Essential Immunology

IVAN M. ROITT

THIRD EDITION

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Acknowledgements

First edition

While not wishing to saddle my colleagues with responsibility for some of the wilder views expressed in this book it would be ungrateful of me not to acknowledge with pleasure the helpful discussions I have had with Jonathan Brostoff, George Dick, Deborah Doniach, Frank Hay, Leslie Hudson, Gerald Jones and John Playfair. I would like to express my appreciation to my secretary, Gladys Stead, who helped to prepare and assemble the manuscript with her usual impeccable expertise and who always encouraged me when my authorship seemed to be faltering. I also wish to acknowledge my debt to Valerie Petts for her excellent help with the photographs. My thanks also to the many people who supplied material for the illustrations; they are acknowledged at the appropriate place in the text. In particular, Bill Weigle kindly let me have unpublished information. Finally let me say that the pain of converting blank paper to written manuscript at home was made bearable by the loving support and understanding of my wife and family.

Second edition

The necessity for a second edition has been dictated by the breakneck increase in immunological knowledge since this book was first written—clearly the subject has too many adherents! My colleagues will know how much I have appreciated their invaluable discussions; particularly I must mention Ita Askonas, Jonathan Brostoff, Deborah Doniach, Arnold Greenberg, Hilliard Festenstein, Frank Hay, M. Hobart, Leslie Hudson, D. L. Brown, John Playfair and Mac Turner. Once again I would have been lost without the admirable help of my secretary, Gladys Stead. Even the publishers have been nice!

Third edition

The indecent speed at which we lurch forward has necessitated radical revision of many sections in this new edition. The

anatomical basis of the immune response, immunity to infection and the biological significance of the major histocompatibility complex have all been given fuller treatment. A summary has been added to the end of each chapter which should be a help to those poor souls for whom circumstances make revision essential. The index has received serious attention and I hope it will be of greater value. I am most grateful to the many colleagues whose wisdom I have sought: Franco Bottazzo, Jonathan Brostoff, Peter Campbell, Debroah Doniach, Hilliard Festenstein, Peter Gould, Frank Hay, Peter Lachman, Ian McConnell, John Playfair and Martin Raff. Finally, my thanks are due to Miss Christine Meats for her most able and cheerful secretarial assistance.

Contents

1 Introduction

Memory, specificity and the recognition of 'non-self'—these lie at the heart of immunology. Our experience of the subsequent protection (*immunity*) afforded by exposure to many infectious illnesses can in fact lead us to this view.

We rarely suffer twice from such diseases as measles, mumps, chicken-pox, whooping cough and so forth. The first contact with an infectious organism clearly imprints some information, imparts some *memory*, so that the body is effectively prepared to repel any later invasion by that organism. This protection is provided by antibodies evoked as a response to the infectious agent behaving as an antigen (figure 1.1). Combination with antibody leads to elimination of the antigen.

By following the production of antibody on the first and second contacts with antigen we can see the basis for the development of immunity. For example, when we inject a bacterial product such as staphylococcal toxoid into a rabbit, several days elapse before antibodies can be detected in the blood; these reach a peak and then fall (figure 1.2). If we now allow the animal to rest and then give a second injection of toxoid, the course of events is dramatically altered. Within two to three days the antibody level in the blood rises steeply to reach much higher values than were observed in the *primary response*. This *secondary response* then is characterized by a more rapid and more abundant production of antibody resulting from the 'tuning up' or priming of the antibody-forming system to provide a population of memory cells after first exposure to antigen.

Vaccination utilizes this principle by employing a relatively harmless form of the antigen (e.g. a killed virus) as the primary stimulus to imprint 'memory'. The body's defences are thereby alerted and any subsequent contact with the virulent form of the organism will lead to a secondary response with an early and explosive production of antibody which will usually prevent the infection from taking hold.

