SIR MACFARLANE BURNET

Auto-immunity AND Auto-immune Disease

AUTO-IMMUNITY AND AUTO-IMMUNE DISEASE

A survey for physician or biologist

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Preface

In 1957 or thereabouts I became impressed with how immunity could be looked at as a process of Darwinian selection amongst the circulating lymphoid cells of the body. The clonal selection theory which grew out of this has been generally accepted in principle by immunologists, but I do not feel that its full implications in relation to pathology have yet been widely realized. In a previous book, *Immunological Surveillance*, I have tried to apply the approach to cancer immunity. This is a basically similar attempt to look at auto-immune disease from the same Darwinian point of view.

Anyone who attempts to produce acceptable general statements about complex biological and clinical phenomena must have a certain sense of guilt. No biological phenomenon can ever be completely, or even adequately, described. There can, at best, only be a progressive improvement in the acceptability, the intellectual elegance, or the practical usefulness of the working generalizations that can be produced. Any attempt to write at this interpretative level can easily be brushed aside as superficial, unnecessary, and liable to be proved wrong or irrelevant by new developments. It is anathema to many good professional scientists and their objections are real enough.

Yet one can still ask whether there is any human significance in what we are doing if it does not help to provide, for those who want it, the best understanding of some corner of the universe in terms intelligible to the non-specialist and not currently at variance with the scientific record. This is not a book for the professional immuno-pathologist, but I hope it will be useful for anyone with a real peripheral interest in auto-immune phenomena to see its problems a little more clearly.

F. M. BURNET

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CHAPTER 1

Introduction

When an academic scientist retires he loses his salary and, unless he is very lucky, his secretary. Many other things that seemed important vanish, but there can be compensation in a new freedom. In a senior scientific post one's overriding concern is to try to ensure a satisfying career for the younger people who have come into research under one's aegis. It will do them harm if their sponsor is not well regarded by his senior contemporaries. In particular he must be seen to conform to the rules. There are rules about research which are vital to its integrity and which must never be broken; but there are others which are mere conventions. One is that the only legitimate activity for a scientist must be firmly centred on experimental work under his own control and in part at least done with his own hands. Another is expressed in Medawar's epigram that science is the art of the soluble: that it is only justifiable to ask questions in such a form that at least in principle they can be solved by acceptable experimental methods. As a working scientist I accepted them all as necessary and socially expedient just as I knew that it was wise to keep doubts about the human relevance of much good scientific work to myself. With retirement I have felt free to question them.

In particular I have relished the opportunity to try to see the outline of some of the woods in which I have spent my life examining twigs, branches, or even sometimes a whole tree. I am not self-disciplined enough to eschew the fascination of trying to correlate phenomena in terms of general concepts. The wider they are the more satisfying they feel. My justification, or rationalization, for applying this attitude to classes of phenomena which are admittedly too complex or too ill-defined to be ripe for generalization can be stated under three heads:

1. Any clearly stated hypothesis can stimulate good new experimental work to disprove, modify or confirm, and extend the theory in

question. I can claim with the support of a number of other immunologists that my (1957) clonal selection theory of immunity acted significantly as a catalyst to immunological research in the next decade.

2. Any generalization that covers a wide range of phenomena, even if its validity subsequently proves to be merely provisional and contemporary, can serve as an important aid to the practical man (usually a physician in my context) when he has to make an individual decision on what is always inadequate information. If someone generalizes that an 'auto-immune disease' has the basic quality of a conditioned malignancy due to one or a small number of mutant clones analogous to those of a lymphoma, the clinician may feel, for instance, that he is justified in testing cyclophosphamide or methotrexate as therapeutic agents.

3. Whenever an important new generalization emerges in one field of science, it is usually possible to see that a new need has risen to look at all the adjacent scientific areas in the light of the new concept. Every advance in organic chemistry (e.g. the use of nuclear magnetic resonance in resolving molecular structure) is soon applied to biochemical matters. In somewhat similar fashion any new enlightenment in theoretical immunology, cytology, or virology may have a bearing on the understanding of auto-immune disease. As long as science advances every major theme will need to be restated continually in the light of relevant advances in other fields.

