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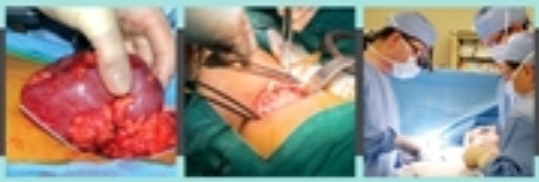
KIDNEY TRANSPLANTATION



Principles and Practice

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ELSEVIER

Kidney Transplantation

Principles and Practice

EIGHTH EDITION

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Video Contents

Preface to the First Edition

Renal transplantation is now an accepted treatment of patients in end-stage renal failure. A successful transplant restores not merely life but an acceptable quality of life to such patients. The number of patients in endstage renal failure in the Western World who might be treated by hemodialysis and transplantation is considerable and comprises some 30-50 new patients/million of population. Unfortunately in most, if not all, countries the supply of kidneys for transplantation is insufficient to meet the demand. Furthermore, hemodialysis facilities are usually inadequate to make up this deficit so that many patients are still dying of renal disease who could be restored to a useful and productive life. Nevertheless, few of us would have imagined even 10 years ago that transplantation of the kidney would have become such a relatively common procedure as is the case today, and indeed well over 30,000 kidney transplantations have been performed throughout the world.

Transplantation of the kidney for the treatment of renal failure has been an attractive concept for many years. As long ago as 1945, three young surgeons at the Peter Bent Brigham Hospital in Boston, Charles Hufnagel, Ernest Landsteiner and David Hume, joined the vessels of a cadaver kidney to the brachial vessels of a young woman who was comatose from acute renal failure due to septicemia. The kidney functioned for several days before it was removed, and the woman regained consciousness. Shortly afterwards, the woman's own kidneys began to function and she made a full recovery. The advent of the artificial kidney at that time meant that this approach to the treatment of acute renal failure was no longer necessary, but attention was soon given to the possibility of transplanting kidneys to patients with end-stage renal failure who were requiring dialysis on the newly developed artificial kidney to stay alive.

Although the first experimental kidney transplants in animals were reported first in Vienna by Dr. Emerich Ulmann in 1902 and then in 1905 by Dr. Alexis Carrel in the United States, the problem of rejection was not mentioned by either author. Later in 1910, Carrel did discuss the possible differences between an autograft and a homograft. The vascular techniques developed by Carrel for the anastomosis of the renal vessels to the recipient vessels are still used today. But in 1923, Dr. Carl Williamson of the Mayo Clinic clearly defined the difference between an autografted and homografted kidney and even published histological pictures of a rejecting kidney. Furthermore, he predicted the future use of tissue matching in renal transplantation.

It is unfortunate that the lower animals, such as the dog, do not possess a blood grouping like that of man. In the future it may be possible to work out a satisfactory way of determining the reaction of the recipient's blood serum or tissues to those of the donor and the reverse; perhaps in this way we can obtain more light on this as yet relatively dark side of biology.

The recognition that allogeneic tissues would be rejected was further established in later years by Drs. Gibson and Medawar, who treated burn patients with homografts in Glasgow during the Second World War. Indeed, it was the crash of a bomber behind the Medawars' house in Oxford during the early years of the war that first stimulated his interest in transplantation, especially of skin.

In his address at the opening of the new Oxford Transplant Unit in 1977, Sir Peter Medawar recounted this event.

Early in the war, an R.A.F. Whitley bomber crashed into a house in North Oxford with much serious injury and loss of life. Among the injured was a young man with a third degree burn extending over about 60% of his body. People burned as severely as this never raised a medical problem before: they always died; but the blood transfusion services and the control of infection made possible by the topical use of sulphonamide drugs now made it possible for them to stay alive. Dr. John F. Barnes, a colleague of mine in Professor H. W. Florey's School of Pathology, asked me to see this patient in the hope that being an experimental biologist I might have some ideas for treatment. With more than half his body surface quite raw, this poor young man was a deeply shocking sight; I thought of and tried out a number of ingenious methods, none of which worked, for ekeing out his own skin for grafting, trying to make one piece of skin do the work of ten or more. The obvious solution was to use skin grafts from a relative or voluntary donor, but this was not possible then and it is not possible now.

I believe I saw it as my metier to find out why it was not possible to graft skin from one human being to another, and what could be done about it. I accordingly began research on the subject with the Burns Unit of the Glasgow Royal Infirmary, and subsequently in the Zoology Department in Oxford. If anybody had then told me that one day, in Oxford, kidneys would be transplanted from one human being to another, not as a perilous surgical venture, but as something more in the common run of things, I should have dismissed it as science fiction; yet it is just this that has come about, thanks to the enterprise of Professor Morris and his colleagues.

Nevertheless in 1951, David Hume in Boston embarked on a series of cadaver kidney transplants in which the kidney was placed in the thigh of the recipient. All but one of these kidneys were rejected within a matter of days or weeks, the one exception being a patient in whom the kidney functioned for nearly 6 months and enabled the patient to leave the hospital! This event provided hope for the future as no immunosuppressive therapy had been used in this patient. At this time, the problems of rejection of kidney allografts in the dog were being clearly defined by Dr. Morton Simonsen in Copenhagen and Dr. William Dempster in London, but in 1953, a major boost to transplantation research was provided by the demonstration, by Drs. Rupert Billingham, Lesley Brent and Peter Medawar, that tolerance to an allogeneic skin graft in an adult animal could be produced by injecting the fetus with donor strain

tissue, thus confirming experimentally the clonal selection hypothesis of Burnet and Fenner in the recognition of self and non-self. The induction of specific unresponsiveness of a host to a tissue allograft has remained the ultimate goal of transplant immunologists ever since.

Then in 1954, the first kidney transplant between identical twins was carried out successfully at the Peter Bent Brigham Hospital which led to a number of further successful identical twin transplants in Boston and elsewhere in the world over the next few years.

There still remained the apparently almost insoluble problem of rejection of any kidney other than an identical-twin kidney. The first attempts to suppress the immune response to a kidney allograft employed total body irradiation of the recipient and were carried out by Dr. Merril's group in Boston, two groups in Paris under the direction of Drs. Kuss and Hamburger, respectively, and by Professor Shackman's group in London. Rejection of a graft could be suppressed by irradiation, but the complications of the irradiation were such that this was really an unacceptable approach, although an occasional relatively long-term acceptance of a graft provided encouragement for the future.

Then came the discovery by Drs. Schwartz and Dameshek in 1959 that 6-mercaptopurine could suppress the immune response of rabbits to human serum albumin. Shortly afterwards, they showed that the survival of skin allografts in rabbits was significantly prolonged by the same drug. This event ushered in the present era of renal transplantation, for very quickly Roy Calne in London and Charles Zukoski working with David Hume in Virginia showed that this same drug markedly prolonged the survival of kidney allografts in dogs. And indeed,6-mercaptopurine was first used in a patient in Boston in 1960. Elion and Hitchings of the Burroughs Wellcome Research Laboratories in New York State then developed azathioprine, which quickly replaced 6-mercaptopurine in clinical practice as it was less toxic. With the addition of steroids, the standard immunosuppressive therapy of today was introduced to the practice of renal transplantation in the early sixties.

Not that this meant the solution of the problems of renal transplantation for this combination of drugs was dangerous and mortality was high in those early years. But there was a significant number of long-term successful transplants, and as experience grew, the results of renal transplantation improved. Another major area of endeavor in renal transplantation at that time was directed at the study of methods of matching donor and recipient for histocompatibility antigens with the aim of lessening the immune response to the graft and so perhaps allowing a decrease in the immunosuppressive drug therapy. Although this aim has only been achieved to any great extent in siblings who are HLA identical, tissue typing has made a significant contribution to renal transplantation, perhaps best illustrated by the recognition in the late sixties that the performance of a transplant in the presence of donor-specific presensitization in the recipient leads to hyperacute or accelerated rejection of the graft in most instances. Nevertheless, the more recent description of the Ia-like system in man (HLA-DR) may have an important impact on tissue typing in renal transplantation. The present decade also has seen an enormous effort directed at immunological monitoring in renal transplantation and at attempts to induce experimental specific immunosuppression. We have solved most of the technical problems of renal transplantation; we have been left with the problem of rejection and the complications arising from the drug therapy given to prevent rejection.

Although the contributions in this book cover all aspects of renal transplantation, certain subjects, as for example immunological monitoring before transplantation, transplantation in children and cancer after renal transplantation, have received considerable emphasis as they do represent developing areas of great interest, and I must take responsibility for this emphasis. For in the seventies we have seen many of the principles and practice of renal transplantation become established and the areas of future investigation become more clearly defined. With an ever-increasing demand for renal transplantation, more and more people in many different disciplines, doctors (surgeons, physicians, pathologists, virologists, immunologists), nurses, scientists and ancillary staff are becoming involved in renal transplantation either in the clinic or in the laboratory. It is to these people I hope this book will be of value.

Sir Peter J. Morris
Oxford, UK
November 1978

Preface to the Eighth Edition

Kidney transplant patients and practitioners benefit from updated knowledge of current and improved practice guidelines and novel techniques, in addition to being familiar with well-established principles. For these reasons we have sought out leading international experts to write the chapters of this 8th edition of the Textbook of Kidney Transplantation. What has not changed over the past 41 years since the first edition is Professor Morris's dedication to the textbook's quality and his personal attention to the details that are included. He has been the lead editor since the first edition, indeed the sole editor of the first five editions. Professor Marson and Knechtle are delighted that he has chosen to include us as editors, as this text remains the most widely circulated authoritative book on the subject of kidney transplantation, used internationally to help develop practice guidelines and train specialists. We are furthermore grateful to the authors who have produced the content of this 8th edition, including its up-to-date outcomes data and analysis of the evidence supporting current practice in the field. Finally, we thank the leadership of Elsevier for its excellent communication with the authors and editors and for their technical assistance with all aspects of the production of this complex project.

In this 8th edition, we have chosen to combine the chapters on azathioprine and mycophenolate based on the relatedness of these compounds as inhibitors of cell proliferation. We have added two new chapters, one addressing kidney allocation because policy varies in the international community, reflecting the ethical and societal values of different countries and populations. Secondly, we have added a chapter on biomarkers of kidney injury and rejection. The latter is in recognition of the need for better monitoring tools for kidney injury and rejection to guide therapy and patient management. Given the large number of candidate assays for injury and rejection and their relatively nascent status with respect to clinical use, we suspect that this will be a rapidly developing field in coming years and will help guide improvement of long-term kidney transplant outcomes. The chapter in the previous edition on belatacept has merged with the chapter on antibody and fusion proteins and includes considerable new data on the clinical use of costimulation blockade.

Some areas of renal transplantation remain challenges for the field and this certainly would include the sensitized patient, antibody-mediated rejection, and management of chronic allograft failure. These topics are addressed in detail in associated chapters. Quite a number of chapters have been completely rewritten by new authors compared with the 7th edition, and we believe that these new chapters offer refreshing perspectives on their respective topics. We acknowledge that our field continues to be guided by new basic and clinical research that in many cases is beyond the scope of this text, despite our desire to treat subjects comprehensively. We have sought to include what is most pertinent to current clinical practice in the field. Our hope is that the coming decades will continue to build on the remarkable record of progress in kidney transplantation that we have witnessed since the first successful kidney transplant in 1954 by the late Joseph Murray at Peter Bent Brigham Hospital in Boston. Ours is an exciting field that offers improved and extended life to many persons with severely impaired renal function. It is also our hope that improved immunosuppressive therapy will further prolong graft survival and reduce the side effects of infection and malignancy, ultimately extending patient survival yet further. Improved preservation techniques offer the prospect of increasing the use of kidneys that were previously considered to be of inadequate quality, and thereby increase the supply of donor kidneys. Improved antiviral agents have made possible the use of HIV-positive and hepatitis C-positive donor kidneys. These innovations are described in this updated text, which we expect will inspire further good work.

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1 Kidney Transplantation: A History

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CHAPTER OUTLINE

The modern period of transplantation began in the late 1950s, but two earlier periods of interest in clinical and experimental transplantation were the early 1950s and the first two decades of the 20th century. Hamilton[1] provides a bibliography of the history of organ transplantation. Table 1.1 summarizes landmarks in kidney transplantation.

