Clinical Aspects of Neuroimmunology

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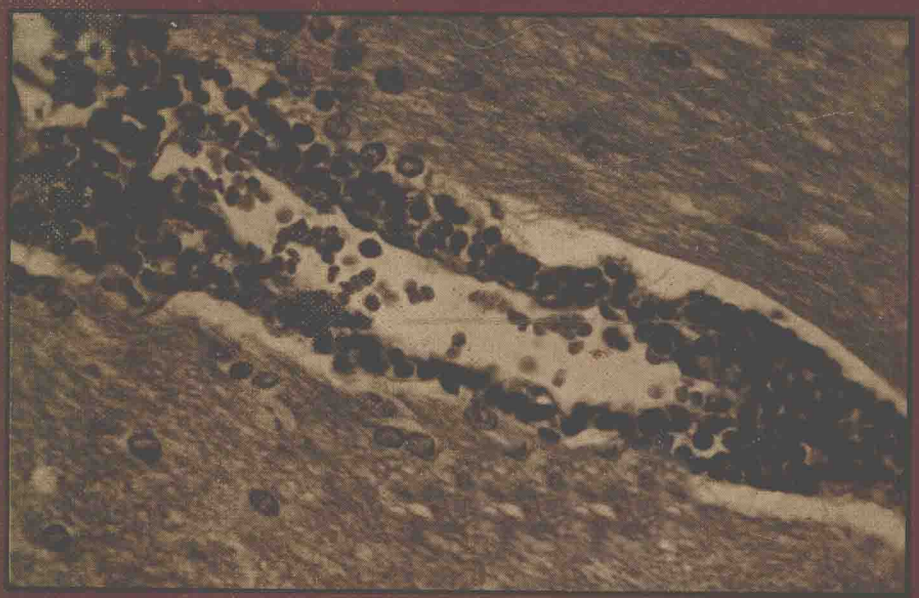

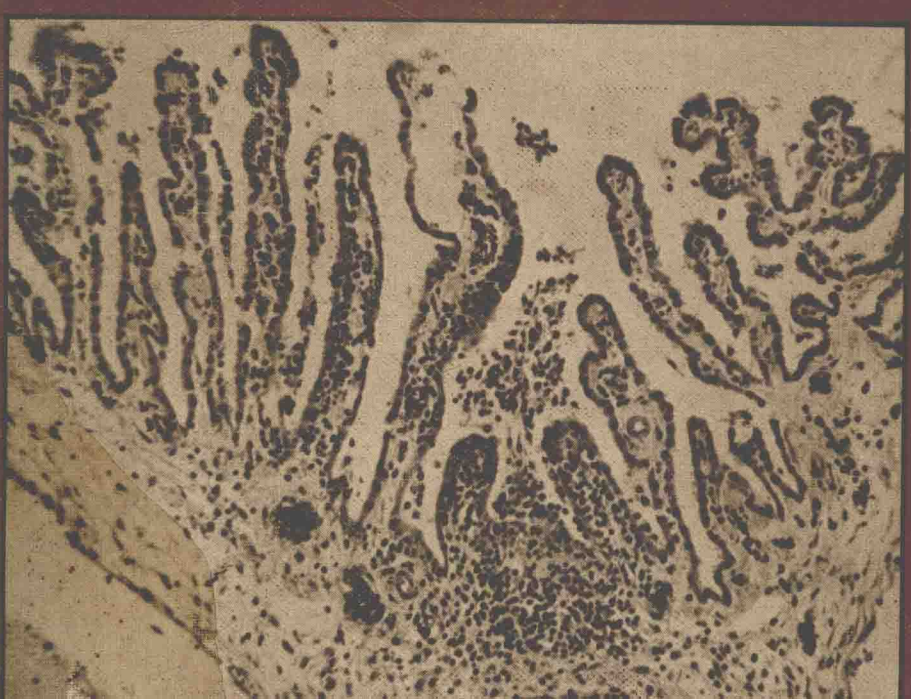

Clinical Aspects of *Neuroimmunology*

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Clinical Aspects of Neuroimmunology

Dedication

This book is dedicated to my beloved parents in honor of their 40th wedding anniversary, and to my former teachers, Dr. Kenneth Bush and Ms. Elsa Stout.

