10. SEROLOGICAL ASPECTS OF INFECTIOUS AND PARASITIC DISEASES

Immunity, or the lack of receptivity to a pathogen, was a well-known phenomenon. Some infectious diseases confer on the body the ability to withstand re-infection by the same germ.

The origin of this phenomenon haunted mankind, but the mechanisms were gradually discovered. They consisted of cellular defense, by phagocytosis (Metchnikoff, 1880), and a humoral mechanism generating substances that bind to the pathogenic agents. The resulting complexes are usually harmless, but can also have harmful effects through the immunopathologic mechanisms of hypersensitivity (anaphylactic shock or allergic reactions) that they trigger or the haematological disorders caused by the antibodies.

The first humoral antibodies to be discovered were the agglutinins (Grüber and Durham, 1896). When placed in the presence of their corresponding antigens agglutinins produce a very specific agglutination reaction that is visible to the naked eye as well as under the microscope. The first serodiagnostic test was applied by Widal to Eberth's bacillus, Salmonella typhi. This test gave rise to many techniques for diagnosing infectious diseases. Serology, or the study of the processes that reveal antigen-antibody affinities through visible reactions, was born.

Other antibodies, the precipitins (Kraus, 1897), were soon identified. Their properties, which are closely related to those of the agglutinins, were applied much later in gel diffusion tests involving the formation of lines of precipitation (Oudin and Ouchterlony techniques) and immuno electrophoretic techniques to identify specific precipitin lines (Grabar, 1955).

The lysins, which were revealed by intraperitoneal lysis of Vibrio cholerae *in the guinea pig (Pfeiffer's phenomenon) in 1894, came into their own when Bordet succeeded in carrying out the reaction* in vitro. *Bordet showed that bacteriolysis could be caused by other substances as well, notably by red blood cells. Their hemolysis could be inactivated by heating at 55-56°C but reactivated by adding fresh serum containing the sensitizing substance. Bordet and Gengou (1901) exploited this discovery to design a complement fixation test based on deviation of the complement (or alexin). The complement fixation reaction occurs in two steps. First the complement binds to the antigen-antibody complex if the latter is present, then a haemolyzed or non-haemolyzed haemolytic system detects the remaining unbound complement. The method is used to screen for a wide range of antibacterial, rickettsial, viral, and fungal antibodies.*

The Bordet-Wasserman reaction is the best known but the least specific application of this test. The first trials used the treponema-rich livers of syphilitic stillborn babies as the antigen source. It was later ascertained that the treponemas were not indispensable and that just as reliable results could be obtained with lipid extracts of liver, even myocardial cardiolipins. This is because the Bordet-Wasserman reaction detects the presence of reagins rather than specific antibodies. It is less useful in tropical Africa because of the presence of non-venereal treponemal infestations and yaws in the humid regions and bejel or endemic syphilis in the dry regions and the production of reagins by totally different infections, such as acute malarial attacks. The Bordet-Wasserman reaction has thus been supplanted by flocculation reactions (Kahn, Meinicke, VDRL), the use of Reiter's antigen, Nelson's immobilization test, and immunofluorescent techniques.

Agglutinins also come in aspecific variants. One example was given by Paul and Bunnel, who showed empirically that the sera of patients with infectious mononucleosis agglutinated sheep erythrocytes.

The prototype of agglutination tests owes its discovery to the property of the sera of patients with rickettsial infections to agglutinate Proteus vulgaris *isolated initially from the urine of patients with epidemic typhus and initially labelled "X," although it was later found that only the "O" variants gave reliable responses.*

Other techniques reveal by purely technical analogy disturbances in the serum globulin composition due to certain disorders, notably liver disease.

The discovery by Landsteiner (1901) of the existence of specific hemagglutinins opened the door to immunogenetics. Knowledge of the three groups (A, B, and O) found in human blood led to the practice of blood transfusions. In 1940 Landsteiner

(cont.)

