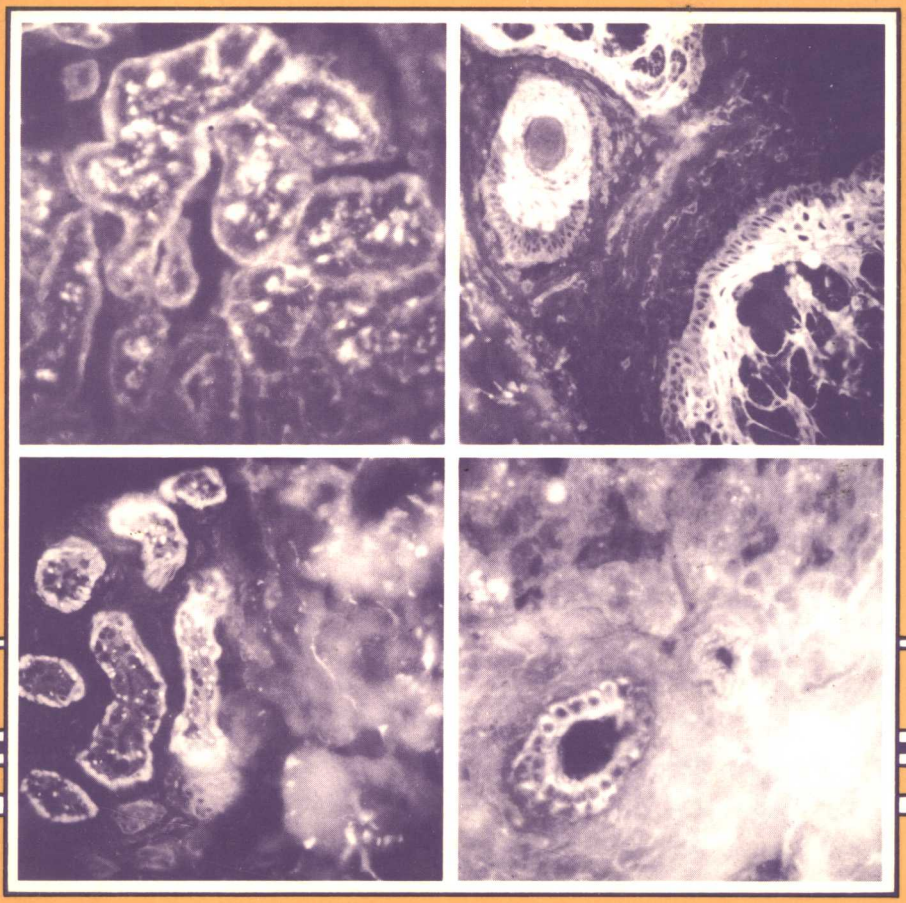
Immunological Aspects of Infectious Diseases

Edited by G. Dick



Immunological Aspects of Infectious Diseases

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Preface

In the first place may I say how grateful I and others are to those who have contributed chapters for this book; all of them are well known for their research on the subject on which they have written and each has indicated the background to his own specialist field by providing an extensive bibliography giving this book a total reference list of over 2,500.

Although there have been enormous advances in immunology over recent years, much of the new knowledge in relation to infectious diseases was scattered over the world's literature and is now brought together in a single volume. Furthermore, while a number of previously unknown infectious diseases have recently been discovered (e.g. Marburg and Lassa virus infections and Legionnaires' disease) to which many new techniques have been applied, there seemed to be no clear statement of the rationale for their use, or for their further exploitation in some of the more common infectious diseases which are discussed in this book.

The host–parasite interaction, as pointed out by R. J. Elin, may be of no consequence, or it may result in colonization, subclinical infection, symptomatic disease, or death, and the first chapter of this book focuses on some of the *non-specific* factors which may affect the response of the host to invading micro-organisms. The normal responses to infections with viruses and bacteria which are the stock-in-trade of those interested in infectious disease have not been separately treated but are summarized in the introduction to Chapter 10. On the other hand, while the host reactions to fungal infections mirrors that to other micro-organisms, the nature of these reactions are largely determined by the *type* of infection, which is discussed by D. W. R. Mackenzie, as well as individual immune responses in some of the commoner mycoses and the application of immunodiagnostic tests to the recognition of the diseases which they produce and their epidemiology.

