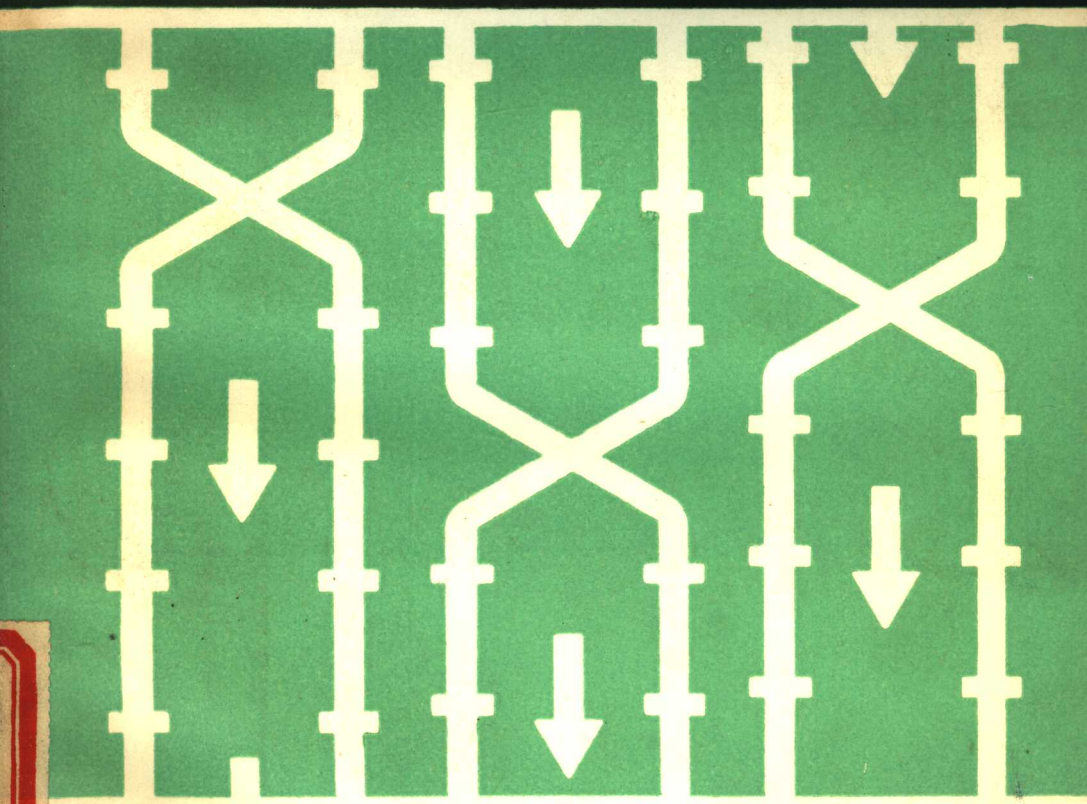
Principles of Immunohematology



PRINCIPLES OF IMMUNOHEMATOLOGY

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PREFACE

This study, part of a collection of similar works, is not a treatise or even a summary; rather it condenses and compiles the principal elements of immunohematology that are indispensable to all concerned with the biological aspects of these problems—students, physicians, transfusion technicians and obstetricians. Thus, our objective in writing this book is essentially practical and, for this reason, a long chapter has been devoted to laboratory techniques. Further, we have emphasized erythroblastosis fetalis, blood transfusion accidents, hemolytic anemias, auto-immune leukopenias and thrombopenias and, particularly, immunological diagnosis.

Even with such specific objectives, our attempt might appear pretentious to specialists, in view of the extreme complexity of this rapidly growing science. However, by condensing into a bibliographical appendix the main theoretical and practical concepts that have been dispersed in numerous surveys or specialized books, we believe this book can facilitate the work of those who must deal daily with immunohematological problems.

Our hope is that the reader will increase his knowledge by delving into the original papers and studies—which we ourselves used very extensively.