*and Wiener added another pillar, the rhesus system, which explained the phenomenon of foetal-maternal incompatibility (*erythroblastosis fetalis*). Other factors, some of which were of particular importance in tropical Africa, were discovered later. For example,* Plasmodium vivax *was found to be unable to survive in erythrocytes lacking the Duffy factor.*

Histocompatibility, while important for skin grafts, became even more important as organ transplants became more numerous. There are incontestable correlations between HLA systems and various diseases (for example HLA B27 and Reiter's syndrome).

Serodiagnosis has acquired great practical importance due to the number of antigens that have become available. The techniques have become more rigorous, more sensitive and specific, or more refined (immunofluorescence, rosette formation, etc.). Whatever the method chosen, its value depends on the antigen's specificity, which is improving due to modern molecular biology. It is no longer a problem to obtain antigens of bacteria and fungi that can be cultured. Even for viruses, obtaining antigen has become easier since the development of tissue and cell culture techniques. Antigens derived from parasites, especially metazoa, must be prepared, except for the ready-made antigen of the hydatic fluid. The antigens specific to the various stages of the parasites' life cycles (eggs, larvae, adults) and their secretions and excretions are of uneven value. Consequently, their use must be specified for each case.

The variability of the moment antigens are produced or their titre and their further evolution in amount and length of persistence according to the way they are induced, require further study. The significant (for example, fourfold) increase in the antibody titre that occurs during the acute and convalescent phases offers a better guarantee than detection of a borderline titre.

While serological diagnosis, if performed correctly, is of undisputed value in individual medicine, it has gained new importance through its applications in community medicine, failing the direct detection of the pathogens. Serodiagnosis has become an important tool for unraveling the epidemiology of communicable diseases.

DIAGNOSTIC TESTS

DIAGNOSTIC TESTS

1. Evolution of immunology and serology

The famous, but still empirical, origins of immunology date back to *Jenner* and *Pasteur*. Their discoveries revealed the true importance of this science by introducing the practice of effective vaccinations to prevent viral and bacterial infections.

Little by little the impressive complexity of the humoral and cellular components of the immune system has been elucidated. We are indebted to *Heidelberger* and *Landsteiner* for the first theories on the molecular structure of antigens and antibodies. *Mechnikoff*'s observations of phagocytes and the demonstration by *Landsteiner* and *Chase* of tuberculin-sensitive cells marked the beginning of our knowledge of cellular immunity.

During the second half of the twentieth century immunology has risen to the rank of a highly specialized discipline involving the collaboration of cytologists, molecular biologists, and geneticists. The number of applications of this discipline in human and veterinary medicine is growing daily. From time to time the steady work in this field receives an added impetus due to new discoveries or the introduction of new techniques, such as is happening today in the areas of genetic engineering and monoclonal antibody production. Everything points to continuing progress in this field, which is expected to make a significant contribution to the fight against numerous tropical diseases.

The importance of immunology is evident on many aspects of infectious and parasitic diseases, namely, antigen identification, immunodiagnosis, inherited immunity, immunoprophylaxis, and immunotherapy. It is even becoming possible to combat haematophagous vector insects by immunological means. Enormous progress has been made in these fields, but a huge unknown territory remains to be explored. It is encouraging to note that, under the impetus of the World Health Organization growing numbers of experts from tropical countries are participating in basic and applied research. Great hope is placed in the results of implementing the newly developed techniques in the field in even the remotest parts of the endemic countries.

The following account will be limited to one of the best-known and most widely used applications of immunology, serology. It will focus on important antigen-antibody reactions involved in the serotyping of infectious agents, serodiagnosis, and measurement of protective antibody levels.

a) In serotyping, one conducts a thorough comparative study of the infectious organisms' antigen configurations by means of conventional antisera or monoclonal antibodies in order to develop a simple serological test for characterization.

b) The aim of serodiagnosis, in the broad sense of the term, is to develop simple, sensitive, and specific tests for detecting antigens or antibodies in the infected host. In antibody screening tests, the quality of the results depends above all on as rigorous a selection of the antigenic reagent as possible. Carefully chosen monoclonal antibodies are used to screen for the antigens.

c) Determining the titres of protective antibodies in infected or vaccinated individuals falls into the areas of immunoprophylaxis and serotherapy. The aim here is to develop tests that reflect the *in vivo* situation as faithfully as possible. One possibility, for example, is to determine to what extent a serum will neutralize the infectious potentials of pathogens or their toxins.

