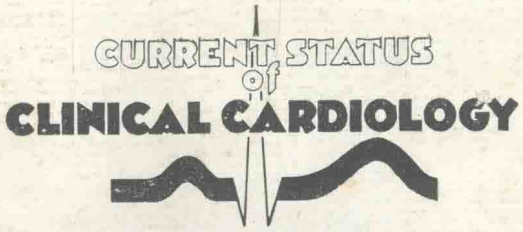

Series Editor J.P. Shillingford

CURRENT STATUS OF CLINICAL CARDIOLOGY 1990

Edited by
D.G. Julian

CURRENT STATUS
of
CLINICAL CARDIOLOGY

Series Editor J.P. Shillingford

CURRENT STATUS OF CLINICAL CARDIOLOGY 1990

Edited by
D.G. Julian

Consultant Medical Director
British Heart Foundation;
Emeritus Professor of Cardiology
University of Newcastle upon Tyne

KLUWER ACADEMIC PUBLISHERS
DORDRECHT / BOSTON / LONDON

Distributors

for the United States and Canada: Kluwer Academic Publishers, PO Box 358, Accord Station, Hingham, MA 02018-0358, USA
for all other countries: Kluwer Academic Publishers Group, Distribution Center, PO Box 322, 3300 AH Dordrecht, The Netherlands

ISBN 0-7923-8936-0

Copyright

Published in the United Kingdom by Kluwer Academic Publishers, PO Box 55, Lancaster, UK.

Kluwer Academic Publishers BV incorporates the publishing programmes of D. Reidel, Martinus Nijhoff, Dr W. Junk and MTP Press.

Printed and bound by Butler and Tanner Ltd., Frome and London

CURRENT STATUS OF CLINICAL CARDIOLOGY 1990

CURRENT STATUS OF CLINICAL CARDIOLOGY

Series Editor J.P. Shillingford

All the chapters in this book, with the exception of Chapter 7, are revised and updated versions of chapters which were originally published in the volumes of the *Current Status of Clinical Cardiology* series, as follows:

Chapters 1, 4, 11: from Spry, C.J.F. (ed.) *Immunology and Molecular Biology of Cardiovascular Diseases* (1987)

Chapters 2, 3: from Camm, A.J. and Ward, D.E. (eds.) *Clinical Aspects of Cardiac Arrhythmias* (1988)

Chapter 5: from Goodwin, J.F. (ed.) *Heart Muscle Disease* (1985)

Chapter 6: from Macartney, F.J. (ed.) *Congenital Heart Disease* (1986)

Chapters 8, 9, 10: from Fox, K.M. (ed.) *Ischaemic Heart Disease* (1987)

List of Contributors

L D Allan
Department of Perinatal Cardiology
Guy's Hospital
15th floor, Guy's Tower
St Thomas Street
London SE1 9RT
UK

D J Betteridge
Department of Medicine
University College and Middlesex
School of Medicine
The Rayne Institute
University Street
London WC1E 6JJ
UK

A Cheng
Department of Cardiology
Harefield Hospital
Harefield
Middlesex UB9 6JH
UK

P Chisholm
Immunology Section
Division of Biomolecular Sciences
Kings College
University of London
Camden Hill Road
London W8 7AH
UK

P A Crean
Regional Cardiology Unit
St James' Hospital
James Street
Dublin 8
Republic of Ireland

S E Humphries
Charing Cross Sunley Research
Centre
1 Lurgan Avenue
Hammersmith
London W6 8LW
UK

C D J Ilsley
Department of Cardiology
Harefield Hospital
Harefield
Middlesex UB9 6JH
UK

D G Julian
Consultant Medical Director
British Heart Foundation
102 Gloucester Place
London W1H 4DH
UK

C M Oakley
Department of Medicine (Clinical Cardiology)
Hammersmith Hospital
Royal Postgraduate Medical School
Ducane Road
London W12 0NN
UK

E G J Olsen
Department of Histopathology (Cardiovascular Division)
Brompton Hospital
Fulham Road
London SW3 6HP
UK

E J Perrins
Department of Cardiology
The General Infirmary at Leeds
Great George Street
Leeds LS1 3EX
UK

A A Quyyumi
National Heart, Lung and Blood Institute
National Institutes of Health, Building 10 7B15
Bethesda
MD 20892
USA

A Tybjærg-Hansen
Charing Cross Sunley Research Centre
1 Lurgan Avenue
Hammersmith
London W6 8LW
UK

D A Zideman
Department of Anaesthetics
Hammersmith Hospital
Royal Postgraduate Medical School
Ducane Road, London W12 0HS
UK

Foreword

D.G. JULIAN

Cardiology has been advancing on a broad front and in recent years we have learned much about the basic mechanisms underlying heart disease, and developed many new methods of diagnosis and treatment. This book discusses in depth some of the most important aspects of these.