EN

and

To Fern

WKC

Foreword

AN INFORMATION EXPLOSION began in the field of experimental immunology in the 1960's, and its sustained and expanding impact is clearly reflected in contemporary clinical medicine. The authors of this text are neurosurgeons who detail the exciting interface of modern experimental immunology with clinical neurology. The topography of this interface is mapped by a few prominances where factual information is strong and has direct application to specific diseases. The best example is myasthenia gravis where an IgG autoantibody to the acetylcholine receptor has been directly implicated in the motor endplate dysfunction. Successful treatment by removal of the autoantibody by extensive plasma phoresis has just been reported. By contrast, the immunopathogenic mechanism, although suspected to be operative in multiple sclerosis, has not been delineated despite a plethora of data. A rational treatment, therefore, is not available. In both these polar instances and other neurological diseases, a detailed exposition of experimental and clinical data is presented and assessments of their present and future relevance provided.

Drs. Neuwelt and Clark have accepted a formidable task in presenting this detailed and cohesive picture of clinical neuroimmunology. They have undertaken it with a refreshing elan by tracing the pathways from the signal, original observations in specific areas such as the paralytic accidents, which occurred with Pasteur's rabies vaccine grown in rabbit brain, to experimental allergic encephalomyelitis. Clinical case histories from the authors' experience are used to illustrate specific areas where neurology and immunology meld together. The book should provide a good introduction to immunology for the neuroscientist and an updated overview of relevant areas of neurology for those interested in immunology. The serious clinician in both fields will find it useful.

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Preface

THERE ARE A number of neurological disorders in which the immune response is being increasingly implicated as playing a key pathogenic role (i.e., muscle disease, tumors, vasculitides, and demyelinating diseases). There are other neurological diseases in which immunology is diagnostically as well as pathogenically important (i.e., CNS infections). Immunotherapy is also being introduced clinically in certain disorders. Many such neurological disorders are reviewed in this volume and, in particular, an attempt is made to relate significant basic science studies which are clinically important and useful.

For those who desire a brief review of general immunology, the first chapter is designed to fulfill this need. Only those areas of general immunology which are pertinent in later chapters are emphasized. In the second chapter are described those methods which are important in neuroimmunology. Technical details of these methods are described in a number of standard texts and are therefore not included. The remaining chapters deal with those categories of disease or specific entities in which neuroimmunology is important. A detailed outline is given at the beginning of each chapter. Since the text is aimed at a readership of diverse background, each chapter begins with a brief review of the clinical, pathological, and pathogenic aspects of the diseases discussed within the chapter. The sections dealing with neuroimmunology then follow. Original data in the form of figures and tables are included frequently. At the end of each chapter is a selected bibliography which is subdivided according to topic. Some but *not* all of the references in the text are included here. Since all the references in the text give the author's name and year in which the work was published, those references not given in the bibliography can be found in the *Index Medicus.* If this proves inconvenient to some readers or undesireable to some authors we apologize. However, we felt that it was necessary to do this to keep the size of the selected bibliography at the end of each chapter to a reasonable length.

Since we are not currently aware of any other introductory text or reference book solely dealing with neuroimmunology, we hope this volume will be useful to its readers.

E.A.N.
and
W.K.C.

Acknowledgments

WE ARE PARTICULARLY indebted to Ms. Elizabeth Neuwelt who carried out a large part of the reference work in this text and coordinated the preparation of the figures and tables. Ms. Keyes, Ms. Eleanor Wheat, and Ms. Betty Lewis provided much appreciated secretarial assistance. Finally, I would like to express our gratitude to Ms. Ruby Richardson, Mr. Bob Och and other staff members of The Williams and Wilkins Co. who gave us expert advice and, at times, much needed encouragement.