These different serological tests are obviously interrelated. Thus, the presence of protective antibodies in a patient's serum often has great diagnostic significance.

2. General principles of serology

2.1. *Antigens and antibodies*

All serological reactions rely on specific binding between antigens and antibodies. The majority of antigens are polysaccharides or proteins containing a large number of different chemical groups called antigenic determinants that are recognized by lymphocytes. Upon antigenic stimulation preexisting clones of B lymphocytes proliferate and transform into plasma cells which produce specific monoclonal antibodies for the various antigenic determinants. A portion of the sensitized B lymphocytes can be held in reserve as undifferentiated memory cells to ensure a rapid secondary antibody response to later exposure to the same antigenic determinant.

Antibodies are immunoglobulins. They are classified into various classes and subclasses on the basis of their different structural and functional characteristics.

IgG accounts for about 75% of the serum immunoglobulins. The class is further subdivided into four subclasses: IgG1, IgG2, IgG3, and IgG4. The

long-lasting antibodies produced by systemic immunization belong to this class.

In contrast, the IgM class consists of early but short-lived antibodies.

A feature common to IgM and the first three IgG subclasses is that, after binding to "their" antigens, the antibodies activate the complement system. This is an enzyme system that carries out various immunological and physiological functions.

The class A immunoglobulins (IgA) make up 10-15% of the circulating serum immunoglobulins, but are especially numerous in body's secretions such as saliva, tears, nasal and intestinal mucus, and breast milk. They are divided into two subclasses, IgA1 and IgA2.

IgD is present only in traces in normal serum. These immunoglobulins act as antigen receptors on the cell membranes of the B lymphocytes. They share this role with IgM.

Finally, the class E immunoglobulins (IgE) are likewise present in serum but only in very low concentrations. The antibodies of this class are directed mainly against allergens. They bind to the surfaces of mastocytes and polymorphonuclear basophils and are the cause of immediate hypersensitivity (allergic) reactions.

Immunization due to a single antigen can thus lead to the production of a huge variety of specific antibodies belonging to various immunoglobulin classes and subclasses, each of which will have different functional properties in the sensitized person.

These basic principles should give an idea of the extreme complexity of the humoral (antibody-mediated) immunity triggered by contact with infectious agents or parasites, each of which has a mosaic of antigens. Moreover, these antigens can undergo profound changes in the course of the infection. The result is directly or indirectly induced polyclonal activation of the B lymphocytes.

2.2. *Antigenic constitution of the pathogens*

The antigen profiles become increasingly complicated as one goes from viruses to prokaryotes, to one-celled eukaryotes (protozoa) or to multicellular pathogens such as helminths.

Representatives of these last two groups can have dozens or even hundreds of antigens. In addition, their antigen structures vary according to their developmental stage. Finally, antigenic diversity is further increased by a number of genetic mechanisms by which mutations can cause sudden changes in the antigenic features of viruses and prokaryotes. In the course of their evolution some protozoa have developed a large set of genes enabling them, through hybridization or other mechanisms of genetic recombination, to produce large alternate antigenic component. This phenomenon, known as antigenic variation, is especially noticeable in the *plasmodia* and African *trypanosomes*.

A systematic inventory of the antigen patterns of the majority of the major pathogens still remains an almost unattainable ideal.

Antisera produced by natural infections are poorly suited for use in serological identification because of their poorly defined composition. Antisera obtained through hyper-immunization are much more easily standardized. A good illustration of this is provided by the comparative immuno-electrophoretic studies of the antigen patterns of various parasites carried out by J. Biguet and A. Capron in Lille. The most refined tools for antigenic dissection are definitely monoclonal antibodies. Besides their maximum analytic potentials, they offer the huge advantage of requiring only very small amounts of antigen with no prior purification. This last feature is crucial when minor antigenic components with special qualities must be recognized.

Antigen identification remains a slow, expensive procedure. Choices must thus be made. It is preferable to look for antigens that are potentially determinant for serotyping, immunodiagnosis, or immunoprophylaxis.

