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RADIOIMMUNOASSAY OF MYELIN BASIC PROTEIN – AS ACTIVE DEMYELINATION.

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ABSTRACT

This article reviews the principles of radioimmunoassay technique, describing both its conventional concepts and current state-of-the-art. The approach is to discuss the various components involved in RIA testing, and how they interact in the reaction tube. Also included are various conditions that affect these reactions and how to deal with them in a normal working environment. A specific test for active Demyelinzation is based on a discovery that free myelin basic protein can be detected in cerebrospinal fluid by the use of a radioimmunoassay, and that the level of basic protein found corresponds to the activity of Demyelinating pathologies. A simple method is utilized, wherein samples of cerebrospinal fluid are assayed and the results of the assay are compared with standard samples of myelin basic protein. Patients with active Demyelinating diseases have high levels (l7-l00ng/ml) of basic protein; those with progressive pathologies have less (6-16 ng/ml); and those in remission have less than 4 ng/ml, comparable to the control population.

KEYWORDS: Demyelinzation, cerebrospinal fluid, pathologies, Radioimmunoassay.

1. INTRODUCTION

1.1 Radioimmunoassay

Radioimmunoassay (RIA) is a sensitive method for measuring very small amounts of a substance in the blood. Radioactive versions of a substance, or isotopes of the substance, are mixed with antibodies and inserted in a sample of the patient's blood. The same non-radioactive substance in the blood takes the place of the isotope in the antibodies, thus leaving the radioactive substance free. The amount of free isotope is then measured to see how much of the original substance was in the blood.

1.1.1 Principle of RIA

It is based on the competition between unlabeled antigen and a fixed amount of the corresponding radio labelled antigen for limited number of antibody binding sites in fixed amount of antiserum.

At equilibrium, the unbound antigen (F) + Antigen-antibody complex (Ag-Ab) are separated and quantified.

Ag(larger) + Ab → Ag-Ab
Ag*(smaller) + Ab → Ag*-Ab

In std. Condition, amount of labeled antigen bound to the antibody decreases as the amount of unlabeled antigen increases in sample.

The reaction between Ab and Ag* is quantified by counting either the bound (B) or the free labeled compound (F).

The amount of labeled compound bound to antibody is dependent on

The amount of ligand (Ag +Ag *) present

The amount of antibody (Ab) present

The equilibrium constant/ affinity constants k1 and K2

Where, k1' = k1/k2 and K2=k3/k4.

Ag* + Ab Ag*-ab and ,
Ag + Ab Ag-Ab

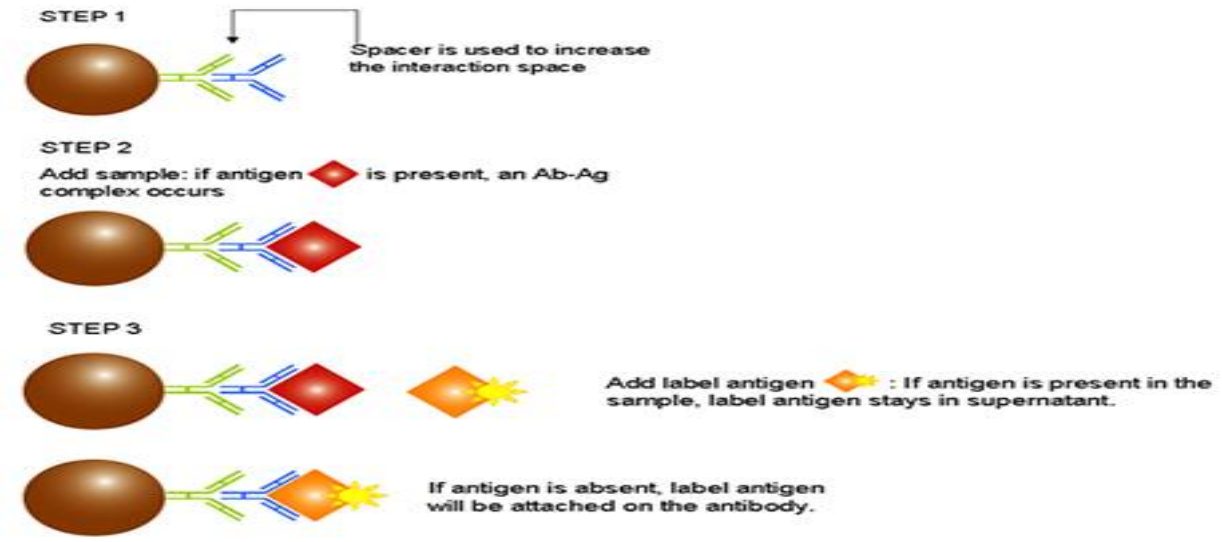
In assay Ag* and Ab are constant as binding of Ag* solely depends on amount of Ag present. **% F = [F/ (F+B)]*100. % B = [B/ (F+B)]*100**

