Diagnostic Immunology and Serology:

A Clinicians' Guide

J.D. Wilson

and

Sandra I. Simpson

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J.D. Wilson and Sandra I. Simpson†*

*Associate Professor of Immunology
†Graduate Technologist
Department of Medicine, University of Auckland School of Medicine, Auckland, New Zealand

With 35 tables and 23 figures

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Foreword

Immunology as an independent discipline is just 100 years old. In the Pasteurian era, it was the direct handmaiden of medical microbiology, but with Landsteiner's discovery of the blood groups in 1901, immunology burst through into other fields. This spreading of immunology into many facets of biology and medicine has continued at an accelerating pace, particularly over these last 20 years. For the physician, immunology is a 'horizontal' specialty, breaking the confines of a single organ system and touching an enormous number of chronic diseases.

This spreading tendency of immunology is both a source of great fascination and great frustration. The research worker in immunology is delighted to be engaged at so many frontiers. The clinician who must use the new research knowledge to help the patient can easily be confused and overwhelmed. The fact that immunology is poorly taught in most medical courses makes things worse. These are the reasons why physicians, clinical pathologists and undergraduate and postgraudate students should hail the publication of 'Diagnostic Immunology and Serology'.

Douglas Wilson and Sandra I. Simpson have achieved a triumph in not only marrying the microbiological and general aspects of diagnostic immunology, but also in achieving an authoritative synthesis in a brief, down-to-earth, eminently readable format. Their book is not a list of recipes — it does not seek to describe the details of the various diagnostic tests used by the clinical pathologist specialising in immunology. Nor is it an erudite treatise of theoretical immunology — we have enough of those. Rather, it sets out the major immunological diagnostic techniques, describes their scientific rationale and gives the clinician a clear indication of the meaning of a positive test, all with a commendable absence of immunological jargon. The book, read cover to cover, serves as a useful guide to those aspects of modern immunology that the clinician most needs to know, and read piecemeal for specific tests, will help a physician to interpret a particular result received from the laboratory with greater perception.

As a subspecialty of clinical pathology, diagnostic immunology is relatively new. This book elegantly fills an important gap. It is a pleasure to recommend it to what I hope will be a wide readership.

G.J.V. Nossal
Director, Walter and Eliza Hall Institute, Melbourne

Preface

The rapid progress of basic immunology in recent years has been followed by a similar expansion in clinical immunology with the recognition of the immune pathogenesis of a number of diseases and the development of a wide range of laboratory assays. Commercial development of antisera, immunodiffusion plates, haemagglutination and radioimmunoassay kits has made relatively sophisticated assays available to most laboratories. While many text books on clinical immunology discuss basic immunology, immunopathogenic mechanisms and clinical features of immunologically related diseases, there is usually a lack of emphasis on the clinical immunological tests and their interpretation. This book is intended to fill that gap by providing a ready reference for the more common immunological and serological tests, with a clear guide to their interpretation. A minimum of space is given to clinical descriptions but some laboratory details of the assays are included to clarify their clinical significance. Finally, an appendix is provided as a precis of the immunological tests of relevance to the diagnosis and management of non-infectious disease.

The book was conceived by John McKay and ourselves and he has contributed much of the material for the chapters on Toxoplasmosis, Leptospirosis, Brucellosis, Hydatids, Syphilis and Acute Phase Proteins. The chapter on Neutrophil Function Tests was written by Cliff Hosking. We have taken liberties with their texts and are responsible for the final presentation, and the errors.

Our thanks are due to Mrs Norma Turner for secretarial assistance, and to Mr David Bree, Dr Ian Simpson, Mr Denis Harding, Dr Lindsay Green of Auckland, and Professor Tony Basten of Sydney for reviewing sections of the manuscript and offering valuable criticisms.

J.D. Wilson
Sandra I. Simpson
Auckland

Contributors

Wilson, J.D.
Associate Professor of Immunology, Department of Medicine, University of Auckland School of Medicine, Auckland, New Zealand.

Simpson, Sandra I.
Graduate Technologist, Department of Medicine, Section of Immunology, University of Auckland School of Medicine, Auckland, New Zealand.

McKay, E.J.
Research Fellow, Department of Clinical Chemistry, University of Lund, Malmo, Sweden.

Hosking, C.S.
Immunologist, Royal Children's Hospital, Melbourne, Australia.

