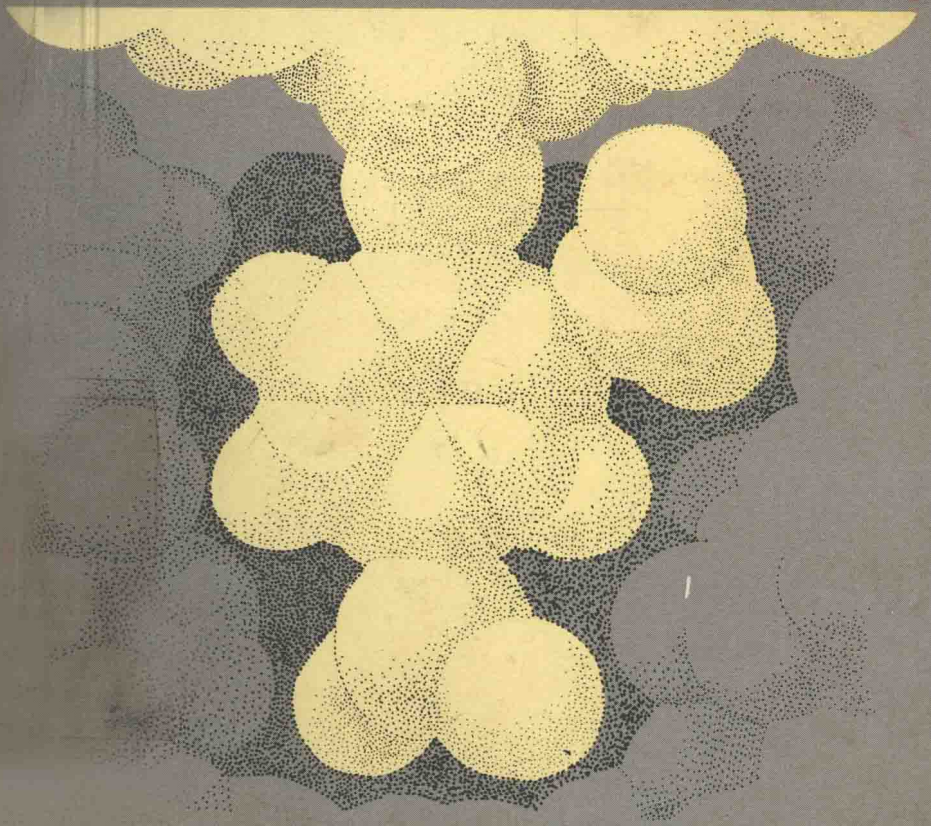
F. M. BURNET

IMMUNOLOGY, AGING, AND CANCER

Medical Aspects of Mutation and Selection



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IMMUNOLOGY, AGING, AND CANCER

A Series of Books in Biology
Cedric I. Davern, Editor

PREFACE

WHEN I ACCEPTED the invitation from students at University of California, Irvine, to give the lectures printed here, I stipulated that I would talk only about what I was currently interested in. This may explain the apparent wide range of topics contained within a very short series. It will be found, however, that there is a common Darwinian theme running through them all, a theme that I believe could provide a useful stimulus toward a fuller understanding of cellular biology as it bears on medicine. I have tried to simplify the discussion as much as possible, and without doubt some of my simplifications will be superseded in the future, but I hope that, till then, they may aid understanding for those interested in the biological borderlands of medicine. The lectures were given at Irvine in April 1975, and, in modified form, at the University of Southampton Medical School in the following month.

May I express my appreciation to the students at Irvine for the opportunity to spend three pleasant weeks on their campus, and especially to my student hosts, Kit Campion and Ken Lehmann, for their friendship and solicitude for my well being.

F. M. Burnet

Melbourne, Victoria
March 1976

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CONTENTS

CHAPTER ONE

THE BASIS OF IMMUNOLOGY

MEDICINE IS A FAR older human interest than biology, and the whole of my formal training was medical, yet in this reshaping of a series of lectures to undergraduates the reader will find much more concern with biology than with medicine. If the book has a central theme, I should like to think that it is a consistent attempt to apply a Darwinian approach to cellular processes within the body. This holds particularly for my approach to immunology in the first four chapters, but a similar evolutionary attitude pervades the later chapters as well.

