. Using single-dose infusions with anti-CD20 monoclonal antibodies, partial or minor tumor regressions were observed in 6 of 15 patients in a Phase I clinical study. Maloney et al., *Blood* 84: 2457-2466 (1994). In Phase II studies, 17 of 37 patients showed complete or partial remissions. In December 1997, the FDA approved the

first antibody-based therapy for NHL. Rituximab (Ritvaxan, IDEC/Genentech) is a chimeric human/murine antibody approved for the treatment of patients with relapsed or refractory low-grade or follicular $CD20^+$ B cell NHL. Maloney et al., *Blood* 90: 2188-2195 (1997).

Combinations of chemotherapy and anti-CD20 therapy have been reported as having better therapeutic efficacy, with 11 of 11 patients showing complete or partial remission. Czuczman et al., Abstract 53, *Ann. Oncol.* 7, Supp. 1: 56 (1996).

While therapeutic regimens using anti-CD20 concepts are potentially effective, all of these therapies have the drawback of being passive therapies, i.e., they do not directly involve the immune system of the patient. Thus, these therapies may require the continued administration of the therapeutic agent for efficacy and do not provide any long-term protection against recurrence. In addition, the passive therapy is monoclonal in nature, therefore escape is possible. It would therefore be desirable to have an active therapy, that is a therapeutic agent which when administered to the patient stimulates an immune response against CD20 found in B-cells.

It is an object of the present invention to provide such a therapy. It is a further object of the invention to provide an active polyclonal therapy that is difficult to evade.

SUMMARY OF THE INVENTION

In accordance with the present invention, NHL is treated, not by administration of an anti-CD20 monoclonal antibody, but by the administration of CD20 itself, or an immunogenic fragment of the extracellular portion thereof, coupled to or administered with an antigenic carrier moiety such as keyhole limpet hemocyanin (KLH). This results in the stimulation of the production of polyclonal antibodies against CD20 (or an immunogenic fragment thereof) which has the affect of reducing the number of B-cells, including malignant B-cells. Thus, the invention provides an active vaccine. The same approach can be used for therapeutics for other diseases and conditions in which target cells possess a transmembrane protein, and is particularly applicable to those diseases and conditions for which administration of antibodies to transmembrane proteins or peptides (i.e., passive therapy) have been shown to provide therapeutic benefits, and especially in the

situations where the target is also capable of transducing or receiving a signal important for cell growth or function. This would include, for example, Her2/neu, VEGF receptor, epidermal growth factor receptor, the CD19 molecule, interleukin-2-receptor, interleukin-4-receptor, and the P-glycoprotein, also known as the multidrug-resistance protein.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A and B show ELISA results for formation of antibodies to human and mouse CD20 in vaccinated mice;

Figs. 2A and B shows results for binding of control B1 antibodies or antibodies in plasma from a mouse treated with human CD20-KLH conjugate with Raji B NHL cells;

Fig. 3 shows $CP19^+$ B cell levels in mice treated with human or mouse CD20-KLH conjugate;

Fig. 4 shows the domain structure of human Her2;

Fig. 5 shows the domain structure of human EGFR;

Figs. 6A-D shows the cross-reactivity of antibodies generated in response to human or mouse CD20 fragments;

Figs. 7A-D show the importance of carrier protein and adjuvant in generating an immune response;

Figs. 8A-D shows the immune response generated using different adjuvants; and

Figs. 9A-I shows $CP19^+$ B cell levels in mice treated with human or mouse CD20-KLH conjugate.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an active vaccine therapy which can be used in the treatment of a variety of cancers and related conditions in which it is desirable to bring about the death of a target group of cells. Conventionally, immunotherapies targeting cells have sought to obtain a cellular immune response (T-cells that recognize the target cells) , since a humoral immune response (antibodies that recognize the target cells) alone is not

deemed sufficient to achieve the desired result of cell death. The present invention departs from this conventional wisdom, and effectively utilizes a humoral immune response against the target cells to provide therapeutic benefit. The targets for therapy include cell surface proteins that when bound by a ligand signal to the cell. The vaccine induced antibody response will mimic ligand binding and cause similar signaling events which can imitate the process of programmed cell death (apoptosis) or halt the cell from growing or change the cancer cell's sensitivity to chemotherapy.

By way of example, the invention is suitably employed in the treatment of NHL and other B cell diseases such as chronic lymphocytic leukemia, auto-immune disorders and B-cell regulatory disorders. In accordance with this embodiment of the invention, a peptide antigen is prepared which contains at least an immunogenic portion of the extracellular domain of CD20 coupled to or administered with an antigenic carrier protein. The CD20 component of the peptide antigen may be syngeneic or it may be xenogeneic. Thus, for example, human patients may be treated with a peptide vaccine containing a human or a mouse CD20-fragment. There is evidence that strong immune responses can be elicited against xenogeneic proteins. Naftzger et al., *Proc. Natl. Acad. Sci. (USA)* 93: 14809-14814 (1996); International Patent Application PCT/US97/22669, filed December 10 1997, incorporated herein by reference. A suitable fragment is the 44 amino acid peptide spanning amino acids 136 to 179 of the sequence of mouse or human CD20. (Seq. ID Nos. 1 and 2) Other immunogenic fragments derived from the extracellular domain of CD20, or the entire CD20 molecule may also be used. Seq. ID. Nos. 3 and 4 shows the nucleic acid and amino acid sequences, respectively, of exon VI (the extracellular domain) of human CD20 as reported by Tedder et al., *J. Immunol.* 142: 2560-2568 (1989).

As used in the specification and claims hereof, an "immunogenic fragment" is a molecule which includes at least a portion of the extracellular domain of a transmembrane protein to direct and immunological response to that transmembrane protein when the immunogenic fragment is coupled to or administered with an antigenic carrier protein effective to break tolerance and administered with an adjuvant. It is not required that the immunogenic fragment alone be effective to stimulate an immune response, although such stimulation would not take a given fragment outside the scope of the present invention.

A preferred antigenic carrier protein is keyhole limpet hemocyanin which can be coupled to peptides using techniques described in Pierce Catalog Protocol. Other antigenic carrier proteins which can be used to break tolerance might be used in the invention include immunoglobulins, tuberculin, tetanus toxin and others well known in the art.

The peptide antigen containing the CD20 component and the antigenic carrier protein is formulated with a pharmaceutically acceptable adjuvant in a liquid carrier and administered to a patient suffering from NHL or another B cell disease. The composition will generally be administered by injection, for example, intramuscular, subcutaneous or intradermal injection, but might also be administered by way of a DNA vaccine (See US Patent No. 5,580,859, incorporated herein by reference) or a viral vaccine, or after mixing with antigen presenting cells (APC's) such as dendritic cells, *ex vivo*. Alternatively, the antigen may be administered without adjuvant by injection into a host prepared by prior or simultaneous injection of an immune adjuvant. Specific amounts to be administered to a patient can be determined by monitoring the titer of anti-CD20 antibodies developed by the patient, or by an average group of patients using well-known technology.

When a peptide of the extracellular domain of human or mouse CD20 is coupled to KLH and administered with an adjuvant to mice, antibodies which react with CD20 are found in plasma. (Figs. 1A and B) These antibodies bind to Raji cells, a human lymphoma cell line, indicating the ability to bind to a cell expressing CD20. (Figs. 2A and B). Moreover, the number of $CD19^+$ B cells present in mice injected with either of the two CD20-KLH conjugates declines substantially (~30% decrease relative to controls). (Figs. 3 and 9). The assay used to quantitate B cell depletion detects CD19 which is also expressed on immature B cells that are $CD20^-$. Thus, the 30% depletion actually underestimates the efficacy of the vaccine against $CD20^+$ B cells. .

Antibodies generated in mice after vaccination with human or mouse-derived CD20 fragments are specific for the peptides used, yet are capable of inducing immunity to the corresponding peptide from other species (Figs. 6A-D). Studies showed that in most instances the peptide, carrier protein and adjuvant are all needed for optimal response, although some responses were detected using less than all of the components. (Figs. 7A-D).