As of 1971 every pathologist and every academically minded physician is aware that a steadily growing number of subacute or chronic diseases are being spoken of as auto-immune, i.e. resulting from misdirected immune responses against tissues or cells in the body. Not everyone believes that by calling a disease auto-immune or auto-allergic anything useful has been accomplished, and this attitude is reflected with various qualifications in most current medical and pathological writing.

In looking over immunological texts published since 1965 I can find no satisfactory general treatment of auto-immune disease based on modern immunological concepts. In many there is a hardly changed attitude that immunity means antibody and that antibody is constructed to a pattern appropriate to deal with antigen. The commonest suggestion is that, under the influence of drug or virus, cell antigens may be changed or previously segregated ones uncovered. The resulting immune response is responsible for the disease. The simple objection to this is

that normal people suffer all types of infections, every variety of trauma, and are given many drugs. At least 99 per cent do not develop diagnosable auto-immune disease. To do so is always rare and most often there is no clear preceding cause or trigger. *A-priori* individual factors, presumably genetic and somatic genetic, would seem the most reasonable explanation for the rarity and individuality of cases of auto-immune disease.

There is a more deep-seated objection to any theoretical approach involving conceptions of somatic mutation or any similar processes. Many physicians and clinical scientists accept with varying degrees of explicitness that no disease condition can be understood other than as a result of some defined or definable impact of the environment on the body. They prefer to look for a slow virus, chronic poisoning by an industrial chemical or psychosocial trauma, rather than accept genetic or somatic-genetic processes, including auto-immune disease, as a valid interpretation of someone's illness. This I believe is an untenable position. There are important immunological anomalies in many disease conditions and in some the auto-immune character is almost self-evident.

My experience in writing at the theoretical level on immunological topics has been to find that most reviewers felt that speculation should stay much closer to the current state of experimental work, and that the only virtue of wider generalization was to suggest new experiments or new approaches to clinical management. I could claim, in extenuation, that the concepts introduced with the phrases 'clonal selection' and 'immunological surveillance' have been accepted as useful by immunologists and that the second is having a significant practical impact on the understanding of the appearance of malignant tumours in patients under immunosuppressive drug regimes. So, in attempting a book of similar type on auto-immune disease, I shall be concerned essentially in elaborating the idea of forbidden clones that from the first development of the clonal selection concept seemed to derive automatically from it. I still believe that, despite my failure to convince most immunologists of the usefulness of the approach, all new work on auto-immune disease is compatible with the idea and is progressively strengthening it.

The basic approach

Perhaps the two diseases which are most widely acceptable as auto-immune are acquired haemolytic anaemia (warm type) and Hashimoto's

disease of the thyroid. Both, however, are still officially diseases of unknown etiology. About ten years ago the mouse strain NZB was shown to develop a reasonably accurate model of auto-immune haemolytic anaemia, and an F1 hybrid with another strain, NZW, derived from the same mixed stock showed regular development of lethal kidney lesions. It seemed that here was the material to establish the nature of auto-immune disease once and for all. After a dozen years of work in many laboratories there is still no interpretation that has been found generally acceptable. Even the self-evident genetic character of the disease has been called into doubt: it could be a vertically transmitted slow virus disease.

The objective in writing this book was to present as far as possible a consistent interpretation of both clinical and experimental phenomena of auto-immunity in terms of the clonal selection approach. To a considerable degree the first development of that approach in 1957 sprang from consideration of a case of macroglobulinaemia (proliferation of primitive lymphoid cells with production of excess immunoglobulin M) in whose blood Gajdusek and Mackay found a high titre of antibody reacting with human tissue extracts. Ever since then auto-immune disease has been a major interest. Much more is now known about the field, but the advances have not been revolutionary ones. My own approach is still expressed as a logical and lineal development of the forbidden clone concept formed within the framework of clonal selection theory in its first extended statement (Burnet, 1959). Like every conceivable interpretation it must constantly be updated and correlated with developments of other aspects of biology, in particular in the fields of general immunology and oncology. The hypothesis in its most generalized form will be as follows:

1. It is necessary for survival that neither immunocytes nor antibodies should exist in the body which are reactive to more than a minimal degree with any accessible body component.