Serological identification of the important antigens is an essential first step. Genetic manipulations or the classic chemical procedures of antigen synthesis can be used to produce the antigens artificially. These techniques automatically lead to biochemical characterization of the molecules.

2.3. *Kinetics of antibody production during infections*

The types and amounts of antibodies produced depend on many factors. First the infective dose, especially in the case of parasitic worms that do not multiply in the host. Next are the intensity, duration, and sites of the infection. As the amount of antigen coming into contact with the immuno-competent cells increases, antibody production is stimulated and the amount of circulating antibody increases. Infections that are restricted to the intestinal lumen produce as a rule little circulating antibody but they may stimulate local production of IgA and IgE. The same applies to infections of the respiratory tract.

Another form of compartmentalized antibody production is seen in the case of central nervous system

infections. The antibodies in the cerebrospinal fluid are the IgM fraction produced *in situ* while the IgG found in the fluid may come from the serum depending on the state of the blood-brain barrier.

In some parasitic infections the kinetics of antibody production depends on the production and migration of various developmental stages of the parasite. Thus, in schistosomiasis, the immune system must deal in succession with cercariae, schistosomula, male and female adult worms, and eggs. In malaria, the sporozoites are followed by the various developmental phases occurring in the hepatocytes and erythrocytes. In African sleeping sickness, the persistent diversity of the antigenic stimuli is the consequence of the development of successive antigenic variants that eventually attack the central nervous system.

Most of the antigenic stimuli associated with living pathogens are produced by their surface antigens or antigenic secretions. Some constituents, coming from the host itself or induced by viruses, can in turn become secondary antigenic stimuli. When destroyed by the host's immune defenses or drug therapy, some pathogens can release a flood of new antigens.

The antibody picture is strongly influenced by repeated infections or superinfections with other pathogens.

Immunogenicity can vary greatly from one antigen to another. The immune system will produce a selective response when confronted with various antigens simultaneously. This phenomenon is known as antigenic competition. Finally, the host's immunocompetence is also a determining factor for antibody production. This competence may be jeopardized in a wide range of circumstances. One should also remember that persistent infections or parasitoses can have an immunosuppressive effect.

Stimulation of the immune response by antigens occurs a few days before antibody synthesis actually begins. Part of the antibodies produced are consumed (fixed) *in vivo* to form immune complexes with the corresponding antigens. These immune complexes may remain bound to the pathogen or to the host's tissues. They can also circulate in a soluble form in the blood plasma or other body fluid. Depending on the intensity and persistence of the stimulation, the corresponding antibodies of the different immunoglobulin classes or subclasses will be produced in large quantities for variable lengths of time.

This general description should give the reader an idea of the numerous facets of the dynamics of the immune response. If one wishes to embark on a serological study of a given model of infection one must find the time for an in-depth analysis of the concepts involved.

3. General features of serological tests

To visualize the reaction between antigens and antibodies a wide range of techniques can be used. They include countless variations and combinations. Those who have mastered the theoretical and practical aspects of serotesting do not limit themselves to routine applications. They try to be creative by imagining other alternatives.

The goal of a serological test is either to detect a given antigen using a reference antibody or to detect antibodies by means of reference antigen. As the antigens or antibodies involved become better defined, more accurate titration techniques become available and the interpretation of findings becomes that much more reliable. In choosing a test, one takes into account a number of factors, including the nature of the problem, the required sensitivity and specificity, the number of tests to be conducted, and the available financial and technical resources. In most cases, several solutions are possible.

The results of some antigen-antibody reactions are directly visible. In other cases they are seen indirectly, through secondary effects or by adding a number of indicators to reveal the immune complexes that are formed. Various tests can serve to detect either the antibody or the antigen, provided that one of these two complementary components are available.

Let us consider a few techniques in general use.

3.1. *Precipitation*

When antigen and antibody bind in a liquid or semiliquid medium they may form a precipitate. The analytic potential of this precipitation is maximized when the reaction takes place in gel or other solid media, possibly combined with physical separation techniques (immunodiffusion or immunoelectrophoresis).