Several different adjuvants were also tested, and QS21 was found to be the most effective of those tested. (Fig. 8A-D).

While not intending to be bound by any particular mechanism, it is believed that the vaccines of the present invention are effective via at least two pathways. First, the generation of a humoral immune response to CD20 is effective to some extent to reduce the numbers of B cells bearing CD20 antigen in a manner consistent with normal immunological response to a target antigen. In addition, however, because CD20 has a signaling function, the binding of antibody to the CD20 moiety activates this signaling function to trigger apoptotic cell death. Such stimulation of apoptosis has been demonstrated to occur *in vitro* following passive treatments with a chimeric anti-CD20 antibody. Maloney et al., *Blood* 88 (Supp. 1): 637a (1996).

It is also possible that T cell mediated effector mechanisms are involved in the immune response. As evidence of this, we illustrate in Table 1 the mouse and human peptide sequences capable of binding to the corresponding mouse and human histocompatability antigens. This information was derived from a search of the NIH Bioinformatics and Molecular Analysis Section HLA Binding Predictions database using the mouse and human CD20 amino acid sequences. (Parker et al., *J. Immunol.* 152: 163 (1994)).

While the method of the invention is illustrated here using CD20 or CD20-derived peptides as the antigen to target B cells, the invention is not limited to this embodiment. Rather, the inventions encompasses the use of vaccine compositions comprising an immunogenic portions of the extracellular domain of transmembrane protein or peptide, particularly a transmembrane protein or peptide having signaling function, coupled to or administered with an antigenic protein and/or adjuvant to break tolerance.

A non-limiting example of another transmembrane protein which can be used in whole or in part in the method of the invention is Her-2/neu. The Her-2/neu oncogene is a receptor-like tyrosine kinase that is expressed on the cell surface of a significant portion of solid tumors. It has been shown that patients with early stage breast cancer have a high titer of antibodies to Her-2/neu. Disis et al., *J. Clin. Oncol.* 15: 3363-3367 (19967). The amino acid sequence and domain structure of human Her-2/neu are shown in Seq. OD. No. 5 and Fig. 4, and isolation and expression of the extracellular domain has been disclosed.

International Patent Publication No. WO 90/14357, which is incorporated herein by reference. There is clinical data showing efficacy of monoclonal antibodies against Her-2-neu in the treatment of patients with Her-2/neu$^+$ tumors, and potential synergism with chemotherapy. Thus, in accordance with the present invention, a vaccine composition comprising at least an immunogenic portion of the extracellular domain of Her-2-neu (amino acids 22 to 652) coupled to or administered with an antigenic protein or peptide such a KLH can be used as a vaccine to provide the same therapeutic benefits using an active as opposed to a passive approach.

A further non-limiting example of a transmembrane protein which can be used in whole or in part in the method of the invention is epidermal growth factor receptor (EGFR). The amino acid sequence and domain structure of human EGFR are shown in Seq. ID. No. 6 and Fig. 5. There is significant data showing that antibodies to EGFR can have anti-tumor activity in breast and prostate cancer, as well as several head and neck tumors. Prewett et al., *J. Immunother. Emphasis Tumor Humoral* 19: 419-27 (1996). The mechanism by which antibody therapy against EGFR may be efficacious can be through the ability to down-regulate vascular endothelial growth factor production by tumor cells and thereby decrease angiogenesis. Petit et al., *Am. J. Pathol.* 151: 1523-30 (1997). In accordance with the present invention, a vaccine composition comprising at least an immunogenic portion of the extracellular domain of EGFR (amino acids 25 to 645) coupled to or administered with an antigenic protein or peptide such a KLH can be used as a vaccine to provide the same therapeutic benefits using an active as opposed to a passive approach. Preferred immunogenic peptides would be selected from regions not deleted in the various types of truncated EGFR mutants associated with some cancers.

A further non-limiting example of a transmembrane protein which can be used in whole or in part in the method of the invention is VEGF receptor. There are significant data showing that antibodies to VEGF receptor can inhibit angiogenesis and thereby halt tumor progression. In accordance with the present invention, a vaccine composition comprising at least an immunogenic portion of the extracellular domain of VEGF receptor coupled to or administered with an antigenic protein or peptide such a KLH can be used as a

vaccine to provide the same therapeutic benefits using an active as opposed to a passive approach.

Still a further non-limiting example of a transmembrane protein which can be used in whole or in part in the method of the invention is the IL-2 receptor. The IL-2 receptor is expressed on most T-cells malignancies, and there is a data showing that antibodies to the IL-2 receptor can be used in the treatment of T-cell malignancies and autoimmune disorders. In the present invention, a composition is made comprising at least an immunogenic portion of the extracellular domain of the IL-2 receptor (e.g., P55 or P75) , coupled to or administered with an antigenic carrier protein or peptide such as KLH. and used as a vaccine.

The vaccine compositions of invention can be used alone or in combination (concurrently or sequentially) with drugs or chemotherapy agents that provide therapeutic benefit for the condition being treated. In the case of NHL, suitable chemotherapy agents which can be used in combination with the CD20 based vaccine include alkylating agents, anthrocyclines, cis-platinum, fludarabine, corticosteroids and vinca alkaloids. These same chemotherapy agents which might be used in combination with other vaccine compositions for other forms of cancer.

EXAMPLE 1

44 amino acid fragments of the extracellular domains of humans and murine CD20 (amino acids 136-179, Seq. ID Nos. 1 and 2) were synthesized using a solid-phase FMOC peptide synthesizer and coupled to KLH using the methodology described in the Pierce Catalog Protocol. The peptide coupled to KLH was then prepared for injection by formulation with QS-21 adjuvant. Balb/c mice were injected according to one of the following protocols on days 1, 8, 15, 22 and 50 of the experiment:

A. Murine CD20 fragment-KLH with QS-21 adjuvant

B. Human CD20 fragment-KLH with QS-21 adjuvant

C. KLH with QS-21 adjuvant

D. QS-21 adjuvant

E. P190 (irrelevant protein) coupled to KLH with QS-21 adjuvant

F. B3A2 (irrelevant peptide) coupled to KLH with QS-21 adjuvant.

The animals were sacrificed on day 62 of the experiment.

Serum samples from the mice were diluted 1:200 and evaluated by BSA-blocked ELISA using goat-anti-mouse antibody conjugated to alkaline phosphatase for antibodies which bind to human CD20, mouse CD20 and KLH. As shown in Figs 1A and B, mice injected with human CD20 coupled to KLH (Fig. 1A) or mouse CD20 coupled to KLH (Fig. 1B) administration of xenogeneic antibody produced a significant polyclonal antibody response to both human and mouse CD20, while the response following administration of syngeneic antibody was principally limited to antibodies to the syngeneic form of CD20. Either xenogeneic or syngeneic peptide can therefore be used to generate an immune response.

To confirm the ability of the antibodies to bind to B cells, Raji cells (a form of human B-cell lymphoma that expresses CD20 on its surface) were blocked with human IgG, washed and then incubated for 30 minutes on ice with a 1:10 dilution of plasma from a mouse vaccinated with P-190-KLH control or huCD20-KLH. As a positive control, Raji cells were incubated with B1 antibody, or IgG2 as an isotypic negative control. After washing, the cells were incubated with goat-anti-mouse antibody, washed and fixed with 1% paraformaldehyde. Flow cytometry analysis was performed in a Becton-Dickinson FACScan. The results are shown in Figs 2A and 3B, wherein the shaded data set are the experimental data set and the outlined data set is the negative controls. As is apparent, there is a strong binding of mouse antibodies and Raji cells, comparable to that observed with B1 antibody .

EXAMPLE 2

To assess the number of B cells present in vaccinated mice, an evaluation was made of cells expressing CD19, a standard phenotypic marker for B cells. Spleens were harvested from the animals vaccinated in Example 1 and put into a single-cell suspension. After counting the total number of cells, the cells were stained with FITC-labeled anti-mouse CD19 and the samples were analyzed by flow cytometry with a FACScan. 10,000 events

were collected. The percentage of CD19 positive cells minus the control gate was multiplied by the total number of cells to determine the number of CD19 positive cells in mice treated with the mouse and human CD20 peptide conjugates, and the P190 irrelevant peptide conjugate control.

