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(54) Title: METHOD FOR PRODUCING MONOCLONAL ANTIBODIES TO AN IMMUNOGENIC ANTIBODY-ANTIGEN CONJUNCTION (57) Abstract A method for the preparation of a monoclonal antibody capable of recognizing the juncture of an antigenic determinant and a corresponding antibody binding site when complexed to each other. Antigen may be subjected to limited proteolytic digest to obtain an immunochemically active fragment. Similarly, a monoclonal antibody directed against the antigenic determinant of interest is enzymatically cleaved to obtain the Fab or F(ab') ₂ fragment. The two fragments are combined so as to form a complex. This immunogenic complex is subsequently injected into recipient animal and the antibodies produced recognize specifically the juncture of the immunogenic complex.		

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METHOD FOR PRODUCING MONOCLONAL ANTIBODIES TO AN
IMMUNOGENIC ANTIBODY-ANTIGEN CONJUNCTIONFIELD OF THE INVENTION

The present invention relates to antibodies and methods for their production. More particularly, the present invention comprises a method for the production of monoclonal antibodies designed to recognize the juncture between an immunogenic antibody:antigen complex.

BACKGROUND OF THE INVENTION

In U. S. Application No. 6,790,077, which is incorporated by reference in its entirety herein, an assay method is described in which a first antibody recognizes and binds to an analyte, typically an antigen, suspected of being in a solution. A labeled second antibody, a monoclonal, recognizes and binds preferentially to the juncture between the first antibody, and any of the analyte present, yet will not bind to analyte alone or antibody alone. The presence or amount of labeled material present is measured and the quantity of analyte present determined. The assay is unique in that it is highly selective: only material bound to the first antibody will be recognized, and only the juncture will be bound by the second antibody. This selectivity eliminates a substantial amount of false or spurious signal resulting from the cross-reactive binding of labeled antibody to materials other than the analyte or to analyte not bound by the first antibody.

SUMMARY OF THE INVENTION

The present invention relates to a method for the production of monoclonal antibodies to a unique antigen. More particularly, disclosed is a method for producing a monoclonal antibody directed
5 against the juncture of the idiotypic determinant of a first monoclonal antibody and the antigenic determinant of an antigen when the monoclonal antibody and the antigen are bound together, comprising: (a) providing an antigen of interest; (b) subjecting the antigen to limited proteolytic digestion to produce one antigenic
10 fragment capable of binding to an antibody directed against the antigen; (c) providing a first monoclonal antibody directed against the antigen and/or antigenic fragment; (d) separating the Fab or F(ab')₂ portion from the Fc portion of the first monoclonal antibody; (e) combining the antigen or antigenic fragment and the Fab or F(ab')₂
15 portion in a manner favoring the formation of an immunogenic complex; (f) injecting the immunogenic complex of step (e) into a host animal; and (g) obtaining the monoclonal antibodies directed against the immunogenic complex capable of recognizing the juncture between the antigen and the first antibody when complexed together.

DESCRIPTION OF THE INVENTION

20 The method of the present invention is useful for the production of highly selective monoclonal antibodies to a unique antigenic entity. Briefly, an antigen of interest is subjected to limited proteolysis so as to provide, among other components, a fragment that
25 contains an active antigenic determinant. A monoclonal antibody

directed against the antigenic determinant is cleaved enzymatically and Fab or $F(ab')_2$ and Fc fragments are separated. The antigen fragment and the Fab or $F(ab')_2$ fragment are then combined so as to produce a complex possessing immunogenic properties. Animal recipients are immunized with this immunogen. Specific antibody-producing lymphocytes are fused with myeloma cells and monoclonal antibodies are selected. The resulting monoclonal antibody will selectively bind to the juncture of the antigen and the antibody, but not the individual components alone.

10 Analyte selection will dictate the extent of proteolytic digestion. Typically, a small antigen, such as a hormone or drug, has one antigenic determinant, i.e., a sequence of amino acids or a molecular structure which is recognized by an antibody. The desired epitope structure on a small antigen or antigen fragment has a higher
15 probability of being recognized than if present on a large antigen with multiple antigenic determinants. Therefore, a large antigen may be degraded into a smaller fragment, which under optimum circumstances possesses only the antigenic determinant desired.