3.2. *Agglutination*

When antigen or antibody-bearing suspended particles are brought into contact with their combining counterparts (antibody or antigen), macro- or microscopic clumps are formed. For direct agglutination tests micro-organisms can be used. It is also possible to coat immunologically neutral particles, such as treated erythrocytes or latex particles, with antigens or

antibodies in order to carry out indirect agglutination tests. The latter have several applications.

3.3. *Complement activation*

As mentioned above, some antibodies activate the complement system as soon as they react with their antigens. This complement activation can be measured in various ways. Classically, a haemolytic system is used as the indicator. However, more recent techniques exist to measure the activation of individual complement components.

3.4. *Tests with labeled reagents*

Both antigens and antibodies can be labeled. As a rule, the antibody is labeled. This can be done, for example, by conjugating the antibody to a fluorochrome, an enzyme, a radioisotope, or to ferritin. The conjugated antibody is then used in immunofluorescence assays, immunoenzyme assays, or radioimmuno assays. The possibilities are unlimited.

Labelled monoclonal antibodies can be used for serotyping or antigen detection. The indirect antibody detection system is the most versatile. Indeed a very broad range of labelled anti-immunoglobulin conjugates are available for this purpose. These conjugates can be specific for the different classes or subclasses of immunoglobulins. These reactions can be carried out in liquid media, but it is also possible to fix the antigens or antibodies to a solid carrier and then expose them to their corresponding soluble component. One can also locate by light or electron microscopy antigens or antibodies attached to cell structures.

3.5. *Tests with living pathogens*

These tests have been performed for years on numerous viruses, prokaryotes, protozoa, worm eggs and larvae. They are ideal to detect protective antibodies in the sera of infected or vaccinated hosts, whether or not involving complement activation. These antibodies are in most cases directed against surface antigens and have an agglutinating, lytic, opsonizing (phagocytosis enhancing), or other action to neutralize the infectivity. Given their great specificity, such tests can also be used for serotyping or diagnosis.

3.6. *Sensitivity and specificity of serotests*

The intrinsic sensitivity of a serotest is measured by the lowest concentration of antigen or antibody that can be detected. Tests using labelled reagents are particularly sensitive.

The specificity of a test is defined as the degree of reliability of a positive result. Theoretically, the outcome of a serotest depends solely on the reaction between the antigenic determinants and the corresponding antibodies. However, in practice, other factors interfere in the majority of cases. The specificity of some serotests is threatened by various interfering physico-chemical reactions giving rise to *background noise*.

Cross-reactions pose a more difficult problem. They result from the overlapping of various parts of the antigen patterns of different pathogens. The closer the micro-organisms are taxonomically, the more similar are their antigen configurations. Heterologous combinations of antigens and antibodies can thus yield *false positives*. Actually, the test provides a true picture of the antigenic determinants and the antibodies present in the mixture, but the interpretation may be misleading.

4. **Serodiagnosis and seroepidemiology**

Serotests have long been used for individual diagnosis or epidemiological surveys of yellow fever, poliomyelitis, rickettsial infections, syphilis, and other diseases. They have been introduced for parasite infestations in the last 20-30 years.

Serotests are especially valuable for diagnosis when the causative agents are difficult to detect because of their small size, small number, or inaccessible site of infection. In the case of large-scale epidemiological surveys, serotests provide rapid information on the prevalence and incidence of infections.

So far, the overwhelming majority of serodiagnostic tests have been based on antibody detection. Tests based on antigen screening are rapidly being developed.

4.1. *Antibody detection tests*

4.1.1. *Collection and storage of blood specimens*

The quality of a serotest depends first and foremost on the quality of the specimens being examined. Pure serum is the ideal reagent for a reaction. However, it is easier in the field to collect a small sample of blood from the fingertip in a heparinzed capillary tube in which the plasma is separated. The blood specimen can also be diluted in a buffer or hemolytic liquid or, alternatively, a drop of blood can be fixed on filter paper and then eluted.

When the tests cannot be carried out immediately, it is imperative that the specimens be stored properly. Antibodies IgM and IgE are particularly delicate.