As shown in Fig. 3, the absolute number of CD19 positive cells was significantly reduced in mice treated with either of the CD20 peptide conjugates. The level of CD19 positive cells is a reflection of the number of CD20 positive B cells, and the number of immature $CD19^+$, $CD20^-$ B cells in the samples. The absolute number of $CD19^+$ B cells actually underestimates the therapeutic efficacy of the treatment for elimination of $CD20^+$ B cells, however, since CD19 is expressed on B cell progenitor cells before expression of CD20.

EXAMPLE 3

Mice were injected five times over two months with one of four treatment protocols as follows:

human CD20 (44 aa fragment)-KLH plus QS1

human CD20 (44 aa fragment)-KLH

human CD20 (44 aa fragment) plus QS21

KLH plus QS21

Blood was collected on week 9 for analysis by ELISA. Sera from the vaccinated mice were diluted 1:200 and incubated on BSA blocked plates coated with msCD20, huCD20, P190 or KLH. Secondary goat anti-mouse antibody conjugated to alkaline phosphatase was added, and the color change of p-nitrophenyl phosphate substrate was measured at 405 nm. The results are summarized in Figs. 7A-D. In most instances the peptide, carrier protein and adjuvant are all needed for optimal response, although some responses were detected using less than all of the components.

EXAMPLE 4

Mice were vaccinated according to the schedule of Example 3 using one of four treatment protocols: human CD20 (44 aa fragment)-KLH plus QS21 adjuvant, mouse

CD20 (44 amino acid fragment)-KLH plus QS21, P190 (irrelevant protein)-KLH +QS21 and KLH and QS21 alone. Mouse serum samples were evaluated by ELISA for the presence of antibodies reactive with msCD20, huCD20, P190 and KLH. The results are shown in Figs. 6A-D. Antibodies generated in mice after vaccination with human or mouse-derived CD20 fragments are specific for the peptides used, yet are capable of inducing immunity to the corresponding peptide from other species.

EXAMPLE 5

Mice were vaccinated five times over two months with huCD20 fragment-KLH conjugate with no adjuvant or in combination with one of three adjuvants: QS21, BCG or Alum. Serum samples from the vaccinated mice were tested by ELISA. The results are summarized in Figs. 8A-D. QS21 was found to be the most effective of those tested.

EXAMPLE 6

To confirm the observations of Example 2, nucleated spleen cells were recovered by centrifugation in a density gradient from mice vaccinated with a CD20-KLH conjugate (human or mouse) in the presence of QS21 adjuvant. 1 X 10^6 cells from each mouse were incubated with 2 μg of rat anti-mouse CD19 FITC-labeled antibody or with isotope-matched FITC labeled rat antibody. Cells were washed, fixed and analyzed with a Becton Dickinson FACScaliber cytometer. Figs 9A-C, D-F and G-I show the results for three exemplary mice of each vaccination group. The decrease in the peak reflecting levels of CD19 positive spleen cells in each of the mice is apparent.

Table 1

Species	Peptide Sequence	HLA Molecule	Score ($T_{1/2}$ of Dissociation of Molecule Containing this Subsequence)
human	NFIRaHTPYI	Kd	1600
human	FIRAHTPYI	Kd	48
human	FLKMeSLNFI	A_0201	21.2
mouse	HFLKmRRLEL	Kd	1600
mouse	IYDCePSNSS	Kd	60
mouse	LIQTSKPYV	A_0201	89.4
mouse	ELIQtSKPYV	A_0201	28.7

CLAIMS

1. A method for active vaccination against autologous cells expressing transmembrane proteins comprising administering to a patient a vaccine composition comprising at least an immunogenic portion of the extracellular domain of the transmembrane protein, or a xenogeneic homolog thereof, coupled to or administered with an carrier protein effective to break tolerance to the transmembrane protein and a pharmaceutically acceptable adjuvant.

2. The method of claim 1, wherein the transmembrane protein is selected from the group consisting of CD20, Her2-neu, VEGF receptor, epidermal growth factor receptor, the CD19 molecule, interleukin-2-receptor, interleukin-4-receptor, and the P-glycoprotein.

3. The method of claim 1, wherein the transmembrane protein is CD20.

4. The method of claim 1, wherein the vaccine composition comprises a peptide having the sequence given by Seq. ID No 1 or 2.

5. The method claim 1, wherein the carrier protein is keyhole limpet hemocyanin.

6. The method of claim 5, wherein the transmembrane protein is selected from the group consisting of CD20, Her2-neu, VEGF receptor, epidermal growth factor receptor, the CD19 molecule, interleukin-2-receptor, interleukin-4-receptor, and the P-glycoprotein.

7. The method of claim 5, wherein the transmembrane protein is CD20.

8. The method of claim 7, wherein the vaccine composition comprises a peptide having the sequence given by Seq. ID No 1 or 2.

9. A method for active vaccination against B cells expressing CD20 comprising administering to a patient a vaccine composition comprising at least an immunogenic portion of the extracellular domain of CD20, or a xenogeneic homolog thereof, coupled to or administered with an carrier protein effective to break tolerance to the transmembrane protein and a pharmaceutically acceptable adjuvant.

10. The method claim 9, wherein the carrier protein is keyhole limpet hemocyanin.

11. The method of claim 9, wherein the vaccine composition comprises a peptide having the sequence given by Seq. ID No 1 or 2.

12. A method for treatment of B cell non-Hodgkin's lymphoma, comprising administering to a patient suffering from B cell non-Hodgkin's lymphoma a vaccine composition comprising at least an immunogenic portion of the extracellular domain of CD20, or a xenogeneic homolog thereof, coupled to or administered with an carrier protein effective to break tolerance to the transmembrane protein and a pharmaceutically acceptable adjuvant.

13. A vaccine composition comprising at least an immunogenic portion of the extracellular domain of the transmembrane protein, or a xenogeneic homolog thereof, coupled to or administered with an carrier protein effective to break tolerance to the transmembrane protein and a pharmaceutically acceptable adjuvant.

14. The composition of claim 13, wherein the transmembrane protein is selected from the group consisting of CD20, Her2-neu, VEGF receptor, epidermal growth

factor receptor, the CD19 molecule, interleukin-2-receptor, interleukin-4-receptor, and the P-glycoprotein.

15. The composition of claim 13, wherein the transmembrane protein is CD20.

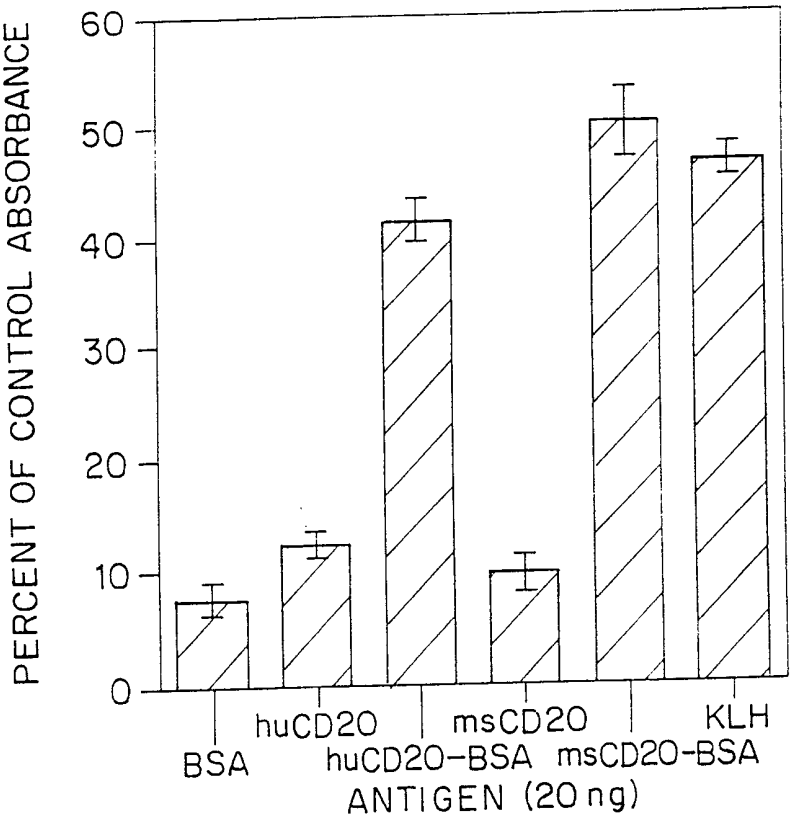
16. The composition of claim 15, wherein the vaccine composition comprises a peptide having the sequence given by Seq. ID No 1 or 2.

