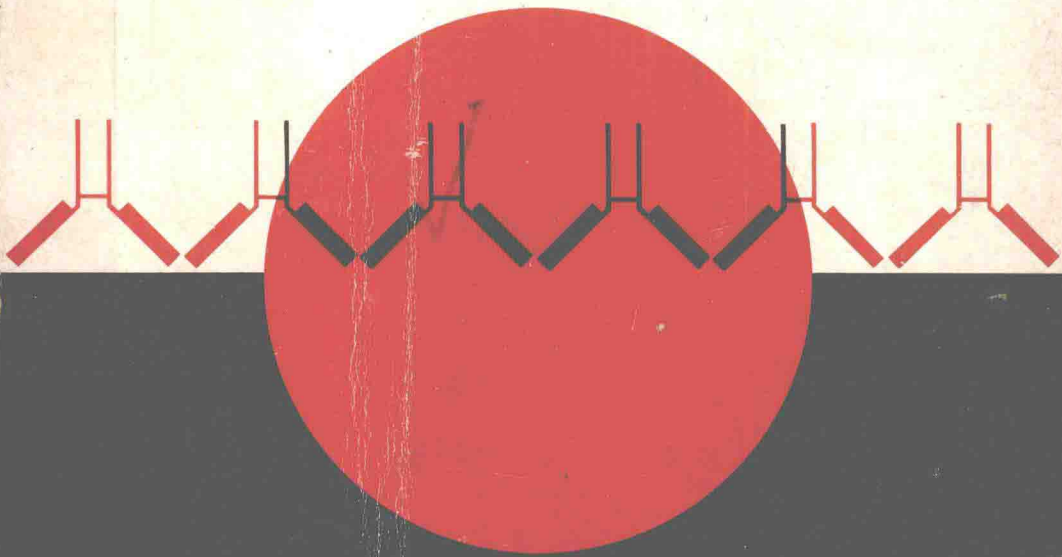
THE IMMUNOBIOLOGY OF TRANSPLANTATION

RUPERT BILLINGHAM • WILLYS SILVERS



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FOUNDATIONS OF IMMUNOLOGY SERIES

Abraham Osler and Leon Weiss, Editors

THE IMMUNOBIOLOGY OF TRANSPLANTATION
Rupert Billingham and Willys Silvers

THE CELLS AND TISSUES OF THE IMMUNE SYSTEM
Leon Weiss

Frontispiece. The chronology of some major discoveries and concepts in transplantation.

To

ELIZABETH RUSSELL and P. B. MEDAWAR,

who initiated us into the field of transplantation biology,

and to all those who have grafted with us.

Foundations of Immunology Series

This series of monographs is intended to provide readers of diverse backgrounds with an authoritative and clear statement concerning significant aspects of immunology. Each volume represents an individual contribution by a distinguished scientist. As a series, they provide a comprehensive view of the field.

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ABRAHAM OSLER
LEON WEISS

Preface

Despite the tremendous burgeoning of interest in the immunobiology of transplantation which has taken place over the decade, and the vast literature on the subject being laid down in scientific journals, reviews, and symposium volumes, there have been few attempts to present a general introductory account of the basic principles and general concepts of the subject and their origins, its unsolved problems, and its future prospects.

We have tried to achieve this goal for the benefit of advanced students and clinicians interested in the biological bases of the "uniqueness of the individual" and in the nature of the processes that have had to be thwarted in order to launch the new era of replacement surgery. We hope this book will enable the genetically, the surgically, and the immunologically unsophisticated reader to get a good insight into the subject commonly known as "transplantation," its methodology, and its own distinctive language, without overburdening him with unnecessary detail.

Limitations of space and the desire to produce an easily readable text precluded crediting many investigators whose contributions we have described and synthesized. To them we tender our apologies and beg their indulgence. We hope that the selected bibliographies at the end of each chapter will provide the means for readers to identify the unsung heroes of the plot and follow their contributions in extenso.

We are deeply indebted to our colleagues for helpful criticism and suggestions, to Mrs. Jean M. Billingham for the illustrations, and to Mrs. Marilee Heffron and Mrs. Martha Lubaroff for help with the manuscript.

It is a pleasure to acknowledge that our own research efforts and the gathering of the material for this book have been made possible largely by grants from the United States Public Health Service.

