Immunological Aspects of

Transplantation Surgery

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PROFESSOR ROY CALNE

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LIST OF CONTRIBUTORS

MARILYN L. BACH, PH.D.
Departments of Medical Genetics, Surgery, Pediatrics and Pharmacology
University of Wisconsin
Madison, Wisconsin 53706, USA

FRITZ H. BACH, M.D.
Departments of Medical Genetics, Surgery, Pediatrics and Pharmacology
University of Wisconsin
Madison, Wisconsin 53706, USA

PROFESSOR L. BRENT
Department of Immunology
Wright-Fleming Institute
St Mary's Hospital Medical School
London W2 1PG, England

PROFESSOR ROY Y. CALNE
Department of Surgery
University of Cambridge
Tennis Court Road
Cambridge, England

ROBERT J. CORRY, M.D.
Harvard Medical School
Massachusetts General Hospital
Boston, Massachusetts 02114, USA

D. A. L. DAVIES, M.A., PH.D., D.SC.
Department of Immunology
Research Division
G. D. Searle and Son
Lane End Road, High Wycombe
Bucks HP12 4HL, England

ERWIN DIENER, PH.D.
MRC Transplantation Unit
University of Alberta
Edmonton 7, Alberta, Canada

DR. WILLIAM L. FORD
Department of Pathology
University of Edinburgh
Medical Buildings
Teviot Place, Edinburgh, Scotland

DR. BASIL M. HERBERTSON
Department of Pathology
University of Cambridge
Tennis Court Road
Cambridge, England

DENNIS W. JIRSCH, M.D.
MRC Transplantation Unit
Provincial Laboratory
University of Alberta
Edmonton 7, Alberta, Canada

THOMAS L. MARCHIORO, M.D.
School of Medicine
University of Washington
Seattle, Washington, USA

GERHARD OPELZ, M.D.
School of Medicine
University of California
Los Angeles, California, USA

L. G. QUADRACCI, M.D.
School of Medicine
University of Washington
Seattle, Washington, USA

PROFESSOR PAUL S. RUSSELL, M.D.
Harvard Medical School
Massachusetts General Hospital
Boston, Massachusetts 02114, USA

DR. ELIZABETH SIMPSON
Immunology Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland, USA

GARY E. STRIKER, M.D.
School of Medicine
University of Washington
Seattle, Washington, USA

PROFESSOR FRANK P. STUART
Department of Surgery
University of Chicago
950 East 59th Street
Chicago, Illinois 60637, USA

PAUL I. TERASAKI, PH.D.
School of Medicine
University of California
Los Angeles, California, USA

ALAN TING, PH.D.
School of Medicine
University of California
Los Angeles, California, USA

DR. E. WHITE
Department of Endodentics
School of Dentistry
University of South Carolina
Charleston, South Carolina 29401, USA

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Introduction

R. Y. Calne

Surgeons are transplanting kidneys in ever increasing numbers—more than 10000 renal allografts have now been reported to the Transplant Registry. With related donors 75% of grafted kidneys continued to function after 2 years, compared with 50% when the donors were unrelated. The therapeutic value is obvious, but the management is largely empirical and results have improved little in the past 5 years. The basic sciences related to tissue transplantation have advanced rapidly. New serological and tissue culture techniques and chemical analysis of antigens and antibodies have produced complicated data that is almost incomprehensible to the non-specialist. Mathematical treatment of genetic probabilities and of immunological kinetics are similarly difficult to follow for those not especially trained.

There has always been a gulf between the practical clinician whose patients do not behave like inbred rodents and the biologist who likes carefully controlled experiments with easily observed results. Both realize, however, that predictable and safe control of rejection must involve close collaboration and co-operation between the laboratory and the clinic. Unfortunately, the different nature of the work and the workers has widened the gap between them. The clinicians tend to improve their techniques and patient care, whilst the biologists seek clearer and more precisely defined experiments which lead them to use increasingly artificial experimental models.

A simple basic dogma of tissue transplantation has become established. Thus, tolerance in the foetus is produced by antigen. Antibody can either destroy grafts, or enhance their survival. Histocompatibility antigens defined serologically determine the fate of grafted tissue. None of these axioms however seems to have much practical relevance. Classical fetal tolerance is of great academic biological interest, but cannot be applied directly. Our inability to differentiate between destructive and enhancing antibodies makes the clinician suspicious of all antibodies. The obvious lack of close correlation between HLA antigens and the fate of unrelated renal allografts has undermined major schemes of national and international organ sharing. It would seem nevertheless to be clear that further understanding of the biology of rejection and donor specific immunosuppression, is of fundamental importance if there is to be clinical progress.