Specificity was mentioned earlier as a fundamental feature of the immunological response. The establishment of memory or

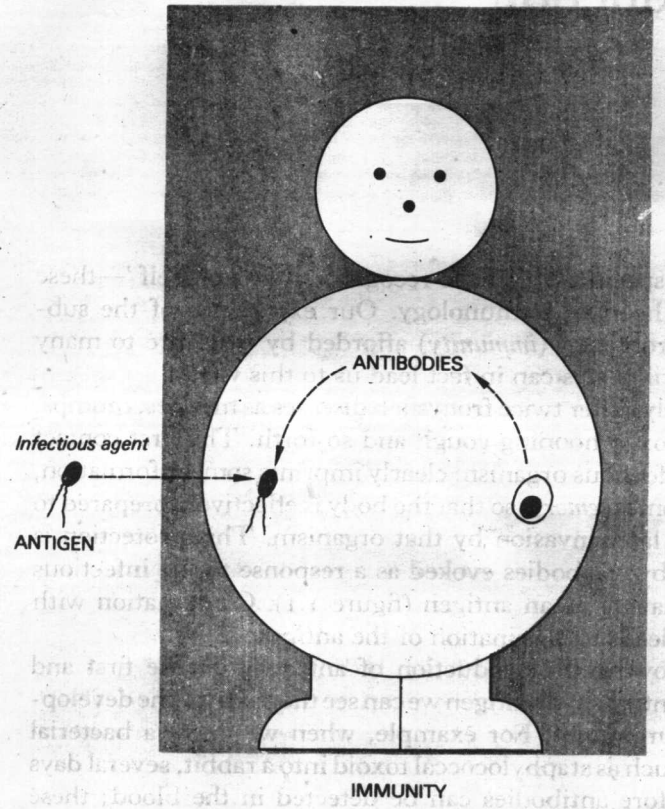


FIGURE 1.1. Antibodies (*anti*-foreign *bodies*) are produced by host white cells on contact with the invading micro-organism which is acting as an antigen (i.e. *gen*erates *anti*bodies). The individual may then be immune to further attacks.

immunity by one organism does not confer protection against another unrelated organism. After an attack of measles we are immune to further infection but are susceptible to other agents such as the polio or mumps viruses. The body can, in fact, differentiate specifically between the two organisms.

This ability to recognize one antigen and distinguish it from another goes even further. The individual must also recognize what is foreign, i.e. what is '*non-self*'. The failure to discriminate between 'self' and 'non-self' could lead to the synthesis of antibodies directed against components of the subject's own body (*autoantibodies*) which in principle could prove to be highly embarrassing. On purely theoretical grounds it seemed to Burnet and Fenner that the body must develop some mechanism whereby 'self' and 'non-self' could be distin-

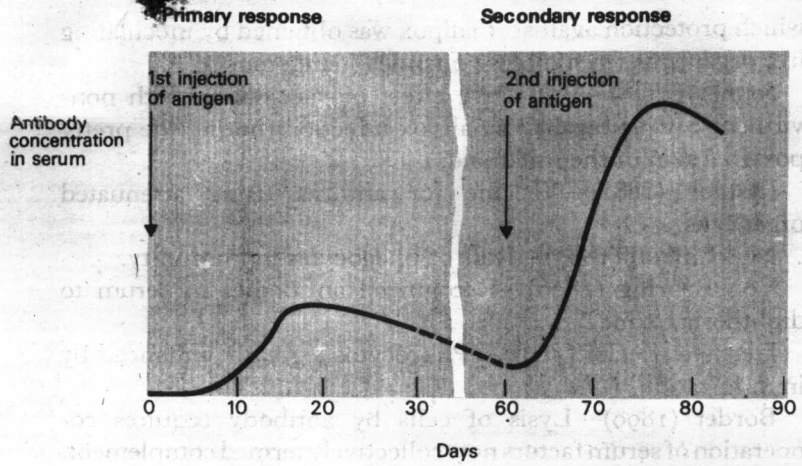


FIGURE 1.2. *Primary and secondary response.* A rabbit is injected on two separate occasions with staphylococcal toxoid. The antibody response on the second contact with antigen is more rapid and more intense.

guished, and they postulated that those circulating body components which were able to reach the developing lymphoid system in the perinatal period could in some way be 'learnt' as 'self'. A permanent unresponsiveness or tolerance would then be created so that as immunological maturity were reached there would be an inability to respond to 'self' components. As we shall see later, these predictions have been amply verified.

