ADVANCES IN
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PREFACE

The primary purpose of *Advances in Immunology* will be to present timely reviews of various topics in immunology that not only will serve to keep investigators informed but will also try to unify the concepts underlying this highly diversified yet technically specialized subject. Each volume will contain about eight reviews on different aspects of immunology, broadly defined so as to include such subjects as immunochemistry, antibody synthesis, biological actions of antibodies, immunological unresponsiveness, mechanisms in innate and acquired immunity not involving antibodies, and specialized immunological techniques. The articles will stress fundamental concepts but at the same time will attempt to evaluate the experimental approaches.

The reviews in this first volume are concerned mainly with some of the more biological aspects of immunology—partly because of intense interest in these subjects at the present time and partly because reviews on more chemical aspects have appeared elsewhere or will be included in subsequent volumes of *Advances in Immunology*.

We hope that immunologists as a whole will welcome Abraham G. Osler's review on "Functions of the Complement System," including its possible role in pathological processes, and Abram B. Stavitsky's survey of "*In Vitro* Studies of the Antibody Response" in relation to theories of induction and to the biochemistry of antibody synthesis. The subject of "Immunological Tolerance" is very thoroughly covered in two reviews, one by Richard T. Smith in respect to nonliving antigens, and one by M. Hašek, A. Lengerová, and T. Hraba in relation to transplantation immunity. Two fields of study, in which rapid progress in our understanding has been made in recent years, are "Delayed Hypersensitivity to Simple Protein Antigens," and the "Fate and Biological Action of Antigen–Antibody Complexes." These are surveyed by P. G. H. Gell and B. Benacerraf, and by William O. Weigle, respectively. The other two reviews concern aspects of immunology which touch upon the interests of workers on cancer research and virus diseases. One on "The Antigenic Structure of Tumors" is by the late P. A. Gorer and is the last scientific paper he wrote before his untimely death. The other one on

the "Duration of Immunity in Virus Diseases" is by J. H. Hale and is discussed in relation to the possible persistence or reintroduction of the viruses.

The editors take pleasure in expressing to the contributors their appreciation for the great effort involved in writing the reviews and for their tolerance of the various frustrations associated with the starting of a new review series.

October, 1961

W. H. TALIAFERRO
J. H. HUMPHREY

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Transplantation Immunity and Tolerance

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I. Introduction

Transplantation immunity and immunological tolerance are, under certain conditions, alternative reactions of the same organism to the same antigenic stimulus—namely, transplantation of tissues or cells from an antigenically different individual. Which of the two alternatives will take place is decided by the circumstances of the first experience by the reacting individual of the given antigens, especially important being the stage of development at which this first experience occurs. In principle, if the first contact takes place early in ontogenesis, when the antigen is tolerated by the organism simply as a consequence of its immunological immaturity, this first experience may cause the capacity for tolerance of this antigen to be retained even after the development of immunological maturity of the organism and, perhaps, throughout its whole life span. By contrast, if an immune reaction is induced by the first introduction of the antigen into the organism, it will as a rule recur at all further contacts. Entry of antigens into an organism which is not yet capable of producing an immune response is normally rather exceptional, and consequently, the first alternative is a much rarer event than the second.

The occurrence of transplantation immunity is a rule and transplantation tolerance is an exception to this rule. In general, the organism

reacts immunologically against the antigens of transplanted homologous tissues, which are thereby destroyed and rejected. The mechanism underlying this regular reaction is the subject of the study of transplantation immunity. The situation in which, under normal conditions, the organism does not react immunologically against tissue antigens of another organism, but tolerates them constitutes an exception to the rule. Such exceptions are the starting points for the study of the mechanism of immunological tolerance.

The fact that the antigen can be tolerated provides an apparent contradiction in terms, since antigens are defined by their capacity to induce an immune response (formation of antibodies) in the recipient. The definition of an antigen is, however, always relative. No substance or complex can be denoted as antigenic in isolation but only in relation to an appropriate recipient, because what is antigenic in one system need not be antigenic in another. For example, the cells of an organism induce the formation of antibodies if administered in a suitable way to another (antigenically different) organism, but they do not elicit a similar reaction in their own maternal organism. It would seem, therefore, that the definition of the antigen must somehow tacitly include a requirement that it be heterogeneic. Even this, however, is insufficient. Under certain conditions the organism tolerates a potential antigen, in the same way as it tolerates the constituents of its own body, and, on the other hand, under different conditions, it can react against a constituent of its own body as it would against a foreign antigen in the usual sense of the word, by forming antibodies. The relationship between experimentally induced tolerance and immunity is, however, not reciprocal. The majority of foreign "antigens" capable of inducing a state of immunological tolerance in a given organism are as a rule able to induce an immune response in it under different conditions, but, in contrast, only a small proportion of antigens producing immunity seems to be able to elicit tolerance as well.