Early Experiments

Interest in transplantation developed in the early part of the 20th century because experimental and clinical surgical skills were rapidly advancing, and many of the pioneering surgeons took an interest in vascular surgical techniques as part of their broad familiarity with the advance of all aspects of surgery. Payr's demonstration of the first workable, although cumbersome, stent method of vascular suturing led to widespread interest in organ transplantation in Europe. Many centers were involved, notably Vienna, Bucharest, and Lyon. The first successful experimental organ transplant was reported by Ullmann in 1902. Emerich Ullmann (1861–1937) (Fig. 1.1) had studied under Edward Albert before obtaining a position at the Vienna Medical School, which was then at its height. Ullmann's article shows that he managed to autotransplant a dog kidney from its normal position to the vessels of the neck, which resulted in some urine flow. The animal was presented to a Vienna medical society on March 1, 1902, and caused considerable comment.[2] At this time, Ullmann was chief surgeon to the Spital der Baumhertigen Schwestern, and his experimental work was done in the Vienna Physiology Institute under Hofrath Exner. Exner's son Alfred had already tried such a transplant without success. In the same year, another Vienna physician, Alfred von Decastello, physician assistant at the Second Medical Clinic, carried out dog-to-dog kidney transplants at the Institute of Experimental Pathology.[3]

Ullmann and von Decastello had used Payr's method, and later in 1902 Ullmann demonstrated a dog-to-goat kidney transplant that, to his surprise, passed a little urine for a while. Neither Ullmann nor von Decastello continued with this work, although von Decastello was noted for his work on blood groups, and Ullmann published extensively on bowel and biliary surgery.

In Lyon, the department headed by Mathieu Jaboulay (1860–1913) had a major influence (Fig. 1.2). In his research laboratories, his assistants Carrel, Briau, and Villard worked on improved methods of vascular suturing, leading to Carrel's famous article credited with establishing the modern method of suturing.[4] Carrel left to work in the United States, and in the next 10 years he published extensively on organ grafting, successfully carrying out autografts of kidneys in cats and dogs and, showing that allografts, contrary to accepted opinion, eventually failed after functioning briefly, established the existence of "rejection" as it was later termed. He made attempts at tissue matching and demonstrated cold-preservation of tissues. He was awarded a Nobel Prize for this work in 1912.[5]

Human Kidney Transplants

Jaboulay, Carrel's teacher, had carried out the first recorded human kidney transplant in 1906,[6] although Ullmann later claimed an earlier attempt in 1902.[7] Jaboulay was later to be better known for his work on thyroid and urologic surgery, but, doubtless encouraged by the success of Carrel and others in his laboratory, he carried out two xenograft kidney transplants using a pig and goat as donors, transplanting the organ to the arm or thigh of patients with chronic renal failure. Each kidney worked for only 1 hour. This choice of an animal donor was acceptable at that time in view of the many claims in the surgical literature for success with xenograft skin, cornea, or bone.

More is known of the second and third attempts at human kidney transplantation. Ernst Unger (1875–1938) (Fig. 1.3) had a thorough training in experimental work and set up his own clinic in 1905 in Berlin, being joined there by distinguished colleagues. He continued with experimental work and by 1909 reported successful

TABLE 1.1 Landmarks in Kidney Transplantation

1902	First successful experimental kidney transplant[2]
1906	First human kidney transplant—xenograft[6]
1933	First human kidney transplant—allograft[54]
1950	Revival of experimental kidney transplantation[4,16,57]
1950–1953	Human kidney allografts without immunosuppression, in Paris[18,19,56,59] and Boston[21]
1953	First use of live related donor, Paris[20]
1954	First transplant between identical twins, Boston[22]
1958	First description of leukocyte antigen MAC[62]
1959–1962	Radiation used for immunosuppression, in Boston[24] and Paris[25,56]
1960	Effectiveness of 6-mercaptopurine (6-MP) in dog kidney transplants[29,42]
1960	Prolonged graft survival in patient given 6-MP after irradiation[34]
1962	First use of tissue matching to select a donor and recipient[44,47,49,56]
1966	Recognition that positive crossmatching leads to hyperacute rejection[29,50,56]
1967	Creation of Eurotransplant[46]
1967	Development of kidney preservation
1973	Description of the transfusion effect[57]
1978	First clinical use of cyclosporine[55]
1978	Application of matching for HLA-DR in renal transplantation[29]
1987	First of new wave of immunosuppressive agents appears (tacrolimus)
1997	Transgenic pigs strategy[63]
2010	Laparoscopic kidney insertion[64]

Fig. 1.1 Emerich Ullmann (1861–1937) carried out the first experimental kidney transplants in dogs in 1902. (Courtesy the Vienna University, Institute for the History of Medicine.)

Fig. 1.2 Mathieu Jaboulay (1860–1913) and his surgical team at Lyon in 1903. Until his death in a rail accident, Jaboulay made numerous surgical contributions and encouraged Alexis Carrel's work on vascular anastomosis. In 1906 Jaboulay reported the first attempt at human kidney transplantation.

Fig. 1.3 A contemporary cartoon of Ernst Unger (1875–1938) at work at the Rudolf Virchow Hospital, Berlin. (Courtesy the Rudolf Virchow Hospital.)

transplantation of the kidneys en masse from a fox terrier to a boxer dog. The urine output continued for 14 days, and the animal was presented to two medical societies. By 1910, Unger had performed more than 100 experimental kidney transplants. On December 10, 1909, Unger attempted a transplant using a stillborn child's kidney grafted to a baboon. No urine was produced. The animal died shortly after the operation, but postmortem examination showed that the vascular anastomosis had been successful. This success and the new knowledge that monkeys and humans were serologically similar led Unger to attempt, later in the same month, a monkey-to-human transplant.[8] The patient was a young girl dying of renal

failure, and the kidney from an ape was sutured to the thigh vessels. No urine was produced. Unger's report concluded that there was a biochemical barrier to transplantation, a view mistakenly advocated by the basic science of the day; his main contributions thereafter were in esophageal surgery. (For a biography of Unger, see Winkler.[9])

These early experiments established that kidney transplants were technically possible. Methods of study of renal function were primitive then; without routine measurement of blood urea and without any radiologic methods, subtle studies of transplant function were impossible. This impossibility plus the uncertainty of the mechanism of allograft rejection led to a diminished interest in organ transplantation after about 10 years of activity. By the start of World War I, interest in organ transplantation had almost ceased and was not resumed in the European departments of surgery after the war. Carrel had switched his attention to studies of tissue culture. Interest elsewhere also was low; in Britain and the United States, scarce research funds were being applied to fundamental biochemistry and physiology, rather than applied projects of clinical relevance. Transplantation immunology faded away after the bright start in the capable surgical hands of Carrel, Murphy's sound grasp of immunosuppression, and Landsteiner's awareness of the serologic detection of human antigens. Carrel, Murphy, and Landsteiner all worked at the Rockefeller Institute in New York.

In 1914, in a remarkable lecture to the International Surgical Society, Carrel did anticipate the future development of transplantation. His colleague at the Rockefeller Institute, J. B. Murphy, had found that radiation or benzol treatment would increase the "take" of tumor grafts in rats, and Carrel realized the potential of these findings:

It is too soon to draw any definite conclusions from these experiments. Nevertheless it is certain that a very important point has been acquired with Dr. Murphy's discovery that the power of the organism to eliminate foreign tissue was due to organs such as the spleen or bone marrow, and that when the action of these organs is less active a foreign tissue can develop rapidly after it has been grafted. It is not possible to foresee whether or not the present experiments of Dr. Murphy will lead directly to the practical solution of the problem in which we are interested. The surgical side of the transplantation of organs is now completed, as we are now able to perform transplantations of organs with perfect ease and with excellent results from an anatomical standpoint. But as yet the methods cannot be applied to human surgery, for the reason that homoplastic transplantations are almost always unsuccessful from the standpoint of the functioning of the organs. All our efforts must now be directed toward the biological methods which will prevent the reaction of the organism against foreign tissue and allow the adapting of homoplastic grafts to their hosts.[10]

The Middle Years

Until the revival of interest in transplantation in the 1950s, the 1930s and 1940s were a stagnant period in clinical science. The great European surgical centers had declined; in North America, only at the Mayo Clinic was there a cautious program of experimental transplantation without building on Carrel's work, notably failing to make attempts at immunosuppression. In transplantation circles, such as they were, there was not even the confidence to counter the vivid claims of Voronoff to rejuvenate human patients via monkey gland grafts, and the endless reports of successful human skin homografts were not examined critically.

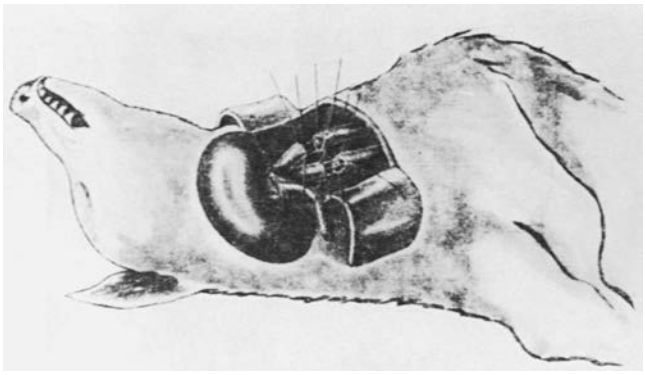

Fig. 1.4 Yu Yu Voronoy (1895–1961) had experience with dog allografts before carrying out the first human kidney allograft in 1933 at Kherson in the Ukraine. His experimental animal model is shown here.

The main event of this period was an isolated and little-known event—the first human kidney allograft. It was performed in the Ukraine by the Soviet surgeon Yu Yu Voronoy.[11] Voronoy was an experienced investigator, and he eventually performed six such transplants up to 1949. Voronoy (1895–1961) trained in surgery at Kiev under Professor V.N. Shamov and obtained experience there with serologic methods of blood transfusion, then in their developmental stage. He used these methods to detect complement-fixing antibodies after testis slice transplants, and later he had some success with the same methods applied to kidney grafts (Fig. 1.4). In 1933 Voronoy transplanted a human kidney of blood group B to a patient of blood group O with acute renal failure as a result of mercuric chloride poisoning. The donor kidney was obtained from a patient dying as a result of a head injury and was transplanted to the thigh vessels under local anesthetic; the warm time for the kidney was about 6 hours. There was a major mismatch for blood groups, and despite a modest exchange transfusion, the kidney never worked. The patient died 2 days later; at postmortem, the donor vessels were patent. By 1949, Voronoy reported six such transplants, although no substantial function had occurred in any. (For a biography of Voronoy, see Hamilton and Reid[12] and Matevossian and colleagues.[13])

Post World War II

The sounder basis of transplantation immunology, which followed Medawar's pioneer studies during World War II, led to a new interest in human transplantation. In 1946 a human allograft kidney transplant to arm vessels under local anesthetic was attempted by Hufnagel, Hume, and Landsteiner at the Peter Bent Brigham Hospital in Boston. The brief period of function of the kidney may have helped the patient's recovery from acute renal failure; it marked the beginning of that hospital's major interest in transplantation and dialysis.[14]

In the early 1950s, interest in experimental and clinical kidney transplantation increased. With a growing certainty

Fig. 1.5 David M. Hume (1917–1973) pioneered human kidney transplantation at the Peter Bent Brigham Hospital, Boston, and the Medical College of Virginia. He died in an air crash at the age of 55.

that immunologic mechanisms were involved, the destruction of kidney allografts could be reinvestigated. Simonsen, then an intern in Ålborg in Denmark, persuaded his surgical seniors to teach him some vascular surgery; using dog kidney transplants, he reported on the mechanism of kidney rejection.[15] Dempster in London also reexamined this question.[16] Both workers found, like Küss in Paris, that the pelvic position of the kidney was preferable to a superficial site, and both concluded that an immunologic mechanism was responsible for failure. Dempster found that radiation, but not cortisone, delayed rejection. Both workers considered that a humoral mechanism of rejection was likely.

In the early 1950s, two groups simultaneously started human kidney transplantation. In Paris, with encouragement from the nephrologist Jean Hamburger, the surgeons Küss (five cases),[17] Servelle (one case),[18] and Dubost (one case)[19] reported on kidney allografts without immunosuppression in human patients, placing the graft in the now-familiar pelvic position. The Paris series included a case reported by Hamburger of the first live-related kidney transplant, the donor being the mother of a boy whose solitary kidney had been damaged in a fall from a height. The kidney functioned immediately, but was rejected abruptly on the 22nd day.[20] In the United States, the Chicago surgeon Lawler had been the first to attempt such an intraabdominal kidney allograft in 1950; it was met with the intense public interest and professional skepticism that were to characterize innovative transplantation thereafter.