1.1.2 General Procedure for Performing a RIA Analysis

- ✓ A known quantity of an Ag is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine attached to tyrosine.
- ✓ This radio labeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two chemically bind to one another.
- ✓ Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from

the serum to compete with the radio labeled antigen for antibody binding sites.

- ✓ As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radio labeled variant, and reducing the ratio of antibody-bound radio labeled antigen to free radio labeled antigen.
- ✓ The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured.
- ✓ With this technique, separating bound from unbound antigen is crucial. Initially, the method of separation employed was the use of a second "anti-antibody" directed against the first for precipitation and centrifugation.
- ✓ Using known standards, a binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived.



1.1.3 Uses for RIA

RIA has many uses, including narcotics (drug) detection, blood bank screening for the hepatitis (a highly contagious condition) virus, early cancer detection, measurement of growth hormone levels, tracking of the leukemia virus, diagnosis and treatment of peptic ulcers, and research with brain chemicals called neurotransmitters.

1.2 Demyelinization

A Demyelinating disease is any condition that results in damage to the protective covering (myelin sheath) that surrounds nerve fibers in your brain and spinal cord. When the myelin sheath is damaged, nerve impulses slow or even stop, causing neurological problems.

1.2.1 SPECIFIC ASSAY FOR ACTIVE DEMYELINIZATION

This invention relates to the specific assay of active Demyelnization. It is more particularly used for the clinical evalution of active Demylein in Cerebrospinal fliud. The invention provides a reliable and expedient means of monitoring active Demyelinating pathologies by assaying the concentration of myelin basic protein in cerebrospinal fluid.

1.3 Myelin Basic Protein

Many diseases and pathologies of the human body are associated with the destruction of myelin, a lipid protein membrane that surrounds and protects components of the central nervous system. One of the degredation products of Demyelinization is a protein known as **myelin basic protein**.

This protein accounts for as much as thirty percent of the protein found in myelin and may be its major structural protein. Myelin basic proteins from various animal species have been purified and their amino acid sequences have been determined. The protein consists of a single chain of 170 amino acids whose sequence is very similar in different species. It is an unusual protein in that it has no disulfide bridges, no secondary helical structure, and does not assume a globular structure in solution. Metabolic studies with the basic protein have involved tissue extraction, electrophoretic separation, and optical measurements to quantitate the protein. Immunological techniques have also been used to measure basic protein.

Recently, a radioimmunoassay for the myelin basic protein has been developed as a marker for myelin extracted from rat brain tissue, which has been shown to be specific, quantitative, and sensitive to as little as 2 ng of basic protein.

One of the major Demyelinating diseases suffered by man is multiple sclerosis. In mutliple sclerosis, patches of destroyed myelin are replaced by scar tissue that interrupts and distorts the flow of nerve impulses.

At the present time, there is no known cure for multiple sclerosis and there is virtually no therapy that can be used against it. Moreover, there are no reliable tests to diagnose or assess the clinical activity of the disease. The diagnosis of multiple sclerosis depends primarily on clinical criteria. The four major approaches that have been taken in the search for a specific diagnostic test includes studies on.