To most of us, the objective of understanding the immunological response to a parasite is to increase our knowledge of the pathogenesis of the disease in question, so that it may be controlled or prevented by treatment or by vaccines. As far as protozoal infections are concerned, J. P. Ackers, in critically reviewing the immune responses to the major protozoal infections of man, reminds us that when the immunological techniques which had been so

successful in the control of microbial infections were applied to the parasitic infections it was soon realized that there was something special about the immune response to protozoa. He discusses these responses in detail with particular reference to antigenic variations and persistence of the parasite in the host. This important contribution leads to many questions on the methods of controlling parasitic infections of man and animals which are such enormous problems in developing countries.

It is only in the last few years that various defects in host response have been identified and their importance defined, particularly in relation to recurrent infections. The *primary* immunodeficiencies are summarized by J. L. Meakins and N. V. Christou and are discussed in greater detail by A. R. Hayward, who has presented a comprehensive study of the clinical picture, cause, and where possible treatment of the known immunodeficient diseases of man. The recognition of these conditions must obviously increase as more and more becomes known of the minutiae of immune responses. Most of the immunodeficiencies which are usually recognized are very often severe and have a fatal outcome from infection unless identified and treated. Some of them are extremely rare and have either a single gene inheritance or sometimes some other characteristic phenotypic manifestation. The importance of less serious immunodeficiencies in allergy and autoimmunity are now becoming apparent.

It is only recently that the importance of *acquired* defects of host defence. such as advanced age, major surgery, trauma, shock and diabetes and uraemia, etc. have been recognized and the part which they play in producing alterations in host resistance in some individuals has been appreciated. The investigations of persons likely to develop sepsis (with its related mortality) is a problem not only for physicians and surgeons, but is of importance to immunologists who may be able to identify the basic alterations in the host defences of these individuals by suitable immunological tests.

The individual family with a primary immunodeficiency is a fascinating but trivial problem compared with that of millions of children in the world who may be suffering from an immunological defect due to malnutrition. A number of immune parameters which are depressed in protein-calorie malnutrition are discussed by R. M. Suskind who points the way for further investigations of this most important problem with particular reference to the effect of protein-calorie malnutrition on polymorphonuclear leukocyte activity.

The recent research work of J. Pepys has provided a much more complete understanding of skin tests in allergy and of their clinical value and interpretation. While IgE is well recognized as the main mast cell sensitizing antibody, evidence is now accumulating of the facilitating effect of IgA in IgE-antigen reactions and also of a heat stable, short-term sensitizing (STS) antibody in the IgG complex which is capable of passive sensitization of mast cells for only a few hours: this observation throws new light on the interpretation of skin-prick tests which are discussed in detail. Further studies

of type III reactions are now leading to much greater understanding of delayed hypersensitivity reactions and the significance of this type of reaction in bacterial, viral, fungal and protozoal disease. To all this has been added the newer techniques which are now available for antibody assays.

At the opposite pole to *allergy* is *anergy*, which was the term which Von Pirquet applied 'quite generally to the absence of clinical manifestation of reaction'. Anergy must be one of the most poorly understood subjects in immunology, however as W. E. Bullock points out, the recent demonstration of an immunoregulatory control system in mammals which exerts suppressor effects on the immune response has provided some insights into its mechanism. This is discussed in detail with reference to infectious diseases, paying particular attention to the clinical phenomenon of delayed-type hypersensitivity and cell-mediated immunity: Bullock concludes that continued investigations along these lines may lead to the concept of 'positive anergy'.

In addition to the tissue injury produced by an infectious agent *per se* or which results from cellular responses to it, there are now many examples of injury associated with humoral antibody which are largely elicited by immune complexes. The nature and detection of these immune complexes and the literature on this subject are discussed and reviewed by P. Casali, L. H. Perrin and P.-H. Lambert.

Infection in the compromised host has been reviewed by E. H. Nauta who after summarizing the normal defence mechanisms of the body describes disturbances of these mechanisms and their clinical manifestations, prevention and treatment with particular reference to bacterial, viral, fungal and protozoal infections in acute leukaemia, in Hodgkin's disease and other myeloproliferative diseases and in organ transplant patients, etc. To all this he brings his personal experience of the diagnosis and prognosis of infections in the compromised host.