Perhaps the potentially most fruitful lines of investigation are the established anomalies. The 'rule breakers' which stick out of the official dogma like sore thumbs—

for example, how is it that patients can retain renal allografts for years with excellent function on modest doses of immunosuppressive drugs despite their being 'full house' mismatches of HLA antigens between donors and recipients? How can a patient with no detectable cytotoxic antibodies reject a kidney from an HLA identical and MLC negative sibling despite immunosuppressive drug treatment? (Dick *et al.*, 1972a; 1972b). How can 25 mg of azathioprine twice a week hold in check a potentially strong allograft reaction 5 years after transplantation (Woodruff, personal communication)? Why is it that a patient with a high titre of cytotoxic antibody, capable of killing 100% of donor leukocytes, can, nevertheless, accept a liver graft from that donor (our own observations)? Why do patients who have received multiple blood transfusions without producing cytotoxic antibodies accept badly matched cadaver kidney allografts more readily than untransfused recipients (Terasaki *et al*, Chapter 4)? What is the mechanism whereby rats, mice and pigs reject violently allografted skin from a given donor source, yet may accept indefinitely organ allografts from the same or similar donors?

The clinician is interested in safe immunosuppression that has a prolonged effect in terms of the life of the organism. Slight prolongation of survival requiring a '*p* value' for its substantiation is likely to be of limited value. Rejection of grafted tissue is a dynamic process in which the dimension of time is often ignored. Biological factors probably play differing rôles in the course of rejection and these may be complicated favourably or unfavourably by attempts at immunosuppressive treatment. Thus, maximal non specific immunosuppression may prevent the development of enhancement. The survival of organ allografts from isologous donors varies greatly in identically treated recipients from highly inbred rodent strains (Brent and Pinto, Chapter 13). This lack of predictability in a controlled laboratory situation points to caution in expecting consistent results in humans receiving allografts from unrelated donors. It is likely that when the relevant factors are known and can be measured, individual repeated titrations will be required in manipulating immunosuppressive regimens for each donor recipient combination.

This volume is not intended to be a comprehensive review, but is a collection of essays aimed at narrowing the gap between basic immunology and experimental organ allografting. The authors have been requested to point out what is factual and what is speculation. Their help has been specifically sought on an analysis of phenomena that do *not* fit in with established theories in the hope that these 'sore thumbs' may point the way, no matter how inelegantly, to new concepts relevant to organ grafting.

Several authors have introduced their subject with remarks on classical experiments and their interpretation. Inevitably, similar ground has been covered by a number of authors and I considered cutting such repetitive material from the definitive text. On

reflection, however, I felt it would be of interest to readers to see how the same investigators and their concepts were interpreted by different contributors to this volume, so that the reader could determine for himself where there was a consensus of agreement and where there was controversy. I would not imply that widespread agreement on a phenomenon indicates that it is probably true, rather it points to current acceptance of a given explanation. A variety of different views on the same experimental findings, however, would indicate uncertainty and ignorance of the mechanisms involved.

It is hoped that the book will be of interest to surgeons working in organ transplantation and also to immunologists who might feel that the immunological aspects of transplantation surgery are worthy of more intensive research.

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1
The Morphology of Allograft Reactions

B. M. Herbertson

INTRODUCTION

The behaviour of transplanted organs and tissues and the morphological changes developing in them are closely inter-related and depend on various controlling factors. The principal circumstances affecting the fate of a graft include the genetic relationship between the donor and recipient, the species to which they belong, the nature of the grafted tissue, the anatomical position of the graft, the condition of the recipient's immune system and the strength and kind of the allergic response it is capable of mounting. With so many factors affecting transplants it is not surprising that their behavior is extremely variable. On the one hand, as a result of tolerance or enhancement, an allograft may be accepted as if it were an autograft and remain normal for an indefinite period. At the other extreme, if the recipient has been previously sensitized to donor antigens, the allograft may be rejected in a rapid and violent fashion. Yet a third possibility is the development of a graft-versus-host reaction causing debility or death of the recipient. If this is to occur, the allograft must contain sufficient immunologically competent cells capable of responding to host histocompatibility antigens and these donor cells must themselves be secure against successful attack by the recipient's immune system. This situation arises when cells of parental strain lymphatic tissue are introduced into F_1 hybrid recipients. These simple examples illustrate the wide diversity of response after grafting between dissimilar members of the same species and emphasize the need for defining the circumstances of any reaction being described.

The purpose of this chapter is to provide a general account of the morphology of allograft reactions and to prepare the ground for the more advanced and specialized topics considered by other contributors. First, a brief outline is given of the events occurring during the rejection of organ and tissue allografts, and this is followed by a

more detailed treatment of certain outstanding features, such as mononuclear cell infiltration, vascular lesions, changes in the graft parenchyma, and the response of host lymphatic tissue.