This science was developed during the era of Pasteur; first, the humoral defense processes of the organism against microbial attack were studied. Immunohematology began in 1900 with the discovery of the ABO blood groups, by Landsteiner. The existence of active allo-antibodies against human red cells was then demonstrated. They correspond to the presence of specific antigens on the cell surface. Almost simultaneously (1904), Donath and Landsteiner reported sudden hemolytic crises of "paroxysmal cold hemoglobinurea" in the presence of an active hemolysin against all human red cells, including the red cells of the patient himself, thus offering the first example of a disease due to "auto-immunization," which was to remain unique for a very long time.

Thus, from the early twentieth century, the two main directions that immunohematology was to follow were outlined very clearly: (1) a study of the allo-immunization processes that subsequently would permit definition

of the innumerable antigens present in the blood elements, and (2) the demonstration of auto-immunization phenomena that can explain the occurrence of various blood diseases as if the organism were sensitized to its own blood constituents.

Within the scope of the study of allo-immunization processes, very numerous erythrocyte antigens were actually demonstrated, but these results were very quickly extended to the leukocytes, platelets and serum proteins, which themselves proved to be carriers of specific antigens. Thus, the antigenic specificities transmitted by heredity define as many hemotypes as were isolated. Moreover, these are becoming more and more numerous, to the point where one day it will be possible to characterize separately the blood of each individual.

It is easy to understand the exceptional importance of blood groups—the true genetic markers—in all fields of genetics and anthropology, to the point where these disciplines can no longer exist without hemotypological controls. It is also the result of the advances made in this field that numerous important points concerning the structure of immunoglobulins, the chemical composition of certain erythrocyte antigens, and the genetic control of protein synthesis could be identified.

However, the importance of immunohematology is not only theoretical. It involves fundamental applications in routine pathology and therapy. In fact, the knowledge of blood groups permitted the first blood transfusions and assured the later advances of this therapy to its remarkable effectiveness. The immunologist continued to guide it step-by-step to prevent its saving effect from becoming a danger, particularly as transfusions became more frequent and repeated, multiplying the risks of immunization. It is no longer the intent to limit the objective to an erythrocyte transplant but also to assure the supply and survival of such things as platelets, white blood cells and bone marrow elements.

The discovery of the Rhesus group in 1940 by Landsteiner and Wiener and its role in causing fetal erythroblastosis (Levine) constituted an important event in the history of immunohematology. It elucidated an entire obscure and dramatic field of perinatal pathology and led to the development of increasingly effective therapy. Certainly, it is not yet possible to block the immunological process when allo-immunization has been produced; however, this immunization can now be avoided by preventive treatment.

Finally, organ transplants are becoming possible again as a result of advances in immunohematology. The histocompatibility of antigens can already be demonstrated, largely because of their identity with leukocyte-platelet antigens, as proved by the studies of J. Dausset. In fact, the choice of donor, control of survival of transplanted organs, and the creation of the most perfect and prolonged tolerance state are entirely results of progress in immunohematology.

It is also the progress of immunohematology as a discipline to which we owe the first demonstration of the concept of auto-immune disease (as we call it). While the very idea of "auto-immune" hemolytic anemia dates back to the beginning of the century, it was subsequently in an eclipse for at least 50 years. We had to await 1945-1946 when, as a result of the development of detection techniques for anti-Rhesus isoantibodies, it became possible to demonstrate the anti-erythrocyte auto-antibodies, which progressively expanded the field of anemias of immunological origin. Subsequently, the discovery of active antibodies to the platelets and white blood cells of certain patients themselves enabled the description of auto-immune thrombopenias and leukopenias, just as isolation of antinuclear antibodies substantiated the diagnosis and physiopathology of disseminated lupus erythematosus.

CONTENTS

CHAPTER I

GENERAL CONCEPTS OF IMMUNOLOGY

Since immunohematology is only one particular aspect of the study of immune processes, we believe it useful to review a few concepts of general immunology which are necessary for our later discussion.