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Chapter I

General Principles

The assays discussed in the succeeding chapters of this book are used:

1) To evaluate the competence of the immune system itself
2) To measure a patient's immune response against foreign or self proteins (antigens)
3) To identify components of hypersensitivity reactions producing disease.

1. The Immune System

The immune system consists of specialised cells and various humoral factors such as immunoglobulins and complement. The central cells in the system are lymphocytes which either circulate between blood and lymph nodes, or are aggregated into specialised units such as the spleen, lymph nodes, tonsils and Peyers's patches. During fetal life developing lymphocytes follow one of two major pathways of differentiation, forming T and B lymphocytes. The T cells are processed in the thymus and then seed to peripheral lymphoid tissues where they serve a number of roles, from regulation of the immune response to direct cytotoxicity. B cells develop within the bone marrow before seeding to peripheral lymphoid tissue where their ultimate task is to differentiate into plasma cells and produce antibodies.

A number of other cells also participate in the immune system, either within lymph nodes or within the reticuloendothelial tissues. Neutrophils and macrophages function as phagocytic cells with the macrophages also interacting with lymphocytes in the development of immune responses. Eosinophils are involved in some antigen-antibody reactions and possibly also in phagocytosis and other scavenging operations. Mast cells provide a package of inflammatory substances which, if released inappropriately, gives rise to some allergic symptoms such as urticaria or bronchospasm.

Identification and measurement of the functional integrity of most of the components of the immune system is now possible. Lymphocyte populations can be recog-

nised and some of their *in vivo* activities can be examined *in vitro*. The competence of neutrophils is detected by various neutrophil function assays. Measurement of patients' immunoglobulins and selected complement components have now, with the development of commercially available immunodiffusion plates, become routine procedures, particularly in the consideration of various forms of immune deficiency or in measuring the aggressiveness of some antibody reactions.

2. The Immune Response

An immune response to an antigen has a number of different components and evaluation of these can provide a wide range of diagnostic information. Both T and B lymphocytes can become effector cells, the former playing a role in cell-mediated immune responses and the B cells and plasma cells secreting immunoglobulin to provide humoral immunity. Generation of an immune response following interaction with antigen is a complicated process. Interactions between various lymphocyte populations, macrophages and secreted factors produce a potent defence response which acts rapidly and selectively. A complex system of regulation limits the duration of the immune reaction and helps protect innocent bystander cells from damage. Breakdown of the regulating process can sometimes lead to host tissue damage and disease.

Under most circumstances the first component of an antibody response is IgM which rapidly declines as IgG antibody develops and this can persist for months or years. Thus demonstration that an IgM antibody against a particular organism is present in a patient's serum usually implies that contact with that organism is recent. Some, but not all, antibodies will fix complement, so analysis of complement-fixing antibodies can give precise clues to the stage of a patient's disease. The presence of antibody against an infectious organism means the patient has had contact with that organism but not necessarily that he is suffering, or has suffered, from clinical infection. The changing levels of antibody, or analysis of the different components of antibody response, are usually able to provide clear information as to present or past infection.

The cell-mediated response depends upon the production of cytotoxic T cells and various factors secreted from lymphocytes (lymphokines) which potentiate the inflammatory process. Antibody is not involved in this response. Measurement of cell-mediated responses is difficult *in vitro* except in specialised laboratories. However, delayed hypersensitivity skin tests such as the Mantoux test will demonstrate *in vivo* a patient's cell-mediated immune reactivity to injected antigens.

3. Hypersensitivity Reactions

On some occasions the immune system can actively damage its own host cells either because the immune responses are too violent, poorly regulated and disrupt

surrounding tissue, or because the immune responses are misdirected against self antigens. Gell and Coombs have devised a classification for the hypersensitivity reactions damaging self tissue.

Type I: Immediate Hypersensitivity

IgE antibodies are bound to mast cell surfaces. The union of two adjacent IgE molecules with an antigen such as protein from pollen, provokes release of a number of vasoactive amines from the mast cell granules with consequent intensive local inflammation. The site of this local inflammation dictates the site of the allergic symptoms, and if this occurs in the nose it is manifest as hayfever, in the bronchi as asthma, or in the skin as urticaria.

Type II: Cytotoxic Antibody

Under some circumstances antibody is directed against self components, uniting with these with resultant tissue damage, either by lysis of host cells, by phagocytosis, or by promotion of inflammation. When the antibody is directed against red cells, autoimmune haemolytic anaemia develops, and if the antibody binds to the basement membrane of kidneys then Goodpasture's syndrome results.

