IMMUNOCYTOCHEMICAL STUDIES USED IN DIAGNOSIS AND CLASSIFICATION OF ACUTE LEUKEMIAS

Review submitted for Partial Fulfilment

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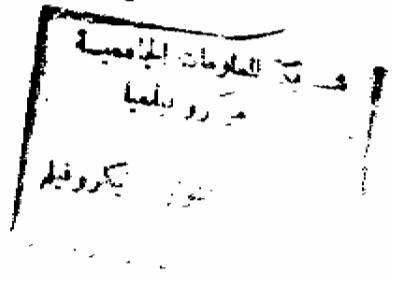

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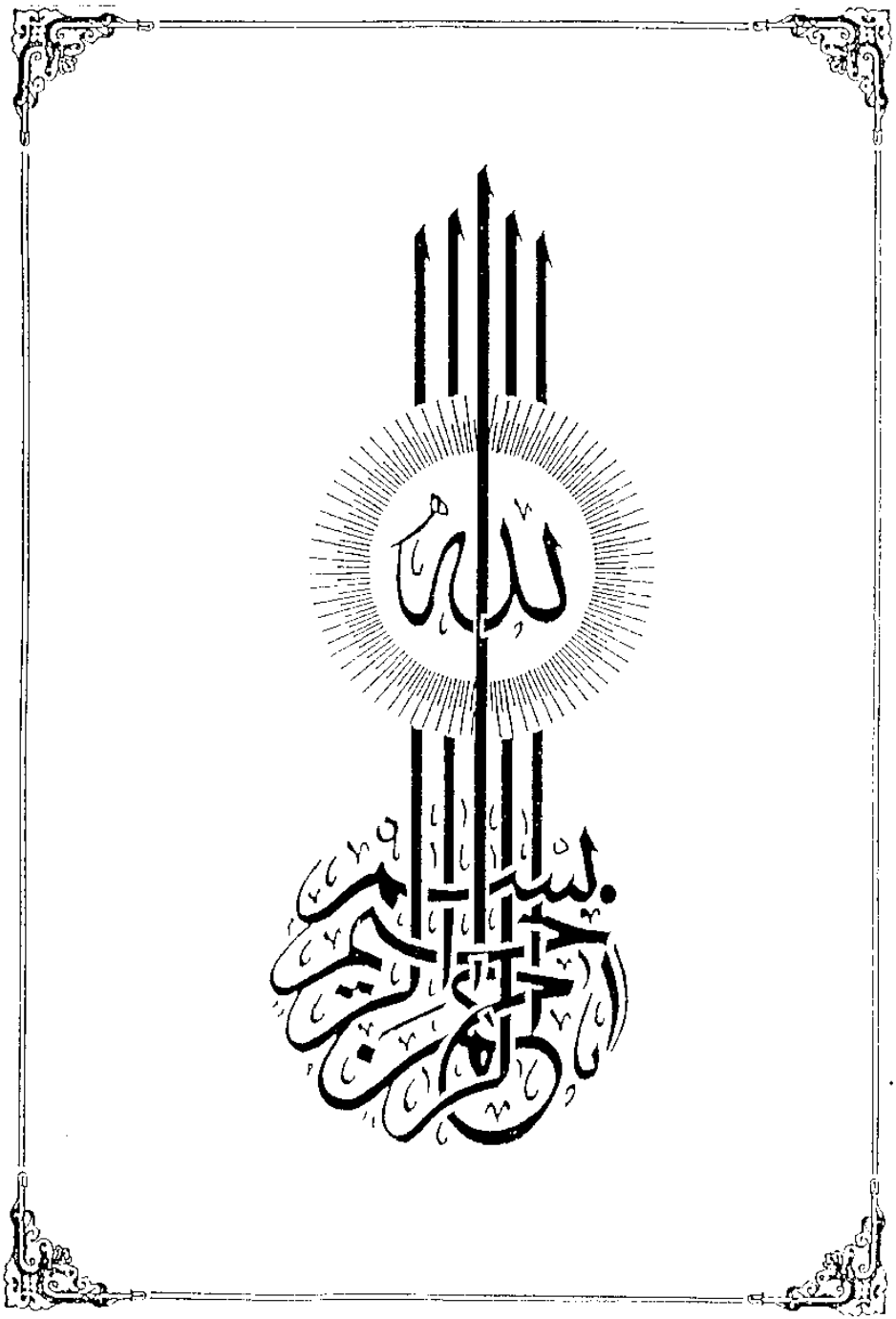

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INTRODUCTION AND AIM OF WORK

It is now clear that three distinct laboratory approaches may be employed to classify acute leukemia. These are Romanowsky stains: cytochemical methodology and the study of differentiation antigens using monoclonal antibodies(William et al. 1985).