2. Since immune pattern is generated by a random process a mechanism must exist by which any 'self-reactive' cells which may emerge can be eliminated or functionally inhibited. More than one mechanism may be needed to establish and maintain this intrinsic immunological tolerance toward self components.

3. There are many genetically determined anomalies in the functioning of the immune system ranging from agammaglobulinaemia, where the

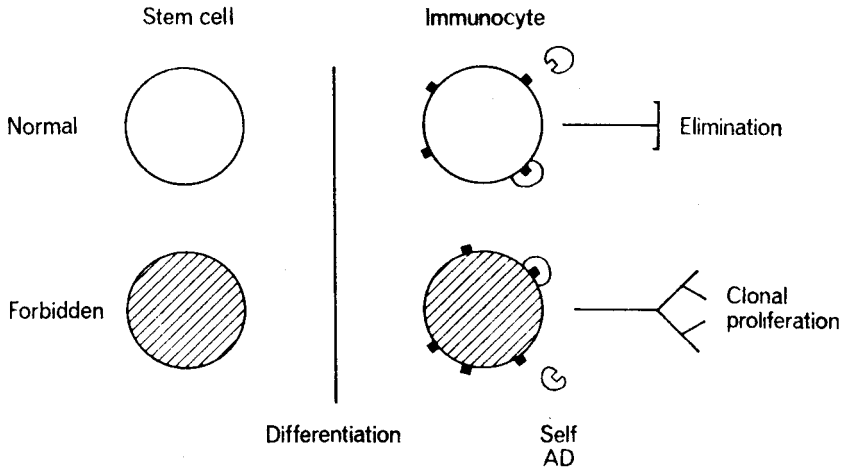

FIG. 1. The essence of the forbidden clone concept. A stem cell on differentiation becomes an immunocyte carrying a specific pattern of antibody-like receptors. If accessible antigenic determinants (AD) are present soon after differentiation any normal immunocyte capable of reacting with them will be eliminated. If, however, the stem line has undergone at some point a somatic mutation, increasing resistance (shaded), the differentiated immunocyte can, on specific stimulation, initiate a forbidden clone.

patient has no capacity to liberate antibody into the blood, to minor degrees of susceptibility to allergic reactions. Genetic susceptibility to develop auto-immune disease undoubtedly exists. Its basis has not been elucidated at either the genetic or the molecular level, but it is reasonable to suggest that it functions by shifting the 'point of decision' determining whether an immunocyte reacting with its corresponding determinant will be stimulated to proliferation and functional activity or will be destroyed or functionally inhibited.

4. Changes due to somatic mutation or to physiological or pharmacological factors may render a newly differentiated immunocyte (the antigen-reactive cell of most investigators) insusceptible to elimination or inhibition by the 'censorship' mechanism concerned. As in virtually every statement one can make in an immunological context, the word 'insusceptible' is not an absolute but simply refers to a range of susceptibility below the level, determined by genetic factors, of the normal newly differentiated immunocyte.

5. When, by somatic mutation, an immunocyte line develops which (*a*) has an undue resistance to elimination by antigenic contact and (*b*) reacts specifically with an accessible body component functioning

as antigenic determinant, it is potentially capable of initiating a forbidden clone of directly or indirectly pathogenic cells. As such they will have all the essential characteristics of a conditioned malignancy.

6. In all probability the clinical manifestations of auto-immune disease are due (*a*) to aggressive T-immunocytes (see p. 51 ff.) producing damage to target cells, (*b*) to the deposition of antigen-antibody complexes in the kidney or elsewhere, and (*c*) to a directly damaging action of antibody, but probably only when the auto-antigen is present in the circulating blood either on cells or as a soluble component.