4.1.2. *Choice of test and antigen*

For convenient reasons, one may choose a commercial test and use a standard technique. It is in the serology laboratory's interest to ensure a certain degree of standardization of techniques and equipment. There is a growing shift to analytical methods that can be automated, such as ELISA. However, only simple systems can be considered for field work and nowadays, agglutination tests answer best such demands, but very simple versions of immuno-enzyme assays are already being developed.

Those who want to prepare their own antigenic reagents can use the existing procedures as a starting point. The many sources of antigens include *in vitro* cultures, experimentally infected animals, and parasites obtained from naturally infected animals or patients. Some antigens may be obtained from WHO.

Most antigen preparations can be stabilized by freezing or lyophilization. If necessary, they can thus be prepared in large quantities.

4.1.3. *Sensitivity and specificity of antibody screening*

In serodiagnosis, sensitivity is defined as the percent of positive tests in a group of people known to be infected. Antibody screening tests have the disadvantage of being positive only several weeks after the onset of infection. In addition, the titres of some antibodies fluctuate during the course of the illness due to changes in their synthesis or metabolism *in vivo*. In this process specificity of the antigen and the immunoglobulin class of the antibody play a determining role.

The specificity of a serodiagnostic test is usually expressed as the percent of false positives in a population that has not been exposed to the infection being studied. To evaluate specificity, one must thoroughly investigate the interference of other infections. The risk of troublesome cross-reactions is great if an unpurified antigen is used. A frustrating example is provided by the serotests for worm infestations, in which one has sometimes to be satisfied with group-specific results.

To enhance a test's specificity it is not always necessary to go through the lengthy process of purifying the antigen. It has been known for years that the titration principle is able to distinguish true from false positives by serial dilutions. Interpretation is even easier when the serum is systematically tested against a battery of different antigen preparations. ELISA is especially well-suited for this procedure. Other tests, such as immunoelectrophoresis, fractionate the various antigens involved. One can also take advantage of the natural fragmentation of the antigenic components into breakdown or secretory products, surface antigens, and internal components bound to cellular or morphological structures. The secretory antigens are often highly specific. They can easily be obtained by *in vitro* culture of micro-organisms or worms surviving in a suitable medium. The surface antigens also deserve special attention. They selectively take part in tests using live or suitably fixed pathogens. Microserotests relying on labelled anti-immunoglobulin antibodies may be carried out to make use of the different antigenic elements associated with gross or subcellular structures. The specificity of an antibody detection test can sometimes be increased fairly simply by selective screening for given immunoglobulin classes or subclasses.

An ideal test for serodiagnosis should be both very sensitive and highly specific. These two criteria cannot always be met by one test. If a single test must be selected, one can only compromise. To avoid this dilemma, two tests may be used – a highly sensitive test and a highly specific test.

4.2. *Antigen detection tests*

There is a tendency in modern serology to complement antibody screening by antigen detection. This trend has been remarkably strengthened by the possibility that we now have of preparing highly specific monoclonal antibodies to specific antigens. Labeled antibodies have long been used to reveal small or scarce pathogens by immunochemical staining. This has gradually led to a system enabling investigators to detect blindly by purely immunochemical means specific antigens in various specimens namely – urine, blood, faecal material, lymph, CSF, biopsy material, a culture medium, etc. The reacting antigen being sought may be either the pathogen itself or its soluble derivatives. The trick is to select the antigens that will be of diagnostic significance. Here, once again, one faces the problem of sensitivity and specificity.

In most antigen screening tests enzyme-linked or radiolabeled antibodies are used. Efforts are being made to develop simple agglutination tests for this. Some are already available on the market (as for hepatitis B, rotaviruses, cryptococci, and schistosomes).

The presence of antigens implies that there is an active infection or infestation. The test may be positive at an early stage of infection and then revert to negative soon after recovery. The specificity of the test can be guaranteed by using carefully selected monoclonal antibodies. These are the many advantages over antibody screening. Serology is moving

towards screening for both members of the antigen-antibody pair simultaneously.

4.3. *Statistical analyses*

Mathematical models and computer programs for organizing and interpreting the data of sero-epidemiological surveys already exist. It is advisable to consult an expert before undertaking large-scale surveys.

An important aspect of serodiagnostic tests is their predictive value. This depends on the sensitivity and specificity of the test used and varies with the prevalence of the infection being studied.