This distinction between antigens to which tolerance can be induced on the one hand and immunity on the other may, perhaps, arise from the different rates at which the two potential responses of the organism come in play under normal conditions. It is possible that under special conditions the development of tolerance to foreign antigens, on the basis of the "first experience," is in principle identical with the mechanism by which, under normal conditions, the organism becomes tolerant of the constituents of its own body. In teleological terms this would mean that the mechanism of tolerance is designed only for a restricted spectrum of the body's own antigens, as a natural defense mechanism against autoimmunity. However, since the antigenic makeup of an individual is the

result of a random event (i.e., of a random recombination of the respective genetic determinants in the formation of a zygote), the spectrum of antigens to which the organism can become tolerant would have to be somewhat wider in order to embrace equally all isoantigens of the given species. The species, which represents all the possibilities of free combination of the antigenic determinants during the process of fertilization, might, therefore, represent at the same time the natural limits for the induction of tolerance under normal conditions. (This begs the question whether experimentally induced tolerance of more remote antigens, if it occurs at all, is the result of a more or less successful application of a mechanism designed normally only for isoantigens, or whether it is a qualitatively different mechanism partially imitating the results of tolerance.) Although such a concept may be far from justified, the fact is that the effectiveness of experimentally induced tolerance of cellular antigens resembles tolerance of the body's own constituents only in some and not all instances when the isoantigens are employed for its induction. Insofar as we are concerned with immunological tolerance and transplantation immunity as two alternative reactions of the organism to the same stimulus, the limited range of tolerance confines this study to the sphere of isoantigens.

While the natural positive role of immunological tolerance in this sphere is readily understandable, the same cannot be said of the role of transplantation immunity. To postulate a natural defense mechanism against surgically transferred foreign tissue seems as inacceptable as the idea that transplantation immunity is an artifact produced by the artifact of transplantation. Nor is it an explanation to regard transplantation immunity as an aberrant form of bacterial allergy directed against the transplanted tissue instead of invading pathogenic microorganisms. The defensive value of bacterial allergy is obscure and to suppose that bacterial allergy is the primary mechanism and that transplantation immunity is derived from it would be quite arbitrary, reflecting evidently the time sequence of their discovery.

It seems far more probable that transplantation immunity and bacterial allergy represent two different manifestations of a more general reaction mechanism based primarily on the ability of the organism to recognize not self from self. What should be, however, the general biological significance of this ability to recognize and destroy "not self"? At first glance it may appear that under normal conditions such an ability would be harmful rather than useful to the organism, e.g., the relation between mother and fetus fulfills the fundamental condition for occurrence of the homotransplantation reaction. Some other mechanism, there-

fore, must be presumed to protect the fetus from the reaction of the mother.

The question of whether the mechanism involved in the homograft reaction plays a positive role in development was discussed recently by Thomas (1959). He suggested two possibilities. First, a mechanism might be involved controlling the uniformity of the cells of the organism and acting against a possible genetic divergence, in other words a mechanism balancing the action of such somatic mutations as might manifest themselves by changes of antigenic specificity. An important part of this biological function could then be the defense against malignant growth based on the immune reaction against a specific tumor antigen produced in the course of malignant transformation.

The second speculation of Thomas is that transplantation immunity could be mediated by a mechanism normally responsible for the "physiological degeneration" of the placenta at the final stage of pregnancy. This concept, therefore, points up a real situation in which under normal physiological conditions the breakdown and rejection of the healthy tissue occur accompanied by pathological changes strikingly recalling the picture of a homograft destruction by transplantation immunity. Both ideas of Thomas are approachable experimentally.

The term "transplantation immunity" was coined to express the discovery that transplantation incompatibility is a phenomenon which is immunological in nature. Although this concept is relatively old (Schöne, 1912), it for long lacked satisfactory experimental evidence owing to failure to detect antibody formation in the organism reacting against foreign tissue. Indirect evidence of basic importance was provided, and older findings rightly interpreted, by Medawar, in the accelerated breakdown of the second graft from the same donor. Such destruction of homografts is commonly thought of in terms of the specific cellular reaction. However, the reactions of the organism against homo- or heterologous tissues can be more varied than this, and the study of their relation to immunological tolerance covers, in fact, a wide field of immunology, namely, not only transplantation of normal and tumor tissues but also transfusion, the relationship between mother and fetus, and autoimmunity. The elucidation of the mechanism of transplantation immunity and immunological tolerance and their relationship is, therefore, not only one of the fundamental problems of any immunological theory, but also a matter of considerable general biological importance.