Introduction

When a host is exposed to a foreign agent, two types of immune responses may result, independently or in concert: a lymphocyte mediated cellular response and/or an antibody mediated humoral response. The importance of both these responses in neurological diseases has been well-known for many decades. An historical account of the development of the rabies vaccine exemplifies both the potential benefits and hazards of humoral and cellular immunity in clinical neurology.

In the 1880's, Pasteur found that serial passage of the neurotropic rabies virus in the rabbit central nervous system resulted in an attenuated virus which was no longer pathogenic in dogs. Furthermore, he found that infecting dogs with this live but attenuated virus rendered them immune to subsequent infection by the "street" rabies virus. Thereby he was able to immunize patients against "street" rabies with dried rabbit spinal cord containing the live but attenuated rabies virus. Unfortunately, soon after the introduction of this life-saving vaccine, "paralytic accidents" (i.e., acute disseminated encephalomyelitis, ADE) were reported as a complication of the vaccine. It was not until 1933 that the pathogenesis of these "paralytic accidents" began to emerge, when Rivers reported that repeated injections of normal neural tissue into laboratory animals results in an experimental allergic encephalomyelitis (EAE). He demonstrated that clinically and pathologically EAE was identical to the ADE associated with the rabies vaccine. Further immunological studies demonstrated that the Pasteur rabies vaccine activated both the cellular and humoral limbs of the immune response. The injected attenuated rabies virus vaccine obtained from rabbit brain did indeed result in the production of the desired antibodies needed for rabies prophylaxis. Paradoxically however, the simultaneously injected neural tissue contained the "encephalitogenic antigen" which activated a cellular immune response and, as will be discussed further in Chapter 9, sometimes resulted in the development of ADE. In response to these experimental findings, a new vaccine was developed from rabies virus serially propagated in duck embryos rather than rabbit spinal cord. Since the introduction of the new duck embryo vaccine which contains little, if any, of the "encephalitogenic antigen", ADE has been associated only rarely with the use of the rabies vaccine.

Contents

1

Basic Immunology

I. HUMORAL IMMUNITY

The ability of the immune system to produce specific antibodies in response to a vast and diverse array of antigens is a major mystery in medical research. Present evidence suggests that a given individual can produce over 100,000 different antibodies and this is probably a conservative estimate. Furthermore, antibody producing cells (i.e., plasma cells), are very highly differentiated, specialized cells. Each plasma cell can only secrete an antibody to a single antigen and this antibody is of a single immunoglobulin class. That is, IgG and IgM antibodies may both be secreted against an antigen, such as bovine serum albumin, yet evidence to date suggests that they are produced by two different antibody producing cells. However, the B-lymphocyte, which is the precursor to the antibody secreting plasma cell, may have more than one class of immunoglobulin on its surface. The most common example of this is the combination of IgM and IgD.

The actual process of antibody production is a particularly complicated and poorly understood process. Furthermore, the process seems to vary a great deal, depending on the nature of the antigen. Three cells are generally involved: the B-lymphocyte, the T-lymphocyte, and the macrophage. The B, or bone marrow derived lymphocyte, is of prime importance in the antibody producing process in contrast to the T, or thymus derived lymphocyte, which is mainly involved in cellular immunity. The production of antibody, however, usually requires the cooperation of both the T-lymphocyte and the macrophage with the B-lymphocyte, except with certain T-independent antigens (i.e., large polymers with many copies of the same antigenic determinant). The function of the T-lymphocyte during antibody production is felt to be one of modulation of the B-cell response to antigen, via direct cell contact and/or via a soluble mediator. The macrophage, by virtue of its "sticky" cell membrane, seems to have a non-specific but important role in enhancing the direct cell-cell interaction between the T- and B-lymphocyte, which is so vital for antibody production against most macromolecules. It also plays a role in T-cell activation.