ANTIGENS

An antigen is any substance which, when introduced into an organism, induces a specific immune reaction to this substance. The immune reaction can be of two forms, immediate or delayed.

Immediate Allergic Reaction

This reaction consists of the production of antibodies, globulins present in the circulating blood with a specific reaction with the corresponding antigen.

These immunoglobulins are produced by plasmocytes which, according to our present knowledge of immunological physiology, result from the transformation of thymus-independent lymphocytes called B-lymphocytes.

This "humoral" immunity is most often involved in the immunohematological processes. Therefore, we will dwell on it more particularly.

Delayed Allergic Reaction

This type of reaction gives rise to a late-onset inflammatory reaction at the point of entry of the antigen which has induced sensitization. This is called "cellular" immunity since it results from the "activation" of lymphocytes (or more generally from so-called "immunologically competent" cells) under the influence of the antigen. The lymphocytes involved are thymus-dependent and are known as T-lymphocytes.

The *tuberculin* skin reaction is a specific example of this type of hypersensitivity. Its role has proved to be of prime importance in numerous other immune processes, including that which controls rejection of grafts.

Moreover, the two types of reaction, humoral and cellular, are not mutually exclusive and may coexist. It even seems that for certain antigens the manifestation of cellular immunity, *i.e.*, sensitization of T-lymphocytes, constitutes an indispensable prerequisite for the induction of humoral immunity, *i.e.*, secretion of antibodies by the plasmocytes resulting from the sensitization of B-lymphocytes.

* *
*

Antigens are characterized as follows:

By their *immunogenic* (or *antigenic*) power, *i.e.*, their power to elicit the formation of more or less potent antibodies;

By their *specificity, i.e.*, the part of their chemical structure which reacts with the corresponding antibody.

Immunogenic or Antigenic Power

The immunogenic potential of antigens is related to various factors.

Nature of the Constituents of the Immunized Subject

An organism can develop immunity only against an antigen which it does not have. It is also said that the individual is tolerant to his endogenous constituents ("self") and can be immunized only against exogenous agents ("not-self"). This fundamental rule of "autotoxic horror" stated by Ehrlich remains valid in the majority of cases.

Genetic Constitution of the Immunized Subject

The synthesis of antigens is under genetic control. This concept which originated from evidence found in blood group research was completed and refined by studies of graft immunology. The greater the difference in genetic constitution between the donor and recipient, the stronger is the antigenic power. In contrast, the greater the genetic similarity between two individuals, the more will their mutual immunization (although difficult) be capable of manifesting minimal antigenic differences. This is why most experiments in immunology are carried out with syngeneic animal strains which all have the same genetic constitution and thus the same characteristics after repeated cross breeding.

Heteroantigens and Isoantigens

Heteroantigens (or species antigens) originate from individuals of different species (certain antigens may be common to several species: the Forssman antigen is the best known of these ubiquitous antigens).

Isoantigens originate from individuals of the same species but who are genetically different. When these isoantigens permit us to define groups of individuals within the species, *i.e.*, an allotypic variation, they are known as alloantigens. In the field of immunohematology, the alloantigens carried by the circulating blood cells usually reveal the difference of genetic constitution between the individual from whom the antigen originates and the immunized individual. Of course, in cases of identical genotypes (homozygous twins), immunization is not possible.

Nevertheless, under pathological conditions the individual seems to become immunized against his own constituents, which might then be compared to *auto-antigens.*

Chemical Structure of Antigens

Generally speaking, antigens are macromolecules (with molecular weight at least greater than 5000). Most often they are of protein nature. Nevertheless, polysaccharides, lipids, and even nucleic acids can have antigenic properties. Moreover, often this is only true in association with proteins, and they then play the role of haptene, *i.e.*, in the complex of the protein and the nonprotein group, the first yields its antigenic power to the molecule, and the second confers its specificity on it. The haptene alone is capable of reacting specifically with the corresponding antibodies but incapable of eliciting their formation.