GENERAL OUTLINE OF ALLOGRAFT REJECTION

As is well known, most organ and tissue allografts transplanted to normal recipients behave for a few days like autografts similarly transplanted. For example, a first-set allograft of skin heals in place in the same fashion as a patch of the animal's own skin transplanted in the same way. Likewise, first-set renal allografts produce urine and hepatic allografts provide the vital metabolic functions of normal liver and secrete bile. On the other hand, when an organ or tissue graft is transplanted to an individual already sensitized to donor tissue, the reaction may be dramatic and result in rapid death of all the grafted tissue. For instance, with an organ graft in which anastomoses are formed and there is full interplay between recipient blood and donor endothelium, the reaction may be virtually immediate. In the more violent forms of 'hyperacute' rejection a renal allograft becomes flaccid and cyanosed within minutes of re-establishing blood flow and perishes during the next few hours. However, with a free graft of skin a reaction of comparable severity only becomes apparent after 2 or 3 days when the graft fails to vascularize in the usual way (the 'white graft' reaction). These examples illustrate the substantial variation in the time interval between grafting and the first macroscopic evidences of rejection.

The course of allograft rejection may also differ in other respects. Although allograft rejection unmodified by treatment is usually regarded as a progressive process which continues uninterruptedly until the transplanted tissue is destroyed, there are circumstances in which a more delicate immune balance spontaneously develops between the recipient and the grafted tissue. For instance, in certain experimental systems rejection of renal allografts may be a distinctly intermittent process with phases of allergic injury alternating with periods of partial recovery. This is most often seen when the antigenic disparity between donor and recipient is relatively slight and a transplant may then survive for long periods, despite occasional episodes of rejection. A rather different and more extreme example of an altered type of relationship is the long-term survival of hepatic allografts between strains of pig which regularly reject skin and renal allografts (Calne *et al.*, 1967). In such animals a relatively slight short-lived allograft reaction is sometimes observed in the liver during the first month or so after transplantation but the recipient later becomes unresponsive to the graft and the reaction completely subsides. Complete or partial

suppression of allograft rejection can, of course, be achieved by the various immunosuppressive measures used in clinical practice but the natural variation in the pattern of allograft reactions in the unmodified animal has perhaps been insufficiently appreciated.

Macroscopic features

In organ and tissue allografts the macroscopic features of rejection consist of a mixture of appearances due to circulatory disturbances, cellular infiltration, edema and parenchymal destruction. The manifestations of vascular change include pallor, congestion, swelling, cyanosis and hemorrhage and the cellular infiltration and edema contribute to the enlargement and pallor of the graft. The kind of parenchymal damage and its effect on the appearance of a graft depend on the form and intensity of the rejection process and on the organ or tissue involved. If rejection occurs rapidly, necrosis of the parenchyma is a major feature and, if not dominated by the deep colors of congestion or hemorrhage, the affected parts will usually appear pale and rather opaque. On the other hand, if rejection occurs much more gradually, atrophy of the specialized tissue with increasing fibrosis may be the major feature. In these circumstances the involved tissue tends to be rather shrunken, greyish-white and may become somewhat tougher than normal. The distribution of these destructive changes varies. Sometimes there seems to be no particular pattern but often, particularly in organ grafts, the lesions have a pronounced vascular arrangement with the development of clearly defined infarcts or wedge-shaped atrophic and fibrotic lesions.

The appearance of an allograft also depends in great measure on the stage which the rejection process has reached. Obviously the changes in orthotopic skin and other superficially placed allografts can be readily seen as the reaction develops but with organ allografts the progress of rejection is less readily observed and a number of similar transplants may have to be examined at intervals before the pattern of the macroscopic features is adequately known. Similarly, the extent to which it is possible for rejection of certain transplants to progress also needs to be considered. For example, if an animal depends for continuing life on a transplanted heart, liver or kidney, the structural changes can develop to a certain degree only before function fails and death ensues. On the other hand, if the animal is not so dependent on the function of the graft, the rejection process can run its complete course. In this event the graft may become completely necrotic and then either slough, or be resorbed or organized. Equally with a slower form of rejection it may be destroyed by a more gradual process but nevertheless ultimately become a fibrous remnant.