The production of antibody against most antigens begins 4–10 days following the initial immunization. This is referred to as the primary immune response.*

The quantity of antibody produced during the primary response can be vastly increased by mixing the antigen with an immune adjuvant, such as complete Freund's adjuvant (killed mycobacteria emulsified in mineral oil, Fig. 1-1). The main immunoglobulin produced during the primary response is IgM, although a less intense, delayed IgG response is also usually seen (Fig. 1-2). If a host is immunized a second time 2 weeks after the initial immunization, the rate of antibody production is greatly accelerated, and this is referred to as an amnestic, or secondary response.* The duration of immunoglobu-

* The terms primary and secondary response should not be confused with primary, secondary, or tertiary manifestation of the antigen-antibody interaction, which will be discussed in Chapter 2.

lin production during the amnestic response is much longer than that following a primary immunization. In addition, the type of immunoglobulin produced in the amnestic response is mainly IgG, in contrast to the predominant production of IgM characteristic of the primary response.

IgG and IgM synthesis in the B-lymphocyte or plasma cell occurs in a manner similar to any other glycoprotein (Fig. 1-3). Messenger RNA is produced against a DNA template (i.e., transcription), and travels out of the nucleus into the cytoplasm. Translation then occurs in cytoplasmic rough endoplasmic reticulum, the result of which is the synthesis of polypeptide chains. These light and heavy polypeptide chains are then transported to the Golgi apparatus via the smooth endoplasmic reticulum. During this period, the polypeptide chains are assembled into intact immunoglobulin molecules and carbohydrate side chains are added. Finally, secretory vesicles snap off the Golgi apparatus and transport antibody to the cell surface, where it is released by exocytosis (Fig. 1-4).

In addition to IgG and IgM referred to above, there are three other classes of immunoglobulins synthesized by activated B cells and plasma cells: IgA, IgD, and IgE. Each of the five types of immunoglobulins are made up of light polypeptide chains designated as kappa (κ) or lambda (λ) and heavy polypeptide chains. Each of the immunoglobulin classes has similar kappa and lambda light chains, but antigenically

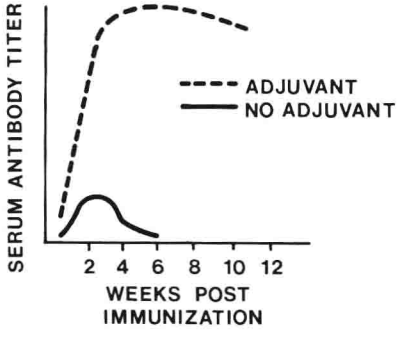

Fig. 1-1. Enhanced antibody production with immune adjuvant. The above diagram is a schematic representation of total antibody production following a single injection of a foreign, soluble protein into a laboratory animal.

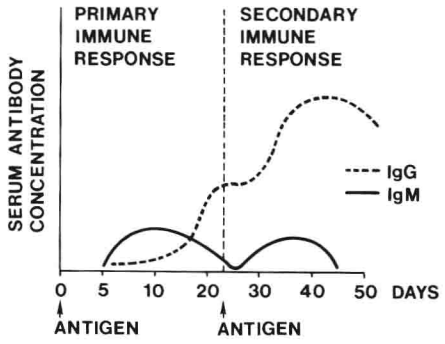

Fig. 1-2. IgG and IgM antibody production following an initial and subsequent antigen injection. The secondary immune response is often referred to as the amnestic response. This diagram is schematic and is representative of antibody production to a number of different antigens.