With the introduction of immunochemical techniques into the routine histology laboratory, a new era of tissue staining evolved. These very sensitive and specific methods, utilizing antigen-antibody complexes, allow visualization of previously undetectable cell components (Bourne 1983).

Immunocytochemical techniques and monoclonal antibody reagents which recognize specific cell markers have improved considerably and made the diagnosis of acute leukemia more accurate. These immunocytochemical studies are relatively simple and do not necessitate expensive equipment. They are applicable to smears of blood and bone marrow, imprints and histologic sections of surgical biopsy specimens, and cytospin preparations of body fluids. Both cytochemical and immunocytochemical studies may be performed simultaneously on the same specimen, and therefore, it is possible to identify types of cells with greater precision (Yam et al., 1971).

Leukemic cells express certain cell surface markers common to normal human lymphocytes and so acute lymphoblastic leukemia (ALL) can be separated into four clinically important immunologically defined subclasses. These subclasses are: common-ALL, T-ALL, undifferentiated ALL (non T and non B), and B-ALL. Relatively simple immunocytochemical staining methods that utilize monoclonal antibodies, are a practical means of recognizing these subsets of ALL (Kersey et al., 1981; Foon et al., 1982).

The aim of this work is to write a review on immunocytochemical methods, used in diagnosis and classification of acute leukemias, making stress on the techniques.

Basic Immunological Principles

BASIC IMMUNOLOGICAL PRINCIPLES

Antibodies

These are proteins, synthesized and secreted by plasma cells in response to an antigenic stimulus. They have the molecular properties of immunoglobulins. The immunoglobulins are glycoproteins composed of four polypeptides chains. Structurally speaking, all antibodies are immunoglobulins but functionally speaking the reverse is not true, not all immunoglobulins are functionally antibodies (Bowry, 1978). The immunoglobulins can be divided into five classes based on their size, weight, structure, function and other criteria. These classes are IgA, IgD, IgE, IgG, and IgM. For immunohistochemical staining, the antibody solution contains mostly IgG type antibodies with lesser amount of the other classes.

Structure of antibodies:

An antibody is made of two types of protein chains:

- Heavy chains: Immunoglobulins are named for their heavy chains , , , and . (fig 1).
- Light chains: For all the five groups, there are only two types: Kappa (K) and Lambda ( ). A single antibody can never have both Kappa and Lambda chains (fig 2) (Weir,

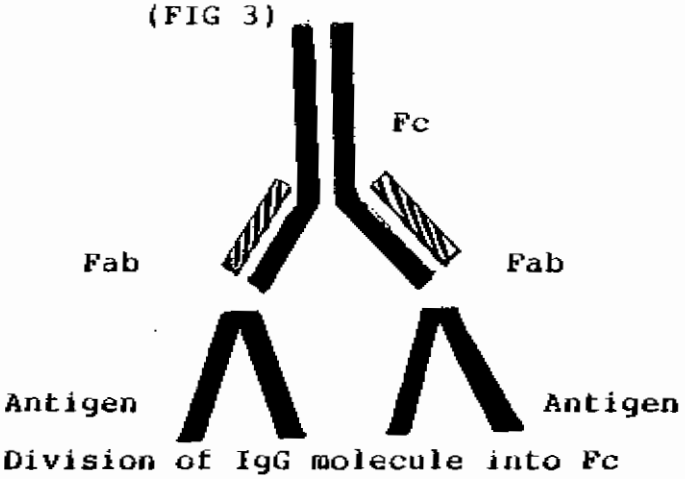
(FIG 1)

*IgG molecule showing paired Heavy chain of gamma type.

(FIG 2)

*IgG molecules showing only possible light chain configurations.

(FIG 3)



Division of IgG molecule into Fc and Fab fragments.

Antigen binding occurs at the two Fab sites.

1983). IgG antibody molecule (fig 3) has two type of binding sites:

Fc portion or the fragment crystalline which will crystallize out upon purification. This portion can fix complement, bind to macrophage, stimulate phagocytosis, cause degranulation of mast cells, and is involved in the transfere of antibody across placenta.