One of the most exciting areas of research has been in molecular biology; Tybjærg-Hansen and Humphries describe how, following the pioneering Nobel prize-winning work of Goldstein and Brown, gene probes are being used to discover the genetic causes of coronary artery disease, especially in the hyperlipidaemias but also in thrombotic states. These developments, together with the introduction of powerful lipid-lowering agents has triggered interest in the primary hyperlipidaemias, which are frequently hereditary. Betteridge discusses their diagnosis and management. Quyyumi describes how a greater knowledge of the physiology and pathology of the coronary circulation has led to a better understanding of the causes of angina pectoris and of 'silent ischaemia'. Crean deals with the difficult topic of unstable angina, which has caused a lot of controversy in the past, but whose investigation and management is now broadly agreed.

Probably nowhere in medicine in recent years has there been a more exciting and important advance than the introduction of thrombolytic agents in acute myocardial infarction. This subject is dealt with in detail by Cheng and Ilsley, but they also consider the role of angioplasty and surgery in this context, as well as the place of drugs including aspirin and β-blockers. It is disappointing that 30 years after the introduction of modern cardiopulmonary resuscitation, so few patients in or out of hospital are saved from cardiac arrest, when the potential is so great. This is partly because many doctors do not know as much as they should about the procedures to be followed. Zideman outlines the modern management of cardiac arrest and cites the latest recommendations of the use of drugs in this situation. Cardiac pacing has become a highly specialised field, as pacemakers become more complex. This certainly has worked for the benefit of patients, but perhaps to the confusion of many doctors. The rate of pacemaker implantation in the United Kingdom is notoriously low and probably reflects both ignorance on the part of the medical profession and financial constraints. Perrins describes the various types of pacemaker available and the indications for their use.

Fetal echocardiography is a technique that is insufficiently well-known and one that deserves much wider application. Lindsey Allan, who is one of the leaders in this field, illustrates very clearly what can be achieved by this method. In the long term it should lead to a substantial reduction in the number of cases of severe congenital heart disease.

Amyloid heart disease is a relative rarity but, as Celia Oakley points out, one of considerable clinical interest.

Immunology has until recently had a relatively minor impact in cardiology, but, as Olsen shows, there are a large number of cardiac conditions from rheumatic disease to cardiomyopathy in which immune processes play an important role.

Heart transplantation is now an accepted form of treatment for advanced cardiac disease and the results today are very much better than they were only a few years ago. Nevertheless, there is still a long way to go in solving the problems of rejection; Chisholm discusses the various models which are being currently studied with the hope that a better understanding of the processes involved will lead to substantial improvements in survival and the quality of life of survivors.

The authorities who have contributed to this book have addressed their subjects in a comprehensible and stimulating way. Readers will certainly find much to interest them amongst the wide range of topics discussed.

Contents

1
Experimental cardiac transplantation

P. M. CHISHOLM

INTRODUCTION

Cardiac transplantation is not of course a cardiovascular disease, but the procedure is carried out as a consequence of disease. The immune response of the recipient animal to the histocompatibility differences between the donor and recipient of the graft also results in cardiac pathology, i.e. the destruction of cardiac tissue and loss of organ function. Experimental cardiac transplantation has as its raison d'etre the need to understand the fundamental nature of the immune response to organ or tissue allografts transplanted between different members of the same species, and the mechanisms by which these allografts are rejected. From this understanding strategies should develop for the diagnosis and prevention of graft rejection which will be applicable in man.