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Introduction

For centuries surgeons have yearned for the day, now close at hand, when it would be possible for them to replace damaged or diseased tissues or organs by grafts from other human beings or, perhaps more ambitiously, from other mammals, as a standard therapeutic procedure.

Since the beginning of this century biologists from a wide variety of disciplines, including experimental oncology, embryology, genetics, and endocrinology, have been making use of transplantation as a powerful analytic tool in the analysis of a wide range of problems. Indeed, a considerable body of workers have found that elucidation of the requirements of grafts for conservation of viability and normal functional activity, and devising means of meeting these, to be interesting and worthwhile research goals in themselves.

The transplantation of living cells, tissues, or organs for experimental or therapeutic purposes entails problems of two entirely different categories. The first is concerned directly with the act of transplantation itself. Mainly of a technological nature, these problems are associated with the procurement, preparation, temporary or long-term storage or "banking," and finally relocation of the grafts in such a manner that the normal healing processes, following appropriate surgical procedures where necessary, such as vascular anastomoses, suffice to ensure their survival and conservation of essential structure and function. In experimental animals and in man, the feasibility of replacement by grafting of blood, bone marrow, corneas, extensive areas of skin, kidneys, hearts, lungs, liver, small bowel, pancreas, and other endocrine tissues is firmly established. This indicates that adequate if not completely satisfactory solutions to these problems have been developed for the tissues and organs listed. For completeness, it is worth mentioning that the slow rate and inefficiency of nerve regeneration remain very serious obstacles that must be overcome before limb transplantation can be regarded as a worthwhile endeavor for the clinician.

The second category of problems is much more formidable and stems from the unrelenting incompatibility of *homografts* (i.e., grafts trans-

planted from one individual to another of the *same* species—also referred to as *allogeneic* grafts or *allografts*).

Homografts normally fail where *autografts* (grafts of which the donor is also the recipient) or *isografts* (sometimes referred to as syngeneic grafts; grafts of which the donor and recipient are of the *same* genetic constitution, as in the case of identical twins or members of the same inbred strain) transplanted in exactly the same manner are permanently successful. For example, if free skin autografts and homografts are placed on a common bed on the side of a rabbit's trunk, the autografts undergo a phase of minor reparative changes, including transient epidermal hyperplasia, and then reassume their original condition with a high degree of perfection. The homografts also heal in, acquire a rich blood supply, and behave as autografts do at first. Soon, however, they become inflamed and edematous and their dermis is heavily infiltrated by leucocytes, predominantly by lymphocytes and histiocytes. Cessation of blood flow, lymphatic drainage, and disruption of vessel walls, accompanied by the disintegration and separation of the entire epidermis and death of the cellular population of the graft, including the immigrant cells of host origin, soon follow. Finally, the necrotic graft is totally transformed into a desiccated, discolored scab which sloughs as a consequence of its being undermined by ingrowth of native host epidermis. This process of destruction, known as the *homograft reaction,* is usually complete within two weeks.

An essentially similar fate overtakes homografts of all other solid tissues (normal or malignant) or organs that establish vascular connections with their hosts of their own accord, or have them established surgically by vascular anastomoses. Of course, different symptoms are associated with the destruction process as it affects different kinds of homografts in different species. There is cessation of urine output in the case of the kidney homograft, altered electrical activity followed by diminishing output in the case of cardiac transplants, bilirubinemia and other biochemical changes in the constitution of the blood in the case of livers, and tumor homografts wane in size and eventually disappear. Cartilage loses its ability to take up radioactive sulfate ions, and ovarian homografts, in ovariectomized hosts, can no longer sustain sexual cycles. Thyroid grafts lose their power to take up radioactive iodine, and parathyroid grafts fail to maintain normal calcium levels in the blood of hosts whose own organs have been removed. Bone marrow grafts cease to contribute red cells of donor antigenic type to the blood stream, and the death of transplanted antibody-forming cells is indicated by the fall in titre of antibody of "adoptive" origin in their hosts. Another useful method for following the rejection of transplanted lymphoid cells entails labeling the cells before

transfer with tritiated thymidine or chromium[51] and then following the elimination of these labels.