A series of nine cases, closely studied, was recorded from Boston, using the thigh position of the graft, and for the first time hemodialysis had been used in preparing the patients, employing Merrill's skill with the early Kolff/Brigham machine. David Hume (Fig. 1.5) reported on this Boston experience in 1953. Modest unexpected survival of the kidney was obtained in some of these cases and served to encourage future careful empirical surgical adventures, despite advice from scientists to wait for elegant immunologic solutions. Although small doses of adrenocorticotropic hormone or cortisone were used, it was thought that the endogenous immunosuppression of uremia was responsible for these results, rather than the drug regimen. Many of Hume's tentative conclusions from this short series were confirmed later, notably that prior blood transfusion might be beneficial, that blood group matching of graft and donor might be necessary, and that host bilateral nephrectomy was necessary for control of posttransplant blood pressure.[21] The first observation of recurrent disease in a graft was made, and accelerated arteriosclerosis in the graft vessels was noted at postmortem. Other cases were reported from Chicago, Toronto, and Cleveland in the early 1950s, but because no sustained function was achieved, interest in clinical and experimental renal allograft transplantation waned, despite increasing knowledge of basic immunologic mechanisms in the laboratory.

The technical lessons learned from the human allograft attempts of the early 1950s allowed confidence in the surgical methods, and in Boston, on December 23, 1954, the first transplant of a kidney from one identical twin to another with renal failure was performed. From then on, many such transplantations were performed successfully in Boston.[22] Although sometimes seen now merely as a technical triumph, valuable new findings emerged from this series. Some workers had predicted that, in the short term, the activity of the inactive bladder could not be restored, and that in the long term, human kidney grafts would decline in vitality as a result of denervation or ureteric reflux. Other workers were convinced that a single kidney graft could not restore biochemical normality to an adult, and that in any case the existing changes caused by chronic renal failure were irreversible. All of these gloomy predictions were neutralized by the success of the twin kidney transplants, and the greatest triumph came when one such recipient became pregnant and had a normal infant, delivered cautiously by cesarean section, with the anxious transplanters in attendance. Many of the twin recipients are still alive today, although the good results were tempered by failures caused by the prompt return of glomerulonephritis in some transplanted kidneys. This complication was later much reduced by immunosuppression. Other lessons learned were that the hazard of multiple donor renal arteries provided a need for pretransplant angiography of the kidneys in living donors, although it still was not thought necessary to perfuse or cool the donor organ. Lastly, there was the first airing of the legal aspects of organ donation, particularly the problem of consent in young, highly motivated related donors. (For an account of this period, see Murray and colleagues.[23])

Immunosuppression and the Modern Era

In 1948, the first patients crippled with rheumatoid arthritis were given the Merck Company's Cortone (cortisone) at the Mayo Clinic, and intense worldwide interest in the pharmacologic actions of adrenal cortical hormones followed. Careful studies by Medawar's group in the early 1950s suggested a modest immunosuppressive effect of cortisone,

but when Medawar shortly afterward showed profound, specific, and long-lasting graft acceptance via the induction of tolerance, the weak steroid effect was understandably sidelined and thought to be of no clinical interest. Induction of tolerance in adult animals (rather than newborns) was accomplished by lethal irradiation and bone marrow infusion, and with this strong lead from the laboratory, it was natural that the first attempts at human immunosuppression for organ transplants were with preliminary total-body irradiation and allograft bone marrow rescue. These procedures were carried out in Paris, Boston, and elsewhere in the late 1950s.

This regimen was too difficult to control, and graft-versus-host disease was inevitable. It was found unexpectedly that sublethal irradiation alone in human patients was quite immunosuppressive, however, and this approach was used until 1962, the year of the first general availability of azathioprine (Imuran). In Boston, 12 patients were treated in this way, but with only one long-term survival in a man receiving his transplant from his nonidentical twin.[24] In Paris, similar success was obtained with sibling grafts.[25,26] These isolated kidney survivals after a single dose of radiation gave further hope and showed again that the immunology of humans, dogs, and mice is different. These cases also showed that if a human organ could survive the initial crucial rejection period, it could be protected or adapted to the host in some way, possibly shielded by new endothelium, by enhancement, or, as suggested later, by microchimeric tolerance induced by mobile cells in the graft.

Chemical Immunosuppression

In 1958, at the New England Medical Center, attempts were made at human bone marrow transplantation for aplastic anemia and leukemia. To enable the marrow grafts to succeed, irradiation of the recipient was used. Results were poor, and mortality was high. Schwartz and Dameshek[27] looked for alternatives to irradiation and reasoned that an anticancer drug, such as 6-mercaptopurine (6-MP) or methotrexate, might be of use for immunosuppression in their patients. (For an account of this period, see Schwartz.[28]) Their important paper in 1959, showing a poor immune response to foreign protein in rabbits treated with 6-MP,[27] was noticed by Roy Calne, then a surgeon in training at the Royal Free Hospital, London, and David Hume, new Chairman of Surgery at the Medical College of Virginia. Calne had been disappointed at the failure of irradiation to prolong kidney allograft survival in dogs and, like others looking for an alternative, he found that 6-MP was successful.[29] Zukoski and colleagues[30] in Richmond found the same effect.

In 1960 Calne visited Boston for a period of research with Murray, and Hitchings and Elion of Burroughs Wellcome, then at Tuckahoe, provided him with new derivatives of 6-MP.[31] Of these, BW57-322 (later known as azathioprine [Imuran]) proved to be more successful in dog kidney transplants and less toxic than 6-MP.[32]

From 1960 to 1961, 6-MP was used in many human kidney transplants. In London at the Royal Free Hospital, three cases were managed in this way, but without success, although one patient receiving a live related transplant died of tuberculosis rather than rejection.[33] In Boston, no lasting human kidney function was obtained, but in Paris, Küss and associates[34] reported one prolonged survival of a kidney from a nonrelated donor when 6-MP was used with intermittent prednisone in a recipient who also had received irradiation as the main immunosuppressive agent (Fig. 1.6). This case was the first success for chemical immunosuppression.

Fig. 1.6 R. Küss (right) and M. Legrain (center) in 1960 with their first long-term kidney transplant survivor. The patient and her brother-in-law donor (center right) are shown with the staff of the unit at the Hôpital Foch. Immunosuppression with irradiation and mercaptopurine was used. (Courtesy Prof. M. Legrain.)

This change in approach, giving lifelong, risky medication with toxic drugs, although an obvious development in retrospect, was accepted with reluctance because it meant leaving aside, at least in the short term, the hopes from the work of the transplantation immunologists for the elegant, specific, one-shot, nontoxic tolerance regimen. Many workers thought that entry into this new paradigm was only a temporary diversion.

In 1961 azathioprine became available for human use; the dosage was difficult to judge at first. The first two Boston cases using the drug did not show prolonged survival of the grafts, but in April 1962 the first extended successes with human kidney allografts were obtained.[35] Shortly afterward, at the bedside rather than in the laboratory, it was discovered that steroids, notably prednisolone, when given with azathioprine had a powerful synergistic effect. The regular use of both together became a standard regimen after reports by Starzl and colleagues[36] and Goodwin and coworkers,[37] and this combined therapy continued to be the routine immunosuppressive method despite many other suggested alternatives, until azathioprine was displaced by cyclosporine much later. Use of the combined immunosuppression and the increasing use of live related donors (rather than occasional twin or free or cadaver kidneys), along with the remarkably good results reported in 1963 from Denver[36] and Richmond,[38] greatly encouraged the practice of transplantation. (For an account of this period, see Starzl.[39])

A Time of Optimism

The mid-1960s was a period of great optimism. The rapid improvement in results seemed to indicate that routine success was at hand. Looking to the future, calculations were

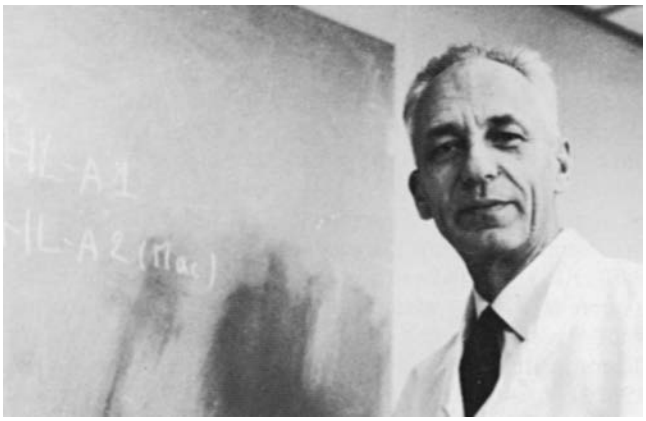

Fig. 1.7 Jean Dausset first described an antigen MAC, later known as HLA-2, defined by numerous antisera from multitransfused patients, and which later was shown to be part of the major histocompatibility complex in humans (HLA).

made that suggested that enough donor organs would be available in the future if all large hospitals cooperated, and such donations did start to come from outside the transplantation pioneer hospitals. Transplantation societies were set up, and specialist journals were started. The improvements in regular dialysis treatment meant an increasing pool of patients in good health suitable for transplantation, and this allowed for better and planned preparation for transplantation. With a return to dialysis being possible, heroic efforts to save a rejected kidney were no longer necessary. Management of patients improved in many aspects, and the expected steroid long-term effects were met and managed (primarily by the demonstration that low-dose steroids were as effective as high-dose steroids). The need for cooling of donor organs was belatedly recognized, many tests of viability were announced, and transport of organs between centers began. Bone disease and exotic infections were encountered and treated, but the kidney units were affected by a hepatitis B epidemic in the mid-1960s, which affected morale and status. The narrow age limit for transplantation was widened, and in Richmond the first experience with kidney grafts in children was obtained.

Recipients of kidney transplants reentered the normal business of life and became politicians, professors, pilots, and fathers and mothers of normal children. Other good news in the United States came when the federal government accepted the costs of regular dialysis and transplantation in 1968. There were always unexpected findings, usually reported from the pioneer units with the longest survivors. Cautiously, second kidney transplants were performed at Richmond when a first had failed; these did well, and the matter became routine. Chronic rejection and malignancy first were reported in kidney transplant recipients from Denver. As a result of the optimism, experimental heart transplantation started, the first human livers were grafted, and there was a revival of interest in xenotransplantation. Although the attempts of Reemtsma and coworkers,[40] Hume,[41] and Starzl[39] at transplantation with chimpanzee or baboon kidneys ultimately failed, rejection did not occur immediately, and the cases were studied closely and described.

In the search for better immunosuppression, there was great excitement when laboratory studies by Woodruff and Medawar produced a powerful immunosuppressive antilymphocyte serum, and production of versions suitable for human use started.[42] Initial results were favorable, but the whole antilymphocyte serum had an unspectacular role thereafter, added to from 1975 onward by the use of monoclonal antibody versions. Hopes for another biological solution to transplantation were raised in 1969 when French and Batchelor[43] found an enhancing serum effect in the new experimental model of rat kidney transplantation made possible by the development of microsurgical methods, but it proved impossible to mimic the effect in humans.

Tissue Typing

The greatest hopes resided in the evolution of tissue-typing methods, which entered routine use in 1962 (Fig. 1.7).[44,45] The increasing identification of the antigens of the human leukocyte antigen (HLA) system seemed to promise excellent clinical results in the future from close matching made possible when choosing from a large pool of patients. Sharing of kidneys in Europe started in 1967 at van Rood's suggestion,[46] and in North America, Amos and Terasaki set up similar sharing schemes on both coasts of the United States. Others followed throughout the world, and these organizations not only improved the service but also soon gathered excellent data on kidney transplant survival. The need to transport kidneys within these schemes encouraged construction of perfusion pumps designed to increase the survival of organs and the distance they could be transported.[47] Much work on perfusion fluids was done until the intracellular type of fluid devised by Collins et al. in 1969 allowed a simple flush and chill to suffice for prolonged storage.[48] Although the hopes for typing were not fully realized, such schemes had other benefits in obtaining kidneys when urgently required for patients with rarer blood groups, for children, or for highly sensitized patients. Such patients had been recognized by the new lymphocytotoxicity testing using a crossmatch between donor cells and recipient serum. First noted by Terasaki and associates[49] and described in more detail by Kissmeyer-Nielsen and colleagues[50] in 1966 and Williams and colleagues,[51] such pretransplant testing explained cases of sudden failure and led to a marked diminution in hyperacute rejection.

The 1970s Plateau

The 1970s was a period of consolidation, of improvements in data collection such as the valuable European Dialysis and Transplant Association surveys, and increased sophistication in HLA typing methods and organ-sharing schemes. Cadaver organ procurement generally increased as a result of wider involvement of the public and medical profession, although the number of patients waiting for transplantation persistently exceeded the organs available, and donation declined transiently during times of public concern over transplantation issues. Governments took initiatives to increase donations; in the United Kingdom, the Kidney Donor Card was introduced in 1971, becoming a multidonor card 10 years later. In hospital practice, methods of resuscitation and intensive care improved, and the concept of brain death was established to prevent prolonged,

pointless ventilation, although its immediate application to transplantation provoked controversy. Despite many new claims for successful methods of immunosuppression, such as trials of splenectomy, thymectomy, thoracic duct drainage, and a new look at cyclophosphamide, no agent except antithymocyte globulin became established in routine use.