17. The composition of claim 13, wherein the carrier protein is keyhole limpet hemocyanin.

18. The composition of claim 17, wherein the transmembrane protein is selected from the group consisting of CD20, Her2-neu, VEGF receptor, epidermal growth factor receptor, the CD19 molecule, interleukin-2-receptor, interleukin-4-receptor, and the P-glycoprotein.

19. The composition of claim 17, wherein the transmembrane protein is CD20.

20. The composition of claim 19, wherein the vaccine composition comprises a peptide having the sequence given by Seq. ID No 1 or 2.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50
40
30
20
10
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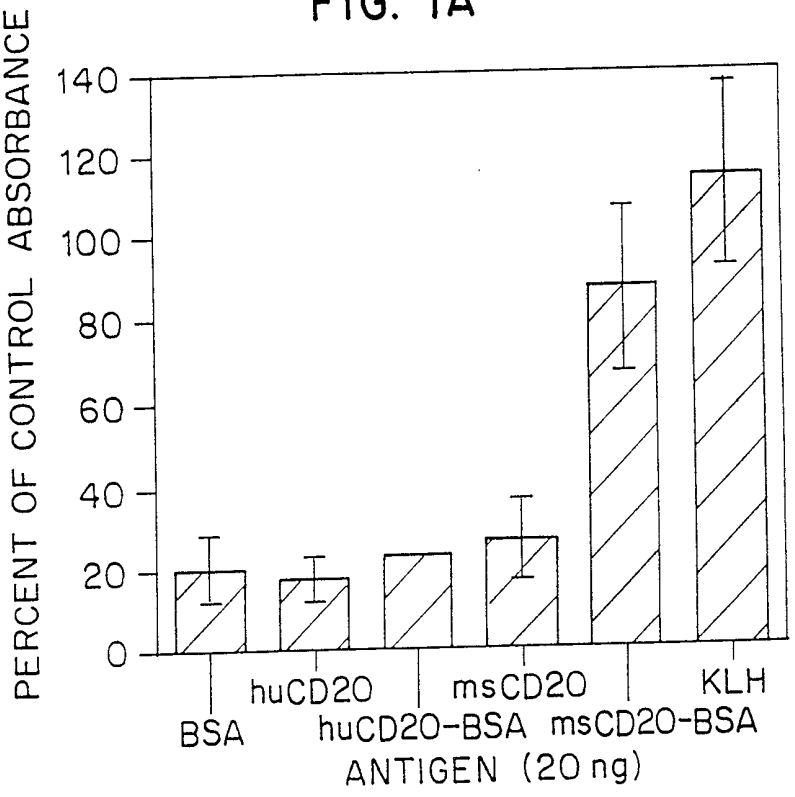
KLH
ANTIGEN (20 ng)

FIG. 1A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120
100
80
60
40
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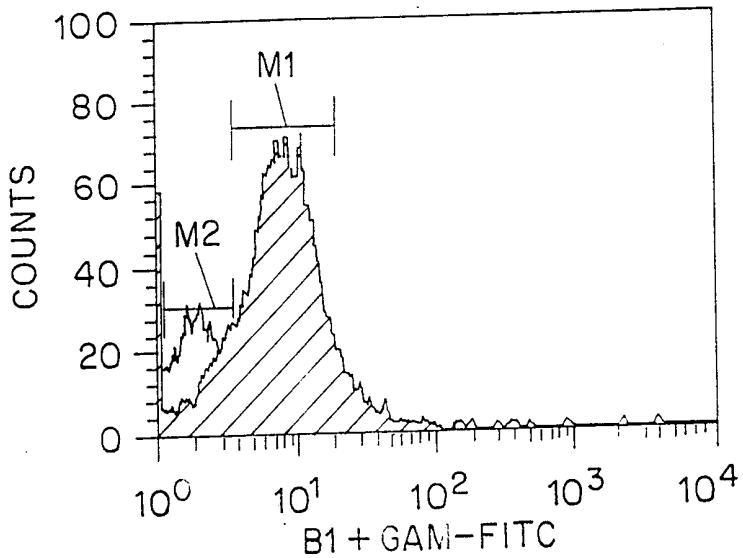
huCD20-BSA
msCD20
msCD20-BSA
KLH
ANTIGEN (20 ng)

