PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 3 :		(11) International Publication Number:
G01N 33/56	A1	WO 83/ 01118
		(43) International Publication Date: 31 March 1983 (31.03.83)

(21) International Application Number: PCT/US82/01245

(22) International Filing Date: 14 September 1982 (14.09.82)

(31) Priority Application Number: 303,573

(32) Priority Date: 18 September 1981 (18.09.81)

(33) Priority Country: US

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(81) Designated States: AT (European patent), AU, BE (European patent), BR, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB (European patent), HU, JP, KP, LU (European patent), MC, MG, MW, NL (European patent), NO, RO, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

Published
With international search report.

(54) Title: MONOCLONAL ANTIBODY DETECTION SYSTEM

(57) Abstract

Use of monoclonal Rheumatoid antibody for assaying biological fluids.

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Monoclonal Antibody Detection System

FIELD OF THE INVENTION

The invention is directed to compositions and methods of detecting monoclonal antibodies and to assay systems based on such methods. More particularly, the invention can be applied in analysis of biological fluids, such as serum or urine, to determine the antibody, antigen and/or antigen-antibody complex content of such fluids.

BACKGROUND OF THE INVENTION

Analysis of biological fluids for antibody, antigen and antigen-antibody complex content is a useful tool for the diagnosis of disease. That is, many diseases are characterized by the presence in the circulation of antigen-antibody complexes. Antigens are proteins or other structures which may be present due to bacteria or virus or due to their release from human tissue or cancer cells. Antibodies are predominantly immunoglobulins of the IgM or IgG class, synthesized by the lymphoid system.

An antibody is said to be specific to a particular antigen, as the antibody will complex with its specific antigen. That complex formation between the antigen and its specific antibody is the basis of present analysis of biological fluids for detection of antigens, antibodies and complexes thereof. Various techniques for detecting antibody, antigen and antibody-antigen complex content in biological fluids are known and are referred to as assays. The quantification techniques are referred as "immuno-assay" procedures. One of such assays depends on the reaction of an antigen-antibody complex with

Rheumatoid factor (RF) or with components of complement (C); such an assay was apparently first described by Agnello et al, J. Exp. Med., 134, 228, 1971 and later developed in U.S. Patent Nos. 4,062,935 and 4,143,124.

Present assay and immunoassay techniques, based on antiglobulin reagents, involve collecting the reagents or their precursors from human serum or immunizing animals with, for example, purified immunogloulin to produce anti-immunoglobulin antibodies, although widely used in various assay procedures, possess various disadvantages. Pools of such anti-immunoglobulin antibodies, produced by animals, are not identical, since each animal may respond to immunization in a slightly different way. Accordingly, use of pools of systems based on such anti-immunoglobulin antibodies involves constant recalibration. Moreover, activity and specificity of such anti-immunoglobulin antibodies vary widely.

A new art is developing based on the discovery that antibody forming cells, plasma cells, can be immortalized and can then produce unlimited amounts of the antibody, specifically the monoclonal antibody. Particularly, fusion of antibody forming cells from mammals, such as mice and rates, leads to the immortalization of the antibody forming cell, a plasma cell, and the ability to obtain unlimited amounts of its antibody, otherwise referred as monoclonal antibody. This development has greatly altered the way in which antigens will be assayed in the future. Research units are now producing monoclonal antibodies to a variety of antigens.

Furthermore, at least two techniques have been developed to detect the binding of conventional or monoclonal antibodies to the desired antigen. In one technique, which is probably the most popular technique, the conventional or monoclonal antibody is reacted with bound

antigen, antigen bound, for example, to plastic dishes or beads; then the mixture is washed to remove unbound antibody; the anti-immunoglobulin reagent is added, and then the mixture is washed again to remove unbound anti-immunoglobulin reagent. The anti-immunoglobulin reagent can be iodinated (I^{125}) or coupled with an alkaline phosphatase or peroxidase for detection in an Elisa System, described in the literature by Engvall, E. and Perlmann, Journal of Immunology, Vol. 109, pg. 129 (1972). Detection in an Elisa System involves having an enzyme coupled to the conventional anti-immunoglobulin reagent. The addition and degradation of the enzyme substrate can be measured photometrically. The color developed is proportional to the amount of unknown antibody present in the test solution.

A second technique involves labeling a monoclonal anti-immunoglobulin antibody, either by internal labeling (C^{14}) or external labeling (I^{125}, alkaline phosphatase, etc.) and using it to detect the first antibody. Both techniques for detecting the unknown conventional or monoclonal antibody involve cumbersome wash procedures which decrease efficiency and potentially decrease accuracy of the technique. Moreover, the latter technique requires the use of a scintillation counter for all isotope tagged samples and requires tagging or labeling an enormous number of reagents.

