IMMUNOPATHOLOGY OF THE EYE

A.H.S.Rahi and A.Garner

Foreword by Norman Ashton F.R.S.

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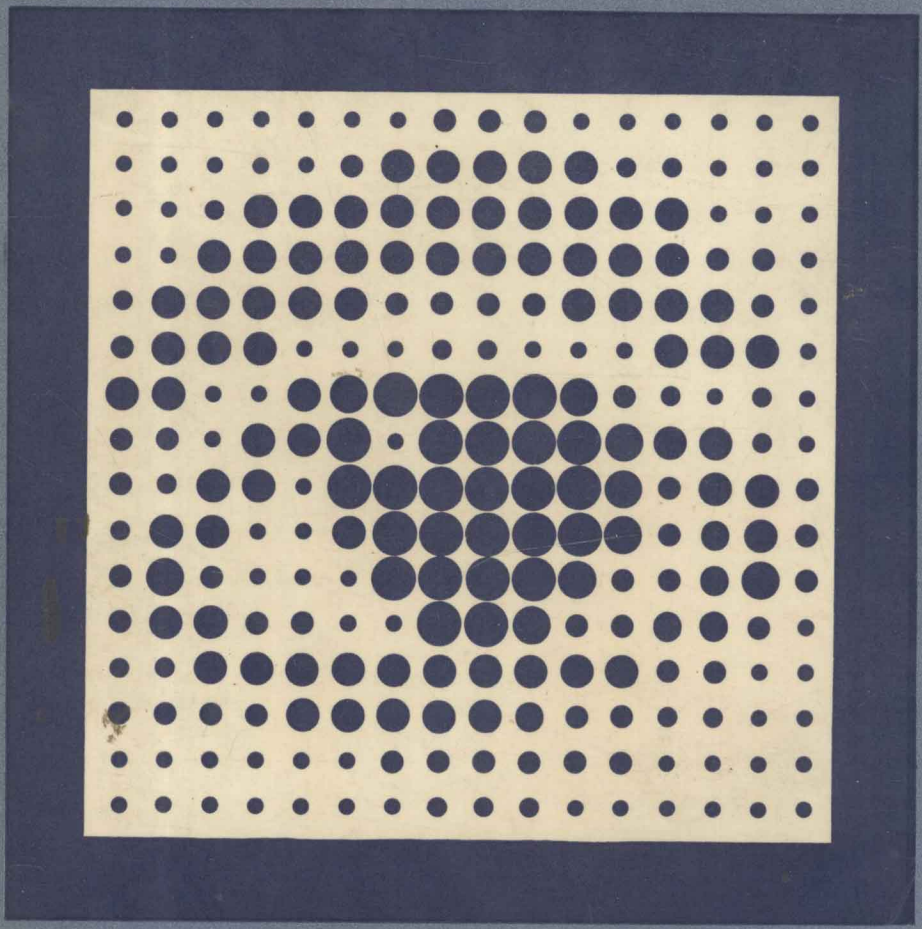

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To our wives
Suarn and Catherine

FOREWORD

While many tributaries to the forward flow of medical knowledge have sprung and continue to spring from the science and practice of ophthalmology, neoteric developments in conceptual and scientific knowledge in the general stream of medicine only slowly filter back. Nor are the reasons difficult to find, for in the past ophthalmologists were more exclusively identified with surgery and more deeply concerned with its techniques than with enigmas of aetiology and pathogenesis, while today, despite the reversal of this tendency, the vast deluge of medical literature with its innumerable reports of highly specialized investigations in widely differing disciplines, each apparently addressed only to its own initiates, has necessarily meant that there are fewer and fewer people with the time and intellectual power to comprehend more than a fraction of the whole. Even those who try with some desperation to do so by scanning the latest publications are soon frustrated by the terminology, which is no sooner introduced than converted into initial abbreviations forming an esoteric jargon, usually without a glossary. So it is that in the modern medical domain some of the most valuable communications, whether they be reviews, chapters or books, are those which set out to address their work, as clearly as may be, to specialists in one sector, informing them of relevant or apparently relevant advances in other sectors. Such communications should, as far as possible, avoid technicalities and trace the subject matter from its basic principles, so arming the reader for the thickets of the later chapters, for it would be idle to pretend that the complexities can be avoided.