Autoimmunity implies loss of tolerance and L. E. Glynn discusses the ways in which infectious diseases may interfere with immunological tolerance and overcome it. T cells are more easily rendered tolerant than B cells and natural tolerance to most autochthonous antigens is of the T-cell variety; for an infection to induce an autoimmune reaction it would only be necessary for T cells to be activated. Probably the most important cause of autoimmunization is due to cross-reactions of antigens of the parasite and the host: the chemical characteristics of these antigens and the enhancement of the immune response by micro-organisms and other substances is discussed in detail. The whole story is exemplified with a discussion of autoimmune phenomena associated with infectious diseases such as those due to *T. pallidum*, and to streptococci in relation to rheumatic fever and to glomerulonephritis. Although infections with viruses (because of the nature of these agents) might be expected to be the most likely micro-organism to be associated with autoimmune disease, so far there is no direct evidence of their role in this respect in many of the virus diseases where they are strongly suspected.

The chapter on chronic infections by J. L. Turk brings together many of the facets of the immunological aspects of infectious disease which are discussed in earlier chapters. Attention is focused on the role of bacteria in chronic diseases, and particular attention is paid to the clinical spectrum of leprosy, tuberculosis, syphilis and also to some parasitic and fungal infections.

In the final chapters C. J. Gibbs and his colleagues and J. M. Adams discuss the role of viruses in chronic, persistent and recurrent viral infections and in slow infections. The pre-requisites for chronic infections are the ability of the infecting agent to avoid host-defence mechanisms and second to be of such low toxicity or invasiveness that the host can survive a prolonged state of parasitism. The recent *in vivo* and *in vitro* studies which have led to a better understanding of persistent viral infections and the role of the immune response in these infections in man are discussed.

J. M. Adams takes us beyond both conventional and unconventional 'viruses' towards the immunology of diseases not yet known to be caused by classical infective agents. The understanding of these conditions has made enormous strides in recent years based on the appreciation that scrapie was caused by an agent which produced a similar histopathology to that of Kuru. Further study of these slow infections may lead to an understanding of diseases such as multiple sclerosis and of the disease which finally affects most of us – old age – if not cerebral dementia.

G.D.

Contents

1
Non-specific Resistance to Infection

R. J. ELIN

1.1 INTRODUCTION

The ability of vertebrates to combat invasion by micro-organisms can be divided into two different mechanisms: a specific immunity based upon the development of humoral (antibodies) or cellular factors and a so-called natural or non-specific resistance that is not unique to the invading micro-organism. The remaining chapters in this book focus on several aspects of

the first mechanism, i.e. the influence of the immune system in host resistance to infectious diseases.

The defence mechanisms of the body against pathogenic micro-organisms that are not mediated by the specific action of the immune system comprise the host's non-specific resistance to infection. These mechanisms include both humoral and cellular components. The inhibition of infection by non-specific mechanisms is affected by substances that alter humoral or cellular factors of the host to induce the non-specific resistance. Usually, these substances do not directly affect the infecting micro-organism. These substances are termed non-specific since they induce protection against a spectrum of micro-organisms. A large number of humoral and cellular factors may enhance the resistance of the host to infection, but only a few of these factors have been studied in sufficient detail to establish a relationship to non-specific resistance to infection. The remainder of this chapter will focus on three groups of compounds—vitamins, minerals and microbial products—and an organ, the spleen, which have clearly been shown to be related to non-specific resistance to infection.

1.2 VITAMINS

The experimental evidence that vitamins A, C and E are required to maintain normal host defence mechanisms against infection or induce non-specific resistance to infection is reviewed below.

1.2.1 Vitamin A

An excess of vitamin A induces non-specific resistance to infection, and a deficiency of vitamin A causes an enhanced infection with a challenge organism in experimental animals. The injection of vitamin A into mice produced significant protection compared with the control group when these animals were challenged with either *Pseudomonas aeruginosa*, *Listeria monocytogenes* or *Candida albicans*[1]. Those animals treated with vitamin A and infected with *P. aeruginosa* cleared the bacteria from the blood more rapidly than the control group. The vitamin A had no effect on the growth of these organisms *in vitro* at concentrations greater than that achieved in the experimental animal. Thus parenteral administration of vitamin A enhances the resistance of mice to challenge with these bacteria and fungal micro-organisms.