It is worth emphasizing that the lower animal forms possess so-called 'non-specific immunity' mechanisms such as phagocytosis of bacteria by specialized cells, which afford them protection from infecting organisms. The *adaptive* immune response in higher animals which we have been discussing, has evolved to provide more effective defence in that appropriate immunological cells concentrate their energies on the particular agents infecting the body at any one time and the specific antibodies which they synthesize greatly speed up the disposal of these organisms by facilitating their adherence to phagocytic cells (see chapter 7). In other words the specific adaptive immune response operates to a considerable extent by increasing the efficiency of the non-specific immunity systems.

Some historical perspectives

Space does not allow more than a cursory survey of some of the outstanding contributions to the development of immunology.

India and China (ancient times)—Practice of 'variolation' in

which protection against smallpox was obtained by inoculating live organisms from disease pustules (dangerous!).

Jenner (1798)—Protective effect of vaccination with non-virulent cowpox against smallpox infection (noting the pretty pox-free skin of the milkmaids).

Pasteur (1881)—Vaccine for anthrax using attenuated organisms.

Metchnikoff (1883)—Role of phagocytes in immunity.

Von Behring (1890)—Recognized antibodies in serum to diphtheria toxin.

Denys & Leclef (1895)—Phagocytosis greatly enhanced by immunization.

Bordet (1899)—Lysis of cells by antibody requires co-operation of serum factors now collectively termed complement.

Landsteiner (1900)—Human ABO groups and natural isohaemagglutinins.

Richet & Portier (1902)—Anaphylaxis (opposite of prophylaxis).

Wright (1903)—Relation of opsonic activity to phagocytosis.

Zinsser (1925)—Contrast between immediate and delayed-type hypersensitivity.

Heidelberger & Kendall (1930–35)—Quantitative precipitin studies on antigen–antibody interactions.

Later work is referred to in subsequent chapters but note in particular the finding that immunization leads to more effective phagocytosis. At this stage we can examine the work of Heidelberger and Kendall and its implications in more detail and with some benefit.

The classical precipitin reaction

When an antigen solution is mixed in correct proportions with a potent antiserum, a precipitate is formed. Quantitative analysis of this interaction by the method shown in figure 1.3 gives both the antibody content of the immune serum and also an indication of the valency of the antigen. This can vary enormously depending on the antigen, its size, and the species making the antibody. With rabbit antisera, ovalbumin may have a valency of 10 and human thyroglobulin as many as 40 combining sites on its surface. By splitting antigens into large fragments with proteolytic enzymes it has become clear that the separate combining areas on the surface of a given protein (called antigenic *determinants* or *epitopes*) are by no means identical.