Nowhere is this need more apparent than in the relationship between ophthalmology and immunology which have for each other both real and potential significance at present relatively unexplored. Not only is this important for the education of students preparing for higher examinations, and for immunologists seeking to explore the ocular field, but, perhaps more especially, for that large group of ophthalmologists holding senior positions with teaching and research responsibilities, who as students, received a training in immunology

which has become largely out-dated. Although they learnt of the exciting early years when discoveries of new biological phenomena seemed to fall like manna from the skies, of the work of the giants of those days—Pasteur, Koch, v. Behring, Metchnikoff, Roux, Ehrlich, Widal, Landsteiner—it was a time when the actual mechanisms of those old discoveries were still quite obscure and the subject was confused by tenuous hypotheses and provisional conjecture. It was an interim period of consolidation with few major discoveries. The new surge forward amounting to a scientific upheaval has come largely within the last two or three decades and, as so often in research, was the harvest of new techniques and experimental methods which have led to a recognition of the nature of immunoglobulins, the cytological basis of antibody formation, the existence of two distinct populations of lymphocytes, the complex nature of complement and its important biological properties, and the role of the macrophage. The problems of immune tolerance, graft rejection and autoimmunity have been greatly clarified and distinct types of hypersensitivity states have been clearly delineated. Although various authors have sought in a limited way to introduce this wealth of knowledge to the ophthalmic literature, there has until now been no treatise available which is specifically devoted to a detailed consideration of this progress in immunology as related to the particular problems of ocular disease.

This pressing need is most successfully met in this excellent volume by my colleagues Dr A. H. S. Rahi and Dr Alec Garner, both of whom have the exceptional qualifications of a specialized training and long experience in general pathology before concentrating on ocular pathology, deriving from the first a sound knowledge of immunology and from the second a perceptive and discerning appreciation of its relevance to ophthalmology. Here can be found the basic principles simply expounded, the avoidance of technicalities, the known and supposed significance of immunological mechanisms in ocular disease, the rationale of therapy and, admittedly, the complexities.

It is my hope and expectation that this book will find a welcoming place, not only in libraries where it will surely serve to bridge the widening gulf between two disciplines, but also in research and clinical laboratories where it may also consolidate and stimulate new ideas in the evolution of eye disease which still remains one of the most challenging areas in medical research today.

Norman Ashton

PREFACE

A glance at the lists of contents of most ophthalmological journals bears rich testimony to the growing contribution of immunology to the understanding and management of eye disease. Already the literature has accrued to such an extent that any attempt to provide a truly comprehensive account of the work undertaken in this field would place impossible demands on the would-be authors and, one suspects, the readers. Consequently, we have tried to select some of the more significant and clinically important developments and to present them in a way which will be intelligible to both trainee and established ophthalmologists whose understanding of basic immunological processes is, at best, limited. In doing so we are conscious that we have excluded many valuable contributions—contributions which the future may show to be vastly more important than many we have included—and that in trying to make complicated and still evolving concepts less complex we have run the risk of dangerous oversimplification. Nevertheless, it is our hope that the reader will derive an improved understanding of the principles of immunological reactivity and an enhanced appreciation of their place in the spectrum of ocular disease, without being blinded either to the huge gaps in knowledge which remain or to the speculative and incomplete nature of some of the concepts upon which immunology is founded.

The book begins with an introduction to the fundamental aspects of immune and allergic reactions before entering on a rather more detailed account of their contribution to ocular pathology. As pathologists, it is beyond our competence to advise on the treatment of allergic conditions in the eye and, apart from a brief closing section concerned with the mode of action of some of the immunosuppressive agents, we have confined our attention chiefly to the nature of the disease processes themselves.