II. Immunology of Homograft Reactions

Surgical transfer of tissues between two individuals of the same species almost invariably ends in the destruction of a homograft by an immune process. The biological basis of this generalization is best illustrated by a few rare but well-defined exceptions. One of these is the success of transplantation between monozygotic twins (K. H. Bauer, 1927; Padgett, 1932; Hume *et al.*, 1955) which is evidently due to their genetic identity. Genetic diversity is on the contrary a practically absolute condition for tissue incompatibility; this reflects in turn the fact that genetic disparity (for this purpose at least) is also complete, or, in other words, that each individual of the given species has a unique makeup of genetically controlled factors by which individual specificity is determined.

A second exception of the rule of tissue incompatibility has helped to elucidate the nature of the genetic disparity between recipient and donor of transplanted tissue which is necessary for the development of transplantation immunity. The F_1 hybrids between two highly inbred strains are universal recipients of transplanted tissues not only from individuals of both parental strains but also from their progeny in the first and further generations. This was demonstrated by Little and Johnson (1922) and denotes clearly that genetic disparity of the graft is not sufficient in itself for manifestation of incompatibility to occur in the recipient. The F_1 hybrids are universal recipients because they represent a complete combination of tissue compatibility factors characteristic of both parental strains, so that any disparity of tissues derived from parents or from their own progeny is a negative one, due to the absence of some of their genetic determinants. A transplantation reaction to a graft can be stimulated only by a positive difference between the donor and recipient, i.e., by the genetically determined presence of some chemical constituent of the donor tissues which is absent from the recipient.

Such constituents, or isoantigens, may broadly be defined as any genetically controlled positive differences between individuals of the same species which are capable of inducing some type of immune reaction. Their physiological role is largely unknown and their chemical nature is also far from being explained satisfactorily. Proof of their existence is based so far only on their ability to induce immune reactions or to combine with specific antibodies.

Specific isoantigens of the given individual involved in homografting seem to be genotypically fully represented in the cells of a different histogenetic origin, although the possibility is not excluded that their phenotypical representation may vary to some extent. Incomplete repre-

sentation of the individual specific antigens on the surface of the cells of certain tissues (e.g., endocrine glands) could account for their apparently greater resistance to transplantation immunity, the latter being, in fact, weaker and directed against only a limited number of the host's individual antigens. Exceptional behavior of homografts of endocrine glands is, however, far from being unequivocally demonstrated and, on the contrary, more detailed experiments conducted by Krohn (1959) with ovarian homografts in mice show that their sensitivity to transplantation immunity is not weaker than that, for example, of skin.

Organ-specific antigenic differences within the individual, capable under certain conditions of inducing serum (Witebsky and Rose, 1956; Roitt *et al.*, 1956) as well as cell-bound (Lipton and Freund, 1953) autoantibody formation, may induce an immune reaction also in other individuals of the same species (Witebsky and Rose, 1956; Rose and Witebsky, 1956). There is no evidence, however, that they act at the same time as isoantigens in this reaction, i.e., that they lead to antibody formation against individual-specific in addition to organ-specific antigens. There is some evidence for the existence of individual-specific differences in organ-specific antigens (Oudin, 1956a,b; Dray and Young, 1958; Dubiski *et al.*, 1959), but not for their participation in the induction of transplantation immunity or, especially, of immunological tolerance. The fact that transplantation immunity to a certain tissue can be produced by means of another tissue from the same donor (Medawar, 1946) does not conflict with the eventual participation of organ-specific antigens; in order to obtain success in inducing and testing transplantation immunity by different tissues, it is only necessary for those two tissues to share at least one antigen in common. By contrast, when attempting to induce and test tolerance with tissues of different origin, a negative result implies that each specific antigen of the challenging tissue is not represented in the antigenic complex of the tissue used to induce tolerance. Tolerance induced with cells of mesenchymal origin, however, also extends to skin (Billingham *et al.*, 1952) and other tissues (Simonsen, 1955a; Medawar and Russel, 1958), suggesting that, if the organ-specific antigens play a role at all, they cannot display any individual-specific differences.