It will be noted from the precipitin curve in figure 1.3 that as

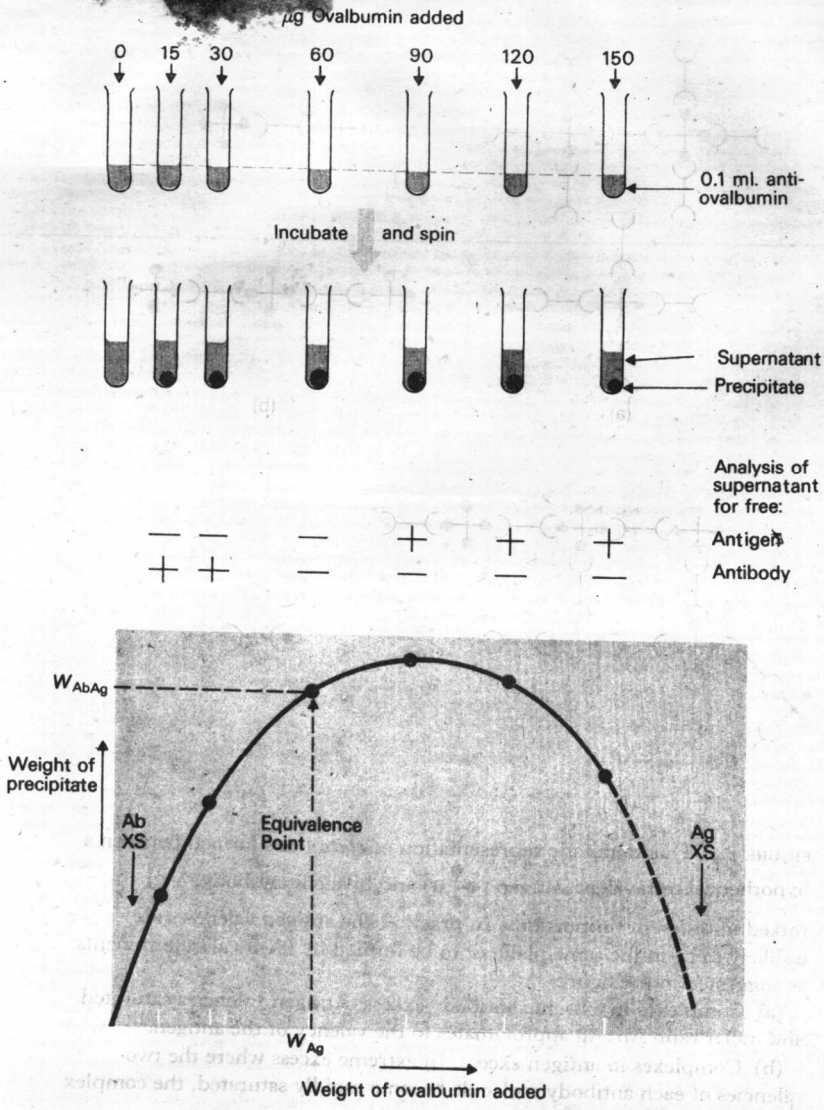


FIGURE 1.3. Quantitative precipitin reaction between rabbit anti-ovalbumin and ovalbumin (after Heidelberger & Kendall). Increasing amounts of ovalbumin are added to a constant volume of the antiserum placed in a number of tubes. After incubation the precipitates formed are spun down and weighed. Each supernatant is split into two halves: by adding antigen to one and antibody to the other, the presence of reactive antibody or antigen respectively can be demonstrated. The antibody content of the serum can be calculated from the equivalence point where no antigen or antibody is present in the supernatant. All the antigen added is therefore complexed in the precipitate with all the antibody available and the antibody content in 0·1 ml of serum would therefore be given by $(W_{AgAb}-W_{Ag})$. Analysis of the precipitate formed in antibody excess (AbXS), where the antigen-combining sites are largely saturated, gives a measure of the molar ratio of antibody to antigen in the complex and hence an estimate of the antigen valency.

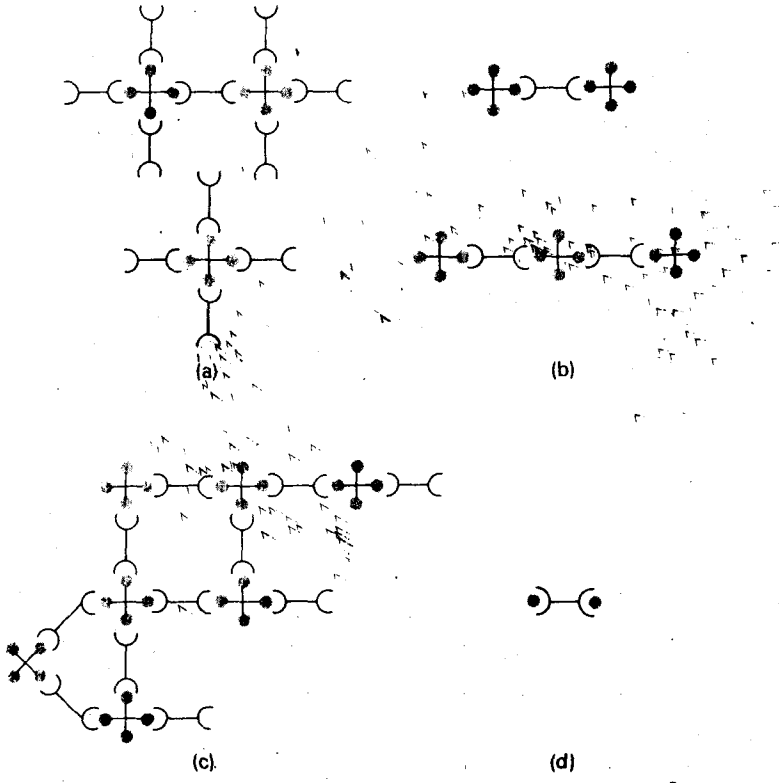


FIGURE 1.4. Diagrammatic representation of complexes formed between a hypothetical tetravalent antigen (●✚●) and bivalent antibody (⟩–⟨) mixed in different proportions. In practice, the antigen valencies are unlikely to lie in the same plane or to be formed by identical determinants as suggested in the figure.