Antigenic Specificity

An antigen elicits the formation of specific antibodies, *i.e.*, those which closely correspond to it.

Antigen-Antibody Congruence

Landsteiner's initial research with artificial antigens, and the numerous studies which followed it, showed that the specificity of the union between antigen and corresponding antibody was the fundamental basis of immunology. This property is related to the presence on the antigen molecule of a certain arrangement of chemical structure called the antigen *site* or specific determinant group. Generally, this "antigenic template" represents only a small part of the total surface of the molecule; it corresponds very exactly to the specific active site of the antibody which, according to Pauling, would reproduce the impressed antigenic template on the surface of the antibody in the same way, in what has become a classical comparison, as a key fits into a lock (Figure 1).

Thus, antigen specificity is related to the presence of constituent determinants of the molecular structure of the antigen. For example, in the

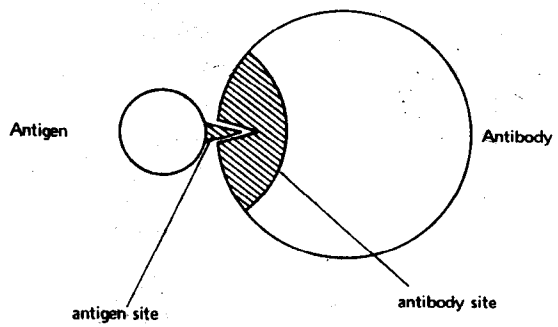


Figure 1. Schematic representation of the "congruence" between an antigen and the corresponding antibody.

case of antigens of blood group ABO, constituted of branched polysaccharide chains on a protein, specificities A or B differ only by the nature of the last "ose" of these chains (Figure 2).

Identical facts are well known for the polysaccharides of *Pneumococci* or *Salmonellae.*

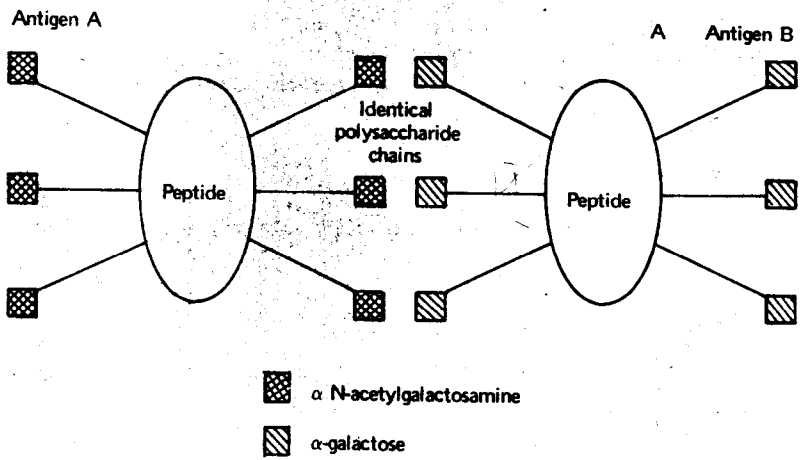


Figure 2. Schematic representation of substances of blood group A and B.

Cross Reactions

A given antigen usually has several distinct antigenic determinants. As a result, the antiserum produced by immunization to a given antigen would contain as many different antibodies as the antigen has antigenic determinants

(heterogeneity of antibodies, Figure 3). This multiplicity of antibodies is responsible for cross reactions.