In deficiency states of vitamin A, bacterial, viral and parasitic infections are more pronounced. A deficiency of vitamin A appears to enhance the virulence of *Mycobacterium tuberculosis* and other bacteria in man and the chick[2–4]. The response of the chick to challenge with Newcastle disease virus is impaired with dietary vitamin A deficiency[5]. The rate of parasitaemia

is significantly increased in vitamin A-deficient rodents challenged with either *Plasmodium berghei* or *Trypanosoma musculi*[6,7]. Thus a deficiency of vitamin A impairs host defence mechanisms for a variety of micro-organisms.

The mechanisms by which vitamin A increases host resistance to infection have not been defined, although vitamin A is an effective adjuvant[8,9] and has been shown to labilize lysosomal membranes[10], both of which may affect the response of a host to an infectious agent. It is well established that a deficiency of vitamin A induces squamous metaplasia of respiratory epithelia which may alter host defence mechanisms for respiratory infections[11]. However, studies of the relationship between vitamin A and infection have been limited almost entirely to experimental animals, and the clinical importance of these experimental findings is unknown. Since a chronic intake of vitamin A greatly in excess of the requirement clearly results in a toxic syndrome in man known as hypervitaminosis A, further investigation will be needed to determine the possible clinical relevance, the spectrum of micro-organisms, and the mechanism of non-specific resistance before this vitamin can be recommended therapeutically[12].

1.2.2 Vitamin C

The medical literature for the past 35 years has reflected the controversy over the efficacy of vitamin C at preventing or altering the symptoms of the common cold and enhancing resistance to infectious diseases. The early clinical trials assessing the value of vitamin C as a prophylactic agent against the common cold and other infectious diseases were more positive than negative[13–18]. Some of these studies would have been considered poorly controlled because they did not employ the techniques of randomization and double blinding[13–15].

In 1970, Professor Linus Pauling published his now famous book entitled, *Vitamin C and the Common Cold*[19]. In the book, Pauling emphasized that vitamin C is indeed a 'vitamin' for only a few species; an exogenous source of vitamin C is required only by man, other primates, guinea-pigs, flying mammals, and certain highly evolved birds[20]. These animals must obtain vitamin C in the food they eat because they lack the enzyme L-gulonolactone oxidase which catalyses the final step in the microsomal conversion of D-glucuronic acid to L-ascorbic acid[21]. It was noted that man has a relatively low concentration of vitamin C compared with animals that can synthesize the vitamin, and these animals with an endogenous source of vitamin C seem to be free from the common cold[22]. Thus the implication is that dietary supplements of vitamin C might alter the response or prevent the common cold.

During the past 7 years, the intense public interest in this area has mandated several clinical trials which have employed techniques of randomization and double blind[23–30]. These trials were conducted by four different

groups of investigators. Three studies have now been published by the group in Toronto, Canada[23–25]. The first study was conducted specifically to evaluate Professor Pauling's claim that the intake of 1 g of vitamin C per day would substantially reduce the frequency and duration of colds[23]. The results of this study showed that the average number of colds per subject was not statistically different between the two groups; however, those on vitamin C experienced 30% fewer days' disability and this difference was significant ($p < 0.001$) compared with the placebo group. The second trial was designed to examine the effect of prophylactic and therapeutic dosages of vitamin C, but the results were less than clear-cut, in part due to the complexity of the experimental design[24]. The third trial assessed dosage. Subjects were randomly allocated to one of three treatment groups: placebo, 500 mg of vitamin C weekly and 1500 mg of vitamin C daily. Subjects in both vitamin groups experienced less severe illness than subjects in the placebo group ($p < 0.05$). The authors concluded that supplementary vitamin C can reduce the burden of winter illness, but dietary supplementation could be done weekly with an amount less than 1 g[25].

A group in England assessed the prophylactic value of placebo and 200 mg or 500 mg of vitamin C in school children over a 9-month period. They found that catarrhal cold symptoms were reduced by over 50% in girls taking 500 mg of vitamin C daily, but there was no consistent effect in boys[26,27].