Limited proteolysis of antigens is an established procedure, and
20 discussed in greater detail in the Examples. Briefly, the antigen is subjected to the action of a proteolytic substance under conditions that allow the extent of degradation to be controlled. The products of this proteolytic cleavage are subjected to sodium dodecyl sulfate ("SDS") polyacrylamide gel electrophoresis ("PAGE") to separate the
25 protein fragments by size. Protein fragments are transferred to nitrocellulose paper by the Western blot technique, which is well known in the art. Blots are developed using a specific monoclonal

antibody in an indirect enzyme-linked immunosorbent assay ("ELISA") to identify the polypeptide fragment that contains the antigenic determinant. The identified fragment with the antigenic determinant is purified by molecular exclusion chromatography, or alternatively, 5 by SDS-PAGE followed by electroelution.

A monoclonal antibody is used that is capable of binding to the antigenic fragment. In order to reduce the size of the antigenic fragment-antibody complex and to again make recognition of the juncture easier, the Fc fragment is removed from the antibody 10 molecule. In a syngeneic system, i.e., monoclonal antibody and recipient animal, the immune complex consisting of a whole antibody molecule bound to an antigen would be cleared by normal mechanisms without generating the desired unique antibody(ies). In an allogeneic or xenogeneic system, removal of the Fc portion would eliminate 15 numerous potential antigenic determinants. Cleavage can be accomplished by one of several established techniques. The most common technique uses the enzyme pepsin which cleaves the IgG molecule just below the hinge region, yielding a Fc fragment and a single $F(ab')_2$ fragment possessing a molecular weight of approximately 100,000 20 daltons and having two antigen binding sites, also called paratopes. Alternatively, the antibody can be cleaved just above the hinge region by using the enzyme papain, yielding a larger Fc fragment and two Fab fragments, each with a molecular weight of 50,000 daltons and each having a single antigen binding site. For the purposes of 25 illustration only, the Fab fragment will be discussed in this application.

The $F(ab')_2$ or Fab fragment can be purified by using either molecular exclusion or affinity (Protein A) chromatography; both methods are discussed in detail in the Examples set forth below.

In order to create the immunogen necessary for immunization an antibody:antigen complex must first be formed. The purified antigen preparation possessing the desired antigenic determinant is combined with the Fab fragment of the antibody under appropriate conditions, i.e., pH, molarity and temperature. The antigenic determinant is recognized and bound by the binding site of the Fab creating a more exposed conjunction site with an enhanced probability of immunogenicity. It is this conjunction that will serve as the new immunogen for the production of the conjunction binding antibody. The complex is purified by a means gentle enough to allow the complex to remain intact; for example, one appropriate method is molecular exclusion chromatography, which will separate the complex from the reactants based upon molecular size.

Immunogenicity, the characteristic of a molecule or substance to evoke a host immune response can be enhanced by various means. Existing methods include chemical cross-linking (glutaraldehyde) and heat-induced aggregation which might create a repeating polymeric structure of the complex. The use of adjuvants and multiple inoculations may also be used to enhance immunogenicity. These methods are all well known in the art.

In order to produce the desired unique antibodies necessary for the invention, it is requisite to use monoclonal antibodies for production of the conjunction site. Moreover, in order to reduce the likelihood that the recipient animal will immunologically react with

epitopes present on the Fab antibody fragment, it is preferable to use a syngeneic system, i.e., any antibodies used to create the conjunction site in the method should be produced from genetically identical animals as those used to make the conjunction binding 5 antibodies. This reduces the possibility that a recipient will process an antibody as a foreign antigen, such as a Balb/c mouse recognizing a C57BL mouse antibody as foreign and producing antibodies to the antibody, rather than to the antigen-antibody conjunction as desired.

10 Monoclonal antibodies are prepared essentially as described by Milstein and Kohler (Nature, 256:495-497, 1975). The process is well known and is reviewed in the Examples set forth below. Briefly, an appropriate animal, such as a mouse, is injected with an immunogen, such as the complex of antigen and antibody fragments. Subsequently, 15 the mouse is sacrificed and cells removed from its spleen are fused with myeloma cells. Fusion results in the formation of immortal hybrid cells, known as "hybridomas," which synthesize antibodies. The hybridoma population produced is screened to identify the desired antibodies. The hybridoma populations thus identified are cloned by 20 limiting dilution to isolate hybridomas derived from a single cell and thereafter are designated as monoclonal hybridoma. The antibody obtained via this procedure is the product of a single B-cell from the immunized recipient animal that is generated in response to a specific antigenic determinant identified on the immunogen. In the case 25 described it is the juncture which is recognized.