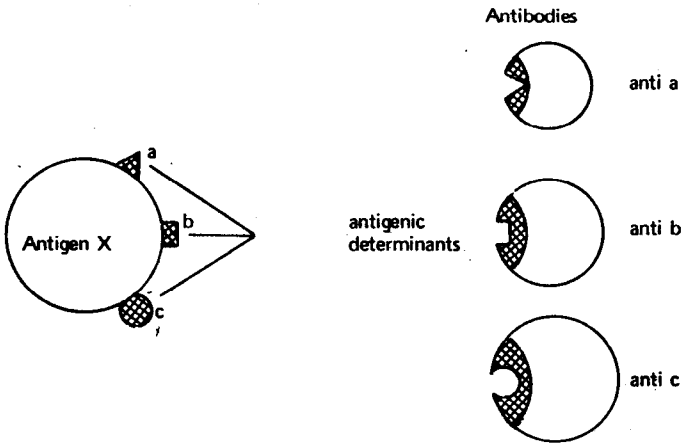
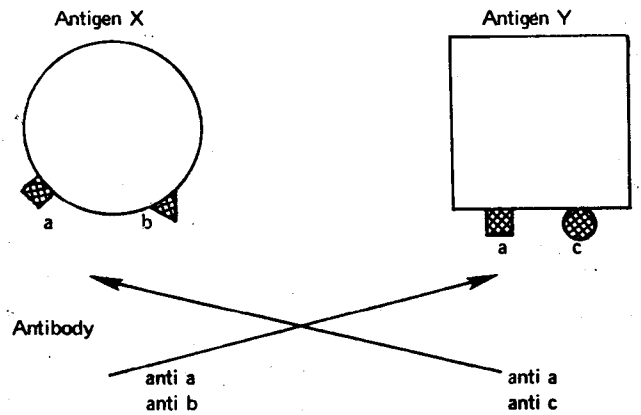
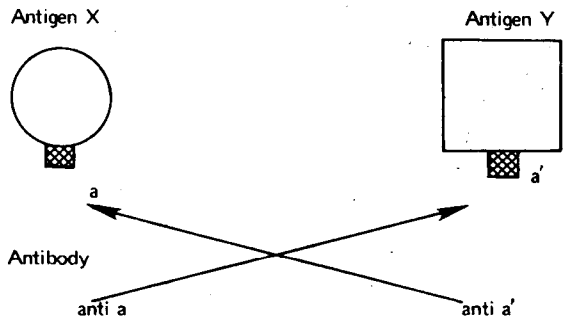


Figure 3. Heterogeneity of antibodies formed against an antigen X.

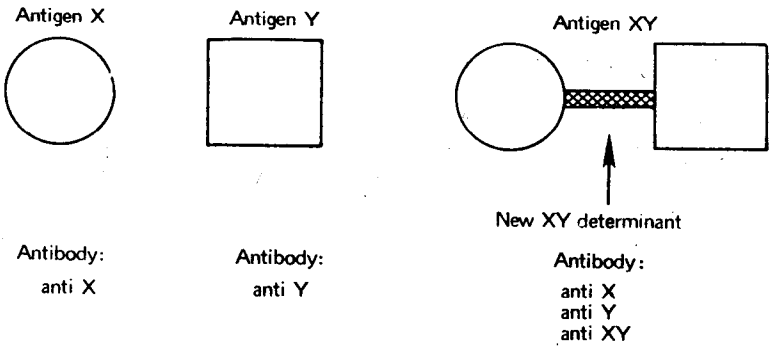
a) If two different antigens have one or more *identical antigenic determinants,* a cross reaction results between the antigens and the corresponding antisera as in the following example:



b) If two antigenic determinants have a *very similar structure*, sufficient doses of the corresponding antibodies can react with the two determinants as demonstrated by Landsteiner and van der Scheer. The principle of this second type of cross reaction is illustrated by the following diagram:



c) A new *antigenic specificity* may appear solely because of the union between two different antigens:



d) Finally, the antibodies formed against the same antigen determinant may exhibit differences related to the quality of the congruent fit of their specific site with the antigenic site. This results in *differences in affinity*, or attraction of the antibody for the antigen.

As a whole, these facts indicate that immunization to a given antigen will lead to the formation of multiple antibodies. Each one of these corresponds to an antigenic determinant (*concept of antibody heterogeneity*). Moreover, they are endowed with variable activity depending on the more or less perfect fit of antigen and antibody sites.