In the published proceedings of the last two International Congresses of the Transplantation Society, held in Helsinki, Finland in 1986[1] and in Sydney, Australia in 1988[2], almost all the papers presented in experimental transplantation described results obtained in rodent models and a substantial proportion of those used cardiac allograft models. This is primarily a reflection of the explosion over the past two decades in research in cellular immunology and immunogenetics using inbred strains of mice and rats, which has resulted both in a thorough understanding of the genetics and biology of the major histocompatibility complex (MHC), the gene complex which codes for the tissue antigens involved in transplant rejection, and in a comprehensive description of the cellular mechanisms responsible for the immunological rejection of organ allografts. It is also a reflection of the recognition that many features of the immune response to MHC-disparate grafts are universally applicable to grafts of different tissues in different species. Thus it has become clear that although the MHC is highly polymorphic, i.e. the extent of MHC-encoded antigenic variation between individuals of a species is very large, the number of effector mechanisms in graft rejection directed at those differences is strictly limited: differences in the fate of different organ grafts in the same or different species can be considered as variations on a single, although complex immunobiological theme.

This great increase in transplantation research has generated an enormous literature. In a number of recent reviews on the immunobiology of transplantation and the mechanisms of graft rejection[3,4] results obtained from experimental cardiac transplantation have made a significant contribution. It is my intention here not to review the field once more, but instead to simply highlight the key features of experimental cardiac transplantation which may be of interest to the non-immunological cardiologist or biologist, and which I believe are of most relevance to the practice and understanding of clinical heart transplantation. The first section comprises a brief consideration of those aspects of tissue immunogenicity which govern the fate of transplanted tissues, and a description of the types of immune mediated injury which occur during the process of transplant rejection. The second section describes the various models of rodent cardiac transplantation in common use. The final section describes the molecular and cellular events which take place within allogeneic cardiac tissue during uninterrupted graft rejection, and shows how these events can be modified by immunological intervention.

THE IMMUNOBIOLOGY OF GRAFT REJECTION

Tissue immunogenicity

The molecules which induce the immune response to organ allografts are the major and minor histocompatibility antigens of the species. The major antigens are coded for by a group of genes located on a single chromosome, the major histocompatibility complex (MHC) and, as their name implies, they provoke the strongest alloimmune responses. They are glycoproteins expressed on cell surfaces and, on the basis of structural and biological differences, are classified as Class I or Class II MHC molecules. There are a large number of minor histocompatibility antigens and these can also be important in transplantation. None of the gene products has yet been identified, but they are coded for by genes distributed throughout the genome and estimates of their number are in the hundreds.

In the context of organ transplantation it is important to realize that all the cellular constituents of a tissue are not equally immunogenic and that, at least partly, this is a consequence of differences in the amount, i.e. numbers, of MHC molecules on the constituent cell types. In the normal heart, which consists basically of myocardial cells, vascular endothelium and interstitial dendritic cells, myocardial cells express very few Class I MHC molecules and no Class II molecules. Vascular endothelium expresses Class I and, in some species only, e.g. human, Class II. Interstitial dendritic cells express both Class I and (relatively large amounts of) Class II. They have their counterpart in most other tissues[5], are bone marrow derived and have a comparatively short turnover time of days or weeks[6]. After transplantation, these cells are lost from the graft by

replacement from the recipient bone marrow: loss of these 'passenger leukocytes' which are extremely potent stimulators of alloimmune responses from the graft leads therefore to a natural reduction in the immunogenicity of the graft.

In complete contrast to this reduction in MHC content which occurs as a result of normal cell turnover, the content of MHC antigen in tissues can be dramatically increased as a result of immune activation. *De novo* or increased expression of MHC antigens on cells *in vivo* was first described in rodents in the target organs of graft-versus-host disease[7,8] and more recently in rejecting cardiac[9] and skin[10] allografts. Numerous *in vitro* studies[11] then showed that the expression of MHC Class I and Class II antigens can be substantially altered on cells cultured from virtually every organ by exposure to immune interferon or to crude tissue culture supernatants obtained from activated lymphocytes. From all these studies has emerged the important concept that the amount of alloantigen contained within a particular tissue is not an unvarying quantity, and that the immunogenicity of a graft after transplantation may change as a consequence of the immune reaction to it. The extent of expression of MHC Class I or Class II antigens within an organ allograft at the time of transplant is of importance because each class of antigen tends to induce different types of immune response: in general, Class I MHC alloantigens induce cytotoxic T-cell responses and Class II antigens induce activation of the T-cells involved in delayed type hypersensitivity responses[12]. Subsequent increases in the expression of MHC molecules on the graft tissue following transplantation as a consequence of that immune activation will also have a profound effect on the susceptibility of the graft to immune-mediated damage. Effector mechanisms involving T cells directed at Class I and Class II differences are both involved in graft rejection[12,13] although the relative contribution of each may differ in different circumstances (see below).