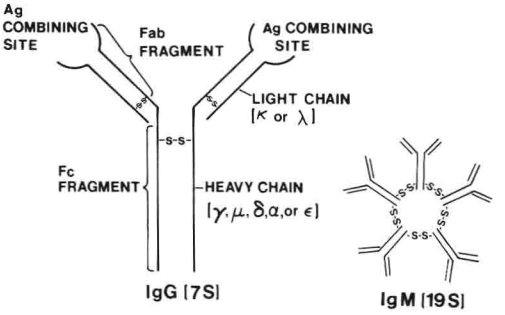

Fig. 1-3. The structure of IgG and IgM. IgG which has a sedimentation coefficient of 7 S is composed of two light chains and two heavy chains covalently bound by disulfide bonds. Following papain digestion, IgG is degraded into two Fab (antigen binding) fragments and one Fc (crystallizable) fragment. Each Fab fragment contains one antigen binding site. IgG contains either kappa (κ) or lambda (λ) light chains and two gamma (γ) heavy chains. The other types of heavy chains, μ, δ, α, and ϵ, are found in IgM, IgD, IgA, and IgE, respectively. IgM has a sedimentation coefficient of 19 S and is decavalent.

specific and characteristic heavy chains. Thus IgG antibody contains γ (gamma) heavy chains, IgM μ (mμ) heavy chains, IgD δ (delta) heavy chains, IgA α (alpha) heavy chains, and IgE ϵ (epsilon) heavy chains (Table 1-1). A given antibody molecule contains only one type of light chain and one type of heavy chain.

Since IgG is the predominant CNS immunoglobulin in both normal and most

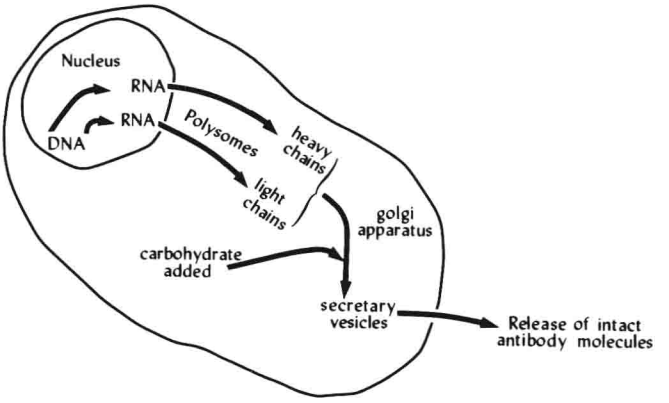

Fig. 1-4. Immunoglobulin production in a plasma cell (i.e., a sensitized B-lymphocyte following exposure to antigen differentiates into a plasma cell). Note that immunoglobulin production is completely analogous to the synthesis of any other glycoprotein.

Table 1-1. Properties of Human Immunoglobulins

Property	IgG	IgA	IgM	IgD	IgE
Average serum concentration (mg/ml)	10	3	1	0.03	0.00015
Presence in CSF	+	+	–	–	–
Complement fixation	+	–	+	–	–
Serum half life (days)	23	6	5	3	2.5
Structure light chain	κ, λ	κ, λ	κ, λ	κ, λ	κ, λ
Heavy chain % carbohydrate	3	7	12	13	11
Valence	2	4 (variable)	10	–	2
Sedimentation coefficient	7 S	9 S (variable)	19 S	6 S	8 S

pathological states, it is appropriate to utilize this immunoglobulin molecule to exemplify antibody structural properties and function. As illustrated in Figure 1-3, IgG is composed of two light chains, which can be either kappa or lambda, and two gamma heavy chains. Ultracentrifugation studies in a dilute salt solution have demonstrated that this class of immunoglobulins has a sedimentation coefficient of 7 S. The molecular weight of IgG has been demonstrated to be about 150,000 daltons. It is the predominant antibody species in serum and, at a much lower concentration, in cerebrospinal fluid (CSF). Detailed structural and sequencing studies of this immunoglobulin have been performed on monoclonal IgG purified from the serum of multiple myeloma patients. Based on these studies, it was found that disulfide bonds play an important role in the secondary structure of this type of antibody. Each light chain is covalently bound to a heavy chain by a disulfide bond and the two heavy chains are covalently linked in a similar manner (Fig. 1-3). When the protein is subjected to proteolytic digestion by papain, each IgG antibody molecule is broken into two Fab (antigen binding) and one Fc (crystallizable) fragments (Fig. 1-3). The Fab fragments, composed of an intact light chain and part of a heavy chain covalently bound together by a disulfide bond, each contain an antigen binding site. Thus, IgG antibody is divalent. That is, one mole of IgG antibody can bind two moles of antigen. The Fc fragment of IgG cannot bind antigen, but plays an important role in certain secondary and tertiary phenomena, such as the fixation of complement and immunoprecipitation. For instance, following the binding of antigen to the antigen binding site on the Fab fragment, which is a non-covalent process,