In this first section I want to present a broad picture of immunity and immunology as an element in the process by which we and all other mammals survive. Survival is the business of evolution, and if

we are to have a satisfying understanding of any life phenomenon bearing on survival, we must have some concept of how it evolved. Let us look first at the sort of phenomena on which immunology is based. The first immunological observations were probably made long ago, when people began to recognize that an individual who had recovered from smallpox was immune against a second attack. Because anyone who had recovered carried the scars of his experience on his face, the relation of immunity to the fact of past infection was very obvious. Out of that observation came the idea that immunity to smallpox might be acquired, without facial disfigurement, by artificial variolation, or by Edward Jenner's vaccination with cowpox. That was the central concept of human immunology from Jenner's time to the present, with effective immunization against polio and measles being the last two significant achievements.

Incidental to such work was the discovery that usually recovery from infectious disease was associated with the presence in the blood of antibody, a modified immunoglobulin, a protein that would combine specifically with the responsible microorganism, and so facilitate its destruction in the body. I shall have a lot to say about antibody, but I should make it clear at once that nowadays we realize we know much less about how antibodies function than we thought we did 20 years ago. Gone forever is the comfortable idea that immunology meant finding the microorganism that causes a disease, making a vaccine, and protecting the child against that sort of infection by the antibody which the vaccine stimulates the body to produce. There is much more to it than that.

Probably the next immune phenomenon to be recognized was discovered by surgeons, especially plastic surgeons. They could patch up a man's injuries in remarkable fashion by taking grafts of bone or skin from one part of the body and implanting them where they could replace defects. But a piece of skin from another person was rejected within two weeks.

A final example which will help to complete the range of immunological phenomena and allow me to define the essential basis of immunity is the Rh baby. It is not uncommon to find a family in

which, after two normal babies, the third is born suffering severe destruction of its red blood cells and, unless it receives an exchange transfusion of suitable blood, may die. Such a situation arises only when the father is Rh positive and the mother Rh negative, i.e., he produces red cells carrying a certain Rh antigen D but the mother does not. The fetus receives half its chromosomes from each parent and can therefore sometimes differ from the mother in having the Rh antigen D on its red cells. If those red cells enter the mother's circulation, she can respond by antibody production against the Rh antigen D, especially if she had been rendered susceptible by a previous pregnancy of the same quality. If the mother develops a high enough concentration of antibody in her blood, enough may leak into the fetus toward the end of pregnancy to give rise to hemolytic disease.

Basically, immunology is concerned with how and why the body reacts actively against almost anything that is foreign, that is genetically different from its own substance. Sometimes, as in rejecting a skin graft, or in a mother's attack on her own infant's red cells, the difference may seem very slight indeed. In immunology we are seeking to understand how the body can recognize the difference between self and not-self, and ensure that, whereas not-self is destroyed or cast off, the body's own self-substance normally provokes no reaction. There are, however, exceptions that will turn out to be important. Well before immunologists began to recognize the difficulties of grafts from other individuals, embryologists found that they could graft a piece of embryonic skin from one chicken embryo to another with ease, and have the recipient hatch and grow up with, for example, a patch of black feathers amongst its own uniform white ones. Workers with insects, too, found that they could transfer glands from one specimen to another without their being rejected. In general, invertebrates show little ability to recognize fine differences, although they will reject tissues from more distant species. Subject to minor qualifications, it is correct to say that vertebrates have a much finer capacity to distinguish self from not-self than invertebrates, and that before a certain stage in embryonic development vertebrates too fail to make these fine

distinctions. But, as I shall have to say repeatedly, it is important to recognize that all general statements in immunology are likely to need qualification sooner or later. Immunology is a soft-edged science, and physicists and mathematicians are neither comfortable nor successful when they dabble in its problems.

I think it will be already evident, in what I have said, how importantly I believe that immunology is linked with genetics. When we use the terms "foreign" or "not-self" about cells or tissues, we mean always "not genetically proper to the individual." Once the concept of protein synthesis being governed by the genetic information in deoxyribonucleic acid (DNA) had been formulated, and especially when the way in which the information was transcribed and translated had been worked out, it was evident to everyone that the specific antibody pattern of immunoglobulin was genetically determined. The clonal selection theory was the first attempt to work out the mechanism by which that could be possible.

Genetic information is expressed in the phenotype only after a lengthy process in which synthesis of protein, including immunoglobulin and antibody as well as all the enzymes and structural proteins of the cell, is only the beginning. The process by which the fertilized egg cell, the zygote, proliferates into a ball of cells and then molds itself into developing organs and supporting tissues is differentiation, the next great problem for theoretical biology. Like the rest of the body, the immune system is a product of differentiation, and we have only a superficial picture of its progress at the cellular level. It is convenient, however, to outline the current convention for naming the main stages. This is a purely provisional schema that is bound to undergo progressive modification, but if I set it out here it will be easier to develop the discussion.