Mechanisms of graft tissue injury

A great deal of work over the past decade has established the cellular basis for the immunological rejection of allografts. This has included detailed descriptions of the ways in which the immune system is stimulated by the presence of an organ or tissue allograft to produce a range of responses, and of the intragraft events which lead to its eventual destruction. Classical experiments in the 1950s and 1960s established that graft rejection cannot be passively transferred by immune serum but the transfer of lymphocytes from an animal which has rejected one allograft of a particular histocompatibility type to a naive recipient of a graft of the same histocompatibility type will result in an accelerated rejection of the graft. If a naive recipient of a graft is rendered incapable of rejecting the graft before transplantation, e.g. by irradiation, then transfer of T-lymphocytes alone from a previously sensitized animal will restore graft

rejection. This type of experiment in a number of animal models including the rat cardiac allograft model[14] has established that graft rejection is primarily a cell-mediated reaction involving T-lymphocytes, although other cell types may be secondarily involved.

There are a number of ways in which the immune system can inflict damage on an allogeneic target organ. Specifically sensitized cytotoxic T-cells kill allogeneic target cells independently of alloantibody and complement: these cells are produced in response to cardiac allografts and can be recovered from the rejecting tissue itself[15]. A different cytotoxic cell, the killer or K-cell, destroys target cells only if they are coated with specific antibody, i.e. in a transplant situation if the graft cells are coated with alloantibody. This antibody dependent cell-mediated cytotoxicity (ADCC) has been demonstrated in cardiac allografts and, interestingly, it has been shown that different cells within cardiac tissue differ in their susceptibility to this kind of immune damage: vascular endothelial cells but not myocardial cells from rat heart were killed by cytotoxic T-cells, whereas the myocardial cells were more efficiently lysed by alloantibody dependent killing[16]. A third cytotoxic effector cell involved in cell-mediated cytotoxicity is the natural killer or NK-cell, and increased levels of NK-cell activity have been reported in rats rejecting cardiac allografts[17].

In addition to these direct mechanisms, graft tissue can be damaged indirectly by a number of inflammatory processes, probably mediated by various cytokines, and triggered coincidentally with and/or as a consequence of the specific immune response. For example, macrophages accumulate within rejecting cardiac tissue and exhibit some of the features of activation which suggest that they may be involved in graft destruction[18].

There has been much debate in recent years on the relative importance of each of these numerous effector systems to graft rejection[13,19]. It is most unlikely that any one mechanism of tissue destruction will always predominate, and highly likely that the importance of one or other will depend on the organ grafted, and the species involved. The relative importance of each mechanism may also change within the same organ with time after transplantation as a result of immune activation. The balance between the different effector mechanisms is also likely to be altered by different immunosuppressive strategies which may selectively inhibit one particular effector function and not another. More important, therefore, than trying to assign a rigid hierarchy of importance to the various mechanisms of rejection is to identify the detailed molecular and cellular events within each organ type in any particular species during uninterrupted graft rejection so that strategies for immune intervention can be designed.

RODENT MODELS OF CARDIAC GRAFTING

The availability of large numbers of different inbred strains of mice and rats has made these the species of choice for experimental transplantation. They are

relatively inexpensive, easy to house, breed rapidly and efficiently. Grafts can be exchanged between individuals of different inbred strains which have well defined MHC disparities, and so the importance of genetic differences to the outcome of transplantation can be assessed. Much more is known about the genetics of the mouse MHC, the H-2 system, than the rat MHC and for this reason most of our knowledge has been obtained in the mouse.

However, although the study of the immune response in mice to allogeneic tissue, in the form of dissociated cell suspensions usually leukocytes, has furnished many of the basic facts of the immunobiology of transplantation, it is difficult to perform organ allografts in so small an animal. The rat is much more amenable to microsurgical techniques and this, together with the recent increase in the number of available inbred rat strains, has resulted in widespread use of rat cardiac graft models. The heart graft can be either adult heterotopic, i.e. placed surgically in an extra-thoracic site such as in the abdomen[20] or neck[21], or it can be neonatal. Fetal grafts can be placed, without microsurgery, in a highly vascularized tissue space such as the pinna of the ear, or the plantar space[22]. The amount of fetal tissue transplanted is very small, and gaseous exchange is achieved by simple diffusion.