We are conscious of the tremendous help we have received from Professor Norman Ashton in reviewing the manuscript, making many constructive suggestions and writing the foreword. But perhaps most of all we are grateful

for the enthusiasm he has shown in encouraging us in our self-appointed task. For secretarial assistance of a very high order we are indebted to a number of people, especially Miss Cheryl Wilson and Miss Evelyn FitzGerald. We also appreciate the kindness of Dr René Barry in providing the specimen of corneal tissue depicted in Fig. 6.3 and of Dr John Ball in making useful comments on the chapter dealing with the eye in systemic immune disorders. Figs 7.6 and 11.4(b) and (c) are reproduced by courtesy of the Editor of the *British Journal of Ophthalmology*, and Figs 11.5 and 11.6 by courtesy of the Editor of the *Transactions of the Ophthalmological Society of the United Kingdom*. It is also a pleasure to acknowledge the patience of our publishers and in particular the assistance provided by their staff in bringing our typescript to print.

Finally we want to record our appreciation of the privations inflicted on our families which the writing of this book has entailed. Theirs was the hardest part and we are all too aware of the lack of attention they have had to endure.

A. H. S. Rahi
A. Garner

CONTENTS

ONE

Basic immune mechanisms

A fundamental truth of the animal kingdom is contained in the statement 'kill or be killed'. A multitude of exogenous microorganisms present a constant threat to higher forms of life, while spontaneous genetic mutations create a risk of potentially harmful cells being developed from within. Correspondingly, the emergence of the vertebrate phyla has been accompanied by the evolution of an elaborate system for the recognition and elimination of both invading pathogens and abnormal mutant cells. The growing appreciation of the central role of lymphocytes, hitherto regarded as having the appearance of phlegmatic spectators in inflammatory processes (Rich 1936), in this primarily protective mechanism has had far-reaching consequences in the understanding and management of many pathological conditions.

Development of the lymphoid system

Phylogenetic studies indicate that the ability to mount a specific immune response to potentially harmful agents is confined to vertebrates and is correlated with the presence of lymphoid tissue (Papermaster *et al.* 1963). Although recently it has been shown that some invertebrates also can produce an immune reaction to foreign proteins in the absence of lymphoid tissue, this property is probably non-specific and appears to be related to the direct activity of phagocytic cells (Hildeman & Clem 1971).

The lymphoid tissues of man and other mammals can conveniently be divided into at least two classes (a third has been postulated but its source is obscure) which have distinct embryological origins and functions (Fig. 1.1).