Some instances of successful homotransplantation are only apparent exceptions to the rule of tissue incompatibility. Thus, the success usually obtained with orthotopic corneal homografts is not conditioned by the special properties of the graft, but by those of the site of implantation. There is unequivocal evidence that corneal homografts transplanted in a heterotopic site (for example, placed on the skin; Billingham and Boswell, 1953) are destroyed, and, on the contrary, skin homografts survive when

placed on the cornea (Bednyakova, 1955) and in the anterior chamber of the eye (Medawar, 1948). Success probably depends on the absence of vascularization, which seems to play an important role in homograft reactions (Mervin and Hill, 1954). An exceptional site for transplantation is also the brain (Murphy, 1926); here even vascularized skin homografts may survive although they would be destroyed by the recipient if grafted orthotopically. The special situation noted in respect of homografts in the brain appears to be due to the absence of lymphatic vessels which constitute the usual channels for the antigenic stimulus to reach the reactive cells. Consequently, when homografts are placed on the brain sensitization of the recipient does not occur.

Similar special properties of certain tissues and transplantation sites, which give rise to exceptions interesting both from practical and theoretical point of view, are not of fundamental importance to a study of basic laws of transplantation immunity, but they can sometimes obscure the interpretation of experimental results. For this reason, skin transplantation became the basic model for the study of transplantation immunity; skin, if transferred orthotopically, does not possess any subtle peculiarities and, moreover, it is endowed with further advantages, such as marked sensitivity to the immune reaction which it induces on being grafted, the ease of the technical procedure, availability of material, etc. The widespread use of skin in tissue transplantation investigations is without doubt also due to the fact that by this technique was worked out the experimental and theoretical foundation represented by the pioneering work of Medawar (1943, 1944, 1945, 1946).

However, if an attempt is made to classify the known categories of transfer of homologous tissue according to the type of actively acquired immunity which is induced in the recipient, then skin grafting represents only one extreme, namely, that in which a specific cellular reaction and cell-bound antibodies are largely or perhaps exclusively involved. At the other extreme we have blood transfusion, where immunity is mediated by serum antibodies. If blood transfusion is at the opposite pole to skin grafting, it is necessary to exclude cases in which natural immunity exists against the transfused blood. Natural immunity has no known analogy in skin homografting, and such unique situations as the human ABO system must be excluded, since they give a false picture of this type of homograft reaction.

As in other attempts to classify biological phenomena, here also there are inherent difficulties. All gradations can be found between the more or less artificially defined extreme categories, and to define strict limits becomes, in fact, meaningless. If the types of immune reactions against

homografts are grouped according to the type of antibodies that they induce, the origin of the most typical tissues, or the nature of antigens responsible, there can always be found intermediate categories which represent a combination or interaction of the extremes. Nevertheless, however imperfect such a classification may be, it serves a useful purpose until we know enough to decide whether there should be more categories or, in fact, only a single category.

A. Types of Reactions against Cellular Isoantigens

The chief evidence for the homograft reaction being an immunological matter appears to be: (a) the second-set phenomenon; (b) the possibility of transferring sensitivity by means of immunologically competent cells, and in some cases by isoimmune serum; and (c) the finding that induced immunological tolerance based on specific inhibition of the immune response makes possible even a permanent take of the homograft.

A piece of fundamental, but indirect, evidence for an immune mechanism is provided by the clear demonstration of the second-set phenomenon (Gibson and Medawar, 1943, Medawar, 1944, 1945), i.e., accelerated destruction of a second skin homograft from a donor of the same genotype. The first graft heals in, forms vascular and lymphatic anastomoses, and has all the appearances of an autograft. Only after some time, as a rule several days, round cell infiltration appears, vascular disorders and necrosis occurs, and finally the graft is rejected. By contrast, the second-set graft has no period of normal take. Vascularization is incomplete, round cell infiltration of the bed and the bottom layers of the graft occurs immediately, and breakdown takes place rather more rapidly than with the first homograft. In any given combination, the survival time of the homograft is exclusively determined by the type and the extent of the isoantigenic differences between the donor and the recipient if other conditions (including the size of the graft) are kept constant. Thus, in the case of transfers between members of two inbred strains, the median survival time of the grafts is nearly constant and exhibits a minimum scatter. In skin homotransplantation between the mice of A and CBA strains, the destruction of the CBA graft on A recipients is accomplished in 10.2 ± 0.3 days, whereas the destruction of the second CBA graft transplanted to the recipient two weeks after the breakdown of the first one is completed in less than 6 days (Billingham *et al.*, 1954a). These quantitative data are strictly reproducible, and, in this host–donor combination, the histological picture of the homograft at a given time after transplantation can be used for the detection of the immune state (Billingham *et al.*, 1956b).