7. The accessibility, amount, and physical character of the (auto-)antigen plays an essential part in determining both to what extent a potentially pathogenic clone is stimulated to proliferate and what opportunity it has to damage cells and tissues in the body.

It will be evident that this hypothesis of auto-immune disease is not one which can be stated bluntly and briefly in any such terms as 'a slow virus disease', 'the result of somatic mutation', 'due to antigen modification by virus or toxin', or 'a collagen disease'. To provide a reasoned argument for, and against, the theory as summarized, it seems necessary and logical to use the introductory chapters to discuss what seem to be relevant aspects of some more fundamental areas of biology.

The first can be spoken of broadly as the stochastic approach in biology which is concerned with the regularities that emerge from the occurrence of rare and random events with continuing consequences. In the field of mammalian pathology this must be concerned primarily with somatic mutation though there is a growing feeling that essentially similar processes are important in many of the normal functions of differentiation in the body. It is accepted by all that the generation of diversity of pattern in antibodies and immunocyte receptors has such an origin and it would be strange if similar principles did not apply elsewhere.

In so far as human disease (including auto-immune disease) is concerned, the most important observable consequence of any process involving somatic mutation is the age-specific incidence of the disease which results. For some diseases it may be possible to express the age incidence in terms of the total population at risk in each age-group. More often it is simpler to assess the percentage of all cases of disease that fall in each age-group. The criterion for selecting the significant

date (and age) for each individual may be either by the onset of clinical disease, the appearance of a significant auto-antibody in the blood, or death from the disease. Burch's important work in this area will require sympathetic, if occasionally critical, discussion.

The second area is that of classical immunology, of which three particular fields bear most closely on auto-immunity. One concerns the apparently unique capacity of the immune system to produce by some type of randomized genetic process an immense diversity of immune patterns. Such immune patterns may be potentially useful, potentially dangerous, or simply irrelevant. This calls for means of getting rid of those that are potentially dangerous. Neglecting for the present some observational and logical difficulties, this is the basis of the important field of immunological tolerance. Auto-immune disease is, in the view to be adopted, the result of a failure to develop normal tolerance to some bodily component. The second field comprises what is probably the most significant development in recent immunology, the differentiation of the T and B immune systems concerned approximately with cell-mediated immunity and antibody production respectively. Both are deeply involved in auto-immune disease.

The third area for preliminary discussion is concerned with the principles of pharmacology in so far as they impinge on the production of cellular damage by immunological processes. Closely related and still far from being completely understood is the drug-like action of antigen (or antigenic determinant) on the specific receptors of the different varieties of immunocyte. It is perhaps significant of the possible breadth of this area that Boyden once put forward the view, admittedly only half seriously, that all inflammation is essentially an auto-immune reaction. It is quite difficult to find a formal disproof of such a thesis.

In undertaking a discussion of auto-immunity and auto-immune disease I have a fairly well-defined objective of the same type as I had in two previous books: *Self and Not Self*, a discussion of general immunological theory based on the clonal selection approach, and *Immunological Surveillance*, dealing with cancer immunity. The aim in all three has been to provide a general account which would be acceptable in outline to professional investigators in each field, but would be directed more to those interested in an attempt to look at clinical and pathological matters from a consistently biological angle. I do not think that it is only a reflection of my own interests to say that in the last two decades it is the biological disciplines concerned essentially.

with abnormality and infection, immunology, oncology, and virology which have contributed most to new understandings of the principles of biology. It is probably equally true to say that, for anyone seeking an understanding of the biology of man, the existence of cancer, auto-immune disease, and ageing are essential components of the whole human situation.