1. Lymphocytes
2. Demyelinating properties of serum
3. Changes in the chemical composition of the CSF
4. Radioimmunoassay of antibodies to myelin basic protein (Binding of Myelin Basic Protein by Serum and Cerebrospinal Fluid, and a cerebrospinal fluid inhibitor cross reactive with myelin basic protein).

1.3.1 Lymphocytes.

This test involves the migration of lymphocytes in an electric field; it has been observed that lymphocytes from multiple sclerosis patients migrate significantly more slowly than do those from healthy individuals. Lymphocytes from patients with other neurological diseases appear to migrate significantly faster. This test has not yet been reproduced.

1.3.2 Demyelinating Properties of Serum

This is based upon the observation that multiple sclerosis may involve a defect in the functioning of unsaturated fatty acids in cellular membranes. The test involves the migration of macrophages in an electric field. When human leukocytes are exposed to an antigen to which they are sensitized, they release a substance that slows the migration of macrophages from healthy guinea pigs. It has been reported that leukocytes from multiple sclerosis patients release more of this substance, and cause the migration of macrophages to be slowed more markedly than by leukocytes from healthy individuals and patients with other neurological diseases. However, this test is difficult to perform accurately and requires at least 8 to 10 days to obtain a result. Moreover, the test is complicated, sensitive to slight alterations, and has not vyet been consistently reproduced.

1.3.3 Changes in the Chemical Composition of the CSF

This test involves the cerebrospinal fluid of multiple sclerosis patients revealed the presence of elevated levels of protein and/or IgG. However, patients with non demyelinative neurologic disease also had increased levels of cerebrospinal fluid protein and/or IgG. Electron microscopy has revealed the presence of myelin fragments in cerebrospinal fluid in two out of three cases of active multiple sclerosis. Patients with other neurological diseases and those in remission did not have such fragments in their cerebrospinal fluid. Because of the probable action of proteolytic enzymes on free myelin, that myelin fragments or myelin basic protein could be identified in cerebrospinal fluid.

1.3.4 Radioimmunoassay Of antibodies to myelin basic protein

A specific radioimmunoassay for myelin ‘basic protein has recently been developed. This assay has been applied to quantitatively measure myelin in neural tissue and subcellular components by assaying the basic protein as a marker for myelin. The present invention has as a primary objective the provision of a radio immunological assay for the detection and measurement of myelin basic protein in cerebrospinal fluid. A more particular object is to provide an assay which is quick easy and inexpensive to administer, and which is accurate and reliable.

The concentration of myelin basic protein in said sample is then measured by a sensitive Radioimmunoassay say that can measure as little as 2 ng of myelin basic protein in cerebrospinal fluid. High levels of basic protein indicate active demyelinating disease, lesser levels indicate progressive demyelinating disease, and median levels comparable to the control population indicate demyelinating disease in remission.

Regarding the assay itself, in the preferred embodiment, 0.05 ml of a concentrated assay buffer (2 M Triacetate, pH 7.5, containing 10 mg/ml of histone) and antisera at the appropriate concentration is added directly to 0.5 ml of spinal fluid. This mixture is incubated for one hour at 37 degrees C. 5,000 to 20,000 cup of ^{125}I labeled myelin basic protein are added and the mixture is then incubated for one to seventy-two hours at temperatures ranging from 4°C to 37° C. The antibody-basic protein complex is precipitated with 0.3 to 0.7 ml of cold ethanol, the pellet and supernatant are separated by centrifugation, and each is assayed for radioactivity, using a Gamma counter or other means for detecting gamma radiation. The percentage of ^{125}I basic protein bound is the determined and the results are compared to a standard curve. Patients with active Demyelinating disease will have high levels of basic protein in the range of 17-100 ng of basic protein per ml. of cerebrospinal fluid, those with progressive Demyelinating disease will have lower levels of basic protein in the range of 6-16 ng of basic protein per ml of cerebrospinal fluid, and patients in remission will have less than 4 ng of basic protein per ml of cerebrospinal fluid.