Fab or fragment antigen binding region:

Fab is the part of the antibody molecule capable of specifically binding to the antigen. IgG molecule has 2 Fab sites and is therefore capable of binding two antigen molecules, one at each Fab site (Bowry, 1978).

Antibody production for laboratory use:

This needs the presence of purified antigen which is obtained by subjecting a source of antigen as serum, urine or tissue to a combination of procedures including precipitation, centrifugation dialysis, chromalography and electrophoresis. The antigen is then injected into an animal of a different species than that of the antigen source. The animal will identify the antigen as foreign matter and produce an antibody directed specifically against it. Antibody production begins within twenty minutes after injection but measurable quantity of antibodies can not be

detected for 5-10 days. Small blood samples are usually obtained and pooled at two weeks intervals. Booster injections of antigen are often adminstered every month to promote consistent antibody production.

The choice of an animal for injection depends upon: the antigen used, housing facilities available, amount of antibody needed and personal preference.

Usually, several animals of the species chosen will be injected with an antigen, after several bleeding are pooled, contaminants must be removed. This is accomplished by either liquid or solid phase antigen absorption techniques (Barrett, 1983).

Purification of Immunoglobulins:

These are two different methods by which immunoglobulins can be purified:

1) Non-specific methods: These methods simply treat immunoglobulins as -globulins and seperate them from other serum proteins on the basis of their unique biophysical properties.

Examples of non-specific methods:

a)precipitation of -globulins by mixing antiserum in equal proportions with saturated ammonium sulfate or sodium sulfate. This precipitate is soluble in distilled water and

can be reprecipitated for additional purification. Dialysis or gel filteration to remove sulfate ions yields a purified antibody preparation.

b) Fractionation of serum by ethanol has been widely used to prepare human and animal serum proteins commercially. This method requires ionic strength and strict temperature, so it is not convenient for occasional use.

c) Precipitation of -globulins from serum by lower concentrations of heavy metal ions than needed to precipitate other proteins (Barrett, 1983).

2) Specific methods: All the specific methods for the purification of antibodies depend on a specific serologic reaction of the antibody with its antigen or hapten, after which the antibody is separated from the complex. This is easiest when the antigen is a polysaccharide, as polysaccharides have grossly different chemical properties from those of globulins.

Antisera to polysaccharides may be purified by first preparing the antigen-antibody precipitate, harvesting it by centrifugation, and washing it free of contaminating serum proteins.If a specific enzyme such as dextranase or lysozyme is available to digest the antigen substrate, the antibody is left in a relatively pure state. If no such enzyme is

available, the antigen-antibody aggregate may be dissociated by increasing the salt concentration, raising the temperature or altering the pH. Dissociation is followed by a chemical procedure adapted to the precipitation of one of the reactants, or it may take place in a medium designed to hold one of them in an insoluble condition. The dissociated antigen-antibody complex can be subjected to electrophoresis, gradient centrifugation, gel filteration,or ion exchange chromatography to seperate the two reactants, provided that they differ appreciably in size or ionic charge (Barrett, 1983).

Affinity purification is the most recent innovation in the purification of antibodies. This means the purification of a mixed antiserum by affinity absorption. The pure antigen is absorbed onto a solid phase such as cyanogen bromide-activated Sepharose beads. The coated beads are then mixed with the antibody which reacts with the antigen. Non attached proteins are washed off and the specific antibody is eluted from its antigen by washing with a low or high pH buffer (Van Noorden and Polak, 1983).

Types of antibody solution (Bourne, 1983):

For immunological procedures, there are several antibody **preparations.**

1) Whole serum:

It is obtained from animal blood containing the antibody by centrifugation to seperate the cells from the serum and any contaminating antibodies are absorbed out. This solution (fig 4) will contain: specific antibody for the antigen (the animal was immunized with) and other antibodies. the bulk of the whole serum fraction is made up of ordinary serum components as enzymes, electrolytes and serum proteins.

Advantage: it is the easiest to produce and therefore the most commonly used and least expensive.

Disadvantage: occasionally the serum elements can cause unwanted background staining in some techniques due to affinity of serum proteins for certain tissue components.

2) Immunoglobulin fraction preparation: (fig 5)

It contains mostly antibodies both specific and naturally occuring, plus a very small amount of residual serum protein.

Advantage: Removal of the majority of proteins will reduce the chances of nonspecific reaction in various techniques.

Disadvantage: Nonspecific reactions may appear.