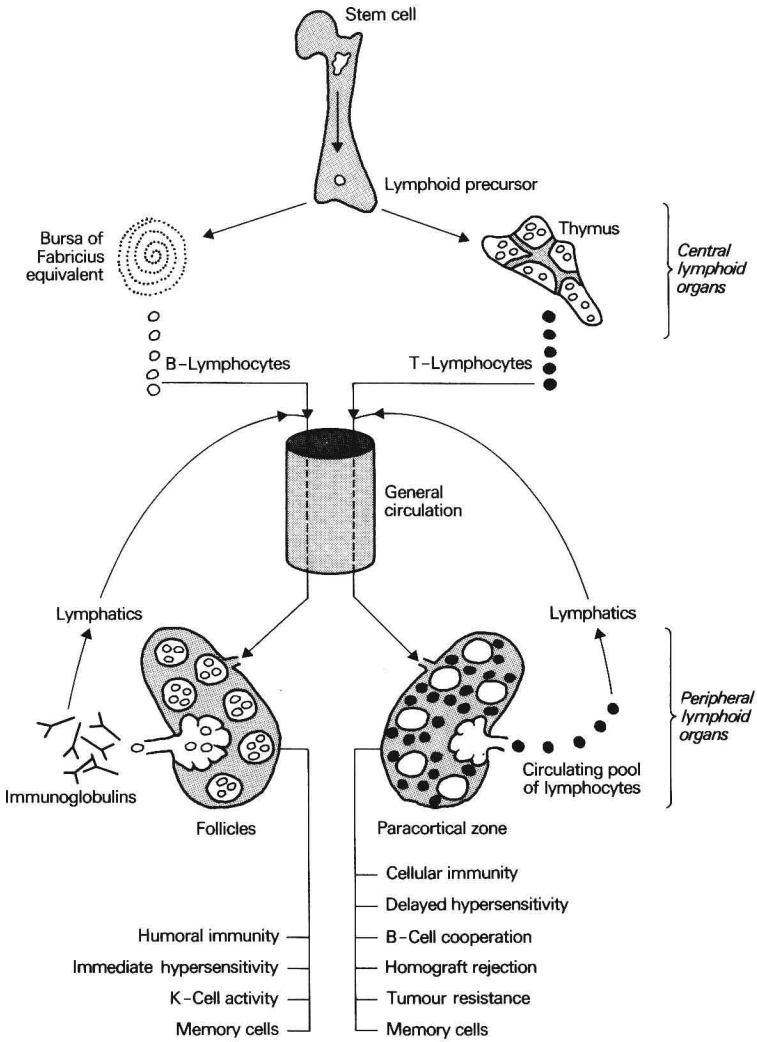

Fig. 1.1. Origin and development of the lymphoid system. The peripheral lymphoid organs include the spleen and lymphocyte aggregates in the gut mucosa in addition to lymph nodes.

THYMUS-DEPENDENT LYMPHOCYTES

It is probable that most lymphocytes originate in the bone marrow during the growth of the fetus. A proportion subsequently come under the influence of the thymus to the extent that they become equipped to be the

mediators of direct cellular immunity. Although the means whereby this modification or instruction is effected is not entirely clear, it appears probable that the lymphocytes need to pass through the thymus and there is some evidence that during their sojourn they are acted upon by a hormone which may be a product of thymic epithelium, known as thymosin (Goldstein *et al.* 1970, Goldstein 1974).

On emerging from the thymic influence the lymphocytes are immunologically competent and capable of responding to antigen. They are then referred to variously as thymus-dependent, thymus-processed or thymus-derived cells, or, more simply, as T-lymphocytes.

From the thymus the lymphocytes re-enter the circulation and many become seeded in the paracortical zones of the peripheral lymph nodes, the periarteriolar regions of the spleen and the interfollicular zones of such submucosal lymphoid aggregates as the tonsils and intestinal Peyer's patches. The activity of the thymus continues beyond the fetal and neonatal periods into adult life, albeit at a much reduced level (Metcalf 1965).

Much of our understanding of thymic function has come from classical experiments on mice showing that neonatal thymectomy is associated with an inability to mount the cell-mediated responses necessary for delayed hypersensitivity reactions and tissue allograft rejection, and with a failure of the paracortical areas of the peripheral lymphoid organs to develop (Miller 1962).

BURSA-DEPENDENT LYMPHOCYTES

The bursa of Fabricius shares with the thymus the property of being derived from embryonic gut epithelium and has an analogous effect on antibody-forming lymphocytes. It is a structure apparently confined to birds and its parallel in mammals has not been identified with certainty, although some observers now suspect that the bursa-equivalent in man is likely to reside initially in the liver and subsequently in the bone marrow (Raff 1973). Bursectomy in the chicken is followed by a marked impairment in the ability to produce antibodies.

When the lymphocytes have been instructed by the bursa, or its equivalent, they migrate to the peripheral sites of lymphoid activity and are responsible for the cortical follicles of lymph nodes and submucosal lymphoid tissue, as well as contributing to the periphery of the white pulp of the spleen. Terms commonly applied to these cells are bursa-dependent, bursa-derived, bursa-equivalent or B-lymphocytes.

OTHER TYPES OF LYMPHOCYTE

There is some reason to believe that there is a third class of lymphocyte which does not itself react to antigenic stimulation but which acts in a non-specific fashion on antibody-coated targets (Perlmann *et al.* 1972). Lymphocytes of this kind have been called null cells, antibody-dependent lymphocytes and killer or K-cells (Harding *et al.* 1971, Greenberg *et al.* 1973). As yet their origin is obscure.

A recent study has concluded that the peripheral blood also includes some lymphocytes which have both B- and T-cell properties but their mode of derivation and functional significance is unknown (Dickler *et al.* 1974).