(a) Complexes in extreme antibody excess. Antigen valencies saturated and molar ratio Ab : Ag approximates to the valency of the antigen.

(b) Complexes in antigen excess. In extreme excess where the two valencies of each antibody molecule become rapidly saturated, the complex Ag_2Ab tends to predominate.

(c) Large three-dimensional lattice obtained in typical immune precipitate.

(d) Monovalent antigen binds but is unable to cross-link antibody molecules.

more and more antigen is added, an optimum is reached after which consistently less precipitate is formed. At this stage the supernatant can be shown to contain soluble complexes of antigen (Ag) and antibody (Ab), many of composition Ag_4Ab_3, Ag_3Ab_2 and Ag_2Ab. In extreme antigen excess (AgXS, figure 1.3) ultracentrifugal analysis reveals the complexes to be mainly of the form Ag_2Ab, suggesting that the rabbit antibodies studied are bivalent (figure 1.4; see also figures 2.6 and 2.7). Between these extremes the crosslinking of antigen and antibody will

generally give rise to three-dimensional lattice structures, as suggested by Marrack, which coalesce to form large precipitating aggregates.

The basis of specificity

Much of our understanding of the factors governing antigen specificity has come from the studies of Landsteiner and of Pauling and their colleagues on the interaction of antibody with small chemically defined groupings termed *haptens*, a typical example being *m*-aminobenzene sulphonate (figure 1.5). Whereas an antigen will both evoke antibody formation and combine with the resulting antibody, *a hapten is defined as a small molecule which by itself cannot stimulate antibody synthesis but will combine with antibody once formed.*

The problem of how to produce these antibodies was solved by coupling the haptens to proteins which acted as 'carriers'. It then became possible to relate variations in the chemical structure of a hapten to its ability to bind to a given antibody. In one experiment, antibodies raised to *m*-aminobenzene sulphonate were tested for their ability to combine with *ortho*, *meta* and *para* isomers of the hapten and related molecules in which the sulphonate group was substituted by arsonate or carboxylate (figure 1.6). The results are summarized in table 1.1. The hapten with the sulphonate group in the *ortho* position combines somewhat less well with the antibody than the original *meta* isomer, but the *para*-substituted compound (chemically similar to the *ortho*) shows very poor reactivity. The substitution of arsonate for sulphonate leads to weaker combination with the antibody; both groups are negatively charged and have

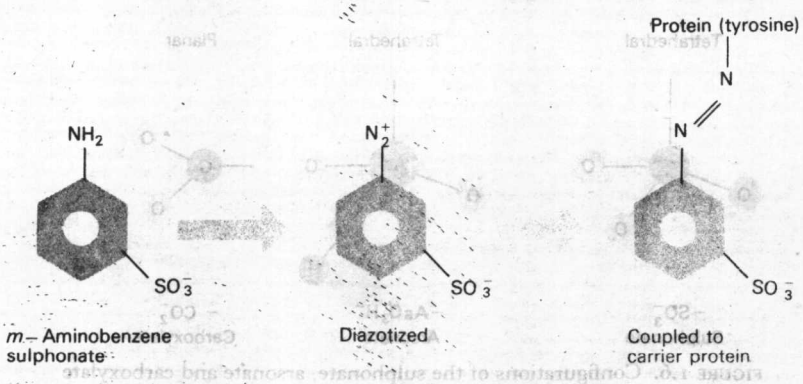
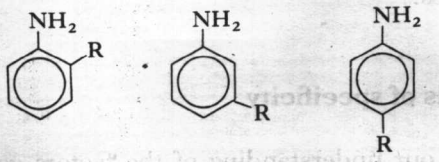


FIGURE 1.5. Coupling of hapten to protein by diazotization.