Type III: Immune Complex or Arthus Reaction

When antibody is bound to antigen in some ratios it leads to the formation of immune complexes which are not readily removed by the phagocytic cells of the reticuloendothelial system, but rather tend to adhere to endothelial surfaces or basement membranes, initiating intense local inflammatory reactions. Immune complex formation provides the explanation for many pathological changes in a number of diseases including many infections, some forms of glomerulonephritis, polyarteritis, and systemic lupus erythematosus.

Type IV: Cell-Mediated Reactions

Under some circumstances a pure cell-mediated immune reaction is either misdirected against self components or is aimed at extrinsic compounds which have bound to self protein. When this occurs in the skin the phenomenon of contact dermatitis results, with a cell-mediated immune reaction directed against a combination of the sensitising agent and skin proteins.

Chapter II

Antistreptococcal Antibodies

Identification of streptococcal infections is important, largely in relation to the differential diagnosis of carditis and glomerulonephritis. Symptoms suggestive of rheumatic fever or post streptococcal glomerulonephritis require laboratory investigation to establish the patient's recent infection with streptococci. Antistreptococcal antibodies rise following infection so, when infection has terminated and bacterial cultures are negative, a diagnosis can still be made on the antibody response alone.

1. Clinical Conditions Associated with Streptococcal Infection

Antistreptococcal antibody assays should be requested wherever there is a clinical suspicion of acute glomerulonephritis or rheumatic fever.

1.1 Acute Glomerulonephritis (AGN)

Although figures vary widely from epidemic to epidemic, a small percentage of patients seem to develop acute glomerulonephritis after a β-haemolytic streptococcal infection. Acute nephritis follows a streptococcal infection within 10 days in most patients, whereas rheumatic fever has a much longer latent period — 18 days. In patients who develop high antistreptolysin-O (ASO) titres after streptococcal infections the incidence of AGN is greater than among those without high antibody titres.

The incidence of glomerulonephritis is greatest between the ages of 3 and 7 years but it can develop in children below the age of 2; in contrast, rheumatic fever rarely affects children under 2 years of age. Some adults get the disease, but the brunt of the attacks fall on school age and pre-school age children. Males are affected twice as commonly as females. 80% of the patients have a preceding upper respiratory tract

infection but a few have skin infections such as impetigo. A proportion of cases occur without any apparent preceding infection and it has been suggested that in these the infection precipitating the attack of acute nephritis may be viral in origin. It is difficult to be certain about the absence of a preceding streptococcal infection unless very strict care is taken to perform all the antibody tests repeatedly, as well as taking cultures of the appropriate areas of the patient.

1.2 Rheumatic Fever

A significant proportion of patients with untreated epidemic exudative streptococcal pharyngitis develop rheumatic fever. In patient groups treated with antibiotics, rheumatic fever usually occurs only among those patients in whom the treatment fails to eradicate the streptococci. Moreover, the attack rate of rheumatic fever is decreased significantly by eradication of the streptococci as long as 9 days after the onset of symptoms, by which time maximum antibody stimulation has already occurred (Cantanzaro et al., 1954). These observations indicate that persistence of the organism in the host is critically important for the development of rheumatic fever.

The attack rate of rheumatic fever following streptococcal infections is much higher in patients who have already had rheumatic fever than in those who have not. The rate is highest in the first few months after the rheumatic attack and declines thereafter. Rheumatic fever recurrences occur only among those patients who develop a streptococcal antibody response. In those patients with antibody responses the recurrence rate per infection increases with the magnitude of the antibody rise and ranges from 15% in infections characterised by the minimum significant ASO rise to 70% in infections characterised by the maximum antibody rise observed.

Low antibody titres may be detected in patients who present with Sydenham's chorea, probably due to a longer latent period of this manifestation. Lower titres can occur in isolated cases of carditis which may have come to medical attention relatively late in their course.

Deceptively high titres may be due to non-antibody inhibitors of streptolysin-O in hepatitis, biliary obstruction and nephrotic syndrome or to monoclonal IgG in myeloma.

2. Diagnosis

Rheumatic fever and glomerulonephritis are suspected by their clinical presentation and history (table I). Establishment of recent streptococcal infection depends firstly on identification of the organism from throat or skin swabs and secondly and most commonly by serological tests.