In accordance with the process, the conjugation immunogen is injected into a suitable animal, such as a mouse. The resulting hybridoma cells are screened, such as by Microtiter ELISA technique, for the production of a monoclonal antibody that binds to an intact (non-digested) antigen-antibody complex, but not the antibody or the intact antigen alone.

The monoclonal antibody thus produced can be utilized in an assay procedure to bind to any complexed antigen:antibody present in the reaction mixture. Either this monoclonal antibody or the complexed monoclonal antibody can be tagged with one of many possible labeling materials, such as an enzyme, fluorescent, chemiluminescent, bioluminescent, isotopic, ferromagnetic, or other common signal generating material. As a result, the sensitivity of the assay procedure will be high because of the unique selectivity of the conjugation binding monoclonal antibody.

An alternative embodiment involves first forming a complex of intact antigen and intact first monoclonal antibody. Then the Fc fragment is cleaved from the antibody using pepsin or papain, followed by limited proteolytic digestion of the antigen under conditions that will not result in digestion of the bound antibody. The purified product is then used as the new immunogen to be injected into the recipient animal. Care must be taken to create reaction conditions conducive to the desired reaction. If the pH is too low dissociation of the antigen:antibody complex is possible. Furthermore, because papain and pepsin are relatively non-specific enzymes (other than the action on sulfhydryl groups) there is the possibility of degradation of the antigen.

While the invention has been described in connection with a preferred embodiment, it is not intended to limit the scope of the invention to the particular form set forth, but, on the contrary, it is intended to cover such alternatives, modifications and equivalents 5 as may be included within the spirit and scope of the invention as defined by the appended claims.

EXAMPLES

Example 1

Using Chlamydia trachomatis major outer membrane protein (MOMP) as the antigen:

5 A. Preparation of antigen fragment containing active antigenic determinant:

1. Procedure for limited proteolytic digestion of antigen:

Purified (MOMP) eluted electrophoretically from gels is dissolved at approximately 0.5 mg/ml in sample buffer which contains 0.125 M Tris/HCl at pH 6.8, 0.5% SDS, 10% glycerol, and 0.0001% bromphenol blue. The samples are heated to 100°C for 2 minutes. Proteolytic digestion is carried out at 37°C for 30 minutes by addition of appropriate amounts of a particular protease. Selection of protease and optimal conditions are determined empirically for each antigen to provide the desired digestion products. Among the preferred proteases include, but are not limited to, endoproteinase Glu-C from Staphylococcus aureus V8, endoproteinase Arg-C from mouse submaxillary gland, endoproteinase Tys-C from Lysobacter enzymogenes, and the like. Following addition of 2-mercaptoethanol and SDS to final concentrations of 10% and 2%, respectively, proteolysis is stopped by boiling the samples for 2 minutes. 20 to 30ul (10 to 15ug) of

each sample is loaded into a sample well of 15% acrylamide gel and the gel is run according to accepted procedures.

2. Identification of epitope-bearing portion of the proteolytic digest preparation:

SDS-polyacrylamide gel electrophoresis of the digest is performed. The preparations are transferred electrophoretically to nitrocellulose paper using the method of Towbin, et al, commonly referred to as Western blot. To identify the polypeptide fragment of interest an ELISA is performed using a selected monoclonal antibody.

3. Purification of chlamydia MOMP fragment:

The MOMP fragment is purified by SDS-PAGE and electroelution.

B. Preparation of Fab antibody fragment

1. Procedure for cleavage of antibody molecule:

(a) 0.5 ml Immobilized papain (pre-washed in buffer) is added to 10 mg IgG in 1.0 ml freshly made 20 mM NaH₂PO₄ + 20 mM cysteine HCl + 10 mM EDTA-Na, pH 6.2.

(b) Incubated 5 hours at 37°C with rocking.

(c) 30 ml 10 mM Tris-HCl, pH 7.5 is added. The reactants are mixed and centrifuged.

2. Procedure for purification of Fab antibody fragment:

(a) The supernatant is applied to a 5 ml Immobilized Protein A column equilibrated with 10 mM Tris-HCl, pH 7.5.

5 (b) The column is washed with 15 ml 10 mM Tris buffer, pH 7.5. The protein eluted is Fab.