5. Immunological techniques applied to the diagnosis of African trypanosomiasis

The large number of immunological studies on African trypanosomiasis had three main aims: to increase the diagnostic capacity, to determine the variation in antigens of the parasite and to clarify the immunopathologic mechanisms during infection.

The habitual procedure for diagnosis lies within the detection of the parasite in lymph node aspirate, blood or cerebrospinal fluid (CSF). Such an examination is tiresome and chancy as the parasites are often in small number and appear irregularly during the evolution of the disease.

Many research workers have explored the diagnostic power of various serotests. In early periods the formol gel-test was already used for its simplicity It was based on the changes in serum proteins in case of trypanosome infection through increase in globulins but was not very specific.

From 1940 onwards, several Belgian investigators as *J. Rhodain, C. Van Goidsenhoven, F. Schoenaers, A. Dubois, F. Evens, A. Kaeckenbeek* and *G. Neujean*, tried to prepare a more specific test, in particular the complement fixation test. The greatest difficulties, as seen by the number of published papers, were the production of pure trypanosomes from blood of highly infected rats and a stable reagent of antigens without anti-complement activity. The isolation of trypanosomes from blood was settled in 1968 when *S. Lanham* introduced the ion exchange technique.

At the end of the fifties, *Charmot, Grabar* and *Burtin* have detected the IgM macroglobulinaemia in the serum of trypanosomiasis patients. Some years later several French research workers have shown the diagnostic value of the method and prepared simple quantitative techniques. In 1966, *G. Binz, G. Timperman* and *M.P. Hutchinson* used this property for a survey on 10,000 people in the sub-district of South Gombe (Zaire) where 200 new cases have been detected.

During the sixties a new serological method has been used by a number of African laboratories, indirect fluorescent antibody test. Among the advantages of this method one must mention its high sensitivity, easy application, low cost and great sampling facility. Blood drops from the tip of the fingers are collected on filter paper and then are sent to the laboratory. This method has been used in Zaire by the department of parasitology of Kinshasa University, supervised successively by *M. Wéry, P. Kageruka, M.C. Henry* and *P. Mulumba*. The technique was largely applied to hundreds of thousand Zaireans by the Central Bureau of Trypanosomiasis headed by *J.F. Ruppol* and *G. Kazyumba*. The serology department at the Institute of Tropical Medicine at Antwerp has solved the antigen standardisation by preparing various lyophilized antigenic reagents of well-defined trypanosomes. The reagents are available for every laboratory worker.

During the seventies the enzyme-linked immunosorbent assay (ELISA) joined the series of tests at hand. Several authors demonstrated its diagnostic value for trypanosomiasis. *T. Vervoort* and colleagues could increase the sensitivity and the reproductibility of the technique by using selected antigens.

However the applicability of the ELISA and even the indirect fluorescent antibody test (IFAT) remains restricted, as there is sometimes a long delay between sampling and reading of the results, entailing difficulties to recall seropositive cases.

In 1975, *G.J. Boné* and *J. Charlier* introduced an indirect hemagglutination test allowing diagnosis on the spot. The antigen is stable and is supplied lyophilised as single doses in capillary tubes. These are easy to handle and reading is done after half an hour. Once on the market this test could have a huge success, but for an unknown reason it was not applied at large scale. According to some authors the test should still be too complicate to carry out.

In 1978, *E. Magnus, T. Vervoort* and *N. Van Meirvenne* reached the limits of simplification by proposing an agglutination test on a card. It can be performed in a very short time by mixing a drop of reagent in which coloured trypanosomes are present with a drop of blood, serum or plasma. A preliminary evaluation organised by WHO in 1983 has demonstrated the potentialities of the method which was gradually applied in Zaire (Testryp-CATT, see p. 282)

5.1. *Antigenic definition of parasites*

Accurate knowledge of the antigenic composition in trypanosomes is necessary to understand the immune relations between host and parasite.