During their growth, Group A β-haemolytic streptococci produce a number of extracellular products and enzymes which are antigenic and these promote the

Table I. Comparison between glomerulonephritis and rheumatic fever. Adapted from Youmans et al. (1973) with permission of the authors and editors

Feature	Glomerulonephritis	Rheumatic fever
Age distribution	Can affect any age	Unusual in infancy
Familial factors	Family contacts	Familial tendency
Sex distribution	Predominantly male	No difference
Incidence (following streptococcal infection)	Variable (up to 28%)	Variable (3%)
Subsequent attacks	Rare: may occur after skin infections	Relatively common
Average latent period between infection and first attack	10 days	18 days
Latent period between subsequent infection and exacerbation	Shortened compared with latent period in first attack	Same as latent period in first attack
Relation between degree of ASO increase and incidence of first attack	No relation	Incidence proportional to degree of ASO increase
Time of ASO increase in relation to onset of relapse	After	Before
Serum whole complement and C3	Decreased	Increased
M-types of initiating Group A haemolytic streptococcus	Pharyngeal: 1, 4, 12, 18 Skin: 2, 31, 49, 52-55, 57,60	Any pharyngeal type (skin strains are not rheumatogenic)

development of antibodies by the patient (fig. 1). The respective antibodies block specific enzymatic activity and thus a reduction in activity of a standard enzyme preparation can be used as an indicator for the antibody.

A battery of antistreptococcal antibody assays is necessary to confirm infection in most cases because:

a) Infecting streptococcal organisms vary
b) Different patients produce different patterns of antibody to the same organism.

A single determination of antistreptococcal antibodies is of limited value as results above the normal range may not necessarily indicate recent infection. Antibody titres are elevated for up to 4 to 6 weeks after infection, so serial tests are necessary to detect this rise. Titres will decline very slowly over 6 months. When titres do not fall, the possibility of recurrent infection must be considered.

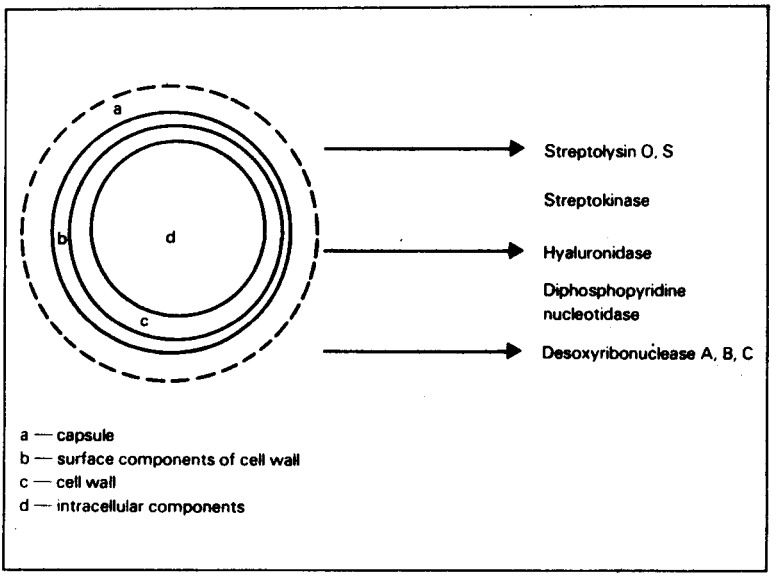

Fig. 1. Diagrammatic representation of the cellular structure and some of the antigenic extracellular products of group A streptococci (adapted with permission of the authors from Wannamaker and Ayoub, 1960).

A single assay, even if used serially, will not detect all patients reacting to streptococcal infection but with multiple tests most patients will be diagnosed. 60 to 80% of Group A streptococcal infections are detected by antistreptolysin O (ASO) alone, about 80 to 90% when both ASO and antihyaluronidase (AHT) are used, and 95% when antistreptokinase (ASK) is estimated in addition.

3. Individual Antibody Assays

3.1 Antistreptolysin-O Test (ASO)

Group A streptococci produce a haemolytic factor, streptolysin-O, which is capable of haemolysing red blood cells. The release of streptolysin-O during an infection stimulates the development of specific antistreptolysin-O antibodies. When a patient's serum containing this antibody is added to streptolysin *in vitro* an antigen-antibody reaction will occur, antistreptolysin neutralising the streptolysin, in part or com-

pletely, depending on the concentration of antibody. This reaction is visualised and measured by addition of red cells to various titres of serum.