Although patient survival after kidney transplantation continued to increase, the 1970s did not show the expected increase in cadaver graft survival. Some groups reported decreased survival figures; this paradox was solved partly by the demonstration that blood transfusion during regular dialysis, which had been discouraged because of the risk of sensitization, was beneficial to the outcome of kidney transplantation,[52] an observation made some years earlier by Morris and coworkers.[53]

The 1970s ended with two innovations that revived hopes of reaching the goal of routine, safe, and successful kidney transplantation. Ting and Morris[54] reported the successful clinical application of HLA-DR matching, and Calne and associates[55] revived memories of the excitement of the early days of the use of azathioprine by introducing into clinical practice the first serious rival to it in 20 years, cyclosporine, which had been discovered to be a powerful immunosuppressive agent by Borel.[56] Cyclosporine replaced the earlier drug regimens and was the dominant agent in use until the 1990s. Transplantation had grown to a sufficiently large clinical service that it was worth the attention of the pharmaceutical companies, and in the 1990s steady production of new agents occurred—tacrolimus, mycophenolate mofetil, rapamycin, FTY720, brequinar, and others. Any drug with promise was marketed aggressively, and sponsored trials became a routine part of clinical life.

The improved results of transplantation meant that the shortage and procurement of organs became a more dominant issue. Living donors were encouraged, to which were added occasional altruistic donations, with use later of "kidney chains" to pass on locally incompatible organs. There was a return to using possibly damaged "marginal" kidneys and organs removed rapidly after cardiac death (DCD). Comparisons of transplantation practice throughout the world showed remarkable differences in attitudes to use of live related donors and cadaver organs, depending on religion and cultural traditions. Kidney transplantation had started as a difficult surgical and scientific challenge confined to a few academic centers in the developed world, but its success had led to the technique becoming a routine service in all parts of the world.[57] In some nations not sharing Western attitudes, the donor shortage meant the appearance of undesirable commercial developments in renal transplantation, such as the purchase of kidneys from living unrelated donors (discussed in more detail in Chapter 41).[58]

Waiting for Xenografts

As the demand for kidney transplants continued to exceed supply, other initiatives appeared and included study of nations and areas with high donation rates (e.g., Spain). As all attempts to increase donor supply fell short of the ever-rising target, the radical alternative of the use of animal organs was examined afresh. Profound immunosuppression alone was ineffective and, at first, methods of removing natural antibody from recipient plasma were tried to deal with the hyperacute phase of xenograft organ rejection. Although the traditional hopes for xenografting of human patients had assumed that "concordant" species such as the monkey would be used, a new strategy using genetic engineering methods first used a line of transgenic pigs, a distant species discordant with humans, with a modified endothelium that reduced the complement-mediated immediate reaction.[59] Hopes continue that these early developments will evolve into a sophisticated successful routine.[60] Meanwhile, the kidney transplanters can only watch, with detached interest, the emergence of stem cell use in cellular transplantation.

These new hopes for xenografts raised old fears among the public and legislators, notably regarding disease transmission. Although this had been a familiar problem in human-to-human transplantation and had been met regularly and dealt with, governments required reassurances about xenotransplantation with the added threat of retrovirus transmission.

Conclusion

Kidney transplantation was the first of the organ transplant procedures to develop because cadaveric donor kidneys revived with time, the availability of live donors increased, and the crucial backup of dialysis was implemented. When radical new ideas are to be tested, pioneers still turn to kidney transplantation. Kidney transplantation is where it all started, with good reason, and it will always be a test bed for major innovation, including laparoscopic and robotic surgery.

Nowhere is the excitement of the early days reflected better than in the recollections of 35 of the pioneers of transplantation gathered together by Terasaki.[61]

References

1. Hamilton D. Organ transplantation: a history. Pittsburgh: Pittsburgh University Press; 2012.
2. Ullmann E. Experimentelle nierentransplantation. Wien Klin Wochenschr 1902;15:281. [For a biography of Ullmann, see Lesky E. Die erste Nierentransplantation: Emerich Ullmann (1861–1937). Munch Med Wochenschr 1974;116:1081.]
3. von Decastello A. Experimentelle nierentransplantation. Wien Klin Wochenschr 1902;15:317.
4. Carrel A. La technique operatoire des anastomoses vasculaires et la transplantation des viscères. Lyon Med 1902;98:859.
5. Hamilton D. The first transplant surgeon: the flawed genius of nobel prize winner, Alexis Carrel. Singapore: World Scientific; 2017.
6. Jaboulay M. Greffe de reins au pli du coude par soudure arte. Bull Lyon Med 1906;107:575. [For a biography of Jaboulay, see Biogr Med Paris 1936;10:257.]
7. Ullmann E. Tissue and organ transplantation. Ann Surg 1914;60:195.
8. Unger E. Nierentransplantation. Berl Klin Wochenschr 1909;1:1057.
9. Winkler FA. Ernst Unger: a pioneer in modern surgery. J Hist Med Allied Sci 1982;37:269.
10. Carrel A. The transplantation of organs. New York Times 1914.
11. Voronoy YY. Sobre el bloqueo del aparato reticulo-endothelial. Siglo Med 1936;97:296.
12. Hamilton D, Reid WA. Yu Yu Voronoy and the first human kidney allograft. Surg Gynecol Obstet 1984;159:289.
13. Matevossian E, Kern H, Hüser N, et al. Surgeon Yurii Voronoy (1895–1961) — a pioneer in the history of clinical transplantation. Transpl Int 2009;22:1132.
14. Moore FD. Give and take: the development of tissue transplantation. Philadelphia: WB Saunders; 1964.

15. Simonsen M. Biological incompatibility in kidney transplantation in dogs: serological investigations. Acta Pathol Microbiol Scand 1953;32:1.
16. Dempster WJ. The homotransplantation of kidneys in dogs. Br J Surg 1953;40:447.
17. Küss R, Teinturier J, Milliez P. Quelques essais de greffe du rein chez l'homme. Mem Acad Chir 1951;77:755.
18. Servelle M, Soulié P, Rougeulle J, et al. Greffe d'une reine de supplicie à une malade avec rein unique congénital, atteinte de nephrite chronique hypertensive azotémique. Bull Soc Med Hop Paris 1951;67:99.
19. Dubost C, Oeconomos N, Vaysse J, et al. Resultats d'une tentative de greffe rénale. Bull Soc Med Hop Paris 1951;67:1372.
20. Michon L, Hamburger J, Oeconomos N, et al. Une tentative de transplantation rénale chez l'homme. Presse Med 1953;61:1419.
21. Hume DM, Merrill JP, Miller BF, et al. Experiences with renal homotransplantation in the human: report of nine cases. J Clin Invest 1955;34:327.
22. Murray JE, Merrill JP, Harrison JH. Kidney transplantation between seven pairs of identical twins. Ann Surg 1958;148:343.
23. Murray JE, Tilney NL, Wilson RE. Renal transplantation: a twenty-five year experience. Ann Surg 1976;184:565.
24. Murray JE, Merrill JP, Dammin GJ, et al. Study of transplantation immunity after total body irradiation: clinical and experimental investigation. Surgery 1960;48:272.
25. Hamburger J, Vaysse J, Crosnier J, et al. Transplantation of a kidney between non-monozygotic twins after irradiation of the receiver: good function at the fourth month. Presse Med 1959;67:1771.
26. Küss R, Legraine M, Mathe G, et al. Prémices d'une homotransplantation rénale de soeur à frère non jumeaux. Presse Med 1960;68:755.
27. Schwartz R, Dameshek W. Drug-induced immunological tolerance. Nature 1959;183:1682.
28. Schwartz RS. Perspectives on immunosuppression. In: Hitchings GH, editor. Design and achievements in chemotherapy. Durham, NC: Burroughs Wellcome; 1976. p. 39–41.
29. Calne RY. The rejection of renal homografts: inhibition in dogs by 6-mercaptopurine. Lancet 1960;1:417.
30. Zukoski CF, Lee HM, Hume DM. The effect of 6-mercaptopurine on renal homograft survival in the dog. Surg Forum 1960;11:47.
31. Calne RY. The development of immunosuppressive therapy. Transplant Proc 1981;13:44.
32. Calne RY, Alexandre GPJ, Murray JE. The development of immunosuppressive therapy. Ann NY Acad Sci 1962;99:743.
33. Hopewell J, Calne RY, Beswick I. Three clinical cases of renal transplantation. BMJ 1964;1:411.
34. Küss R, Legraine M, Mathe G, et al. Homologous human kidney transplantation. Postgrad Med J 1962;38:528.
35. Murray JE, Merrill JP, Harrison JH, et al. Prolonged survival of human kidney homografts by immunosuppressive drug therapy. N Engl J Med 1963;268:1315.
36. Starzl TE, Marchioro TL, Waddell WR. The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet 1963;117:385.
37. Goodwin WE, Mims MM, Kaufman JJ. Human renal transplant, III: technical problems encountered in six cases of kidney homotransplantation. Trans Am Assoc Genitourin Surg 1962;54:116.
38. Hume DM, Magee JH, Kauffman HM, et al. Renal homotransplantation in man in modified recipients. Ann Surg 1963;158:608.
39. Starzl TE. Personal reflections in transplantation. Surg Clin North Am 1978;58:879.
40. Reemtsma K, McCracken BH, Schlegel JU, et al. Renal heterotransplantation in man. Ann Surg 1964;160:384.
41. Hume DM. Discussion. Ann Surg 1964;160:409.
42. Wolstenholme GEW, O'Connor M, editors. Antilymphocytic serum. London: J&A Churchill; 1967.
43. French ME, Batchelor JR. Immunological enhancement of rat kidney grafts. Lancet 1969;2:1103.
44. Dausset J. The challenge of the early days of human histocompatibility. Immunogenetics 1980;10:1.
45. Hamburger J, Vaysse J, Crosnier J, et al. Renal homotransplantation in man after radiation of the recipient. Am J Med 1962;32:854.
46. van Rood JJ. Histocompatibility testing. Copenhagen: Munkgaard; 1967.
47. Belzer FO, Ashby BS, Dunphy JS. 24-Hour and 72-hour preservation of canine kidneys. Lancet 1967;2:536.
48. Collins GM, Bravo-Shugarman M, Terasaki PI. Kidney preservation for transportation: initial perfusion and 30 hours' ice storage. Lancet 1969;2:1219.
49. Terasaki PI, Marchioro TL, Starzl TE. In: Amos DB, van Rood JJ, editors. Histocompatibility testing. Washington, DC: National Academy of Sciences; 1965:83.
50. Kissmeyer-Nielsen F, Olsen S, Peterson VP, et al. Hyperacute rejection of kidney allografts. Lancet 1966;2:662.
51. Williams GM, Hume DM, Hudson RP, et al. Hyperacute renal-homograft rejection in man. N Engl J Med 1968;279:611.
52. Opelz G, Sengar DPS, Mickey MR, et al. Effect of blood transfusions on subsequent kidney transplants. Transplant Proc 1973;5:253.
53. Morris PJ, Ting A, Stocker J. Leucocyte antigens in renal transplantation, I: the paradox of blood transfusions in renal transplantation. Med J Aust 1968;2:1088.
54. Ting A, Morris PJ. Matching for B-cell antigens of the HLA-DR (D-related) series in cadaver renal transplantation. Lancet 1978;1:575.
55. Calne RY, White DJG, Thiru S, et al. Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet 1978;2:1323.
56. Borel JF. Comparative study of in vitro and in vivo drug effects on cell mediated cytotoxicity. Immunology 1976;31:631.
57. Burdick JF, DeMeester J, Koyama I. Understanding organ procurement and the transplant bureaucracy. In: Ginns LC, Cosimi AB, Morris PJ, editors. Transplantation. Boston: Blackwell; 1999. p. 875–94.
58. Morris PJ. Problems facing the society today. Transplant Proc 1987;19:16.
59. van den Bogaerde J, White DJG. Xenogeneic transplantation. Br Med Bull 1997;53:904.
60. D'Apice A, Cowan PJ. Gene-modified pigs. Xenotransplantation 2008;15:87.
61. Terasaki PI. History of transplantation: thirty-five recollections. Los Angeles: UCLA Tissue Typing Laboratory; 1991.
62. Dausset J. Iso-leuco-anticorps. Acta Haematol (Basel) 1958;20:156.
63. Oriol R, Ye Y, Koren E, Cooper DK. Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation. Transplantation 1993;56:1433–42.
64. Rosales A, Salvador JT, Urdaneta G, Patiño D, Montlleó M, Esquena S, et al. Laparoscopic kidney transplantation. Eur Urol 2010;57:164–7.