certain conformational changes in the Fc portion of the IgG molecule seem to occur, which subsequently enable the molecule to initiate the cascade of reactions referred to as the fixation of complement. In summary then, IgG is a divalent 7 S antibody which is present in both plasma and CSF, and which can initiate secondary immunological phenomena, such as complement fixation and immunoprecipitation.

In contrast to IgG, IgM or macroglobulin is a decavalent (Fig. 1-3) 19 S antibody which, probably because of its size, is not normally able to penetrate the blood-brain barrier, and thereby is only present in the CNS under pathological conditions. Indeed, the presence of CSF IgM is characteristic of certain specific CNS disorders, such as trypanosomiasis, although IgM is also sometimes found in the CSF merely as the result of non-specific disruption of the blood-brain barrier, as in bacterial meningitis. Like IgG, immune complexes containing IgM can fix complement and undergo immunoprecipitation (Table 1-1).

IgA, whose concentration classically is reduced markedly in the plasma of patients with ataxia telangiectasia (Chapter 5), is considered to be mainly a secretory immunoglobulin. It is an important effector of the immune response in respiratory and intestinal epithelium, and in such bodily secretions as milk, saliva, and tears. A low level of IgA is a normal finding in the CSF. The molecular weight of IgA is quite variable, since it exists in a variety of polymeric states. In external secretions, tetravalent antibody with a sedimentation coefficient of 9 S is the most common polymer (Table 1-1). Unlike IgG and IgM, it cannot fix complement.

The other two immunoglobulin classes, IgE and IgD, at present, have not been directly implicated in neurological diseases. IgE is a cytophilic antibody which plays an important role in atopic disorders.

The physiological significance of IgD is poorly understood; however, it is known to exist on the surface of B-lymphocytes and it can be secreted into serum as a monoclonal spike in multiple myeloma (Table 1-1).

II. THE FIXATION OF COMPLEMENT

The finding of antigen to IgG and IgM antibody can initiate a cascade of enzymatic reactions known as complement fixation. At least nine different complement components (11 different proteins) are involved, and each component has been assigned a number according to the order in which it was discovered, rather than according to its order of activation. The sequence of activation of each of these components of complement, as well as some of the properties of each component, is listed in Figure 1-5.