TABLE 1.1. Effect of variations in hapten structure on strength of binding to *m*-aminobenzene sulphonate antibodies



	ortho	*meta*	*para* isomers
R = sulphonate	++	+++	±
R = arsonate	−	+	−
R = carboxylate	−	±	−

Strength of binding is directly graded from negative (−) to very strong (+++). Since free haptens can only combine with one antibody-combining site and cannot therefore cross-link, they form only soluble complexes; their binding strength was assessed through their ability to inhibit precipitation by antibody of a new carrier protein substituted with several of the original hapten (*m*-aminobenzene sulphonate) groups per molecule (from Landsteiner K. & van der Scheer J. *J.exp.Med.* 1936, **63**, 325)

a tetrahedral structure but the arsonate group is larger in size and has an extra H atom (figure 1.6). The aminobenzoates in which the sulphonate is substituted by the negatively charged but planar carboxylate group show even less affinity for the antibody. It would appear that the overall *configuration* of the hapten is even more important than its *chemical* nature, i.e. the hapten is recognized by the overall three-dimensional shape of its outer electron cloud as distinct from its chemical reactivity. The production of antibodies against such strange moieties as benzene sulphonate and arsonate becomes more comprehensible if they are thought to be directed against a particular electron-cloud shape rather than a specific chemical structure. This view is consistent with the nature of antigen–antibody binding which is known not to involve covalent linkages.

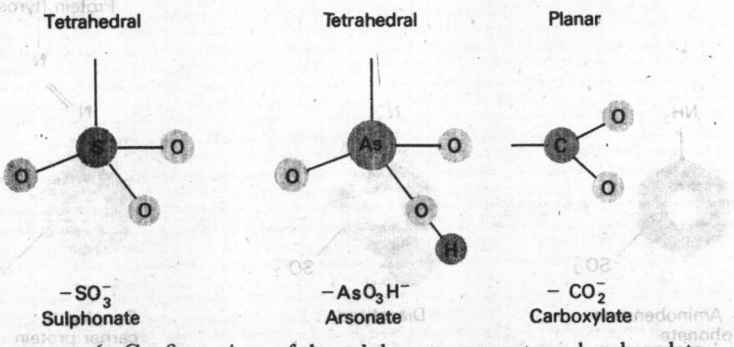


FIGURE 1.6. Configurations of the sulphonate, arsonate and carboxylate groups.

THE FORCES BINDING ANTIGEN TO ANTIBODY

It should be stressed immediately that the forces which hold antigen and antibody together are in essence no different from the so-called 'non-specific' protein–protein interactions which occur between any two unrelated proteins (or other macromolecules) as, for example, human serum albumin and human transferrin. These intermolecular forces may be classified under four headings:

(a) *Electrostatic*

These are due to the attraction between oppositely charged ionic groups on the two protein side chains as, for example, an ionized amino group (NH_3^+) on a lysine of one protein and an ionized carboxyl group ($—COO^-$) of, say, aspartate on the other (figure 1.7a). The force of attraction (F) is inversely proportional to the square of the distance (d) between the charges, i.e.

$$F \propto 1/d^2$$

Thus as the charges come closer together, the attractive force increases considerably: if we halve the distance apart, we quadruple the attraction. Dipoles on antigen and antibody can also attract each other. In addition, electrostatic forces may be generated by charge transfer reactions between antibody and antigen; for example an electron-donating protein residue such as tryptophan could part with an electron to a group such as dinitrophenyl which is electron-accepting thereby creating an effective $+1$ charge on the antibody and -1 on the antigen.

(b) *Hydrogen bonding*

The formation of the relatively weak and reversible hydrogen bridges between hydrophilic groups such as .OH, $.NH_2$ and .COOH depends very much upon the close approach of the two molecules carrying these groups (figure 1.7b).

(c) *Hydrophobic*

In the same way that oil droplets in water merge to form a single large drop, so non-polar, hydrophobic groups such as the side chains of valine, leucine and phenylalanine, tend to associate in an aqueous environment. The driving force for this hydro-