Despite the wide variations in the macroscopic appearance of allografts during

rejection, for most organs and tissues certain broad groups can be distinguished. For example, with renal allografts there are several common forms. First, there is the large pale kidney, up to three times its original weight, with an edematous bulging cut surface (Figure 1.1). Sometimes such kidneys have a blotchy appearance with ill-defined congested patches irregularly scattered in their otherwise pale fawn to

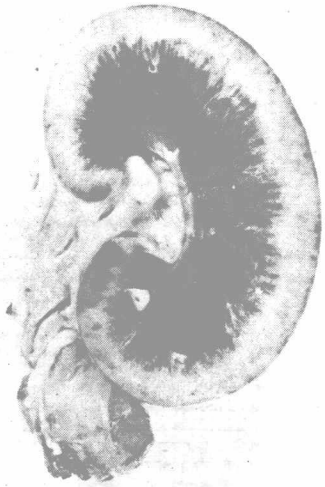

Figure 1.1 *Dog renal allograft (first-set) 17 days after transplantation. No immunosuppression. A considerably enlarged kidney with a moist pale greyish-white cortex and congested medulla. A few small hemorrhages in cortex. Microscopically: intense mononuclear cell infiltration, considerable edema, acute arterial lesions and focal parenchymal necrosis. Half natural size*

whitish-grey substance (Figure 1.2). A second group comprises the massive deep reddish-purple kidneys, even five times their original weight, with hemorrhages distributed throughout their substance and in the wall of the edematous and congested pelvis and ureter (Figure 1.3). In addition there may be some irregular pale opaque necrotic patches in the cortex or typical well-defined infarcts. A third group consists of the renal allografts which as a result of rejection are totally or mostly necrotic. These kidneys vary considerably in their other features some being large and rather hemorrhagic, others being of about normal size and having a uniform opaque

brownish cut surface. In a fourth group the kidneys are of about normal size or somewhat smaller, have an uneven rather coarsely scarred and pitted pale outer surface and irregularly narrowed rather tough fibrotic cortex (Figure 1.4). However, although these divisions serve some purpose in helping description, it would be a mistake to believe that they are more than parts of a broad spectrum which merge imperceptibly with one another. It should also be emphasized that the macroscopic features of an allograft depend on the pattern of rejection in the particular individual concerned. For instance, during the early stages the kidney may become large, pale and edematous; later it may become intensely congested and hemorrhagic, and

Figure 1.2 *Human renal allograft (first) 121 days after transplantation. Immunosuppression with steroids and azathioprine. A slightly enlarged kidney (180 g) with a blotchy appearance. Microscopically: atrophy and fibrosis and recent necrosis of cortex, fibrinoid necrosis of arteries and arterioles and slight mononuclear cell infiltration and edema. Half natural size*

finally, especially if the recipient is not dependent on the function of the allograft, it may become completely necrotic. Of course, a renal transplant could become

completely necrotic by a rather more direct route, for example, as a result of hyperacute rejection. The macroscopic features would then usually be rather different from the necrotic and hemorrhagic kidney seen as the final stage of the sequence described above.

Figure 1.3 *Human renal allograft (first) 29 days after transplantation. Immunosuppression with steroids and azathioprine. A greatly enlarged deep purple-red kidney (610 g). Main renal artery and vein free from thrombus. Microscopically: extensive recent parenchymal necrosis, fibrinoid necrosis and thrombosis of intrarenal arteries and arterioles. Substantial edema and interstitial hemorrhage and only slight mononuclear cell infiltration. Two-fifths natural size*

Microscopic features

The principal microscopic changes developing during allograft rejection include mononuclear cell infiltration, edema, vascular lesions and destruction of the parenchyma of the graft. However, the precise character, timing and severity of the lesions in individual grafts depend, like the other features, on the various donor and host factors already mentioned.

Mononuclear cell infiltration is the first morphological change during rejection of most first-set grafts and usually begins a day or two before there is any macroscopic evidence of an allograft reaction. The tissue soon becomes slightly edematous. As the mononuclear cell infiltration increases, vascular lesions develop but their type and time sequence vary considerably from graft to graft. In some transplants the

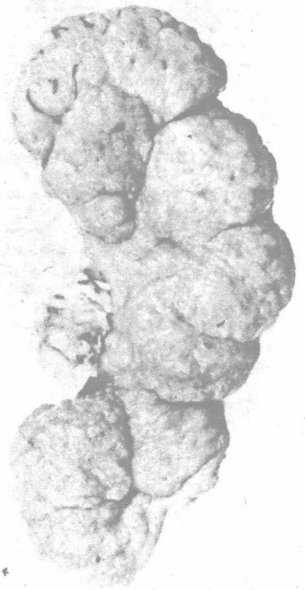

Figure 1.4 *Pig renal allograft (first-set) 483 days after transplantation. No conventional immunosuppression but given 1 litre donor blood during transplantation. A small coarsely scarred and fissured fibrotic kidney. Microscopically: focal parenchymal atrophy and fibrosis with intimal thickening of related arteries. No evidence of pyelonephritis.*
Half natural size

capillaries, venules and veins are principally involved but in others arterial changes become prominent. Sometimes the arterial lesions are of a proliferative kind but on other occasions fibrinoid necrosis of arteries and arterioles occurs and platelet–fibrin