SUMMARY OF THE INVENTION

The invention is directed to compositions and methods for detecting either conventional or monoclonal antibodies bound to antigens. In accordance with the invention, the reagent used for detection includes monoclonal Rheumatoid antibodies.

The methods of the invention involving the use of monoclonal Rheumatoid antibodies obviate multiple wash

stages in the assay. Moreover, the methods of the invention are highly specific and selective with respect to the antigen to be detected. Recalibration of the reagent, in accordance with the invention, is substantially, if not completely, eliminated, and the invention allows quantitative antigen assays.

DETAILED DESCRIPTION OF THE INVENTION

Complex formation between an antigen and its specific conventional or monoclonal antibody results in conformational changes with the heavy chain portion of the antibody molecule and thus in a number of sites which can bind other substances. At least one of those sites can bind monoclonal Rheumatoid antibodies; at least one of those sites can bind components of complement (C). Neither monoclonal Rheumatoid antibodies nor components of complement can bind the antigen, the conventional or the monoclonal antibody in the absence of those sites. Rheumatoid antibodies are circulating antibodies that react with sites on activated immunoglobulin (immunoglobulin bound to its antigen). Parenthetically, they are called Rheumatoid factors (RF) because they were originally discovered in patients with Rheumatoid Arthritis.

The methods of detecting conventional or monoclonal antibodies, in accordance with the invention, involve inducing conformational changes in the respective antibodies to produce sites which will bind Rheumatoid antibodies and providing monoclonal Rheumatoid antibodies which will bind at such sites. The methods of the invention differ from each other in the step of detecting the binding of monoclonal Rheumatoid antibodies to those sites. Those sites can be produced by combining the conventional or monoclonal antibody with its specific antigen to form an activated antigen antibody complex,

which expressed the site that allows the monoclonal Rheumatoid antibody to bind.

Thus, the invention provides an assay, a method of analyzing a biological fluid sample for conventional or monoclonal antibody, for antigen or for monoclonal antibody-antigen complex content thereof which includes the step of adding to the sample, before or after the addition of other reagents, a solution containing monoclonal Rheumatoid antibody to bind with the antigen:antibody complex. Thus the assay method includes

providing a source of antigen, such as a sample of biological fluid, for detecting the presence of a specific antigen therein; adding to the antigen source an amount of conventional or monoclonal antibody specific to the antigen to be detected to allow the formation of the complex between said monoclonal antibody and its specific antigen; and adding a known amount of monoclonal Rheumatoid antibody to bind with a complex of said monoclonal antibody and its specific antigen.

Lastly, the method includes detecting the binding of the monoclonal Rheumatoid antibody to said complex. The methods of the invention differ with respect to the means of detecting the binding of the monoclonal Rheumatoid antibody to said complex, as will be seen in the following discussion.

Many cultures of fused cells secreting monoclonal antibody have been produced. The methods of producing the monoclonal antibody generally involve fusion of antibody-forming cells which are plasma cells to hybridoma tumors, leading to the immortalization of the plasma cell and the ability to obtain unlimited amounts of its antibody, i.e. the monoclonal antibody. By way of illustration, it is noted that tissue culture cell lines which secrete monoclonal anti-sheep red blood cell antibodies

were made by the fusion of a mouse myeloma and mouse spleen cells from an immunized donor and described in NATURE, Vol. 256, pp. 495-497 (August 7, 1975) by Kohler et al. Since the Kohler et al description, monoclonal antibodies have been made by the spleen cell fusion technique of Kohler et al against a variety of antigens, as reviewed in Curr. Topics Microbiol. Immun., 81 (1979), and adopted by Secher et al, NATURE, Vol. 285, pp. 446-450 (1980). The preparation of a monoclonal Rheumatoid antibody involves immunizing the donor by injecting the donor with aggregated immunoglobulins, and then isolating the spleen cells of the donor and fusing them to a tumor; these aggregated immunoglobulins are heat aggregated purified gamma globulin molecules from any species that are to be utilized in the test system. In general these would be human, mouse, rabbit, etc.

The method involves adding an amount of the monoclonal Rheumatoid antibody to the antigen source, prior to, during or after addition of the conventional or monoclonal antibody specific to the antigen to be detected, to allow binding of the monoclonal Rheumatoid antibody to the complexed antigen. A known amount of monoclonal Rheumatoid antibody is generally used, and preferably that amount is in excess of that amount needed to bind to all antigen antibody complexes. Preferably, the monoclonal Rheumatoid antibody will be used in solution form, although the invention embraces its use in insolubilized form. For practical reasons, preferably the amount of antibody specific to the antigen to be detected is used in an amount in excess of that amount necessary to complex all of that antigen. Although the amounts of specific conventional or monoclonal antibody and of monoclonal Rheumatoid antibody are preferably in excess of those amounts necessary to complex all antigen to be detected and to bind the said complex, it is understood

that determination of the presence or the absence of the antigen to be detected is not critically dependent on those amounts; for non-quantitative determination, those amounts are only dependent on the sensitivity of the equipment used in detection.