The fixation of complement is analogous

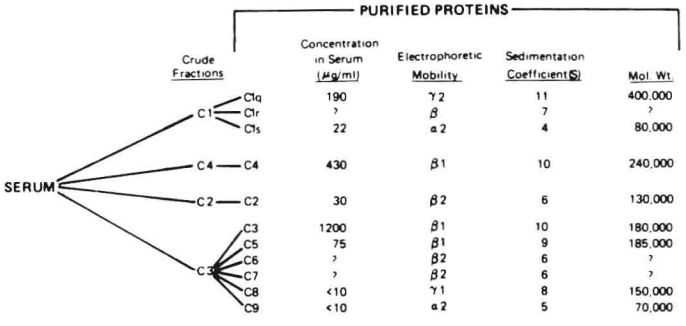

Fig. 1-5. Some properties of purified human complement proteins. The four crude fractions (C1 to C4) were originally characterized by conditions that inactivated or removed them from fresh serum. C1 and C2 are extremely heat-labile: they are inactivated in a few minutes at 56°C, whereas inactivation of C3 and C4 at this temperature requires 20 to 30 minutes. C1 is precipitated with the "euglobulins" by dialysis against H_2O at pH 5, whereas C2, a "pseudoglobulin," is soluble under these conditions. C3 is inactivated by zymosan and C4 by exposure to ammonia or hydrazine. C1 to C4 are numbered in the order of their discovery, which unfortunately is not the same as the order in which the components react in the complement cascade. (Reprinted with permission from B.D. Davis et al., Microbiology, p. 516, Harper & Row, Hagerstown, Md., 1973.)

to the cascade of reactions that leads to the development of a fibrin clot. That is, the first component of complement (C1) is first activated following antigen binding by a process which involves the Fc end of an immunoglobulin molecule. Then, the activated C1 in turn activates the next component, and so on, until all of the components are activated. If the antigen which initiates this process is located on a bacterial cell wall or on a cell membrane, cell lysis occurs upon the fixation of all the complement components.

Aside from the classic sequence of activation of complement components described above, there is an alternate pathway known as the properdin pathway. Certain substances such as IgA and bacterial lipopolysaccharide can activate this pathway which bypasses the need to activate the initial three complement components.

In addition to cell lysis, there are a number of biologically active polypeptides which are released during this autocatalytic process (Fig. 1-6). These protein fragments can result in localized anaphylaxis, chemotaxis, phagocytosis, and immune adherence, in addition to cytolysis. That is, anaphylotoxin (C3a, C5a) induces the release of vasodilators such as SRS-A (slow releasing substance-A) and histamine from basophils and mast cells. These agents are important in promoting the egress of polymorphonuclear leukocytes from the intravascular space of regional capillaries. Once a polymorphonuclear cell has escaped from the vascular lumen, a concentration gradient of chemotactic factors (C3a, C5a, C6a, C7) then draws the inflammatory cells to the actual site of complement fixation. After the arrival of the inflammatory cells, fragment b of C3 (i.e., C3b) which has already bound to the immune complex, is able to accelerate phagocytosis. Immune adherence may also contribute to the accelerated phagocytosis because of the binding affinity of C3b for the cell membrane of polymorphonuclear leukocytes and macrophages. Thus the very immune complexes which fixed complement are subsequently eliminated by the inflammatory response that results from complement fixation.

The main physiological role of complement in man appears to be as part of the body's humorally mediated defense mechanism against infection, particularly against bacterial infection. However, the fixation of complement does not always have a beneficial effect. It can result in pathological tissue damage in disease processes such as systemic lupus erythematosus and other immune complex disorders (see section on immunopathogenic mechanisms below). In patients with such dis-

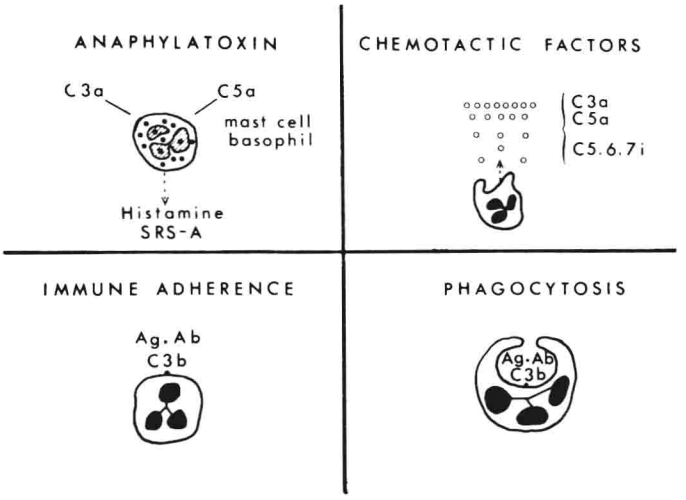

Fig. 1-6. Complement dependent activities. The inflammatory response evoked by Ag-Ab complexes is mediated by activation products bound to the complex (such as C3b) and by free or fluid phase products (C3a, C5a, C5, C6, C7i). (Reprinted with permission from P. Kohler, Medicine, 52:419–29, 1973.)

orders, serum complement levels are often abnormally low, a fact that can be of diagnostic importance.