The sequence, then, begins with the hematopoietic stem cell from which all the mobile cells of blood and lymph are thought to develop. From one line of descendants come the lymphocytes, divided into T cells and B cells. At a certain stage, lymphocytes of both groups develop specific receptors which allow them to respond to contact, for each cell, with one particular type of antigen. I find it convenient to follow William Dameshek and call these immu-

nocytes. "T cells" are so called because in mice they develop only in the thymus, which is also the dominant, but perhaps not the only, source in other mammals and birds. The name "B cells" originally referred to origin of these cells in the bursa of Fabricius of chickens, but nowadays the group is defined by the active production of immunoglobulin easily recognized on the cell surface by immunofluorescence. The final, mature, postmitotic state of the B cell takes the form of the plasma cell, a specialized factory for rapid immunoglobulin production.

This is probably enough to remind you of the general outline of vertebrate, and particularly mammalian, immunology as an introduction to saying something about the probable ways in which the immune system evolved. The main problem, and perhaps the only one which has a chance of being resolved, is how the effective but not very versatile defense mechanisms of most or all invertebrates developed into the refined and flexible immune system of the higher vertebrates. There are two aspects of vertebrate immunology that are common to all types above the most primitive forms. The first is the ability to produce recognized antibody and the second is exemplified by the fact that a man, a chicken, or a goldfish can recognize—by rejecting it—the foreignness of a piece of skin from another individual of the same species. No antibody has been observed in any invertebrate, and although slow rejection of foreign tissue grafts has been described in earthworms it is much less rapid and effective than rejection in mammals and birds. There are still many problems to be solved about both these aspects of immunity, and much of what I have to say in later chapters will be concerned with them. In this preliminary outline it is probably best to start by looking broadly at the situation in invertebrates.

Just as much as mammals, all invertebrates must have an effective set of mechanisms to avoid microorganismal invasion. Every living organism is a potential source of nutrients for the ever-present microorganisms in its environment, and effective defense is essential. It is conventional to put most responsibility on the wandering mesenchymal cells present in most or all metazoa. These are phagocytic for any microorganisms or particles of damaged

tissue and are doubtless of importance for defense. However, this merely pushes the problem one step farther back. The capacity of the phagocytic cell to recognize the microorganism as foreign, and to digest it without damage to its own constituents, is presumably just a specialized example of a general capacity of living cells that may remain beyond analysis at the molecular level for a very long time.

I find that the more insight one gains into detailed processes within and between cells, the more difficult it is even to conceive how the almost infinite number of processes that are going on manage to avoid serious interference with one another. At all levels there must be "recognition" that the situation is as it should be or that something is wrong and calls for some correcting response. I can recognize that situation in the chromosomal mechanism of *Escherichia coli* or any other cell, in the reaction of the cytoplasm of a phagocytic cell to an ingested particle, or in the immune system as a whole, and I am dubious whether we shall ever reach the stage of interpreting it in molecular terms. I suspect that we may always have to be content with the use of general terms like recognition, specific response, activation, toxic damage, and so forth, in the explanation of most immunological phenomena, and attempt a deeper study only in a few specially favorable situations.

With this modest objective, let us start with invertebrate reactions that show at least a capacity to differentiate between self and not-self. It is well known that a variety of colonial marine organisms will fail to fuse with a portion of any colony that is not genetically similar. At the interface a damaging interaction takes place and a clear line of demarcation develops. Theodor's analysis (1971) of this in *Gorgonia* assumes the existence of a bifunctional killer molecule in the tissues of each which is held inert by being specifically bound to a self-type inhibitor. When the killer molecule complex diffuses into the foreign tissue, it is liable to dissociate, and the absence of a specific inhibitor of the right type allows its toxic quality to be exercised. The essential feature in these organisms, and probably in all metazoa, is the existence of a variety of mechanisms with the common quality of being able to recognize

the "rightness" of the local situation. Evolution has found ways to ensure the development of sterically complementary molecules or structures which, when mutual recognition occurs, can generate a signal that in essence says "all's well" and inhibits any response evolved to protect the integrity of cell or tissue. It seems likely, in fact, that wherever cells are related to each other as part of the same organism, they must be capable of recognizing the "rightness" of any cell with which they are in persisting or transient contact. This idea could be elaborated considerably in reference to the wandering coelomocytes or hemocytes of the more advanced invertebrates, but for the present all I want to emphasize is that some way must have been invented early in evolution for recognizing self. Implicit in this invention was the potentiality that it could also be used to recognize foreignness by the randomization mechanism, which I shall speak about in the second chapter.