When the monoclonal Rheumatoid antibody binds with the complex, discernible agglomeration may occur. The invention includes other additional ways of detecting the binding of monoclonal Rheumatoid antibody to the complex. Accordingly, those agglomerates can be centrifuged and washed and the monoclonal Rheumatoid antibody content of the agglomerate measured.

In accordance with the invention, detection and/or measurement of the monoclonal Rheumatoid antibody bound to the complex can be undertaken in the following ways. The monoclonal Rheumatoid antibody may be measured directly if it is labeled, tagged and/or contains a developing marker, all of which can be detected by conventional means. Thus, the monoclonal Rheumatoid antibody may contain internal C^{14} or I^{125} labeling or a fluorescent or a co-enzyme label. Preparation of monoclonal antibodies containing radioactive isotopes is described in Hybridoma Technology with Special Reference to Parasitic Diseases, pg. 73 (for 3_H) and 74 (for C^{14}). Undr/World Bank/Who World Health Organization Publication, (1980), Switzerland 80/4675 - Reggiani - 1000. Preparation of monoclonal antibodies containing I^{125} labeling is described by Yallow, R. S. and Berson, S. A., 1960, J. of Clinical Investigation, Vol. 13, pg. 1157 and Marchalonis, J. J., 1969, Biochemistry Journal, Vol. 113, pg. 299. Preparation of monoclonal antibodies containing a fluorescent is described in the Handbook of Experiment of Immunology, Vol. 1, Immunochemistry Section 18.9, edited by D. M. Weir, Backwell Scientific Publications, London, second edition. Preparation of monoclonal

antibodies containing co-enzyme labeling is described by Nakane, P. K., Annals of New York Academy of Sciences, Vol. 254, pg. 203, 1975 and Engvall, E. and Perlmann, P., 1971, Immunochemistry, Vol. 8, pg. 871. Thus, the monoclonal Rheumatoid antibody bound to the antigen-antibody complex may be detected by a scintillation counter or a spectrophotometer. The agglomerate may be treated with a substrate for detection in the Elisa System.

The binding of the monoclonal Rheumatoid antibody to complexed antibody may be detected by complement (C) or complement component activation. The "complement" system is the primary humoral mediator of antigen-antibody reactions and consists of at least 15 chemically and immunologically distinct serum proteins which may interact with each other, with antibody and with cell membrane. In accordance with this aspect of the detection method of the invention use of both synthetic and naturally occurring complement and complement components are contemplated. Activation of the complement system is well document, e.g., "Basic and Clinical Immunology," second edition, Lange Medical Publications, Los Altos, California, pp. 66-77 (1978).

Thus, detection can be based on the capacity of certain monoclonal Rheumatoid antibodies (1) to undergo conformational changes on binding and (2) to induce, in the presence of complement, activation of complement components. Such activation of the complement can be measured by providing a substrate which undergoes changes as a direct result of activation and measurable by conventional means. The changes may be enzymatic and/or visible color or fluorescent changes which can be measured by spectrophotometers. For example, certain peptides generate a fluorescent compound, amino methyl coumarin, such as the 7-amino-4-methylcoumarin, upon cleavage by enzymes generated during complement activa-

tion; cleavage of the substrate can be followed by measuring absorbance of the coumarin derivative at 360 nM in a Cary 219 spectrophotometer, J. Immun., Vol. 126, No. 5, pp. 1963-1965 (1981). In this alternative procedure, the monoclonal Rheumatoid antibody bound to the complexed monoclonal antibody need not be isolated for treatment with complement; but rather the complement may be added directly to it as it forms, prior to, during or after addition of said substrate. Complement and complement component are produced from serum components. Fresh animal serum contains the essential complement proteins necessary for such assays. Alternatively, such components are available for purchase. Buxted Rabbit Complement Co., East Grinstead, England is such a commercial supplier. The monoclonal Rheumatoid antibody which undergoes conformational changes which induce complement activation are, Human IgM, IgG_1, IgG_2, IgG_3; Mouse ^{IgM}, $IgG2_a$, IgG_{2b}; Rabbit; as well as antibodies from other species.

The methods described herein provide quick means for antigen or monoclonal antibody detection. The method obviates recalibration of reagents used in said detection, obviates multiple wash stages and obviates variations in specificity of the reagent compositions. Although the invention has been explained and illustrated by reference to specific elements of the invention, it is intended that equivalents and obvious modifications of those specific elements are embraced by the appended claims.