Antigen

Substances which provoke a specific lymphocyte response are called antigens. Usually they are of exogenous origin but occasionally may be derived from the body's own tissues. Also, while the response they elicit is generally positive and aimed at their elimination, in some circumstances, especially when present in excessively high or low concentrations, antigens can inactivate or paralyse the immune response (Dresser & Mitchison 1968).

GENERAL PROPERTIES

The vast majority of antigens are proteins, although high molecular weight polysaccharides can also fulfil this function. Proteins with molecular weights as low as 4000 can be antigenic (Sela *et al.* 1962), examples of clinical importance being insulin (mol. wt 6000) and glucagon (mol. wt 3800), but in general antigenicity is most evident among the larger proteins. Polysaccharides with molecular weights of less than 100 000 are not normally antigenic. Lipids as such are never antigenic although they may form part of antigenic lipoproteins and lipopolysaccharides.

Additionally, there are certain chemical substances which, while not antigenic in themselves, can bind to body proteins to form a complex that will stimulate an immune response against the non-proteinous moiety. This type of substance is usually of low molecular weight, dinitrochlorobenzene (mol. wt 203) being a typical example, and is known as a hapten or incomplete antigen. In theory it is possible to render any small molecule

antigenic by linking it covalently to a protein or polypeptide and much of our knowledge of the requirements for antigenicity and the nature of antibody has resulted from the use of such conjugates.

It is considered that, to be antigenic, a molecule must possess structural rigidity and the inclusion of aromatic components such as tyrosine is of importance in this respect (Pappenheimer 1938, Sela & Arnon 1960). For this reason, possibly, ovalbumin is a strong antigen, haemoglobin is a weak one, and gelatin is non-antigenic. Antigenicity is also a function of the size, shape and electrochemical forces of the side chains of the amino acid components of the protein. Furthermore, it is necessary to appreciate that antigenicity is a property created by the interaction between the substance in question and the host immune mechanism and can vary, not only from species to species, but also within a species. Thus, an individual's own tissue proteins usually provoke no response whatsoever but if given to another subject, as in tissue transplant surgery, the same proteins can give rise to a marked immune response.

If the antigenicity of a molecule can be thought to reside in the possession of rigid electrically-charged side or end chains its specificity probably depends on the spatial distribution of these determinant sites. In other words, the possession of certain amino acids determines the capacity to evoke an immune response while their sequence in the protein molecule governs the overall specificity of the response. In the case of tissue polysaccharides, such as blood group antigens, specificity is conferred by minor differences in the composition of the component oligosaccharides.

CLASSIFICATION

Because of modifications to the responsiveness of the immune mechanism the body's own proteins and polysaccharides do not normally elicit any reaction. Exceptionally, however, this tolerance of self is lost and the host's tissues behave as *autoantigens*. Differences in genetic constitution between the members of a species are reflected in minor but important differences in a majority of tissue proteins and these behave as *homologous antigens* if allowed to come into contact with the lymphoid tissues of other members of the same species. *Isoantigens* are a special type of homologous antigen present in some but not all members of a species and, as such, include blood group substances and transplantation antigens. Between one animal species and another there is frequently a wide difference in protein structure and this results in *heterologous antigens* which are liable to elicit a more marked response than attends many homologous antigens. Thus,

injections of horse serum usually meet with a more vigorous immune response in man than do injections of homologous serum.

Some antigens, such as those responsible for tissue transplant rejection, are common to all the cells of an individual but others, such as thyroglobulin, are *organ-specific*. Also, antigens may be either *species-specific* or, as in the case of many lens antigens, be shared within a range of sbecies. *Individual-specific* antigens, that is antigens peculiar to the host and not present in even closely related members of the same species, do not occur under normal circumstances governed by the genetic transmission of protein coding patterns. They can develop, however, as a result of genetic mutation and be a feature of some malignant neoplasms.

Antigens which are identical or very closely related have been described in the tissues of widely different species. Known as *heterophile antigens*, an example is the Forssman antigen which gives rise to an antibody able to combine with tissue extracts from many species as disparate as mice and men. Closely related antigens have also been recognized in patients with rheumatic fever wherein antibodies formed against a specific strain of streptococci react with an analogous antigenic constituent of human myocardium: this type of behaviour is referred to as a cross-reactivity.