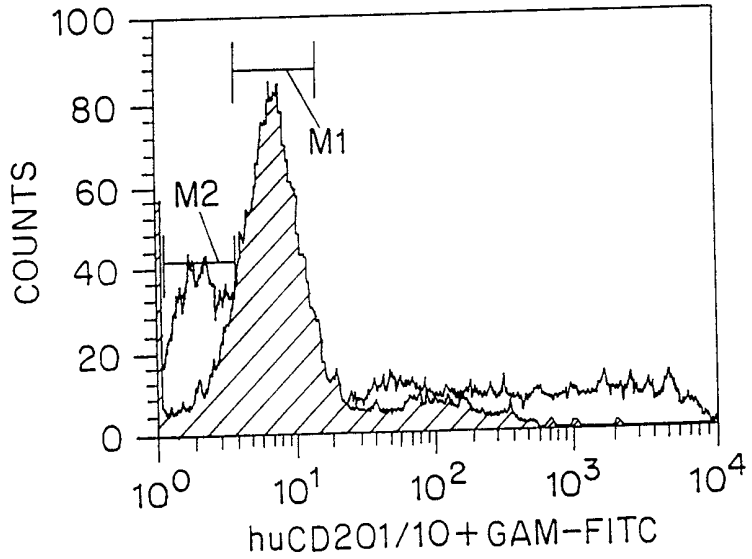
FIG. 1B



TOTAL EVENTS: 10000

MARKER	LEFT, RIGHT	EVENTS	% TOTAL
ALL	1, 9910	10000	100.00
M1	3, 17	7525	75.25
M2	1, 3	1201	12.01

FIG. 2A



TOTAL EVENTS: 10000

MARKER	LEFT, RIGHT	EVENTS	% TOTAL
ALL	1, 9910	10000	100.00
M1	3, 12	7327	73.27
M2	1, 4	1454	14.54

FIG. 2B

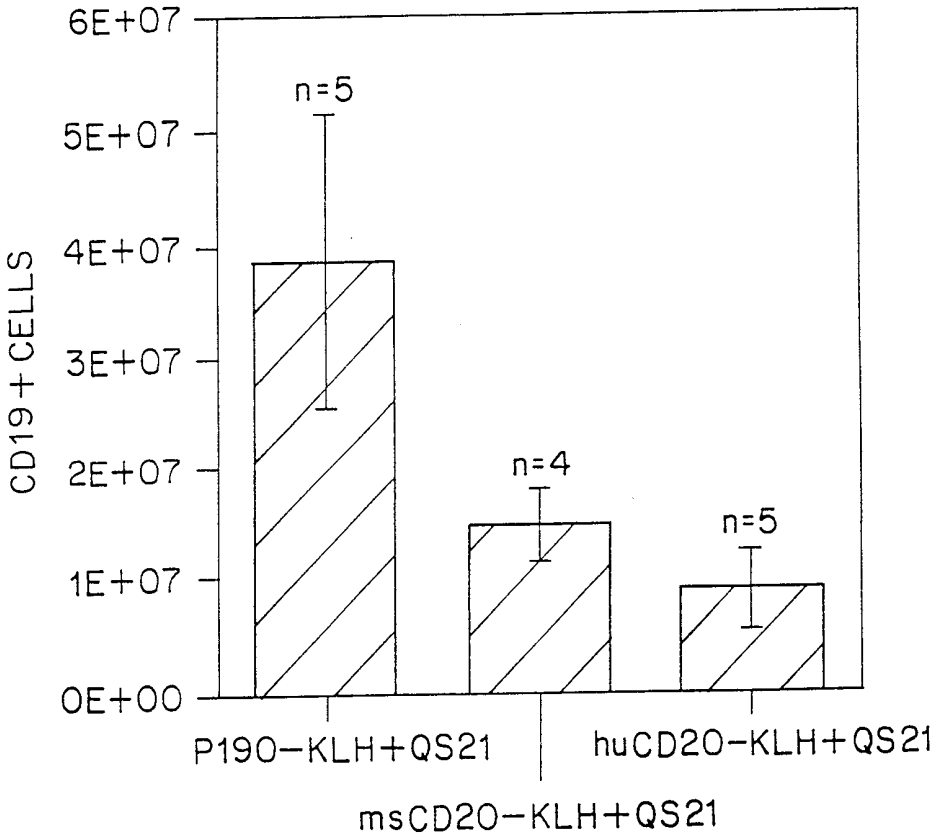
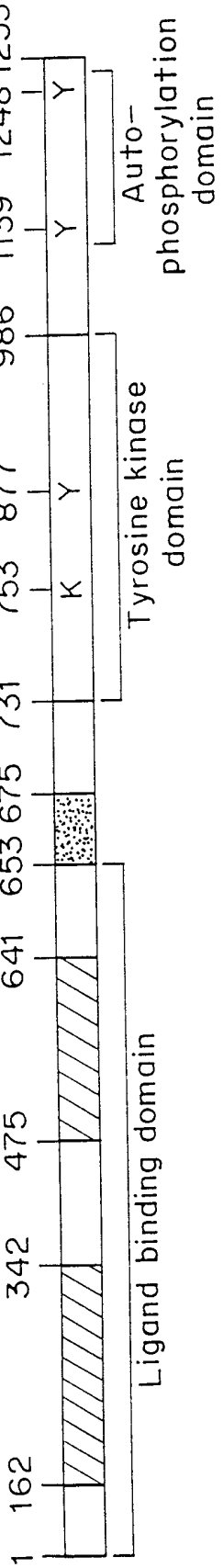

6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0E+00
CD19+CELLS
n=5
n=4
n=5
P190-KLH+QS21
msCD20-KLH+QS21
huCD20-KLH+QS21

FIG. 3


1
162
342
475
641
653
675
731
753
877
986
1139
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1255
Y
Y
K
Y
Y
Auto-
phosphorylation
domain
Tyrosine kinase
domain
Ligand binding domain

FIG. 4

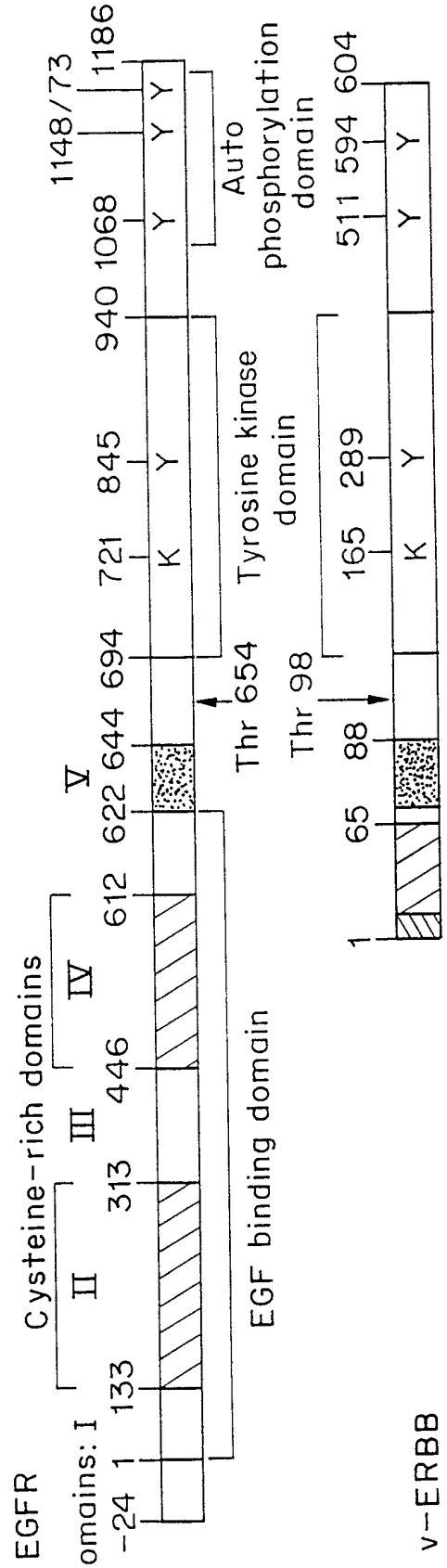
EGFR
Domains: I
-24
1
133
II
313
III
446
Cysteine-rich domains
IV
612
V
622
644
694
721
845
940
1068
1148/73
1186
Y
Y
Y
K
Y
Auto
phosphorylation
domain
Tyrosine kinase
domain
EGF binding domain
Thr 654
Thr 98
1
65
88
165
289
511
594
604
K
Y
Y
Y
v-ERBB