2 The Immunology of Transplantation

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CHAPTER OUTLINE

Introduction

Solid organ transplant requires the removal of an organ from one individual, the donor, and its placement in the recipient. Whether the donor is living or deceased, this process inevitably requires a temporary cessation of circulation and hence oxygenation, with attendant cellular dysfunction and damage. Thus when the blood supply is restored to the allograft in the recipient, and the recipient's immune system can access the transplant, there are broadly two main stimuli that may be recognized: damage-associated signals that activate the innate immune system, and differences in cell surface molecules (such as human leukocyte antigens [HLA] or blood group antigens) between donor and recipient that can activate the adaptive immune system. In the past 50 years, the increased understanding of cellular adaptive immunity has transformed our ability to suppress this arm of the immune system, such that T cell-mediated rejection (TCMR) is now uncommon, occurring in less than 20% of kidney transplant recipients, for example. However, the control of innate and humoral adaptive immunity remains challenging, and efforts to achieve this will need to be underpinned by a greater understanding of the basic biology of these important systems. Of note, attempts to understand the immune response to an allograft have historically relied on rodent and nonhuman primate models. Although useful, such studies do not always accurately reflect the alloimmune response in humans, and there is an increasing emphasis on the need for experimental medicine studies in transplantation to enable advances in genomic, transcriptomic, and proteomic technologies to be harnessed toward this goal.[1] In this chapter, we will provide a description of the various arms of the immune system and consider how they contribute to the immune response to transplanted organs (Fig. 2.1).

Activation of the Immune System Peritransplant

During the process of organ retrieval and reimplantation, there is an inevitable period of ischemia. The cessation of oxygen supply renders the cells unable to generate sufficient energy to continue homeostatic processes that maintain cellular

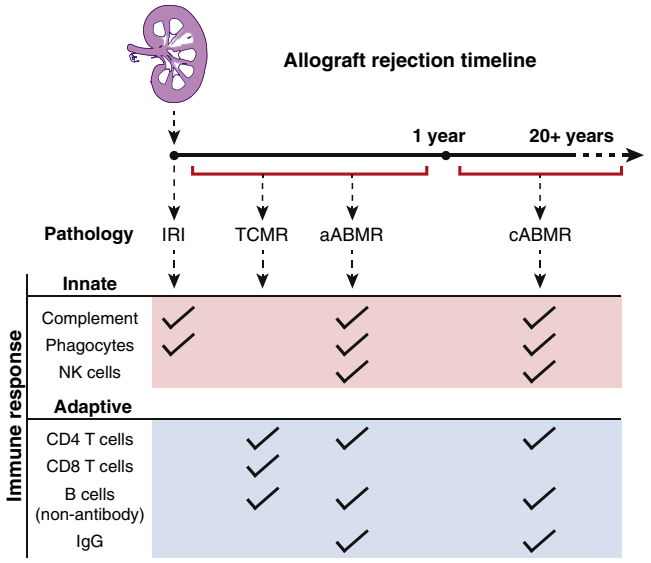

Fig. 2.1 Timeline of allograft rejection. During transplantation and beyond, allografts are subjected to a variety of stresses that cause graft damage and destruction. Indicated in the schematic shown here is a timeline of the pathologic insults potentially experienced by a transplanted organ and the types of immune cells that contribute to each pathology, stratified based on innate and adaptive immunity. *aABMR*, Acute antibody-mediated rejection; *cABMR*, chronic antibody-mediated rejection; *IgG*, immunoglobulin G; *IRI*, ischemia reperfusion injury; *NK*, natural killer; *TCMR*, T cell-mediated rejection.

integrity, leading to damage or even death of some cells. This cellular damage or death is associated with the release of molecules that can be detected by both the innate and adaptive immune system. During organ reperfusion, it is the innate immune system that is principally activated. This ancient system includes a soluble arm—the complement system and a variety of opsonins that have evolved to facilitate pathogen recognition, for example, C-reactive protein (CRP), complement activation products (C3b), natural immunoglobulin (Ig) M antibody, and a cellular arm, composed of phagocytes and innate lymphoid cells, including natural killer (NK) cells.

DAMAGE SIGNALS AND THEIR RECEPTORS

The innate immune system has evolved to recognize molecules expressed by pathogens, known as pathogen-associated molecular patterns (PAMPs), including specific carbohydrates, lipopolysaccharide (LPS), flagellin, lipoteichoic acid, and double-stranded ribonucleic acid (RNA). This is achieved by an array of receptors, so-called pattern recognition receptors (PRRs), some of which are surface bound and survey the extracellular environment, and some of which are located within the cell, in the cytoplasm or endosomal compartments (Fig. 2.2). PRRs include cell-associated receptors, such as toll-like receptors (TLRs),[2] retinoic acid inducible gene-1–like receptors,[3] and nucleotide-binding oligomerization domain (NOD)-like receptors,[4] and soluble molecules, including CRP, ficolins, and mannan-binding

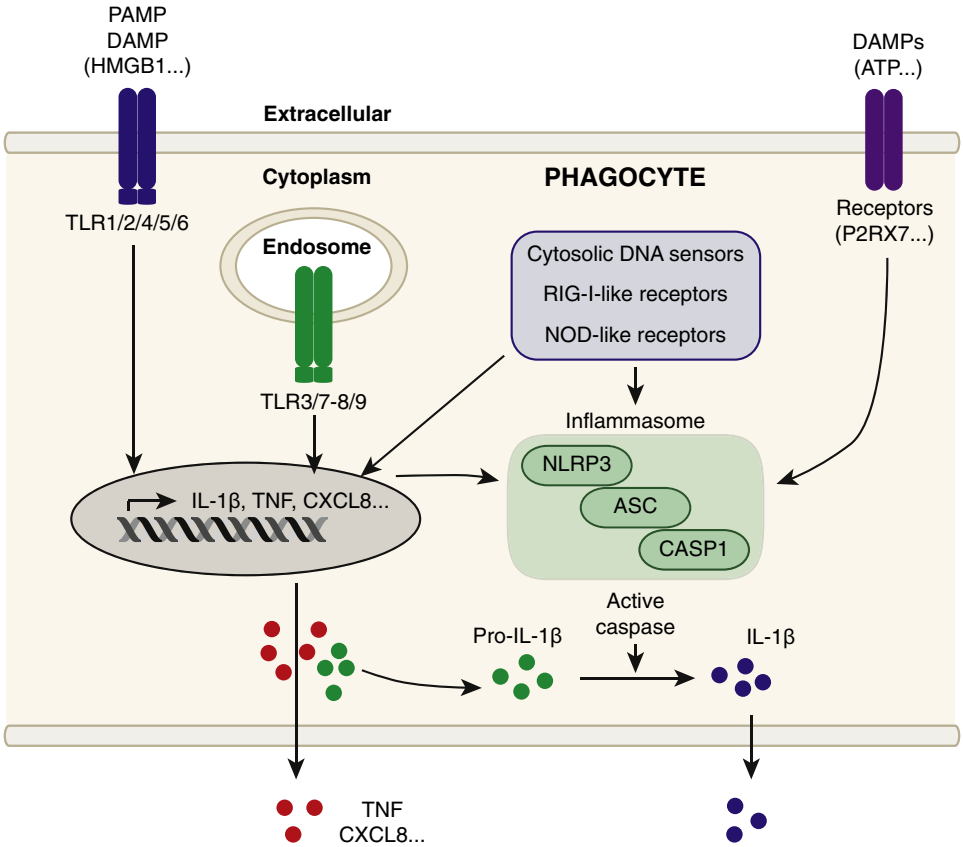

Fig. 2.2 Mechanisms of immune sensing and inflammatory cytokine production during sterile inflammation. Schematic of the different classes of pattern recognition receptors and their potential to response to danger-associated molecular patterns (DAMPs) released during sterile inflammation and ongoing allograft destruction. For example, high mobility group box 1 *(HMGB1)* binds to toll-like receptors (TLRs) to induce expression of inflammatory mediators, while extracellular adenosine triphosphate *(ATP)* can engage cell surface receptors, such as P2RX7, leading to inflammasome assembly (e.g., the nucleotide-binding domain leucine-rich repeat containing protein 3 *(NLRP3)* inflammasome), caspase activation, and mature interleukin (IL)-1β and IL-18 production. These mechanisms are particularly important in innate immune cells, such as macrophages and neutrophils.

lectin (MBL).[5] Matzinger first proposed that the immune system may have the capacity to respond to damage signals, even in the absence of microbes—the danger hypothesis—and may have even evolved in response to these stimuli.[6] It is now clear that many cell-damage or death-associated signals (termed *danger-associated molecular patterns* [DAMPs]) are recognized by the same PRRs that mediate responses to PAMPs.[7] These DAMPs include extracellular adenosine triphosphate (ATP),[8] hyaluronan,[9] uric acid, heat-shock proteins (HSPs), and high-mobility group box 1 (HMGB1).[10] These molecules are normally hidden from the immune system or are derived from degradation products of extracellular matrix components generated during ischemia reperfusion injury (IRI) and inflammation.[11] Similarly, falling intracellular potassium and oxidative stress can act as intracellular danger signals.[12–14]

SOLUBLE INNATE IMMUNITY—COMPLEMENT

The complement system is a series of protein kinases that are sequentially activated and culminate in the formation of the membrane attack complex (MAC).[15,16] The MAC comprises complement components C5 to C9, which are inserted into the cell membrane (pathogen or host), disrupting integrity and causing cell lysis (Fig. 2.3). In addition, many proximal complement components may augment the immune response to the allograft.

The complement system may be activated by three pathways: the classical pathway, the alternative pathway, and the MBL pathway. IgM or IgG immune complexes activate the classical pathway, and hence this pathway may become activated during antibody-mediated rejection (see section on B Cell Activation). The alternative pathway is constitutively active and must be controlled by a series of regulatory proteins. The mannose-binding pathway is activated by carbohydrates present on pathogens or by damaged endothelium. The net result of activating any of the three pathways is the formation of a C3 convertase (either C4bC2a or C3bBb), which cleaves C3. The resulting C3b cleaves C5 and activates a final common pathway resulting in MAC formation. Complement activation also leads to the formation of anaphylatoxins (C3a and C5a), which activate neutrophils and mast cells, promoting inflammation. In addition, C3b can opsonize pathogens for uptake by complement receptors CR1 and CR3 on phagocytes and can activate B cells; the latter may promote B cell activation in transplantation.