As we shall see, destruction of homografts is one of the most striking and reproducible of all immunological phenomena, manifested by all species of vertebrates so far tested. It is the outcome of a highly developed capacity of animals to recognize and treat as foreign and unwanted, cells from genetically dissimilar donors—a consequence of a cell surface allotypy determined by an extremely subtle and complex genetic polymorphism, the biological significance of which has yet to be elucidated.

Although the central core of transplantation immunology still comprises the classical themes of the genetic determination of homograft incompatibility and the nature of the processes which put it into effect, the subject embraces many additional facets and subdivisions which have arisen in large part from chance observations and their interpretation.

For example, Dr. Ray D. Owen's brilliant recognition and interpretation of the phenomenon of red cell chimerism in dizygotic twin cattle in 1945 prepared the way for the discovery of the principle of immunological tolerance in 1953, the basic principles of which were worked out, at least in broad outline, by means of the homograft immunity system. Studies with X rays, certain steroids, and other agents had indicated the feasibility of weakening the homograft reaction nonspecifically, thus initiating the field of immunosuppression on a systematic basis. However, work on immunological tolerance indicated that a *complete* solution to the homograft problem was attainable, at least at the laboratory level. Apart from its impact upon immunology, this work recruited many additional workers, including surgeons, to transplantation immunology.

Another important and largely accidental discovery was that grafts which contained significant numbers of lymphoid cells could, in experimentally definable situations, mount destructive reactions against their alien hosts, i.e., so-called *graft-versus-host* (GVH) reactions. Apart from uttering a caveat concerning possible dangers associated with the use of grafts of the lymphohematopoietic tissue system for therapeutic purposes, GVH reactions have afforded the basis of important, highly discriminatory, and quantitative "tools" in transplantation immunology. They have provided simple assays for immunologically competent cells, revealing their presence as normal components of the cell populations of blood, peritoneal exudates, and thoracic duct lymph. They have also shed a great deal of light upon the sequence of cytological events responsible for the destruction of homografts in conventional host-versus-graft reactions.

It is also worth mentioning that not only have the procedures of transplantation played an indispensable role in the elucidation of the functional significance of the thymus and the bursa of Fabricius, but also the homo-

graft reaction has proved to be one of the most important immunological responses with which to evaluate their role.

The success which has attended deliberate attempts to devise effective means of tissue typing and to characterize at least the macromolecular carriers of the specificities involved (i.e., the chemistry of transplantation antigens) has been dramatic. It has drawn into the field workers whose prime interests are genetic polymorphisms, the relation of genes to their specific products, as well as membrane physiologists, since these products are evidently integrated components of cell membranes.

Also relevant to an understanding of the tremendous increase in popularity, effort, and accomplishment in the field of transplantation which has occurred in little more than a decade are the important two-way exchange of information and ideas between the laboratory and the clinic and the increasing tendency of biologists and surgeons to work together in either location. It is alleged that Pasteur once said that every surgical operation represented an experiment in bacteriology—happily this is no longer true. What *is* true is that, despite the degree of success that now attends renal transplantation, and to some extent that of liver and cardiac transplants in man, every clinical organ transplantation still represents an "experiment" in transplantation immunology.

Important discoveries can still be made and problems investigated in transplantation biology by procedures which, though sometimes demanding in terms of manual dexterity on the part of the investigator (as, for example, kidney, heart, or liver transplantation in rats) and in the genetic constitution of the experimental subjects, are very inexacting in terms of expensive equipment. Elaborate equipment and complex techniques are admittedly indispensable for the detailed elucidation of many problems and phenomena in transplantation immunology, such as the biochemistry of the antigens involved or quantitation of homograft reactivity at the cellular level in vitro. Yet the assertion which Simonsen made a few years ago that "in the whole field of modern transplantation biology there are few significant contributions which were not made with techniques of utter simplicity" still holds good.

As we shall see, transplantation biology is rich in those "exceptions" which Bateson adjured us to treasure. When there are none, he said, "the work gets so dull that no one cares to carry it further." Notable among these exceptions, and one that is currently receiving a great deal of attention, is the mammalian fetus which in outbred populations represents a homograft intimately united to the tissues of a mother who, in theory, would confidently have been expected to reject it.