FIG. 5

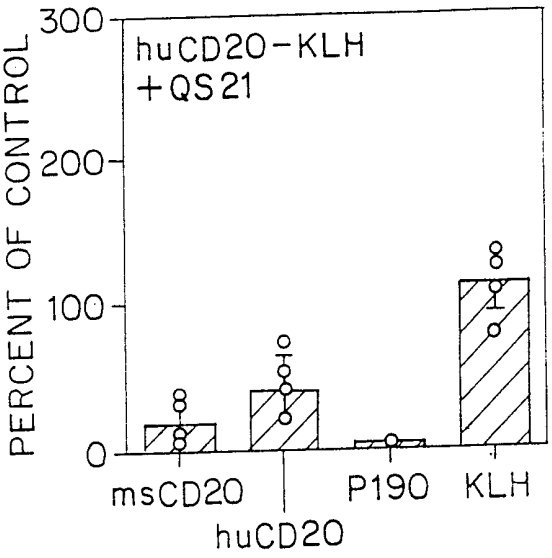

FIG. 6A

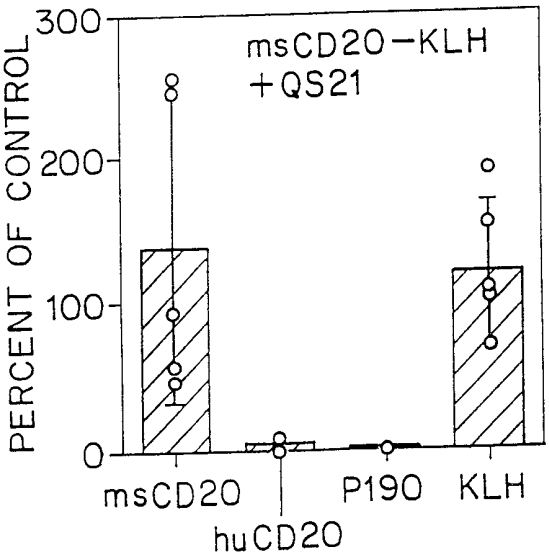

FIG. 6B

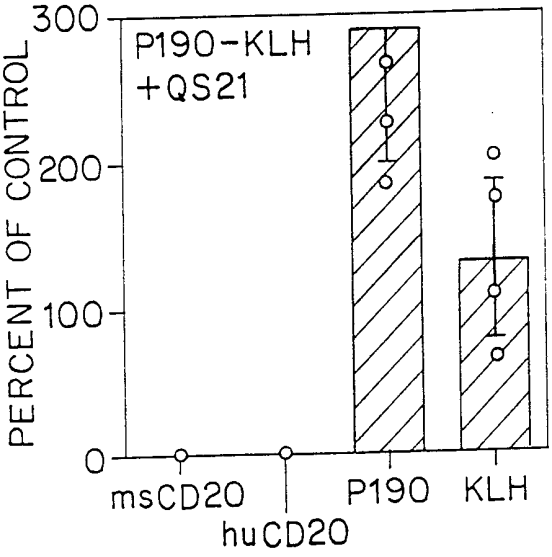

FIG. 6C

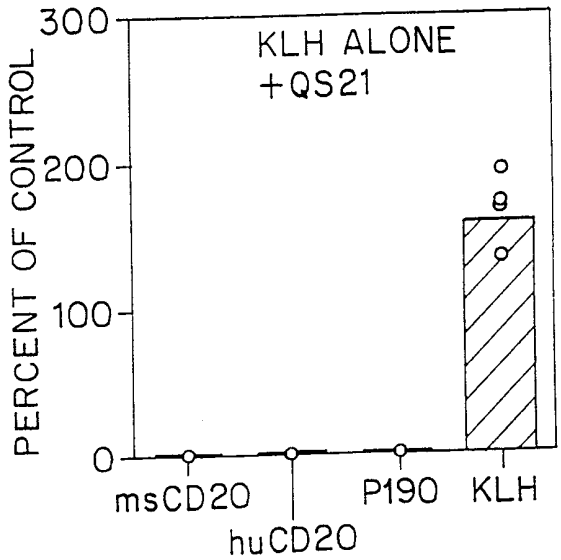

FIG. 6D

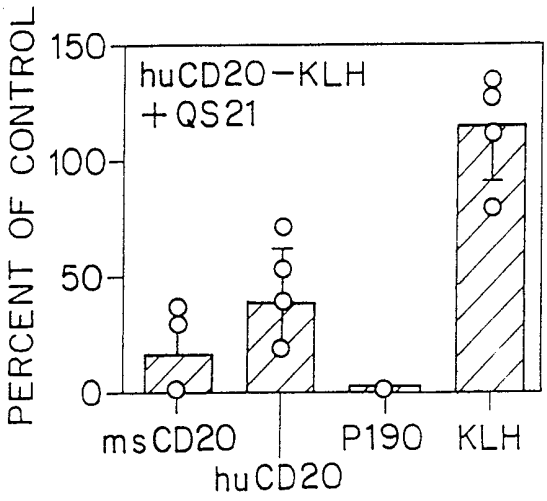

FIG. 7A

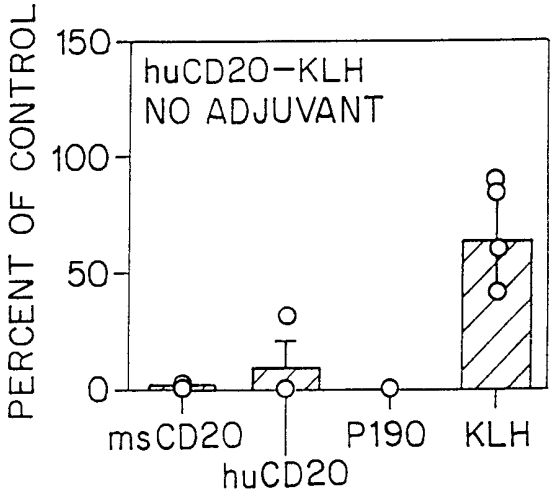

FIG. 7B

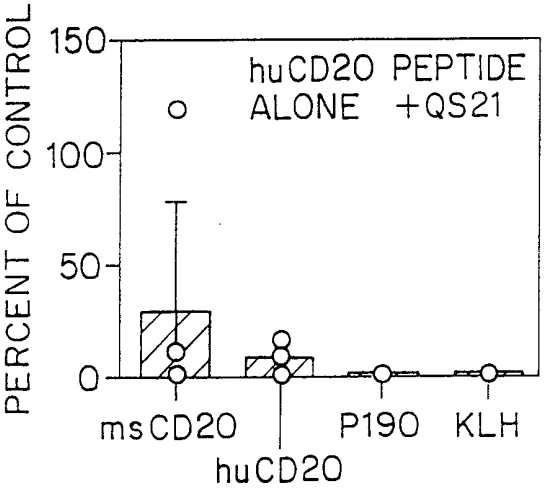

FIG. 7C

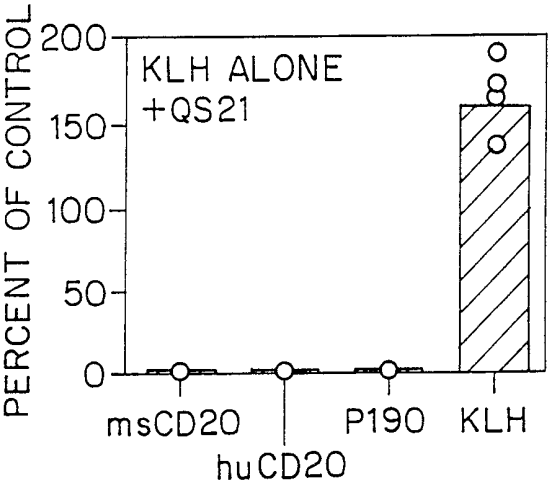

FIG. 7D

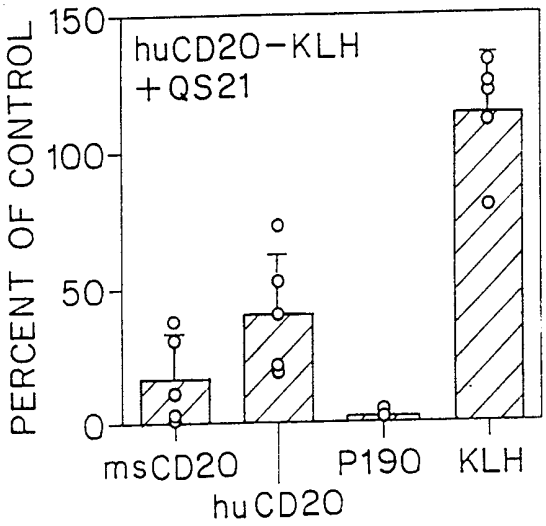

FIG. 8A

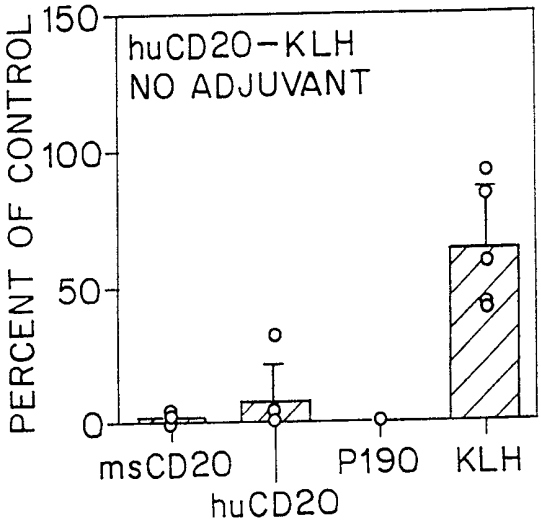

FIG. 8B

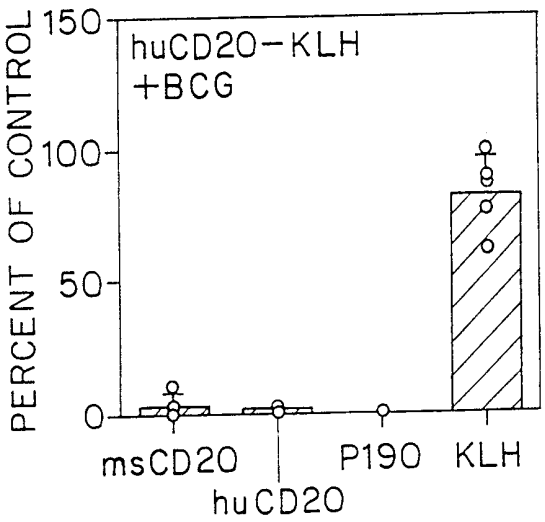

FIG. 8C

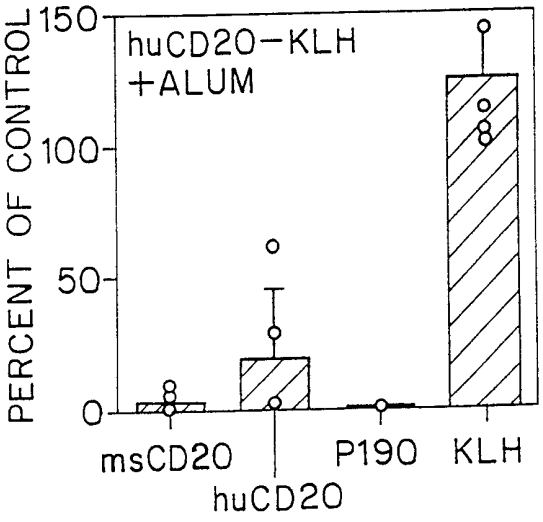

FIG. 8D

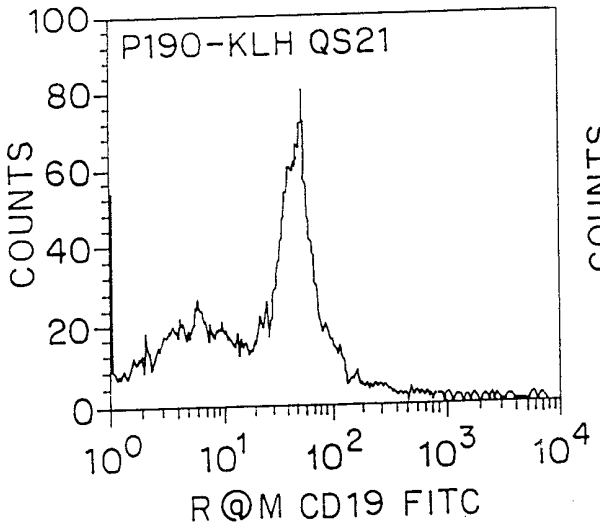

FIG. 9A

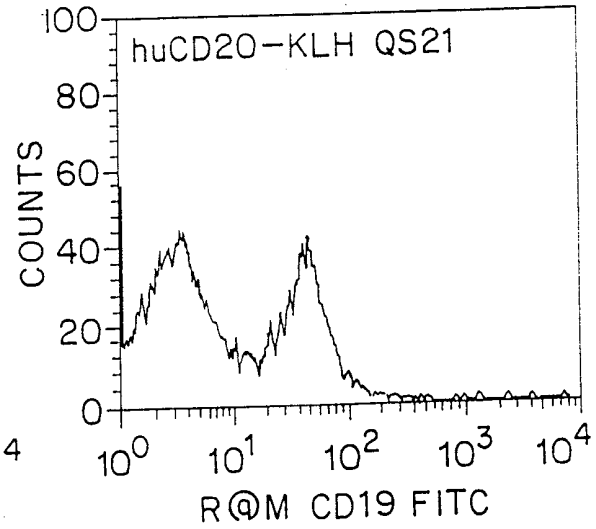

FIG. 9D

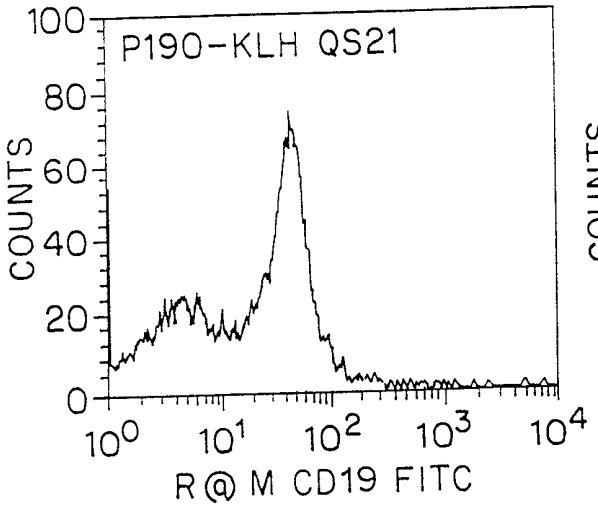

FIG. 9B

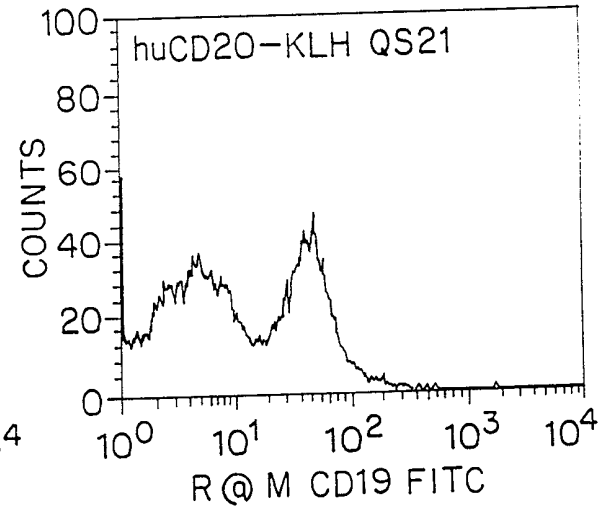

FIG. 9E

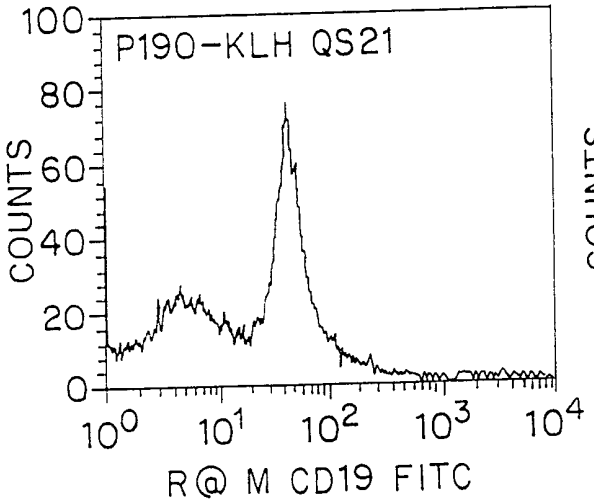

FIG. 9C

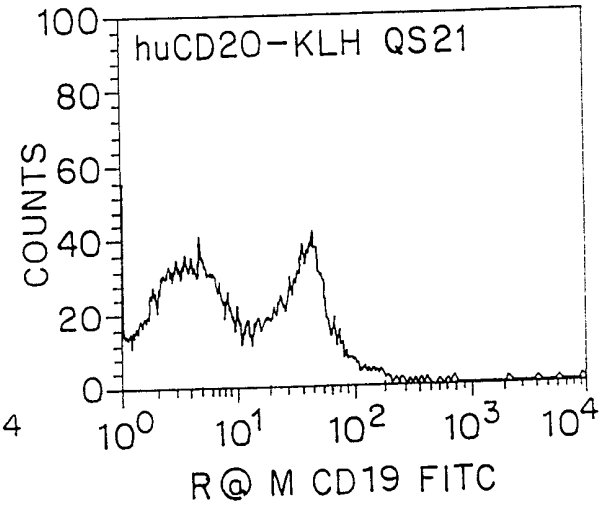

FIG. 9F

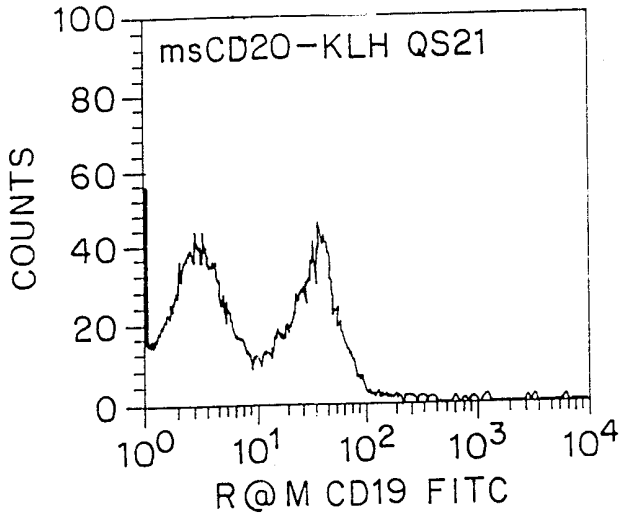

FIG. 9G

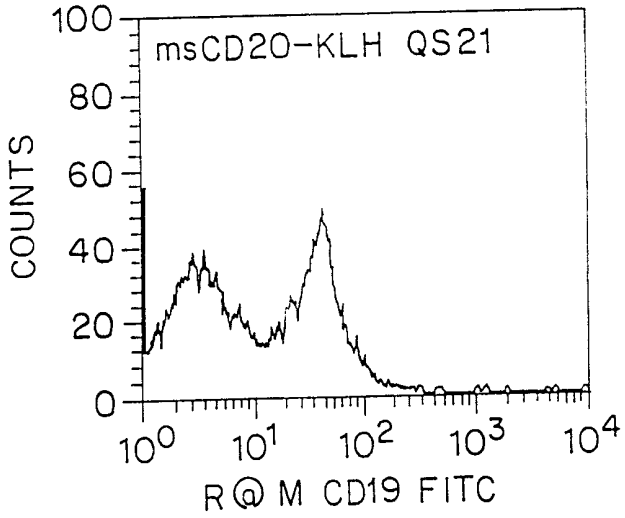

FIG. 9H

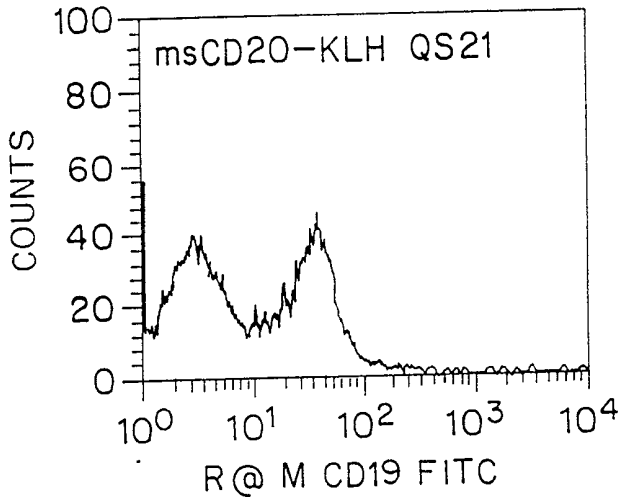

FIG. 9I

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Zelenetz, Andrew D.
Roberts, Wendy

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His Phe Leu Lys His Glu Ser Leu Asn Phe Ile Arg Ala His Thr Pro
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Tyr Ile Asn Ile Tyr Asn Cys Glu Pro Ala Asn Pro Ser Glu Lys Asn
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Ser Pro Ser Thr Gln Tyr Cys Tyr Ser Ile Gln Ser Leu Phe Leu
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35 40 45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
50 55 60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65 70 75 80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
85 90 95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
100 105 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
115 120 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
130 135 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145 150 155 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
165 170 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
180 185 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser

195 200 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
210 215 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
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His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
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Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
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Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
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Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305 310 315 320

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
325 330 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
340 345 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
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Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
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Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385 390 395 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
405 410 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
420 425 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
435 440 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly

450 455 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
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Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
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500 505 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
515 520 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
530 535 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545 550 555 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
565 570 575

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580 585 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
595 600 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
610 615 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625 630 635 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
645 650 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
660 665 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
675 680 685

Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
690 695 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu

705 710 715 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