Because the alternative pathway is continuously activated, effective regulation is critical to prevent inappropriate activation. Regulatory proteins may be circulating or membrane-bound. Circulating inhibitors include C1 esterase inhibitor and factors H and I. Membrane-bound regulatory proteins include membrane cofactor protein (MCP), CD55 (decay accelerating factor [DAF]), and CD59 (protectin). Defects or mutations in complement regulatory proteins can result in severe renal pathology, for example, atypical hemolytic uremic syndrome (HUS), which can recur in the transplanted allograft.[17,18] The C3 glomerulopathies, including dense deposit disease and type I and III mesangiocapillary glomerulonephritides, are also underpinned by complement mutations.[19,20] Small case series suggest a recurrence rate of around 60% in the transplanted organ.[21] The C5 inhibitor eculizumab may well have efficacy in both primary and recurrent forms of these diseases.[22]

The endothelial cell damage associated with ischemia-reperfusion injury during transplantation leads to MBL and alternative complement pathway activation.[23] Histologic evidence of complement activation (C3d deposition) is present in animal models and in human kidneys with

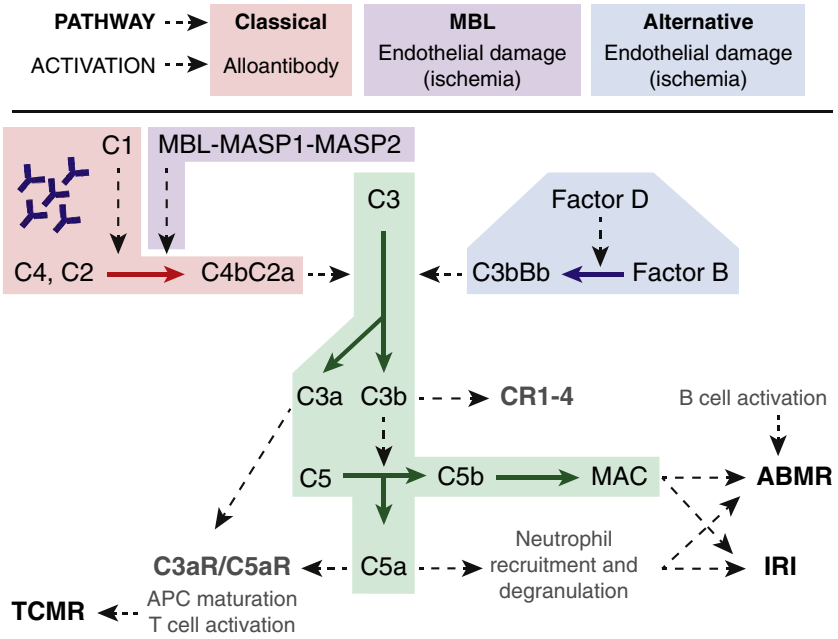

Fig. 2.3 The complement cascade. Activation of the complement cascade can contribute to tissue destruction directly via formation of the membrane attack complex (MAC) and through complement receptor engagement and subsequent immune cell activation. This includes the maturation of antigen-presenting cells and neutrophil activation. Three pathways are possible for complement activation and each may contribute to tissue destruction in rejection. The classical pathway is mediated by antibodies and may be driven by donor-specific IgG and IgM immune complexes during ABMR, whereas activation of the alternative and MBL pathways results from damage to allograft endothelial cells as a result of IRI. All pathways converge on the cleavage of C3 and the generation of the C5-cleaving fragment C3b and the anaphylatoxin C3a. Subsequently, C5a and C5b act as an anaphylatoxin that binds to C5aR and a component of the MAC, respectively, leading to cell death.

acute tubular necrosis (ATN).[24] Factor B-deficiency and a factor B-blocking antibody are protective in a murine model of IRI,[24,25] suggesting alternative pathway involvement. Biopsies in murine and human kidneys with ATN also demonstrate MBL deposition,[26] likely triggered by endogenous ligands expressed by dying cells, and MBL-deficient mice are protected from IRI.[27] Transplantation of a kidney from a C3-deficient mouse into a C3-sufficient recipient results in significant attenuation of IRI, in contrast to the reciprocal transplant, suggesting that local C3 production in the kidney rather than circulating C3 is the major player in IRI.[28] In human kidneys, cold ischemia may alter the methylation state of the C3 promoter, resulting in increased local expression of C3 after reperfusion,[29] which is associated with a diminished graft survival.[30] Silencing of the gene encoding C3 using small interfering RNA (siRNA) has been shown to reduce C3 expression, histologic and biochemical parameters of kidney injury, and mortality in an animal model of IRI.[31] The terminal pathway products C5a and C5b–C9 appear to be critical in mediating cellular injury.[32–34] A C5-blocking antibody and C5a receptor antagonist have both been shown to abrogate IRI[35] and gene silencing of the C5a receptor also protects mice from IRI.[36] Gene silencing may provide a promising tool in renal transplantation, because siRNA-to-complement components might be applied to the allograft during cold storage, before implantation.[37] Similarly, other strategies to inhibit local complement activation may have utility in limiting allograft IRI.[38,39] Ongoing clinical trial in renal transplantation to prevent IRI and delayed graft function include the use of C1 esterase inhibitors (https://clinicaltrials.gov/ct2/show/NCT02134314) and the C5 inhibitor eculizumab (https://clinicaltrials.gov/ct2/show/NCT02145182). However, although there was a reduced rate of delayed graft function in a small pediatric trial ($n = 57$) using eculizumab in kidney transplantation, there was an unexpectedly high rate of graft loss because of thrombosis in eculizumab-treated subjects,[40] necessitating caution in its future use in this context.

INNATE IMMUNITY—CELLULAR COMPONENTS

Cellular innate immunity comprises a variety of hematopoietic myeloid and lymphoid cells, often poised within tissues for the rapid nonspecific detection of invading microorganisms and transformed cells. However, innate immunity also encompasses various nonhematopoietic cells, such as the gastrointestinal, respiratory, and urogenital epithelium, which, in addition to forming a physical barrier, also express PRRs and orchestrate local immunity (Fig. 2.4).

Neutrophils

Although often viewed as nonspecific effector cells, granulocytes, such as neutrophils and eosinophils, are likely to play a significant role in transplant pathology through their potent effector functions and rapid recruitment to sites of inflammation during IRI and rejection. It is also increasingly appreciated that there may be tissue-resident populations within a variety of organs.

Neutrophils are the dominant circulating phagocyte in humans, and their recruitment into the graft involves a complex multistep process requiring a series of interactions between the surface of the leukocyte and the endothelial cell or its extracellular matrix.[41,42] The proteins involved fall into three groups: the selectins, and members of the integrin and Ig superfamilies. Initial interaction and rolling of neutrophils along the endothelium allow the leukocyte to sample the endothelial environment, while maintaining its ability to detach and travel elsewhere. This step is largely controlled by the selectins, although α_4 integrins may also play a role. Endothelial cells express interleukin (IL)-8 and platelet-activating factor, which induces strong neutrophil adhesion. This interaction leads to signaling to the neutrophil, slowing and arresting the rolling process. Shedding of L-selectin by leukocytes allows their detachment and extravasation.[43] The latter stages of leukocyte transmigration are regulated mainly by the β_2 integrins and adhesion proteins of the immunoglobulin superfamily.

The expression of adhesion proteins involved in these interactions is upregulated by proinflammatory cytokines. Ischemic damage alone results in increased expression of several cytokines that upregulate the expression of selectins.[44,45] Other adhesion proteins, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 of the immunoglobulin superfamily and E-selectin (endothelial-specific selectin), are upregulated by cytokines induced by donor brain death[46] and implantation.

After exit from the vasculature, neutrophil PRR engagement by DAMPs can induce the production of reactive oxygen species, hydrolytic enzymes, and cytokines,[47,48] with graft neutrophilia linked to alloreactive T cell responses and disease activity in mouse models.[49–51] Neutrophils can also undergo a form of programmed cell death known as NETosis, whereby activated neutrophils form so-called extracellular traps (NETs).[52] These have been observed in human lung transplant recipients and in mouse models of allograft IRI, although how they contribute to inflammation remains controversial.[53] Perhaps less appreciated is the potential role of neutrophils in the resolution of inflammation in alloimmunity: efferocytosis of apoptotic neutrophils leads to the production of antiinflammatory mediators, such as IL-10, while proresolving factors, including lipoxins and resolvins, are important in wound healing and may suppress ongoing rejection.[54]

Macrophages

Tissue-resident macrophages represent the major innate leukocyte population in most tissues.[55] Through their widespread expression of PRRs, these sentinel cells are specialized in antigen phagocytosis and cytokine production, being key drivers of inflammation in numerous settings. During inflammatory conditions, the macrophage pool is further reinforced by recruited monocytes from the bloodstream, with several macrophage- and monocyte-derived cytokines capable of contributing to tissue damage.[56] For example, tumor necrosis factor (TNF)α can drive cellular necroptosis and the concomitant release of intracellular contents and DAMPs,[57,58] in addition to augmenting angiogenesis, matrix metalloprotease (MMP) production, immune cell activation, and germinal center formation, all with particular importance in the context of transplantation.[59] Furthermore, via production of IL-1β

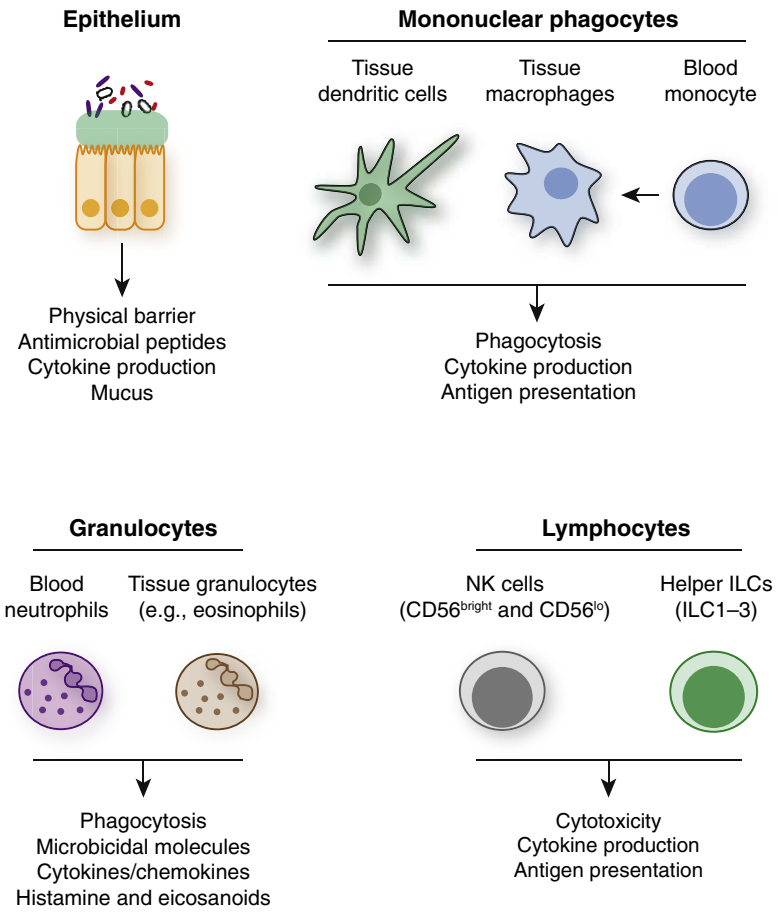

Fig. 2.4 The innate immune system. A schematic of the major components of the innate immune system and a brief summary of their respective functions in inflammation and homeostasis. Epithelial cells form the physical barriers of mucosal gastrointestinal, respiratory, and urogenital tracts, and play a critical role in host-microbe interactions at these environmental interfaces. Mononuclear phagocytes represent tissue-resident dendritic cells and macrophages in addition to those recruited to tissues during inflammation. In most circumstances, these cells play essential roles in tissue homeostasis, but can also prime alloreactive adaptive immune responses, are major sources of inflammatory mediators, and participate directly in tissue destruction. Granulocytes include neutrophils, normally circulating in the blood but rapidly recruited to sites of inflammation, and tissue-resident cells, such as eosinophils. The workhorses of innate immunity, they are increasingly appreciated for their roles in influencing adaptive immunity within tissues and lymphoid organs. Innate lymphocytes encompass the well-characterized NK cells, which participate in receptor-mediated cell lysis and IFNγ production, and the recently discovered class of helper innate lymphoid cells. Helper ILCs are enriched at mucosal surfaces and are potent sources of T cell-associated cytokines, and also serve as APCs through expression of MHC class II molecules.

and IL-8, macrophages play a strategic role in the recruitment of neutrophils to inflamed sites through the induction of adhesion molecules on endothelial cells and direct chemotactic activity, respectively.[60]

Endogenous ligands with the capacity to engage macrophage PRRs are generated during transplantation, either through IRI or as a consequence of ongoing rejection.[61] Detection of DAMPs leads to the association of nucleotide-binding domain leucine-rich repeat containing protein (NLRP)3 with apoptosis-associated speck-like protein (ASC), and recruitment of procaspase-1, forming a complex known as the NLRP3 inflammasome[48,62,63] (Fig. 2.3). Inflammasome activation results in the cleavage of procaspase-1 to caspase-1, which subsequently cleaves IL-1β and IL-18 from their precursors. Of note, in vitro data suggests that in macrophages, inflammasome activation is a two-step process. First, macrophages must be "primed" by TLR stimuli, resulting in NFγB-dependent pro-IL-1β production and upregulation of NLRP3 expression. A number of DAMPs are thought to signal via TLRs; for example, HMGB1 activates TLR4.[64] The second signal is provided by DAMP receptors, for example the ATP receptor, P2X7R.

IL-1 plays a pivotal role in initiating and amplifying sterile inflammation, as evidenced by experiments showing that mice deficient in the IL-1 receptor (IL-1R) or in the adaptor protein MyD88 (which is required for IL-1R signaling), demonstrate minimal neutrophilic inflammation after challenge with necrotic cells.[62] IL-1β has multiple actions, including stimulation of nonhematopoietic cells to produce the neutrophil chemoattractants chemokine (C-X-C motif) ligand (CXCL)2 (also known as macrophage inflammatory protein [MIP]-2) and CXCL1 (also known as keratinocyte chemoattractant [KC]).[65] DAMPs may also act directly as chemotactic agents for neutrophils.[47,66] IL-1β also increases the expression of cell adhesion molecules (e.g., ICAM-1 [CD54]) on endothelial cells.[67] ICAM-1 interacts with integrins (CD11 and CD18) on neutrophils and monocytes to promote endothelial adherence and subsequent entry into tissues.