One of the most successful and widely employed therapeutic homografts is the blood transfusion. The practicality of injecting fluids into veins was demonstrated by the famous British architect, Sir Christopher

Wren, in 1657. Within the next few years both indirect and direct transfusions of blood were performed in dogs. The first really successful clinical blood transfusion was performed in 1829 by a Guy's Hospital physician and obstetrician, James Blundell, on a patient suffering from postpartum hemorrhage. Sporadic transfusions were performed after this, the procedure being complicated by problems of coagulation and unrecognized incompatibility. The existence of blood groups in man was revealed by Karl Landsteiner in 1900. By about 1920 clinical transfusion was becoming fairly commonplace, though for a long time it remained an individual operation—a single donor, usually a relative, being chosen for each patient. Largely under the impetus of World War II, blood banks were instituted in the early 1940s when transfusion became a commonplace procedure. The discovery of the Rh blood group system in 1940, and recognition that it was responsible for erythroblastosis fetalis, initiated clinical interest in the immunobiology of the maternal-fetal relationship.

The flourishing and highly important discipline of tumor immunology has a history which is almost inextricable from that of the immunology of normal tissue grafts. It had an abortive beginning at the turn of the century. Nearly all the early workers labored under the delusion that they were studying cancer when they were, in fact, using tumors to study transplantation. As Woglom put it in 1929, "the tumor problem . . . was a tissue problem, resistance being directed against the tumor graft *as a strange tissue merely,* and not connected with any neoplastic qualities which the graft happened to possess."

Not until the immunogenetic principles of transplantation biology had been firmly established, properly inbred strains of mice had become available, and the need to study tumors of very recent origin was recognized, was the setting conducive for a fruitful reconsideration of the immunology of cancer. Following Foley's classic work in 1953, application of the techniques devised to study immunity to homografts revealed that the cells of tumors induced by chemicals, by some viruses, and other agents differ qualitatively from normal cells by antigens at their surfaces which behave like weak transplantation antigens and are capable of inciting immune responses.

One of the most exciting developments in transplantation immunology has resulted from the reinvestigation of heterologous *antilymphocyte serum,* or ALS, pioneered by the work of Woodruff and Anderson in 1963. Apart from its remarkable capacity to abrogate homograft reactions and other so-called cellular immunities, this relatively harmless biological agent has also been found to be highly effective in overcoming reactivity to *heterografts* (i.e., grafts of which donor and recipient belong to *different* species), enabling human skin, for example, to thrive on mice for many weeks. Previously little attention had been focused on the immunology of hetero-

grafts because of their exceedingly short life spans. With most species combinations they rarely healed in properly and barely displayed any functional activity at all. With a few exceptions, immunosuppressive procedures which were highly effective in extending the lives of homografts proved to be only marginally effective with heterografts. Thanks to ALS, interest in heterografts has recently been revived. Sir Peter Medawar pointed out recently that greater success is now being obtained with grafts *between* species than was obtained within species fifteen years ago. Guarded optimism now prevails that "zoografting" may eventually afford a solution to the increasingly acute logistic problem of donor organ procurement.

One thing that the pioneers of transplantation could not have envisaged was the tremendous influence their field was destined to exert upon immunology in general. Like the early students of delayed hypersensitivity to microorganisms and other agents, the early transplanters owed very little to "orthodox" immunologists who listened to their communications with bored politeness and often wondered whether the homograft reaction had anything to do with immunology at all. The only advice they gave was always directed toward further attempts to reveal the humoral antibodies which, ex cathedra, they felt must be the agents of graft destruction. Inability of dead cells to elicit sensitivity was just another shortcoming that encouraged some of them to believe that the homograft reaction was a phenomenon *sui generis*.

The chapters that follow describe the discovery of the principles of immunological enhancement and of immunological tolerance, the demonstration that immunologically competent cells are *normal* ingredients of the blood, the revelation of the functional significance of the thymus and, most recently, the discovery of the potency of ALS as an immunosuppressant. These are only a few of the contributions of transplantation biologists, or results of the application of their procedures, which have played a major role in revitalizing immunology. Especially important are the attention and effort which transplanters have drawn to the underdeveloped and neglected areas of immunology, notably the so-called "cellular immunities" to which the homograft reaction is so closely related.

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