725 730 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
740 745 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
755 760 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
770 775 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785 790 795 800

Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
805 810 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
820 825 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
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Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
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Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
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Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
885 890 895

Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
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Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
915 920 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
930 935 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
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Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe

965 970 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
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Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
995 1000 1005

Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
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Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
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Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
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Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
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Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
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Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
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Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65 70 75 80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
85 90 95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
100 105 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
115 120 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
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Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
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Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
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Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
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His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
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Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
580 585 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
595 600 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
610 615 620

Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625 630 635 640

Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
645 650 655

Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
660 665 670

Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
675 680 685

Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
690 695 700

Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705 710 715 720

Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
725 730 735

Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
740 745 750

Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
755 760 765

Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
770 775 780

Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785 790 795 800

Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
805 810 815

Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
820 825 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
835 840 845

Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
850 855 860

Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865 870 875 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
885 890 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
900 905 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
915 920 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
930 935 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945 950 955 960

Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
965 970 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
980 985 990

Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
995 1000 1005

Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
1010 1015 1020

Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
1025 1030 1035 1040

Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
1045 1050 1055

Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
1060 1065 1070

Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
1075 1080 1085

Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
1090 1095 1100

Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
1105 1110 1115 1120

Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
1125 1130 1135

Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
1140 1145 1150

Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
1155 1160 1165

Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
1170 1175 1180

Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
1185 1190 1195 1200

Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
1205 1210

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10065

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 37/18
US CL :514/2, 12

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12

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

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

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database BIOSIS, AN 1997:22684, HOOJBERG et al. Lysis of Syngeneic Tumor B Cells by Autoreactive Cytotoxic T Lymphocytes Specific for a CD19 Antigen-Derived Synthetic Peptide. J. Immunoth. 1996, Vol. 19, No. 5, pages 346-356, see especially the Abstract.	1-11
X	US 5,550,214 A (EBERLEIN et al.) 27 August 1996, see especially cols 17-22.	1,2,5,6
X	US 5,726,023 A (CHEEVER et al.) 10 March 1998, see especially cols 3, 13, 14.	1,2,5,6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

10 AUGUST 1999

Date of mailing of the international search report

29 SEP 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-11

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10065

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

GENESEQ, SWISS-PROT, SPTREMBL, APS, EMBASE, BIOSIS, MEDLINE, CAPLUS, DRUGU, PROMT, SCISEARCH, CANCERLIT, LIFESCI, TOXLINE, PHIN
search terms: vaccin?, CD20, her2, neu, erbb2

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-11, drawn to a method for active vaccination against autologous cells expressing transmembrane proteins.
Group II, claim(s) 12 drawn to a method for treatment of B cell non-Hodgkin's lymphoma.
Group III, claim(s) 13-20, drawn to a vaccine composition.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups I-III appears to be that they all relate to a method for active vaccination with at least an immunogenic portion of the extracellular domain of a transmembrane protein.

However, Hooijberg et al (J. Immunother. Emphasis Tumor Immunol, 1996, 19(5), 346-356 specifically teaches a method of active immunization with at least an immunogenic portion of the extracellular domain of a transmembrane protein wherein that protein is CD19, wherein that protein is CD20 (see abstract).

Therefore, the technical feature linking the inventions of Groups I-III does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of Group I is considered to be a method for active vaccination.

The special technical feature of Group II is considered to be a method of treatment.

The special technical feature of Group III is considered to be a vaccine composition.

Accordingly Groups I-III are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Group I

Claim 1 is generic to a plurality of distinct species which are transmembrane proteins that are different in structure and function wherein the transmembrane proteins are:

Species A - CD20 (claims 2-11)
Species B - Her2-neu (claims 2 and 6)
Species C - VEGF Receptor (claims 2 and 6)
Species D - epidermal growth factor receptor (claims 2 and 6)
Species E - CD19 (claims 2 and 6)
Species F - interleukin-2-receptor (claims 2 and 6)
Species G - interleukin-4-receptor (claims 2 and 6)
Species H - P-glycoprotein (claims 2 and 6)

Group III

Claim 13 is generic to a plurality of distinct species which are transmembrane proteins that are different in structure and function wherein the transmembrane proteins are:

Species A - CD20 (claims 14-20)
Species B - Her2-neu (claims 14 and 18)
Species C - VEGF Receptor (claims 14 and 18)
Species D - epidermal growth factor receptor (claims 14 and 18)
Species E - CD19 ((claims 14 and 18)
Species F - interleukin-2-receptor (claims 14 and 18)
Species G - interleukin-4-receptor (claims 14 and 18)
Species H - P-glycoprotein (claims 14 and 18)