There is a significant body of evidence that suggests that sterile inflammation contributes to the severity of IRI;

neutralization of the DAMP HMGB1 with a monoclonal antibody attenuates renal injury after IRI, whereas recombinant HMGB1 exacerbates it.[68] Some DAMPs stimulate TLRs, and mice deficient in TLR-2 and TLR-4 are protected from IRI with a reduction in neutrophil and macrophage infiltration.[69,70] Furthermore, NLRP3, ASC, and caspase-1 deficient mice are protected from renal ischemic injury.[71–73] Pharmacologic inhibition of caspase-1 has been shown to reduce renal IRI[74] and may therefore be a viable therapeutic strategy in transplantation. In rodent models, treatment with monoclonal antibodies directed against ICAM-1, CD11a, or CD11b also protect against IRI.[75–77] In a human phase I trial, ICAM blockade using a murine antibody BIRR1 was associated with a reduction in delayed graft function in renal transplant recipients.[78] However, a randomized controlled trial of anti-ICAM-1 antibody in renal transplantation failed to demonstrate any significant improvement in delayed graft function.[79] Blockade of another adhesion molecule, P-selectin, also attenuates leukocyte recruitment and IRI in rodent models[80,81] and in humans.[82]

Innate cells may also drive an adaptive alloimmune response. In TCMR, macrophages may act as antigen-presenting cells (APCs), and the IRI-associated inflammation may induce upregulation of major histocompatibility complex (MHC) class II (MHC-II) on resident cells, augmenting their antigen-presenting functions. In antibody-mediated rejection (ABMR), IgG and complement are deposited in peritubular capillaries, facilitating monocyte, macrophage, and neutrophil activation via their Fcγ receptors (FcγR) and complement receptors. Indeed, the presence of neutrophils within peritubular capillaries is one of the diagnostic features of ABMR,[83] and increased numbers of intraglomerular monocytes and macrophages have been observed in C4d+ ABMR.[84] Macrophages may also contribute to chronic ABMR; early macrophage infiltration is predictive of chronic allograft nephropathy and long-term graft survival.[85]

NK Cells and Innate Lymphoid Cells

In the context of organ transplantation, it is increasingly clear that NK cells play a significant role.[86–88] NK cells are a distinct class of cytotoxic lymphocyte characterized by the production of perforin, granzymes, and IFNγ that play a role as effector cells, lysing sensitive targets according to the presence or absence of specific target antigens. Two subsets of NK cells exist in humans, $CD56^{bright}$ cells and $CD56^{dim}$ cells, with $CD56^{dim}$ NK cells comprising approximately 90% of blood and spleen NK cells. This subset expresses FcγRIIIA (CD16) and undergoes antibody-dependent cell-mediated cytotoxicity (ADCC), the targeted release of cytotoxic molecules in response to FcγR ligation by IgG-opsonized cells. The relevance of ADCC to organ rejection will be discussed in more detail when discussing mechanisms of ABMR. NK cells also express an array of other activating and inhibitory cell surface receptors that dictate cellular activation depending on the microenvironment encountered by the cell. Although the importance of NK cells in bone marrow transplantation has been long established,[89,90] their role in solid organ transplantation has taken longer to be recognized. Several laboratories using different experimental models found that grafts survive indefinitely in the presence of demonstrable NK effector activity,[91,92] although more recently CD28-independent rejection in mouse models of transplantation has been shown to be NK dependent and sensitive to blockade of NKG2D.[93] The activating receptor NKG2D is engaged by MHC class I polypeptide-related sequence (MIC) A (MICA) and MICB, that are induced in allografts during acute and chronic rejection.[94] The binding of these ligands to NKG2D activates NK cells to enhance effector functions, whereas the engagement of killer immunoglobulin-like receptors (KIRs) by KIR ligands such as HLA-C (KIR2DL1 and KIR2DL2) and HLA Bw4 (KIR3DL1) generally inhibit function. Genetic studies of donor and recipient HLA-C type (grouped as C1 and C2 depending on polymorphisms at position 77 and 80 and which seem to exhibit differential NK cell inhibition) suggest that long-term outcomes may be influenced by donor or recipient interaction with KIRs.[86,87] This has also been observed when KIR HLA mismatches are analyzed in HLA-compatible transplantation.[95]

Inhibitory receptor function underlies the phenomenon of responses to "missing self,"[96,97] which contributes to tumor immunity,[98] the killing of stem cells,[99] and hybrid resistance in experimental models of transplantation.[88]

Beyond NK cells, another class of innate lymphocyte are the recently described "helper" innate lymphoid cells (ILCs). The subject of intense research in the last decade, ILCs are characterized by their similarity to helper T (Th) cell subsets, with the notable absence of somatically recombined antigen-specific receptors or classical lineage markers.[100] ILCs can subdivided into ILC1s, ILC2s, and ILC3s, which mirror Th1, Th2, and Th17 subsets in terms of transcription factor dependency and effector cytokine profile. The phenotype and dynamics of donor and recipient helper ILCs after transplantation remains poorly understood. However, it is likely that the nature of the transplanted organ dictates the relative contribution of ILCs to transplant phenomena: ILCs may be expected to have significant influence on transplanted mucosal tissues, because they are particularly enriched at these sites. For example, the production of homeostatic IL-22 and amphiregulin by ILC3s and ILC2s, respectively, may limit detrimental tissue destruction and reinforce antimicrobial defense at the mucosal epithelium in the gut and lung.[101–103] Indeed, ILC3-derived IL-22 production has been implicated in reduced disease progression and intestinal tissue damage in murine models of graft-versus-host disease.[104,105] Conversely, the transition to ILC1-like phenotypes is associated with increased inflammation and may promote early graft dysfunction.[106] Curiously, the absence of ILC reconstitution in severe combined immunodeficiency (SCID) patients after hematopoietic stem cell (HSC) transplantation, including NK cells, was not associated with any overt susceptibility to disease.[107] Therefore more investigation into the precise nature of ILCs within allografts is needed.

CELLS AT THE INTERFACE BETWEEN INNATE AND ADAPTIVE IMMUNITY

Although sufficient for initial protection against most microorganisms and sterile insults, innate immunity plays a crucial role in shaping adaptive immune responses according to the context in which antigen is encountered. Indeed,

complex mechanisms have evolved to ensure optimal targeting of different effector mechanisms against viruses, bacteria, fungi, protozoa, and multicellular parasites, while maintaining immunologic tolerance toward innocuous self and foreign antigens.[108]

A critical class of innate immune cell mediating this cross-talk between innate and adaptive immunity is the dendritic cell (DC). DCs pick up antigen within tissues and migrate to local draining lymph nodes for MHC-mediated presentation to antigen-specific T cells and the initiation of adaptive immunity.[109] Furthermore, DCs integrate a variety of secondary cues, such as PRR or cytokine stimulation, to dictate the fate of T cell activation. For example, it is not surprising that mucosal-resident DCs are locally primed for the homeostatic induction of peripheral regulatory T cells (Tregs) via production of TGFβ and retinoic acid,[110–112] whereas those DCs elicited in the context of infection can skew T cell activation toward inflammatory Th1, Th2, or Th17 subsets, depending on the nature of the pathogen.[113,114] A similar set of considerations can be applied to monocytes and tissue-resident macrophages. Although less efficient at antigen presentation than DCs, several macrophage-derived cytokines can influence T cell polarization and activation, including IL-1β, IL-23, IL-12, and TNFα.[115–117] This communication with T and B cells is bidirectional. T cell-derived IFNγ and IL-4 or IL-10 are classically associated with the differentiation of monocytes and macrophages to so-called M1 and M2 phenotypes, respectively.[118] M1 macrophages produce high levels of reactive oxygen species and proinflammatory cytokines and chemokines, whereas M2 macrophages produce high levels of IL-10 and tissue-remodeling factors. However, this remains an oversimplification of the complex macrophage phenotypes in vivo. Similarly, macrophages express high levels of FcγRs, cell surface receptors that bind to the Fc portion of IgG antibodies, and mediated potent cellular responses to opsonized microbes, immune complexes, or deposited IgG.[119]

In recent years, there has been increasing appreciation for the role of certain subsets of granulocytes in the activation of adaptive immunity, particularly with regard to B cell activation and maintenance. Neutrophils have been described to promote antibody production by splenic marginal zone B cells via their production of B cell activating cytokines and costimulatory molecules, such as IL-21 and CD40L, respectively.[120] Furthermore, these cells express FcγRs, with the potential for IgG-mediated feedback. There is also evidence that neutrophils can traffic to lymph nodes (LNs) for presentation of antigen to T cells[121] or licensing DCs for T cell activation by TNFα-mediated maturation.[51] Eosinophils have also been demonstrated to be a major determinant of B cell maintenance within the bone marrow and mucosal tissues via similar mechanisms.[122–124] Given their residency and recruitment to numerous tissues, it is likely that these cells can influence the induction or progression of alloimmunity.

ILCs are critically dependent on, and influence the activity of, neighboring immune cells, including those of the adaptive immune system. Indeed, seminal work by Sonnenberg and colleagues has demonstrated that ILC3s are capable of MHC class II-mediated antigen presentation and the suppression of antigen-specific T cells within the gut.[125,126] Similarly, others groups have shown that lung-resident and systemic ILC2s and ILC3s are capable of driving T cell activation in a MHC-II-dependent manner.[127,128] Furthermore, human splenic ILCs support B cell antibody production through the production of B cell activating molecules, including a proliferation-inducing ligand (APRIL) and B cell activating factor (BAFF), and maintenance of B cell-helper neutrophils.[129]

Stimulation of Adaptive Alloimmunity

The antigen-specific or adaptive immune response to a graft occurs in two main stages. In the afferent arm, donor antigens stimulate recipient lymphocytes, which become activated, proliferate, and differentiate while sending signals for growth and differentiation to a variety of other cell types. In the efferent arm, effector leukocytes migrate into the organ and donor-specific alloantibodies are synthesized, both of which cause tissue damage. To initiate adaptive immunity, the graft must express antigens that are recognized by the recipient as foreign, and these include ABO antigens, HLA, and non-HLA "auto-antigens" that are polymorphic.

ABO BLOOD GROUP ANTIGENS

When allocating an organ to a potential recipient the first consideration is to ensure that it is compatible for the ABO blood group antigens. ABO antigens are expressed by most cell types in organ allografts and, were an ABO incompatible transplant to be performed, the presence of naturally occurring anti-A and anti-B antibodies in recipients will likely cause antibody-mediated hyperacute rejection and rapid graft loss. Organs from blood group O donors may be safely given to recipients of any blood groups ("universal donor") and recipients who are blood group AB may safely receive organs from donors of any blood group ("universal recipient"). In practice recipients of organs from deceased donors receive ABO blood group identical organs to avoid inequity of access to organs, although recipients of kidneys from living donors often receive an ABO compatible but non-ABO identical kidney.

HLA MOLECULES

Histocompatibility antigens differ between members of the same species and are therefore targets of the immune response in allogeneic transplantation. In all vertebrate species, histocompatibility antigens can be divided into a single, albeit multigenic, MHC and numerous minor histocompatibility (miH) systems. Incompatibility between donor and recipient for either MHC or miH leads to an immune response against the graft, more vigorous for MHC than miH. Indeed rejection of MHC-compatible organ grafts is often delayed, sometimes indefinitely, although in some mouse strain and organ combinations miH differences alone can result in acute rejection similar to that observed across full MHC mismatch.[130] On the other hand, the outcomes of allogeneic stem cell transplantation between HLA-identical siblings can be significantly affected by miH mismatches causing graft-versus-host disease.[131]

Major Histocompatibility Antigens

MHC class I proteins are cell surface glycoproteins composed of two chains—the alpha chain, which is highly polymorphic and encoded by a class I gene, and a nonvariable β_2-microglobulin chain (molecular weight approximately 12 kD). MHC class I proteins are expressed on most nucleated cells, albeit at variable levels, and they are generally responsible for activating cytotoxic CD8 T cells. MHC class II proteins are encoded entirely within the MHC and are composed of two membrane-anchored glycoproteins, an alpha and a beta chain. MHC class II molecules present peptides and activate CD4-expressing helper T cells. The tissue distribution of MHC class II proteins is far more restricted than that of class I, being expressed constitutively only by B lymphocytes, DCs, and some endothelial cells (particularly in humans). During an immune or inflammatory response, many other cell types may be induced to express MHC class II proteins.[132–136]

Both MHC class I and MHC class II molecules have the capacity to present peptides but the origin of these peptides differs between the two. In the case of MHC-I, they are largely acquired from the intracellular environment, whereas MHC-II largely present peptides acquired from the extracellular environment. Nevertheless, so called "cross-presentation" between these pathways may occur, particularly in the context of specialized antigen presentation by DCs.[137,138]

A combination of MHC and peptide forms a compound epitope that is engaged by the antigen-specific T cell receptor (TCR). The peptide-binding groove is usually occupied by many different peptides, derived from self-proteins (often those from the MHC) which, during infection, are replaced by those derived from pathogens.[139] The TCR repertoire is subject to negative thymic selection so that autoreactive cells are purged and positive thymic selection for TCRs that engage with peptides presented by autologous MHC occurs. When a pathogen invades, MHC proteins become loaded with foreign peptides that are engaged by TCR in a self-restricted immune response.