Claims

1. A method of detecting the presence or absence of a specific antigen via antibody binding to said antigen in a fluid comprising:

providing a known amount of that fluid; adding to the fluid a known amount of an antibody specific to said antigens, which will thus form a complex with said antigen in the presence of said antigen, to allow formation of a complex between said antibody and said antigen; adding to the fluid a known amount of monoclonal Rheumatoid antibody, wherein said monoclonal Rheumatoid antibody will bind to said complex.

2. The method of Claim 1, wherein said monoclonal Rheumatoid antibody is adapted to be detected by scintillation counting and wherein the amount of monoclonal Rheumatoid antibody which is bound to complex is determined by scintillation counting.

3. The method of Claim 1, wherein said monoclonal Rheumatoid antibody is adapted to be detected by U.V. spectroscopy and wherein the amount of monoclonal Rheumatoid antibody which is bound to complex is determined by U.V. spectroscopy.

4. The method of Claim 1, wherein said monoclonal Rheumatoid antibody is adapted to exhibit a visible color change when bound to said complex.

5. The method of Claim 4, which further includes the step of adding complement component in their natural enzymatic state in combination with peptide which generates a fluorescent compound upon cleavage by enzymes generated during complement activation.

6. The method of Claims 1, 2, 3, 4 or 5 wherein said antibody is a monoclonal antibody.

7. The method of Claim 1 further includes adding complement to the fluid wherein said monoclonal Rheumatoid antibody activates the decomposition of the complement.

8. The method of Claim 7 which includes a substrate which will undergo change when said complement undergoes activation.

9. The method of Claims 1, 2, 3 or 4 wherein the amount of said conventional or monoclonal antibody is in excess of that amount effective to form said complex and wherein the amount of monoclonal Rheumatoid antibody is in excess of that amount effective to bind all complex in said fluid.

10. A composition for the detection of a complex of a conventional or monoclonal antibody bound to its specific antigen comprising a monoclonal Rheumatoid antibody.

11. The composition of Claim 10, which is adapted to be detected by scintillation counting.

12. The composition of Claim 10, which is adapted to be detected by U.V. spectroscopy.

13. The composition of Claim 10, which is adapted to exhibit a color change in the presence of said complex.

14. The composition of Claim 10, which includes complement, complement components or admixtures thereof.

15. The composition of Claim 14, which includes a substrate which will undergo changes when said complement undergoes activation, said changes being detectable by conventional means.

16. A method of detecting the complex of a conventional or monoclonal antibody and its specific antigen in a sample, comprising adding to the sample an amount of

the composition of Claim 10, effective to bind to said complex.

17. A method of detecting the complex of a monoclonal antibody and its specific antigen in a sample, comprising adding to the sample an amount of the composition of Claim 11, effective to bind said complex.

18. A method of detecting the complex of a monoclonal antibody and its specific antigen in a sample, comprising adding to the sample an amount of the composition of Claim 12, effective to bind said complex.

19. A method of detecting the complex of a monoclonal antibody and its specific antigen in a sample comprising adding an amount of composition of Claim 13, effective to bind said complex.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 82/01245

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

I.P.C: 3 G01N 33/56 U.S. 424/1.5

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	424/1,1.5,8,85 260/112R 23/230B, 915,920 435/7, 172, 240, 241

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Biosis Previews: 1977-1982
Chemical Abstracts: 1967-1982

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [15] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	US, A, 4,048,298, Published 13 September 1977, NISWENDER	1-19
X	US, A, 4062,935, Published 13 DEcember 1977, MASSON et al.	1-19
X	US, A, 4,138,213, Published 6 February 1979, MASSON et al.	1-19
X	US, A, 4,143,124, Published 6 March 1979, MASSON et al.	1-19
X	N, Nature, Volume 287, Number 5781 Issued 1980, pages 443-445, Steinitz et al, "Continuous Production of Monoclonal Rheumatoid Factor by EBV Transformed Lymphocytes".	1-19

* Special categories of cited documents: [16]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
22 October 1982	27 OCT 1982
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Margaret Moukewitz

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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]
X	N, Immunoassays Eighties, Publised 1981, pages 133-153, Catty et al, "Antiserums in Immunoassays with Special Reference to Monoclonal Antibodies to Human Immuno-Globulin."	1-19
X	N, Proceedings of the National Academy of Sciences, U.S., Volume 68, Number 11, Issued 1971, pages 2846-2851, Warner et al, "Antibody Activity of a Monoclonal Macroglobulin	1-19
X	N, Human Immunology, Volume one, Number Two, Issued 1980, pages 111-120, Tonkonogy et al, "Monoclonal Antibodies Specific for Kappa Chain, Lambda Chain, and IgG1 Human Gammaglobulin."	1-19