Adjuvants

Adjuvants are substances which enhance the immune response to antigen and their presence enables many weak antigens to evoke high orders of antibody production. Among the first applications of adjuvant was the use of alum to improve the antigenicity of diptheria vaccines and, subsequently, many other substances with an adjuvant effect have been employed in both clinical immunization programmes and experimental immunology.

TYPES OF ADJUVANT

A variety of substances have adjucant properties. Some, such as alum, are inert particulate agents which become coated with antigen. Bacteria or their products can also behave as adjuvants and one of the most frequently used adjuvants in experimental situations is Freund's complete adjuvant, which consists of dead tubercle bacilli suspended in a water-in-oil emulsion. There is also a rather less effective form of Freund's adjuvant which is devoid of the mycobacterial component.

Because of the efficacy of adjuvants in promoting immunological responsiveness, there have been many attempts to identify an analogous effect in the emergence of spontaneous autoimmune disease and, although there is no conclusive data, it is possible that viral infection, which may often be subclinical or cryptogenic, can act in this capacity (Witebsky 1964). There is, moreover, a frequent association of autoimmune phenomena with granulomatous bacterial infections, such as syphilis, and it is possible that these disorders simulate the behaviour of Freund's adjuvant. The presence of a range of autoantibodies in leprosy, including antinuclear factors, rheumatoid factor and thyroglobulin antibody, also lends support to this hypothesis (Allison 1973).

MODE OF ACTION

The functioning of adjuvants is only poorly understood although some factors of importance have emerged.

Depot effect

It is well-recognized that poorly soluble antigens produce a more pronounced immunological response than those which are readily soluble. Correspondingly, it has been suggested that the effect of some adjuvants is to retain antigen in the tissues and prolong the period of lymphoid stimulation (Holt 1951). Such retention of antigen is largely associated with the formation of a granuloma at the site of injection, whereby the antigen is sequestered within macrophages and only slowly released, and it has been shown that antigen in the presence of adjuvant will persist for 20–30 days as compared with 5–10 days when given alone.

The importance of slow release of antigen is further demonstrated by the good antibody response to foreign protein injected into the vitreous or cornea of an experimental animal (Thompson & Olsen 1950, Parks *et al.* 1961).

Carrier effect

As previously remarked, immunologically inert substances (haptens) can sometimes be made strongly antigenic by attaching them to a carrier protein and, whereas the specificity of the resultant antibody is determined by the hapten, it is the carrier which controls the ability of the immune recognition system to respond. Thus it appears possible that

receptors on lymphocytes responsive to the carrier protein (belonging to the T-cell series) are responsible for the initial reaction and that the carrier–hapten complex is then transferred to the antibody-forming cells of the B-lymphocyte series. Conceivably adjuvant could act similarly to deliver potential antigen to the antibody-forming cells by behaving as a carrier. Protein adjuvants, including mycobacteria, could possibly come into this category.

Increased antigen exposure

By adherence to particulate matter the surface area covered by antigenic determinants available for interaction with the lymphoid system can be increased. Alum-precipitated diphtheria toxoid may operate in this way.

Preferential lymphatic drainage

The use of water-in-oil emulsions as adjuvants may succeed because of the delayed diffusion of soluble antigen and consequent facilitation of dispersion through the lymphatics. This serves to promote increased contact between the antigen and responsible lymphocytes in the draining lymphoid tissues.

Thymus-dependent lymphocyte stimulation

Adjuvant appears to have a non-specific stimulant effect on thymus-dependent lymphocytes and to enhance the cell-mediated responses to a majority of antigens.

Removal of tolerance

Autologous proteins are generally recognized by the immune recognition system as self and evoke no response. This tolerance can, however, be broken down in the neonatal period by the use of adjuvants and recent studies suggest that in these circumstances there is non-specific stimulation of the thymus-dependent lymphocytes, which then proliferate and cooperate with the antibody-forming cells to produce a range of autoantibodies (Allison 1973).

B-Lymphocyte function

Immunologically competent B-lymphocytes and their plasma cell