(c) To recover Fc and undigested IgG, the gel is washed with 0.1 M glycine-HCl, pH 2.8.

10 C. Preparation and purification of complex consisting of Chlamydia antigen fragment and Fab fragment:

Purified antigen fragment and Fab preparations are mixed in a 20 mM phosphate buffer pH 7.4, with 150 mM NaCl. The mixture is allowed to incubate for a period of time until equilibrium is reached for the binding of the components. The incubation time is determined by empirical observation. When equilibrium has been reached the mixture is subjected to molecular exclusion chromatography using, for example, Sephadex G-75 superfine gel filtration beads (Sigma, St. Louis, MO). Since the molecular weights of both the antigen fragments and the Fab fragments are known, fractions from a calibrated molecular exclusion column containing the complex are easily located by determining protein content using a spectrophotometric reading at 280 nm. The appropriate fractions are pooled and may be concentrated for use as an immunogen.

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D. Preparation of monoclonal antibodies to purified conjugation immunogen:

This antibody is prepared according to the accepted procedure described by Milstein & Kohler (Nature 256:495-497, 1975).

5 The monoclonal antibodies are prepared by fusing spleen cells, from a mouse immunized against the chlamydia antigen:antibody complex, with an appropriate murine myeloma cell line (P3X63-Ag8.653). The resultant product is then cultured in a standard hypoxanthine, aminopterin, and thymidine (HAT) medium. Immunoassays are utilized to screen
10 the specific antibodies produced by the growing hybridoma cultures. Typical immunoassays that may be used include microtiter ELISA and dot-blot ELISA. The first is done in 96 well microtiter plates and the second is done on strips of nitrocellulose paper. The antibody alone, the antigen
15 alone and the antibody:antigen complex are applied to the plates or paper as the antigen source for the conjugation binding antibody. After the antibody of interest has been allowed to react with the three potential antigen sources a second antibody, i.e., enzyme conjugated anti-mouse IgG, is
20 added to indicate where the desired first antibody:antigen complex and not the antibody or antigen alone will be selected.

25 The immunized spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be described in connection with mice. The mouse is immunized by several injections of the

immunogenic complex generally over a period of several weeks. When the mouse shows sufficient antibody titre against the immunogenic complex, as determined by conventional assay methods, it is given a booster injection of the immunogenic complex, then sacrificed and the spleen is removed. The fusion is then initiated utilizing spleen cells from immunized mice and an appropriate myeloma cell line (For Example, P3X63-Ag8.653).

The hybridomas yielding antibodies specific for the immunogenic complex are selected and cloned utilizing standard methods. The monoclonal antibodies from the clones are then tested to determine their specificity for the particular immunogenic complex. Selected monoclonal antibodies specific for the immunogenic complex are then conjugated with an appropriate label. Amounts of antibody sufficient for labeling and subsequent commercial production are obtained from monoclonal hybridomas by the accepted techniques, such as a batch or continuous tissue culture or culture in vivo in mammals, such as mice. The monoclonal antibodies may be labeled with signal generating material, such as an enzyme, fluorescent compound, luminescent compound, radioactive compound, ferromagnetic label, and the like. Examples of enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, urease, and the like. Conjugation with enzymes can be accomplished by any one of the conventional and accepted

methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, the periodate method, or a biotin-avidin bridge.

Example 2

5 The procedures according to Example 1 are followed except that in Step B the $F(ab')_2$ antibody fragment is prepared rather than the Fab fragment using the following procedure:

$F(ab')_2$ preparation and purification:

10 (a) 0.25 ml Immobilized Pepsin (pre-washed in buffer) is added to 10 mg IgG in 1.0 ml 20 mM sodium acetate buffer, pH 4.5.

 (b) Incubate 2 hours at 37°C with rocking.

 (c) 3.0 ml 10 mM Tris HCl, pH 7.5 is added. Mix and centrifuge.

15 (d) Supernatant is applied to 5 ml Immobilized Protein A column equilibrated with 10 mM Tris and HCl, pH 7.5.

 (e) The column is washed with 15 ml 10 mM Tris buffer, pH 7.5. The protein eluted is $F(ab')_2$.

20 (f) To recover Fc and undigested IgG, the gel is eluted with 0.1 M glycine HCl, pH 2.8.