The fixation of complement can also be used in vitro as a means of detecting the presence of such serum antigens as the hepatitis associated antigen, and as a means of detecting specific antibody (i.e., antiherpes simplex virus antibody). Further details concerning complement fixation assays are given in Chapter 2.

III. CELLULAR IMMUNITY

Although the T-lymphocyte, the B-lymphocyte, and the macrophage are all involved in humoral immunity, it is mainly the T-cell which is responsible for cellular immunity. An appreciation of this concept is best demonstrated by a brief discussion of immune deficiency states.

Agammaglobulinema, which results in an almost complete absence of humoral immunity, is a rare sex-linked genetic disorder in man, and can be produced in chickens by either hormonally or surgically resecting an organ unique to the chicken, known as the bursa of Fabricius. In either case, not only are immunoglobulin levels markedly depressed or undetectable, but B-lymphocytes and plasma cells are essentially absent. Rampant pyogenic infections are frequent complications in agammaglobulinemia. On the other hand, both bursectomized chickens and patients with agammaglobulinema have normal numbers of T-cells which also behave normally when examined in vitro (see Chapter 2). In vivo, T-cell functions such as allograft rejection, skin tests, and resistance against viral infection, are also normal. Thus patients lacking humoral immunity due to the absence of B-cells can have normal T-cells and, thereby, normal cellular immunity.

The antithesis of agammaglobulinema is thymic aplasia or neonatal thymectomy, which results in absent cellular immunity. Di George syndrome and Nezelof syndrome are classical examples of thymic aplasia, and "nude" mice provide a laboratory model of this state. Whether in patients or in animal models, thymic aplasia is invariably associated with the absence of the T-lymphocyte in both blood and lymphoid tissue.

Thus, in vitro cultured lymphocytes from athymic individuals do not undergo blastogenesis in response to T-cell mitogens, as occurs in lymphocyte cultures prepared from the blood of normal individuals. In vivo, skin tests are invariably negative to all antigens, allografts are not rejected, and severe and progressive viral and fungal infections are common. Yet immunoglobulin levels are normal, antibody production to T-cell independent antigens relatively intact, and pyogenic infections much less common than viral infections. In short, these studies of immunodeficiency disorders illustrate the key role of the T-cell in cellular immunity, and that cellular immunity can remain intact in the absence of the B-cell.

Although larger antigenic determinants are required to ignite a cellular as opposed to a humoral response, T-cell sensitization, like antibody production, follows exposure of the immune system to a foreign antigen. Thus an antigenic determinant composed of 5–6 amino acids which might evoke a humoral response would probably not evoke a cellular response. As with antibody production, on the other hand, a cellular response can usually be detected 7–10 days following primary immunization. In addition, although cellular immunity is more difficult to quantitiate than humoral immunity, there is a cellular response, referred to as the second set phenomenon, which is roughly analogous to the amnestic antibody response. That is, if an initial skin graft from a C3H to a C57 mouse requires 12–14 days to be rejected, a second skin graft from the same donor to the same host is rejected much more rapidly. Alternatively, the second set phenomenon can also be seen by passive transfer of lymphocytes, sensitized to the skin of a C3H mouse, to an unsensitized C57 mouse. When this is done, a subsequently placed C3H skin graft on the passively sensitized C57 mouse also demonstrates the second set phenomenon (Fig. 1-7). This technique of passively conveying cellular immunity with viable lymphocytes is referred to as adoptive transfer and should not be confused with passive immunization, which refers to the transfer of antibody.

Although cellular immunity is known to play an important role in host defenses