In 1975 *D. Le Ray* published a detailed study of the immuno-electrophoretic structures of *Trypanosoma brucei* within its life cycle. A total of 48 components were detected, divided in specific and heterospecific antigens, the latter being common to other parasites and harbouring components of the cultivation broth or of the host's serum. The specific antigens are subdivided in two groups according to their continuous appearance within the life cycle or their limitation to metacyclic or blood stages. This study has also proven that only the variable specific antigens are able to induce a protective immunity.

The property of African trypanosomes to carry different antigens is known since the beginning of this century and has drawn the attention of dozens of research workers as *N. Van Meirvenne, P.G. Janssens, E. Magnus* and *D. Le Ray*. These scientists have made the systematic catalogue of antigens by new methods for serotyping. This approach has made possible to identify main variable antigenic types to be used for serodiagnosis.

From 1978 onwards, several molecular biologists started to study the genetic mechanisms of variation in antigenic type. At the department of molecular biology of the Flemish- and the French-speaking free Universities of Brussels (*Université Libre de Bruxelles* and *Vrije Universiteit Brussel*) *M. Steinert, E. Pays, R. Hamers, G. Matthijssens* and their co-workers contributed outstandlingly to this study. Nowadays research is aiming mainly at serotyping the metacyclic forms of trypanosomes, as injected by the puncture of tsetse flies. It was already possible to state that these forms have a relatively fixed mixture of antigenic variants with less heterogeneity.

5.2. *Immunopathology*

The immune component within the pathogenesis of sleeping sickness could be foreseen by analogy to fundamental immune mechanisms. Nevertheless it is only since 1973 that experimental data could demonstrate what had only been formulated as hypotheses by P. Boreham, B.M. Greenwood and L.G. Goodwin. However immunological research requests a thoroughly equipped laboratory as the one used by P.H. Lambert, M. Berney and G. Kazyumba in Zaire, which has analysed sera and cerebrospinal fluid.

More recently at the Institute of Tropical Medicine at Antwerp, P. Mulumba, M. Wéry, E. Van Marck and P. Gigase have performed histopathological and immune research on brain lesions by experimental trypanosomiasis.

5.3. *Perspectives*

The control of sleeping sickness due to *Trypanosoma gambiense* requires case detection and systematic treatment of infected individuals. Early screening can no more be considered without serological tests. Presently direct and indirect agglutination tests are the simplest methods that can be used at field level. Techniques using isotope markers, fluorescent stains or enzymes can only be used in well-equipped laboratories. Enzyme linked immunosorbent assays (ELISA) could possibly be adapted to field conditions, while other recently designed simple tests allowing to visualize immune-complexes on gel or hard material deserve an evaluation of the applicability.

In general the sensitivity, the specificity or the simplicity of all tests could be improved. The use of monoclonal antibodies will enable to design very specific tests to detect circulating antigen.

As there are many antigenic variants it seems quite unlikely that a traditional vaccine could be produced against African trypanosomiasis.

Immunopathology of sleeping sickness deserves thorough studies and therapeutic trials against the inflammatory processes.

6. **Conclusions**

Diseases caused by viruses, bacteria, protozoa, worms, and fungi continue to threaten the health of man and animals. Vector control, improved hygiene, and new possibilities for chemoprophylaxis and drug therapy can resolve many of these problems. Immunology can make important contributions to prevention, diagnosis, and therapy.

Serology's contribution to the future of medicine can be outlined as follows. First of all, it is necessary to categorise systematically the antigenic structures of the main pathogens by means of monoclonal antibodies. To reach this objective in a reasonable length of time a major part of the analyses will have to be automated. The major antigens could be better characterized by means of existing technology. If these antigens are difficult to isolate from the natural environment, it is possible to synthesize them either by genetic manipulations or by classical methods. Characterizing the antigens will automatically lead to simple serotyping methods relying increasingly on monoclonal antibodies.

The sensitivity and specificity of serodiagnostic tests can be expected to improve. The large-scale use of such methods will be encouraged by the commercialization of simple, standard tests. Antibody screen-

ing tests can be significantly improved notably by using better-defined antigen preparations and differentiating immunoglobulin isotypes. However, one can expect the most spectacular progress from the development of antigen screening tests.

Finally, there is the hope that serological research will contribute to the identification of protective antigens for the production of vaccines.

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