ANTIBODIES

Antibodies may be defined as plasma proteins newly formed by an organism in response to introduction of an antigen.

The protein nature of antibodies was demonstrated by the studies of Tiselius and Kabat who identified their localization in the γ-globulin zone by electrophoresis at pH 8.2. Immunoelectrophoresis, introduced by Grabar and Williams, showed that the antibodies were distributed in the γ-, β_2M- and β_2A-globulins, which have been redefined in one group as *immunoglobulins* by Heremans. Five individual groups of immunoglobulins have been defined thus far.

Antibodies have been the subject of a large number of studies which led to an elucidation of their physical and chemical properties, structure and biological characteristics. Here we will only summarize the essential points of the present state-of-the-art.

Table 1 lists the present international nomenclature with their former designations.

Table 1. International Nomenclature of Immunoglobulins (Ig = immunoglobulin)

International Nomenclature	Synonyms
I_gG or γG	$\gamma 7S - \gamma_2 - \gamma SS$
I_gA or γA	$\beta_2 7S - \gamma_1 A - \beta_2 A$
I_gM or γM	$\beta_2 19S - \gamma_1 M - \beta_2 M$ β_2-macroglobulins
I_gD or γD	
I_gE or γE	

Immunoglobulin Groups–Physical and Chemical Properties

IgG or γG

These are the best known immunoglobulins and constitute their major fraction (75%). Consequently, they have served as the model for a structural study of these molecules. They are defined by their sedimentation constant of 7S, their molecular weight of 150,000, their slow migration in electrophoresis at pH 8.6, and their immunoelectrophoretic pattern extending from the cathode end up to the zone of the α_2-globulins.

They are precipitated by zinc salts (Kunkel test). This involves glucoproteins containing 2.6% glucides. Their serum level in adults normally ranges from 8 to 12 g/l.

IgA or γA

Two categories should be distinguished: serum IgA and secretory IgA present in external secretions.

Serum IgA molecules are glucoproteins with a sedimentation constant of 7S and a molecular weight of 160,000. In addition to their structural and immunological characteristics, they differ from the IgG group as follows:

- –Presence of minor fractions with a higher sedimentation constant of 9S, 11S, 13S, 15S, polymerized forms of the 7S molecule;
- –They are not precipitated by zinc salts;
- –High glucide concentration, on the order of 10%;
- –Electrophoretic migration in the β_2-globulin zone.

In normal subjects, they constitute 3-4% of the serum proteins. Their average level is about 2 g/l.

Moreover, IgA is the principal exocrine immunoglobulin. The *secretory IgA* molecules with a molecular weight of 400,000 and a sedimentation constant of 11S, consist of two dimerized 7S units. They also have a special constituent called the secretory fragment or SC, a glucoprotein rich in sugars of molecular weight 58,000. This SC fragment is synthesized by secretory epithelial cells.

IgM or γM

Macroglobulins with a sedimentation constant of 19S and a molecular weight of 900,000, they are constituted of 5 monomeric units of molecular weight 180,000 and have a general structure analogous to that of IgG.

Glucide-rich glucoproteins (approximately 12%) have a very marked euglobulinic character (precipitation in distilled water, known as the Sia test). They represent 1-2% of the serum proteins (average level 1 g/l) and in electrophoresis they migrate in the β_2-globulin zone. In immunoelectrophoresis they form a characteristic dual curvature near the starting trough toward the cathode.

IgD or γD

Described by Rowe and Fahey (1965), these are immunoglobulins with a sedimentation constant 7S and of molecular weight 170,000. Rich in sugars (12%), particularly galactosamine, they are present in normal serum in very low concentrations.

IgE or γE

More recently identified in the serum of three myeloma patients, these are immunoglobulins with a molecular weight of 200,000 and a sedimentation