In my opinion there is no convincing evidence of antibody production or of immunological memory in any invertebrate, but there is plenty of evidence that, where it is biologically significant, self can be differentiated from not-self, and "defensive" responses initiated by failure to achieve self-to-self recognition. This, I should point out at once, is a completely different process from the various responses seen in vertebrate immunology as a result of positive recognition by cells of not-self. In a typical invertebrate we have (a) an effective protection against the multiplication of casual microorganisms in the body in which phagocytosis by wandering cells appears to play a part; (b) a variety of not very effective responses to specifically pathogenic microorganisms and metazoan parasites. In general it seems that the invertebrate strategy for species survival is to produce enormous numbers of offspring and accept very large losses from predation, parasitism, and so forth; (c) a limited capacity to recognize self tissue or cells, in the sense that a positive protective or compensatory response occurs when this fails to be achieved. These, then, may have been the basic qualities common to invertebrates from which the vertebrate immune system had to be evolved.

My picture of the way in which the vertebrate system evolved is based to a large extent on Marchalonis's formulation of the facts

(1975) as far as they can be obtained from present day forms. My ideas about the evolutionary process that gave rise to the changes are not dissimilar to his, but I must accept the responsibility for the more picturesque, not to say outrageous, quality of some of my speculations.

I might first state the essential qualities, the parameters of vertebrate immunology, as tabulated by Marchalonis.

1. There are circulating lymphocytes in all vertebrates, and plasma cells in advanced sharks and higher forms.
2. All can produce antibodies.
3. All can reject allografts without the necessity of previous sensitization or immunization. There is, however, quite a sharp difference between the acute rejection seen in mammals and birds and the much slower chronic responses seen in most of the more primitive vertebrate forms.
4. The lymphoid system develops progressively from a minimal requirement of diffuse lymphatic tissue around the gill region—a presumptive anlage of thymus.
5. The ability of antigens bound to antibody to fix complement is seen in all forms above the cyclostomes.

Apart from some equivocal examples of tissue rejection, none of these are found in invertebrates.

Let me begin by stating briefly the general approach to vertebrate immunology that I shall adopt so that I can avoid the necessity of a cumbersome approach from first principles. The justification for adopting one particular set from the variety of alternative interpretations will, I hope, emerge in the course of the chapters that follow. In summary, then:

Most immune reactions are mediated by antibody, i.e., specifically reactive immunoglobulins, in the form either of soluble molecules or as receptors attached to the surface of lymphocytes and other mobile circulating cells. It is assumed that all antibody receptors are synthesized by B cells but that passively acquired immunoglobulins can function as receptors to T lymphocytes, macrophages, and mast cells.

A second distinct system of immune reactivity is concerned not with foreign microorganisms or proteins but with cells of the same species that are differentiated by their major histocompatibility antigens (MHCA's). The corresponding receptors have not yet been chemically characterized and will be referred to as allogeneic receptors (AR's). They are present on and may be limited to T lymphocytes.

The development of the antibody-immunoglobulin system was one of the most fantastically ingenious inventions of evolution. The immunoglobulins seem to be derived from some primitive recognition mechanism based on a rather small protein type referred to now as $\beta 2$ microglobulins. In any modern diagram of a typical antibody, the antibody is shown to be composed of a series of segments or "domains." Each of these has a closely comparable structure of about 110 amino acid residues and a loop formed by a disulphide link between half-cystine residues around positions 30 and 90. The suggestion that each segment represents the result of tandem duplication of a single ancestral gene is compelling. It created great excitement a few years ago when it was found that the major histocompatibility antigen, in both mice and men, was a complex of the antigen proper and a $\beta 2$ microglobulin of essentially similar structure to an immunoglobulin segment. Similar $\beta 2$ microglobulins are widespread in cells, and quite obviously proteins of this sort must resemble closely the gene product of the ancestral gene from which the immunoglobulins evolved.

One can make a few suggestions as to how the immunoglobulin system may have arisen. Even the most primitive stem cell has a reactive cell surface, and it will react more with some chemical structures than with others. No doubt there is at least some potential for specificity in those reactions. The next step in my speculative scheme is a self-recognizing configuration which, though completely symmetrical, has the potentiality of evolving either to (1) a specific individuality marker, a major histocompatibility antigen, or (2) a recognition mechanism, an Ig receptor, or soluble antibody. Like other immunologists, I am puzzled by the nature of the T cell receptor, and at times I see no reason why both sides should not be