The heterotopic adult abdominal model pioneered by Ono and Lindsey[20], and used in a modified form by many groups, involves the microsurgical end-to-side anastomosis of the aorta and pulmonary artery to the abdominal aorta and the inferior vena cava, respectively. It is this method which has been successfully adapted to the mouse[23] although there are only a handful of laboratories in which this extremely exacting technique is routinely carried out. One variation to the model in the rat uses end-to-end anastomoses, following unilateral nephrectomy, between the renal and left carotid arteries and between the pulmonary artery and the renal vein[9]. In a different heterotopic model, cuffing techniques are used to establish the vascular connections and the graft is placed in the neck[21]. The advantages are that difficult microsurgery is avoided, and the cervical site also makes the monitoring of gross graft function (visibly) easy. Both the abdominal and cervical sites have been used sequentially in the same animal to compare the immunological parameters of first and second set cardiac graft rejection[24].

As is the case with all experimental models, the questions asked of these cardiac allograft models must be carefully chosen. In many respects the immune response to allogeneic cardiac tissue is the same irrespective of the particular model used. For example, the rejection time is very similar for the same graft tissue placed in different anatomical sites if there are not substantial differences in blood flow. The antigenicity of adult compared to neonatal tissue is, at least where MHC differences are involved, essentially the same. Without exception, allogeneic cardiac tissue, whether adult or neonatal and irrespective of the transplant site, is infiltrated with leukocytes before rejection, and some or all of the immune effector functions described above have been identified in all the models described. However, in some respects the models differ from each other

and the results obtained are not comparable.

Additionally, it should be remembered that in none of the experimental models described does the graft function as a normal heart, so although an assessment can be made of myocardial function at the cellular level, one cannot ask questions concerning organ function which are physiologically or clinically relevant. For the same reason results obtained from these experimental models can be of only limited usefulness in assessing for example which parameters of cardiac function might be most useful in the early diagnosis of clinical rejection: the criteria for defining useful cardiac function are very different for the orthotopic compared to the heterotopic auxiliary graft. Nevertheless, despite their limitations, these experimental models have furnished the basic facts of cardiac transplantation biology as described in detail in the following section.

INTRAGRAFT EVENTS DURING REJECTION

Uninterrupted rejection

One of the strategies used to identify which lymphocytes are involved in cardiac allograft rejection and to assign specific roles for the different populations of T-lymphocytes (viz. helper/inducer or suppressor/cytotoxic cells) has been to compare the capacity of the different subpopulations to restore graft rejection in grafted animals which are rendered unable to reject their grafts. The results obtained from a number of laboratories can be summarized as follows: purified populations of unsensitized T-cells, i.e. taken from naive animals, were found to be sufficient to restore acute first set cardiac graft rejection in lethally irradiated grafted recipients; B-lymphocytes were not effective[14]. In a similar system using T-cells sensitized to histocompatibility antigens of the same type as the graft, it was found that purified T-cells of either the helper/inducer T-cell subpopulation (also responsible for delayed-type hypersensitivity reactions[25]) or of the cytotoxic/suppressor subpopulation could, when transferred alone, restore first set cardiac rejection[26]. Subsequent studies, using monoclonal antibodies with specificity for the different T-cell subpopulations[27] to purify the T-cell subsets before transfer, confirmed that T-helper/inducer (CD4-positive) cells alone could bring about graft rejection[25] although, as had been described previously in mouse skin allografts[28], the grafts at rejection contained large numbers of the cytotoxic/suppressor (CD8-positive) cells[29], probably of host rather than donor origin. Using a different model it was shown that small numbers of sensitized T-cells injected together with IL2-containing lymphokine supernatants could adoptively restore heart graft rejection in T-cell-deprived rats[30]. More recently the same group reported that although CD4-positive T-cells alone restored rejection, they were less effective than unfractionated T-cell populations also containing CD8-positive cells[31]. Taken all together these studies suggest a necessary role for both the helper/inducer or DTH T-cell and the