1. *Circulating Antibodies*

It is not quite easy to appraise the importance of serum antibodies in transplantation immunity; first because their occurrence (as detected by usual methods at least) is not general, and second because the mere presence of serum antibodies, revealed by serological reactions *in vitro*, provides no evidence of their participation in homograft destruction. A relevant role may be attributed only to those serum antibodies whose biological effectiveness has been clearly demonstrated.

a. Antibodies Demonstrable by Serological Reactions in Vitro. In homotransplantation most attention has been paid to the formation of hemagglutinins. Other serological reactions performed *in vitro* with tissue homogenates or fractions gave mostly negative or unreliable results. Our limited knowledge of antibodies giving such reactions may perhaps lie not in their absence but in the inadequacy of detection systems so far used (unsuitable antigenic preparations, insufficiently sensitive reactions, etc.).

The occurrence of hemagglutinins is, however, regular after transplantation of tissues in mice (Gorer, 1937, 1947), in which the tumor transplant seems to provide an even better stimulus than immunization with blood. Moreover, their formation is induced in some, but not all, cases not only by full thickness skin grafts (which could contain some erythrocytes) or mesenchymal tissues of the dermis, but also by grafts of isolated epidermis (Amos *et al.*, 1954). Such hemagglutinins are, as a rule, of the incomplete type.

Hemagglutinins seem to occur less regularly (perhaps as a result of less intensive investigation) after homotransplantation of tissues in other species. However, Zotikov (1956) unlike Medawar (1946) has found the formation of complete hemaglutinins after homotransplantation of skin in rabbits which are known, of course, to be weak producers of isoagglutinins.

b. Antibodies Demonstrated by Their Biological Effect. The second group of antibodies occurring as a reaction against the graft are those demonstrated by their biological effect on the cells of the graft. Such antibodies were detected by Gorer (1942). Leukemic cells incubated *in vitro* with the serum of the animal immunized with a tumor graft lose their ability to induce tumor growth after inoculation into a sensitive recipient. These neutralizing antibodies are unlikely to be identical with hemagglutinins, because the latter can be removed from an immune serum by absorption without decreasing its neutralizing capacity. Billingham and Sparrow (1954) also found a neutralizing effect on isolated

epidermal cells, demonstrated by a decreased capacity to grow in the original donor after temporary contact *in vitro* with the serum of animals which had rejected a skin graft of the same origin.

Other methods have since been employed to demonstrate antibodies in homograft reactions, such as the demonstration of cytotoxic antibodies *in vitro* (Gorer and O'Gorman, 1956) and passive transfer of homograft immunity by serum (Gorer and Amos, 1956; Amos and Day, 1957; Siskind and Thomas, 1959). These two methods have largely been used for study of transplantation immunity to tumor tissues. It was shown that insofar as serum exhibits a cytotoxic effect *in vitro*, it can as a rule transfer passive immunity (Winn, 1960). However, great differences are found in the reaction of different tumors against cytotoxic sera (Gorer and Kaliss, 1959). Whereas leukemia and other lymphoid tumors are inhibited or destroyed by the action of antibodies detected by the aforementioned tests, other tumors remain not only unimpaired, but their growth in incompatible hosts is often enhanced. Such immunological enhancement (Kaliss, 1958) has been most studied with sarcoma 1 as a typical representative of the second group of tumors.

Between these two groups of tumors which are destroyed by immune serum in the first instance and enhanced in the second, there exists an intermediate group in which different doses of antiserum display a different effect. On the whole, the greater the dose of antiserum the better the inhibition of the tumor growth, and the smaller the dose the greater the probability that enhancement will occur. The difference in the reaction of tumors to the action of antiserum seems to be determined by the properties of the normal tissue from which the tumor originated, but differences in sensitivity to passive transfer of isoimmune serum has, however, been studied so far only in a few normal tissues.

2. *Specific Cellular Reaction*

The failure of attempts to provide evidence for a general role of serum antibodies has no bearing on the concept of homograft reaction as an immune process. There is ample evidence for the decisive part played by the second fundamental immune mechanism—hypersensitivity of the delayed type. The homograft reaction resembles the best known representatives of this category (tuberculin hypersensitivity and drug allergy) in the following features: the time of the onset of the reaction; the histological picture; and, especially, in the fact that each is transferable to other individuals by lymphoid cells of the sensitized donor. This was shown by Mitchison for tumors (1953) and also demonstrated for normal tissues (Billingham *et al.*, 1954b). This evidence is, however, not entirely