Evaluation of the ASO Titre [Normal level less than 300 international units (iu)/ml]

Group A streptococcal infections are common, especially in school-aged children, and significant levels of antistreptococcal antibodies are found in healthy individuals. The new born child has titres similar to the mother but they fall significantly by 6 months of age. Streptococcal infections are uncommon under 2 years of age and children in this age group usually have ASO titres less than 50 iu/ml. School-aged children, particularly in the 5 to 12 year age group, are repeatedly exposed to streptococci and often have ASO titres of up to 300iu/ml in the absence of recent infection. In adults the upper limit of normal is slightly lower, about 125iu/ml. This same pattern is seen with all the antistreptococcal antibodies.

In adults the accepted upper limit of normal for the ASO test is considered to be 200iu/ml. Titres of 300iu/ml or more, rising over 2 to 4 weeks, indicate infection. It must be remembered that rising titres indicate β-haemolytic streptococcal infection and not necessarily rheumatic fever or acute glomerulonephritis.

Deceptively high titres may be due to non-antibody inhibitors of streptolysin-O as in hepatitis, biliary obstruction and conditions with high cholesterol levels or with myeloma protein. Low ASO titres may be found in patients with immunoglobulin deficiencies. Patients with non-suppurative complications of streptococcal infections (e.g. acute rheumatic fever and acute glomerulonephritis) have a higher incidence of elevated ASO titres (80 vs 60%) and higher numerical titres, than patients with uncomplicated streptococcal disease (Glynn, 1975). An important exception to this is in streptococcal pyoderma in which few individuals (25%) demonstrate an elevated ASO titre even if acute glomerulonephritis occurs.

3.2 Antihyaluronidase Test (AHT)

This test is based on the inhibition of streptococcal hyaluronidase by antihyaluronidase in the patient's serum. Excess hyaluronidase is measured by its ability to hydrolyse potassium hyaluronate. The antihyaluronidase titre is the reciprocal of the highest serum dilution showing a clot *in vitro*. The antibody titre of hyaluronidase rises in the second week after infection and falls in 3 to 5 weeks.

Evaluation of the AHT (Normal levels less than 1 : 500)

AHT shows elevated titres in approximately 60% of streptococcal respiratory tract infections.

By itself the AHT is less helpful than the ASO titre but when they are used in association, 80 to 90% of persons with an antecedent streptococcal respiratory infec-

tion will show an elevated titre to at least one of the antigens. Healthy people may exhibit titres of up to 1 : 500 and following infection titres of greater than 1 : 1000 are frequently seen.

3.3 Antistreptokinase Test (ASK)

This test is prepared by using a mixture of streptokinase and streptodornase which has been absorbed onto previously fixed sheep red cells. Addition of serum containing antistreptokinase antibody produces agglutination of the red cells. The antibody level can then be titred.

Evaluation of the ASK Titre (Normal levels are 1 : 640 or below)

The rise in antistreptokinase titre following a streptococcal infection, with or without complications, follows the same general pattern as the ASO titre although studies show that antibody responses occur less frequently to streptokinase. Titres of 1 : 1000 or more are common after infection. ASK values are not affected by hypercholesterolaemia.

3.4 Deoxyribonuclease Test (Anti-DNAse-B)

To determine anti-DNAse-B titres, the patient's serum is inactivated to remove its own DNAse and then diluted and added to standard amounts of antigen. After incubation a DNA substrate is added to each tube and a dye is used as an indicator of remaining intact DNA.

Highly polymerised DNA forms a mucin-like clot and the presence of a clot indicates inhibition of the enzyme DNAse-B (i.e. presence of antibody). Results are read as a colour change in the dye and are expressed as a titre.

Evaluation of the Anti-DNAse B Test (Titres of up to 250 units are found in normal subjects)

In patients with rheumatic fever about 80 % of cases show raised anti-DNAse-B titres and some workers find this figure corresponds to results obtained using the ASO test.

In glomerulonephritis following streptococcal pyoderma 60 % of patients have a positive anti-DNAse test but only about 25 % have elevated ASO titres (Bisno and Ofek, 1974). Some workers have found AHT and ASK are raised more frequently than ASO in patients with pyoderma. As yet no comparison between anti-DNAse-B and ASK or AHT in pyoderma is available. In contrast, when glomerulonephritis follows streptococcal pharyngitis, about 75 % of patients show elevated anti-DNAse-B. If this test is performed in conjunction with other antistreptococcal antibody