In any writing of this sort there are two related difficulties which in one, always unsatisfactory, way or another must be overcome. The relevant literature is far too large for anyone to read *in toto*, and reference to all the papers one has read and which seem relevant would hopelessly clog the sort of discussion required. The solution adopted is to state everything which appears to be broadly non-controversial without references unless there is special historical interest in quoting the circumstances of some discovery or new conception. Where a controversial situation has to be mentioned and either left unresolved or one of the alternatives accepted, a few key references will be given. It is inevitable that in the preliminary chapters a good deal of what was said in the earlier books will be paraphrased or summarized, but it will always be specifically slanted towards its relevance for the auto-immune phenomena with which we are concerned.

This is by no means a comprehensive clinical text and the discussion of clinical features is much more concerned to exemplify immunological principles than to offer help in diagnosis or treatment. However, most of the important auto-immune diseases of man are discussed at some length as well as a number of rarer conditions and some whose nature is still problematical. Considerable use will be made of experimental work both on normal animals and on genetically predisposed strains of mice, but nothing at all will be said about the examples of auto-immune disease that may be met with in veterinary practice.

CHAPTER 2

The stochastic approach to disease

In 1957–8 I was already deeply interested in what was coming to be called auto-immune disease and it became immediately obvious that if a clonal selection theory of immunity was to be accepted it must be applicable, with appropriate modifications, to pathological manifestations of immunity as well. The concept of the 'forbidden clone' emerged almost at once and I have seen no reason to withdraw the phrase or the idea since. It is a concept which can accept infinite elaboration to fit specific instances but yet remain basically very simple. In essence, a forbidden clone arises from a stem cell line in which two types of genetic individuality develop in the course of differentiation and somatic mutation. One is concerned with the nature of the 'immune receptor'. It must allow the immunologically reactive cell (immunocyte) to react specifically with some accessible component of the body acting as an antigenic determinant. The second is a genetic anomaly that makes the cell more resistant than normal to the processes which should result in the elimination of such autoreactive cells from the body. Instead, when conditions are appropriate, it is stimulated to proliferate to form a forbidden clone with potentially pathogenic effect.

In all essential respects a forbidden clone is equivalent to a clone of malignant cells. It is being stimulated to multiply by the constant availability of the corresponding antigen, although for reasons to be discussed later there is almost always some secondary control which prevents it developing into a full-blown malignant leukaemia or a near malignant macroglobulinaemia.

Much of what has been written about malignant disease is also applicable to auto-immune disease. In particular, the basic rule determining whether or not a somatic mutation is of any significance to the organism as a whole still holds. All mutations (i.e. all genetic changes which leave a cell still capable of mitosis) are rare, and random both in respect of time and of the informational content of

the genome. There are sufficient cells in every functioning organ or system for functional abnormality of any sort in a *single* cell to be of no account. A functional abnormality only becomes significant when it endows the cell and its descendants with a capacity to build up an excessive clonal population.

Somatic mutation as exemplified in mammalian skin

It is almost conventional to label anyone postulating that such and such a phenomenon results from somatic mutation as an armchair theorist too lazy to analyse the phenomenon experimentally. There may be a faint basis for the accusation, for somatic mutation is a highly flexible concept which in the limit is almost equivalent to saying that by mutation a cell can lose or gain any qualities that the investigator cares to postulate. Yet exactly the same objection could be levelled at any discussion of the part played by point mutation, deletion, gene duplication, and other intragenomic processes in the germ line, in providing the raw material for evolution. Every observable quality of a living organism is accepted as being determined by, or at least deeply influenced by, genetic processes. At the somatic level everything suggests that very much the same processes can occur in the nucleus as take place in that of a germ-line cell. There is even some evidence that mutation can involve the same genes and take place with similar frequency at both levels.

It is quite impossible to understand auto-immune disease, or malignant disease, without making use of the concept of inheritable change in somatic cells.

In an attempt to clarify ideas on somatic mutation it seems expedient to discuss the one tissue of the body in which the effects are visible to any observer: the skin and its pigment cells (melanocytes).