A third group studied Navajo school children in Arizona[28,29]. The first trial showed no difference between treatment groups in the number of respiratory illnesses, but those children receiving vitamin C had fewer days of morbidity[28]. A second trial to corroborate the first trial showed no differences in the number of children becoming ill, the number of episodes or the mean illness duration between the placebo and vitamin C groups[29].

The fourth group conducted a trial among employees at the National Institutes of Health[30]. In this trial, the incidence of colds was not statistically different between the two groups, but the group receiving vitamin C had a significant reduction in morbidity. The validity of this study is questionable since many of the subjects were aware of their treatment group.

The results of these several clinical trials fail to give a clear mandate for the prophylactic use of vitamin C. The data somewhat suggest that vitamin C does not alter the incidence of upper respiratory infections, but that it may reduce the severity of symptoms and the duration of illness.

Studies in experimental animals support the theory that vitamin C induces non-specific resistance to infection. Vitamin C deficiency in guinea-pigs creates a higher incidence of bacterial pneumonia and septicaemia[31]. Mortality in these animals is increased following challenge with *Staphylococcus aureus*, *Streptococcus pneumoniae* or *Streptococcus pyogenes*. In addition, increased dietary vitamin C markedly reduces mortality in guinea-pigs infected with *Mycobacterium tuberculosis* and increases resistance in

these animals to re-infection[32]. Thus in the guinea-pig vitamin C appears to afford a degree of protection to a variety of bacteria.

What are the possible mechanisms by which vitamin C enhances resistance to infection? Vitamin C potentiates chemotaxis of normal polymorphonuclear leukocytes[33]. Vitamin C has been shown to stimulate hexose monophosphate shunt activity in resting polymorphonuclear leukocytes and macrophages *in vitro* to a similar activity to that seen following phagocytosis[34]. These enhancements of leukocyte function by vitamin C may correct the defect in Chediak-Higashi syndrome[35], in which patients suffer frequent and severe pyogenic infections that are secondary to abnormal function of polymorphonuclear leukocytes. The impaired bacterial activity of the leukocytes appears to be related to delayed delivery of lysosomal contents into phagosomes[36], a functional defect which may be related to abnormal microtubular assembly in polymorphonuclear leukocytes in this syndrome[37]. The treatment of leukocytes from patients with Chediak–Higashi syndrome with agents that increase cyclic 3′,5′-guanosine monophosphate (cyclic GMP) concentration will improve microtubular function[37]. Studies *in vitro* have demonstrated that vitamin C will increase cyclic GMP concentrations in monocytes[33]. In a case report, a patient with Chediak–Higashi syndrome was treated with vitamin C, and the defect in chemotactic migration and bactericidal activity of leukocytes was corrected to normal[35]. Thus there is evidence that vitamin C affects leukocyte function, which is a prime element in host-defence mechanisms against infectious diseases.

1.2.3 Vitamin E

A relationship of vitamin E to resistance to infection has been documented. Supplementing a standard chick ration with either vitamin E or vitamin A increased the protection of 6-week-old immunized chickens against *E. coli* infection by decreasing mortality from about 40% to 5%[38]. Interestingly, the combination of the two vitamins did not give as much protection as either vitamin alone. Further studies will be needed to define the significance of vitamin E in non-specific resistance to infection.

1.3 MINERALS

Serum concentrations and metabolic homeostasis for several minerals (iron, zinc, copper, chromium, cobalt, gallium, iodine and manganese) are altered in infectious processes[39,40]. For several of these minerals additional research is needed to establish a possible link to host-defence mechanisms. However, studies concerning infection-induced alterations in iron, zinc, and copper metabolism have now advanced beyond the purely descriptive stages and suggest that these elements have a role in host-defence mechanisms.

1.3.1 Iron

There is a growing body of experimental and clinical evidence to suggest that the iron status of an individual may affect his resistance to infection. I will discuss initially some of the studies *in vitro* which indicate the importance of iron in infection and then evaluate hyperferraemia, hypoferraemia and resistance to infection.

Iron is essential for bacterial growth. Iron-chelating agents cause a selective inhibition of DNA synthesis in living cells *in vitro* which suggests that iron plays a crucial role in mitosis[41]. Since iron is needed for cell division, it is not surprising that comparable quantities of iron are required for the growth of plant, animal and microbial cells[42]. Thus, if micro-organisms are going to invade a host successfully, they must acquire iron from the host for continued normal growth.