Example 3

 The procedure according to Examples 1 or 2 above is followed wherein substituted for the antigen is thyroxine and step A is eliminated, thereby permitting the use of the undigested

thyroxine molecule as the antigen with which it will form the complex with the first monoclonal antibody, which itself is directed against thyroxine.

Examples 4-7

5 The procedure according to Examples 1 or 2 is followed wherein substituted for Chlamydia trachomatis is one of the following antigens and substituted for the first monoclonal antibody directed against Chlamydia trachomatis is a monoclonal antibody directed against the selected antigen: Candida
10 albicans, Human T-Lymphotropic Virus - III, Giardia lamblia, or glycosylated hemoglobin.

WHAT IS CLAIMED:

1. A method for producing a monoclonal antibody directed against the juncture of the idiotypic determinant of a first monoclonal antibody and the antigenic determinant of an antigen when said monoclonal antibody and said antigen are bound together, comprising:

- (a) providing an antigen of interest;
- (b) providing a first monoclonal antibody directed against said antigen and/or antigenic fragment;
- 10 (c) separating the Fab or $F(ab')_2$ portion of the Fc portion of said first monoclonal antibody by enzymatic cleavage;
- (d) combining said antigen and said Fab or $F(ab')_2$ fragment of said first monoclonal antibody in a manner favoring the formation of an immunogenic complex;
- 15 (e) isolating said immunogenic complex;
- (f) injecting the immunogenic complex of step (e) into a host animal; and
- (g) obtaining said monoclonal antibodies directed against said immunogenic complex capable of recognizing the juncture between said antigen and said first antibody when complexed together.
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2. The method of Claim 1 comprising the further step of subjecting said antigen to limited proteolytic digestion to produce at least one antigenic fragment capable of binding to an antibody directed against said antigen.

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3. The method of Claim 2 wherein said antigen or said antigenic proteolytic digest fragment and said Fab or $F(ab')_2$ fragments of said monoclonal antibody are combined in a manner favoring the formation of an immunogenic complex between the antigenic determinant of said antigen or digest thereof and said Fab or $F(ab')_2$ fragments.

4. The method of Claim 2 wherein said antigen fragment containing the desired antigenic determinant is identified comprising the further steps of:

- (a) performing polyacrylamide gel electrophoresis upon the products of said limited proteolytic digestion;
- (b) performing a Western blot on the product of step (a); and then
- (c) performing an immunoassay technique on the product of step (b) with a monoclonal antibody.

5. The method of Claim 4 wherein the immunoassay technique of step (c) is an enzyme-linked immunosorbant assay.

6. The method of Claim 5 wherein said first monoclonal antibody is contacted with an effective amount of papain so as to cleave said first monoclonal antibody into at least three fragments such that the Fab fragments remain intact and retain their immunochemical activity.

7. The method of Claim 5 wherein said first monoclonal antibody is combined with an effective amount of pepsin so as to cleave said first monoclonal antibody into at least two fragments such that the $F(ab')_2$ fragment remains intact and retains its immunochemical activity.

8. The method of Claims 6 or 7 wherein said fragments are purified by an affinity chromatography method.

9. The method of Claims 6 or 7 wherein said fragment are purified by molecular exclusion chromatography.

10. The method of Claim 2 wherein said immunogenic complex is injected into a recipient animal so as to induce the production of
5 antibodies directed against said immunogenic complex.

11. The method of Claim 10 wherein immune lymphocytes producing said antibodies are removed and hybridoma cells producing monoclonal antibodies are prepared thereto.

12. The method of Claim 1 wherein a syngeneic system of animal
10 recipients is used.

13. The method of Claim 12 wherein said monoclonal antibodies are prepared for a murine recipient.

14. A method for producing a monoclonal antibody directed against the juncture of the idiotypic determinant of a first
15 monoclonal antibody and thyroxine when said first monoclonal antibody and said thyroxine are complexed together, comprising:

- (a) contacting a first monoclonal antibody directed against thyroxine with an effective amount of pepsin in a manner so as to effect the separation of Fab from Fc fragments;
- 20 (b) recovering the Fab fragment of step (a) by using Staphylococcus Protein A affinity chromatography;
- (c) combining said Fab fragment with an effective amount of thyroxine in a manner favoring the formation of an immunogenic complex;
- 25 (d) purifying the immunogenic complex of step (c) by molecular exclusion chromatography;