Normal changes

There is almost a social obligation on the young in Australia to develop as extensive a sun-tan as convention and comfort will allow. The process is easier in some people than others but is possible to some extent in everyone who is not an albino. This can be regarded as an adaptive device involving all or most of the melanocytes and probably set in action by the mild inflammation of sunburn. This in turn is due to the shorter wavelengths of sunlight which impinge on the area exposed. As pigmentation increases, the effective

wavelengths are absorbed before they can produce any inflammatory effect. The adaptive value of the response, which probably involves both an increase in the number of melanocytes and in their activity as melanin producers, is immediately evident.

Recently there has been some interesting discussion about the evolutionary processes that led to the appearance of the very lightly pigmented North European from the ancestral African hominids who must have been black- or brown-skinned. The suggestion is that the chief metabolic function of the skin is to convert inert sterols into calciferols with vitamin D activity. The near ultra-violet is necessary for this and in a high latitude both the amount reaching the skin and the amount of skin that can be exposed to light are reduced. So a minimum of shielding against the ultra-violet was desirable and the pigmentation of the skin grew progressively less. Obviously the melanocyte system must be capable of adaptation to the local environment at the level of the individual and of undergoing evolutionary changes dependent in the last analysis on germ-line mutations in the controlling genes.

In children and adults with genetically poor development of skin pigmentation—traditionally the small boy with red hair and thin, white skin—exposure of skin to sunlight results not in uniform tanning but in freckles. A freckle is a pigmented area resulting from the proliferation of a single melanocyte, originally located at the very centre of the circular freckle, to produce a clone of more than ordinarily active melanocytes. To the best of my knowledge no one has had the curiosity to apply modern scientific methods to find out the details of the freckling process, and its interpretation as resulting from somatic mutation is no more than a deduction from appearances. The facts are straightforward enough. Freckles are circular and enlarge peripherally, their intensity of pigmentation is uniform over the whole circular area. In some children there are freckles of different intensities of pigmentation, as many as five different types being sometimes recognizable. The very dark brown freckles sometimes seen are smaller than the others and probably represent accumulations of more than one layer of melanocytes. Freckles develop preferentially in some areas of skin, but only in skin exposed to sunlight. They vary greatly in their subsequent history and probably most often fade into the general background of skin pigmentation.

I believe that a consideration of the basis of these appearances is directly relevant and rather helpful toward an understanding of what is involved in auto-immune disease. In both we adopt the hypothesis that somatic mutation is the basic process. In accounting for freckles we reach a number of conclusions that seem to be of general interest to biology and pathology. The first is that the mutation produced is such that its functional effect is very similar to what happens in all melanocytes of non-freckling individuals. It proliferates to some extent, produces on the average more melanin, and its descendants of the new clone move in such a fashion as to maintain a single layer with each melanocyte approximately the same distance from its neighbours. The mutation is induced by exposure to sunlight and, since ultra-violet light is a well-studied mutagenic agent in the laboratory, we assume that it is the shorter wavelengths that are responsible. Since the commonest thing to be observed as a result of mutation closely parallels an adaptive response, we must assume that what has been inheritably modified is that part of the somatic genome which determines the normal adaptive reaction. Some very interesting genetic problems (concerning the nature of 'phenocopies') arise here but are not relevant enough for discussion. We know that up to five pigmentary mutations can occur in the freckling process and it is a legitimate speculation that simultaneously a many times greater number of invisible and irrelevant mutations of other types occur in other cells of the melanocyte population. We can be certain that melanocytes move about, divide occasionally, and occasionally vanish. There is scope for competitive survival between clones and it is simplest to assume that non-proliferative mutations are either neutral or disadvantageous in the 'struggle for survival'. If such a mutant is in the area to be occupied by an active 'freckle' clone it will go to the wall.

The final point to be made is probably the most important of all: that the proliferating melanocytes are still under effective control. No freckle expands to cover more than a square centimetre and malignant melanoma never supervenes on a child's freckle. We do not know the nature of that control, but it impresses one as evidence that somatic mutation has been too important a factor in human and vertebrate evolution to be left without control. There is probably what in relation to auto-immune disease I called a 'fail-safe system' available for every important type of somatic