Although the quantity of iron in some host fluids, such as milk and plasma, is more than adequate for microbial growth, the amount of free ionic iron in these body fluids available to micro-organisms for normal growth is too low by a factor of several thousand, because iron-binding proteins present in healthy human hosts (such as lactoferrin and transferrin) are present in sufficient quantity to bind all iron. This is due to the very high association constant (10^{30}) for iron which is characteristic of these iron-binding proteins[42]. Over three decades ago, Schade and Caroline were first to describe the prevention of bacterial growth by iron-binding proteins in egg white and plasma which was reversed by the addition of iron in excess of the binding capacity of these proteins[43,44]. Several investigators have confirmed that the inhibition of growth of a variety of bacteria and fungi by normal serum can be reversed by the addition of iron[45,46]. Thus the availability of free iron in body fluids determines the growth potential for most micro-organisms in these fluids.

Many pathogenic bacteria synthesize iron-chelating agents (siderophores) which can remove iron from the iron-binding proteins. These microbial siderophores have association constants for iron of 10^{30} or more, and at least some of them are capable of withdrawing iron from 30% saturated transferrin[42]. Some of the siderophores have been chemically characterized as phenolates, hydroxamates, or lipopolysaccharides[47]. Thus the resolution of the contest between the invading micro-organism and the defence mechanisms of the host depends upon the iron concentration and iron metabolism of the host.

From the above *in vitro* observations it could be postulated that states of increased iron availability might predispose to infection. Conversely, iron deficiency might protect the individual against infection. The actual clinical situation is considerably more complex and not yet clear.

In a variety of clinical conditions marked by iron overload, the incidence of infection appears to be increased. Hyperferraemic states occur in

individuals who have the following: (1) occult or overt haemolytic anaemia, (2) destruction of liver cells containing ferritin, and (3) an overload of iron from exogenous sources. One of the best examples of haemolytic anaemia is sickle cell disease. Bacterial infection is still the single most common cause of death in sickle cell disease, and the incidence of bacterial meningitis is 300 times greater than in normal siblings[48]. Increased susceptibility to salmonella infections, bartonellosis and malaria is known to be associated with the haemolysis present in sickle cell disease[49]. Systemic salmonellosis is common in persons with viral hepatitis[42]. Following a single injection of iron sorbitol citrate, patients with chronic pyelonephritis had an exacerbation of their infection as documented by increased numbers of white blood cells in urine[50]. A similar increase in white blood cells did not occur in non-infected controls or in patients with non-infectious renal disease[50]. On the other hand, patients with an iron overload due to haemochromatosis or multiple blood transfusions are not notably prone to infection[51].

Hypotransferrinaemic states with an increased percentage saturation may predispose to infection. The incidence of bacterial infection is far higher in children with kwashiorkor (reduced transferrin concentration) than in normal children[52]. There is evidence that the administration of iron to patients with abnormally low transferrin concentrations may result in overwhelming infection and death[52]. Thus in several diseases an increase in the percentage iron saturation of transferrin predisposes the host to infection.

The incidence of infection in iron-deficient human populations is increased rather than decreased. The *in vitro* data suggest that hypoferraemia should be advantageous to the host and disadvantageous to the microbial invader. However, there is widespread belief with some supporting data that iron deficiency predisposes to infection[53].

Several clinical surveys have attempted to correlate the iron status of an individual with the risk of infection. Infection has been reported as the most common symptom for which iron-deficient children seek medical advice[54]. The World Health Organization has reported that individuals with nutritional anaemia tend to have a higher incidence of infections[55]. Several studies have been made of iron supplementation; the first longitudinal study of the effects of iron supplementation in the first year of life was reported in 1928 among infants from low-income families in London[56]. A modest decrease in the number of episodes of bronchitis and gastroenteritis in iron-supplemented children was reported, but, as might be expected, the study was not double blind and defects in the design of the study permit alternative interpretations[56]. The frequency of respiratory infections was reported to be significantly less during the first year of life in infants from Chicago's inner city who were given an iron-fortified formula[57]. However, criteria for the diagnosis of respiratory tract infection were not defined, and precautions to minimize bias on the part of the observers were not taken. Other studies have failed to show a relationship between iron deficiency and the