- (c) contacting said MOMP with an effective amount of a protease under conditions favoring the limited proteolysis of said MOMP while retaining at least one intact active antigenic determinant on said MOMP;
- 5 (d) purifying the antigenic determinant of step (c);
- (e) contacting the said purified Fab or $F(ab')_2$ of step (b) with said purified fragment of said MOMP containing said antigenic determinant in a manner favoring the formation of an immunogenic complex;
- 10 (f) purifying the immunogenic complex of step (e) by molecular exclusion chromatography;
- (g) chemically cross-linking with an effective amount of glutaraldehyde said immunogenic complex so as to enhance the immunogenicity of said immunogenic complex by formation of
- 15 polymers of said immunogenic complex;
- (h) immunizing a Balb/c mouse recipient syngeneic with said first monoclonal antibody of step (a) with the product of step (g);
- (i) harvesting the spleen cells produced in response to said
- 20 immunogenic complex;
- (j) fusing said spleen cells of step (i) with an immortal myeloma cell line; and
- (k) identifying and purifying the monoclonal antibodies that are directed specifically against the juncture of said immune
- 25 complex of said MOMP and said first monoclonal antibody rather than against said first monoclonal antibody or against said MOMP alone.

- (e) chemically crosslinking with glutaraldehyde the immunogenic complex of step (f) so as to enhance its immunogenicity by formation of polymers of said immunogenic complex.
- (f) immunizing an animal recipient syngeneic with said first monoclonal antibody with said immunogenic complex of step (e);
- (g) harvesting the immune spleen cells produced in response to said complex;
- (h) fusing said spleen cells of step (g) with an immortal myeloma cell line; and
- (i) isolating and purifying the monoclonal antibodies formed that are directed specifically against the juncture of said complex rather than said first monoclonal antibody or said thyroxine alone.

15 15. A method for producing a monoclonal antibody directed against the juncture of an antigenic determinant on the Chlamydia trachomatis major outer membrane protein ("MOMP") and the idiotypic determinant of a different monoclonal antibody directed against said MOMP when complexed together, comprising:

- 20 (a) contacting a first monoclonal antibody directed against said MOMP and derived from a Balb/c mouse with an amount of papain so as to cleave the $F(ab')_2$ fragments from the Fc fragment;
- 25 (b) recovering the $F(ab')_2$ fragments of step (a) by using affinity chromatography;

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/01295

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC
IPC (4): A61K 39/00, A61K 39/118, G01N 33/535
U.S. CL: 424/85, 424/87, 435/7

II. FIELDS SEARCHED

Minimum Documentation- Searched 4

Classification System	Classification Symbols
U.S.	435/7, 172.2 530/387, 808 436/507, 512, 543, 548; 935/90, 110 424/85, 87

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

COMPUTER SEARCH: CHEMICAL ABSTRACTS 1967-1987; BIOSIS
1977-1987

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y	US, A, 4,544,640 (SOMA) 01 October 1985, see Column 1, lines 46-58, Column 2, line 67 - Column 3, line 7 and Column 4, lines 3-15.	1-15
Y	H. Eisen, "Immunology" Second Edition, Published 1980, by Harper & Row, Publishers (Philadelphia, Pennsylvania, USA), see page 343, Column 2 and Figure 17-8.	6,8,9,15
Y,E	US, A, 4,670,383 (BAIER) 02 June 1987, see Column 3, lines 36-61.	1-15
Y,P	US, A, 4,636,478 (SIEBERT) 13 Jan. 1987, see Column 7, lines 30-52.	14

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search ¹	Date of Mailing of this International Search Report ²
06 August 1987	19 AUG 1987
International Searching Authority ¹	Signature of Authorized Officer ²⁰
ISA/US	 Esther M. Kepplinger

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
Y	US, A, 4,427,782 (CALDWELL) 24 January 1984, see Column 1, line 64- Column 2, line 13.	15
Y	Proceedings of The National Academy of Science, USA, Vol. 79, issued June 1982 (Washington, D.C., USA), D.A. Nemazee et al, "Enhancing Antibody: A Novel Component of The Immune Response," see page 3832, Column 1.	1-15
Y	The Journal of Biological Chemistry, Vol. 252, No. 3, issued 10 February 1977, (Bethesda, Maryland, USA), D.W. Cleveland et al, "Peptide Mapping By Limited Proteolysis In Sodium Dodecyl Sulfate And Analysis By Gel Electrophoresis", see page 1105, Column 1.	2-11,15