Early applications of Radioimmunoassay on cerebrospinal fluid were negative for myelin basic protein. The present invention is thus a significant advance, in that it quantitatively measures myelin basic protein in cerebrospinal fluid. An even greater advance is the 1 discovery that such measurements can serve as an index of pathologies involving active Demyelination.

2. SUMMARY

Unknown quantity of antigen is compete with known quantity of radiolabeled antigen for antibody binding sites in the serum. As the concentration of antigen is increased, more of it binds to the antibody, displacing the radio labeled variant, and reducing the ratio of antibody-

bound radio labeled antigen to free radio labeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured.

Myelin basic protein is a lipid protein membrane that surrounds and protects components of the central nervous system. One of the degredation products of Demyelinization is a protein known as myelin basic protein. A Demyelinating disease is any condition that results in damage to the protective covering (myelin sheath) that surrounds nerve fibers in your brain and spinal cord. When the myelin sheath is damaged, nerve impulses slow or even stop, causing neurological problems. Assay of active demyelination is used for the clinical evalution of active Demylein in Cerebrospinal fliud. The assay of active demyelination provides a reliable and expedient means of monitoring active Demyelinating pathologies by assaying the concentration of myelin basic protein in cerebrospinal fluid.

The four major approaches that used for a specific diagnostic test includes studies on.

1. Lymphocytes
2. Demyelinating properties of serum
3. Changes in the chemical composition of the CSF
4. Radioimmunoassay of antibodies to myelin basic protein (Binding of Myelin Basic Protein by Serum and Cerebrospinal Fluid, and a cerebrospinal fluid inhibitor cross reactive with myelin basic protein).

The Radioimmunoassay has been applied to quantitatively measure myelin in neural tissue and subcellular components by assaying the basic protein as a marker for myelin. High levels of basic protein indicate active demyelinating disease, lesser levels indicate progressive demyelinating disease, and median levels comparable to the control population indicate demyelinating disease in remission. Regarding the assay itself, in the preferred embodiment, 0.05 ml of a concentrated assay buffer (2 M Triacetate, pH 7.5, containing 10 mg/ml of histone) and antisera at the appropriate concentration is added directly to 0.5 ml of spinal fluid. This mixture is incubated for one hour at 37 degrees C. 5,000 to 20,000 cpm of ^{125}I labeled myelin basic protein are added and the mixture is then incubated for one to seventy-two hours at temperatures ranging from 4°C to 37° C. The antibody-basic protein complex is precipitated with 0.3 to 0.7 ml of cold ethanol, the pellet and supernatant are separated by centrifugation, and each is assayed for radioactivity, using a Gamma counter or other means for detecting gamma radiation. The percentage of ^{125}I basic protein bound is the determined and the results are compared to a standard curve.

Patients with active Demyelinating disease will have high levels of basic protein in the range of 17-100 ng of basic protein per ml. of cerebrospinal fluid, those with progressive Demyelinating disease will have lower levels of basic protein in the range of 6-16 ng of basic protein per ml of cerebrospinal fluid, and patients in remission will have less than 4 ng of basic protein per ml of cerebrospinal fluid.

3. CONCLUSION

Presenting a broad overview of the concepts of RIA testing would require an article of even greater length than this one. In an effort to conserve space, and keep it "simple," this article has discussed the following four components in an RIA procedure.

a) Production techniques and selected aspects of quality control for various component types and sources;
b) Interaction of components in the test tube to produce an RIA test;
c) An encompassing look at some of the more widely used reactions available;
d) A comparison of available techniques as alternatives to RIA. Although this article is not a compendium, it does provide a view of the basic principles of RIA, and it is written with the intention of providing a solid foundation for more in-depth studies.

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