In humans, the HLA class I molecules are HLA-A, -B, and -C; MHC class II molecules are HLA-DR, -DP, and -DQ. Their role in presenting antigenic peptide to the TCR has led to the evolution of a high level of genetic diversity such that there are thousands of variants of both MHC class I and class II genes in the human population. This is likely to have evolved in response to their role as restriction elements in the response to pathogen-derived peptides. Certain cohorts of animals within species that have limited polymorphism at MHC loci have been devastated by infections that are cleared without difficulty in closely related species with polymorphic MHC.[140] This genetic diversity in the MHC loci is an important driver of alloimmune sensitization stimulated by pregnancy, blood transfusion, and prior transplantation. The immune mechanisms involved in these responses are not fundamentally different from those involved with any other antigen. The cellular immune response to alloantigen is, however, fundamentally different at least in magnitude, because MHC molecules bind a diverse range of endogenous peptides, which are therefore normally presented at the cell surface. Allogeneic MHC generate a correspondingly wide range of compound epitopes distinct from the repertoire generated by syngeneic MHC. These are therefore recognized as foreign and engaged by the TCR in the so-called "direct alloimmune response." The cellular immune response to MHC alloantigens is consequently unique in its diversity and therefore the number of T cells that can be recruited to an immune response.[141,142] Clinically, we currently assess and attempt to optimally match transplant donors and recipients according to the number of HLA-A, -B, and -DR mismatches, with a minimum of 0 mismatches (0-0-0) and a maximum of 6 mismatches (2-2-2) considered in the algorithm. In general, a greater emphasis is placed on matching at DR loci because of the capacity of MHC-II mismatches to activate CD4 T cells.

Minor Histocompatibility Antigens

Several genes within the class I and class II regions do not encode classical MHC proteins. In addition to those involved in antigen processing, others encode nonclassical MHC proteins that are similar in structure to classical MHC proteins but are nonpolymorphic. These may have antigen-presenting capacity for specialized antigens, such as lipids (e.g., mycolic acid and lipoarabinomannan from *Mycobacterium*) or peptides of different sequence but with common characteristics (e.g., with N-formylated amino termini). Others such as HLA-G play a role in immune regulation[143] particularly at the feto-maternal barrier.[144]

The class III region of the MHC is large and contains genes encoding proteins with a wide range of functions including many with roles in the immune system, including TNFα and TNFβ.[145] Polymorphisms that determine the production of such cytokines have also been linked to certain immune responses, including transplant rejection.[146]

Although the highest degree of genetic polymorphism within a species lies within the MHC, many other loci encode proteins with a lower degree of variability. It is clear from genetic studies that these proteins can act as transplantation antigens; they are miH antigens. Their structure and distribution were for many years elusive. Although T cells could recognize and respond to cells from MHC-identical individuals, it was almost impossible to raise antibodies against the antigens involved, making biochemical characterization difficult. The knowledge that T cells recognize small peptides, together with the application of molecular genetic techniques, allowed the characterization of the prototypic miH antigen, the male antigen or H-Y.[147,148] From such work, it is clear that miH antigens generally represent peptides from low-polymorphic proteins presented in the MHC groove, in the same way as a conventional antigen derived from infectious agents. The so-called H-Y antigen is actually derived from a group of such proteins encoded on the Y chromosome.[147–150] The first observation explains why it has been difficult to raise antibodies to miH antigens: because the combination of autologous MHC with allogeneic peptide constitutes a relatively poor conformational determinant for antibody binding, despite being an adequate determinant for TCR engagement.

MiH antigens can play a prominent role in rejection in a recipient who is given an MHC-compatible graft but in whom preexisting sensitization to miH antigens exists. This situation can be shown in the rat and mouse[151,152] and probably explains the occurrence of rejection episodes (which rarely result in graft loss) in renal transplants performed between HLA-identical siblings. Multiple miH

differences have been shown to represent an immunogenic stimulus equivalent to that of the MHC in a nonsensitized recipient of a cardiac allograft in the mouse[151] but it is difficult to gather similar data in clinical transplantation. Tissue-specific polymorphic protein antigens have also been described, for example, in mouse skin[153] and rat kidney.[154] An endothelial-monocyte antigenic system has been shown in humans, and it has been suggested that cells sensitized to these antigens can cause graft damage. This area has been reviewed by other authors.[155–157]

ANTIGEN PRESENTATION

As discussed, donor antigens are presented to T cells in the context of MHC. Activation of CD4 T cells is of particular importance, given their ability to promote both cellular and humoral adaptive responses, and has been demonstrated in a number of experimental transplant systems.[132,158–161] CD4 T cell activation requires the presentation of antigen in the context of MHC-II and is carried out by professional APCs, namely DCs, B cells, and some macrophages. DCs in particular, have received great attention in this context.

Dendritic Cells

DCs are present in all organs, including those that are routinely transplanted, and in secondary lymphoid organs.[162–164] Much of the work on DC function has emerged from murine studies, with more limited data from human tissues, because of the challenge of obtaining fresh clinical samples for in-depth analysis. However, expression of CD11c and MHC-II are useful markers to identify classical DCs (cDC) in human tissues. cDC can be further subdivided into the cDC1 subset that are CD141 (THBD) and XCR1$^+$, the cDC2 subset that express CD1c$^+$, and a further CD1c/CD141$^-$ subset. All three subsets have recently been isolated in the human kidney using flow cytometric analysis.[165]

After transplantation, these cells migrate out of the transplanted organ, into the bloodstream, and will subsequently encounter the recipient lymphoid system, where they are able to interact with and stimulate the host immune response.[166,167] Tissue-resident DCs have an immature phenotype,[168] but activation with PAMPs or DAMPs results in their rapid maturation into potently immunogenic APCs[169–172] with high expression of MHC class I and class II antigens together with a range of costimulatory molecules and cytokines (see section on Direct, Indirect, and Semidirect Antigen Presentation).

DCs with an immature or partly mature phenotype deliver tolerogenic rather than activating signals and play a crucial role in the induction of Tregs, T cell anergy, and deletion.[171,173] These effects are mediated by secretion of cytokines such as IL-10 and TGFβ, which promote the emergence of Treg cells and through the expression of "negative costimulatory molecules" such as programmed death-ligand (PD-L)1/2, inducible T cell costimulatory (ICOS) ligand, Ig-like transcript (ILT)3/4, and Fas ligand.

Direct, Indirect, and Semidirect Antigen Presentation

Allogeneic MHC on DCs derived from the graft can present a wide range of endogenous peptides derived from donor tissue, both from nonpolymorphic proteins[174] but also from MHC proteins themselves.[175,176] These combinations

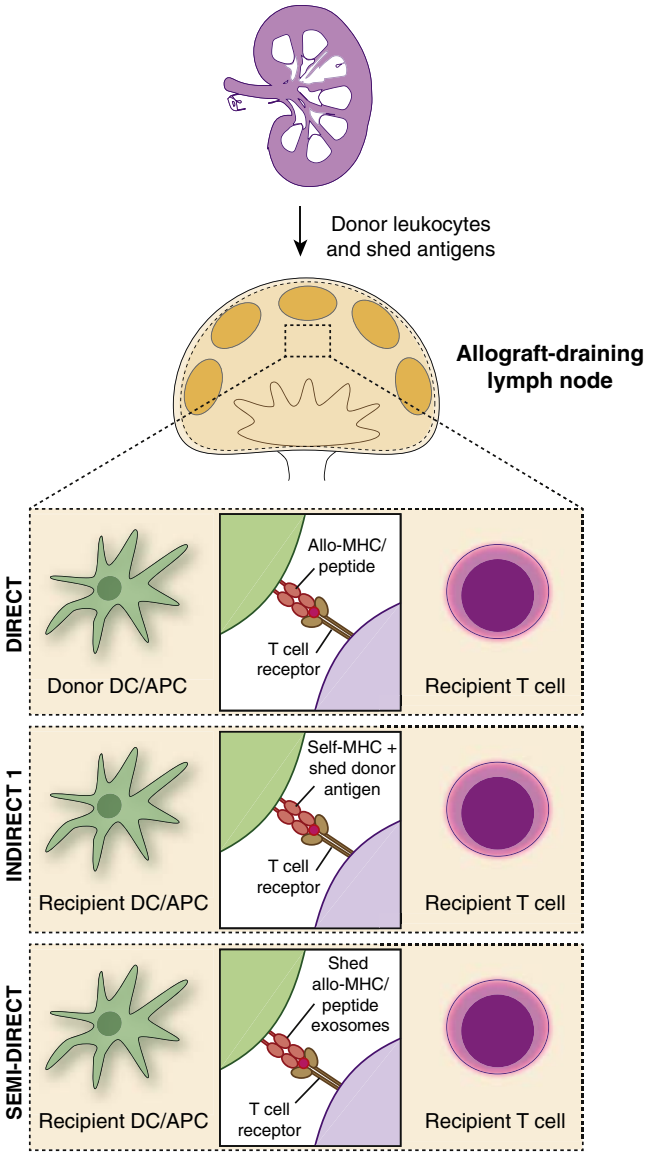

Fig. 2.5 Mechanisms of antigen presentation by dendritic cells in alloimmunity. Donor tissue-resident DCs and shed antigens derived from transplanted allografts can activate recipient T cells for alloreactive immunity. Direct antigen presentation is mediated by migrating donor-derived DCs within local lymphoid tissue. Indirect antigen presentation arises from recipient DCs acquiring shed antigens from damaged tissue. Semidirect antigen presentation results from shed allo-MHC/peptide exosomes being acquired by recipient DCs for presentation to recipient T cells.

of peptide and MHC can be engaged by recipient TCR, in a process termed *direct* antigen presentation (Fig. 2.5). The T cell response to allogeneic MHC occurs at a remarkably high frequency—in the order of 1 in 100. This relates in part to the wide range of endogenous peptides that occupy the MHC groove, generating an equivalent number of compound epitopes. It is also dependent on the capacity of the TCR to recognize multiple distinct ligands, that is to say polyspecificity is a feature of TCR engagement.[141,177]

Alloantigens can also be processed and presented conventionally by recipient antigen-presenting cells. This is termed *indirect* antigen presentation (see Fig. 2.5). The indirect pathway accounts for the fact that, in animal models, elimination of passenger leukocytes from the graft does not

abolish although it may alleviate rejection. Indeed, skin grafts from class $II^{-/-}$ mice transplanted onto normal mice were rejected in a $CD4^+$ T cell dependent manner.[178] These and other experiments demonstrated the potential role of self MHC class II restricted presentation of exogenous alloantigen to stimulate T cell responses through the indirect pathway.[179–181]

In clinical transplantation, and in particular in the context of chronic allograft dysfunction, evidence for the role of indirect allorecognition has been presented in various reports using peptides derived from polymorphic regions of MHC antigens[182,183] or cytoplasmic membrane protein preparations.[184,185] These studies need careful interpretation, however, because such assays present particular difficulties for reproducibility and standardization in an outbred population.[186,187]

It has been proposed that the direct allogeneic response dominates acute rejection and indirect allogeneic response allows for ongoing class II restricted responses after the loss of donor DCs.[188–190] This is almost certainly an oversimplification,[191,192] because there are significant differences between the expression of MHC molecules on the endothelium of mouse versus human, with long-term tonic expression of MHC-II on human endothelium even in the absence of inflammation. Therefore mouse transplant experiments may not reflect the response in humans. Nevertheless, these models have implicated indirect allorecognition in providing cognate help for B cell alloantibody formation,[193,194] and are supported by the observation of an increased risk for graft loss associated with nondonor-specific and donor-specific antibody.[195,196]

In addition to direct and indirect antigen presentation, there is evidence that intact proteins can be exchanged between cells in cell culture systems, that MHC proteins transfer in vivo[197] and that transferred MHC stimulates allogeneic responses in vitro.[197–200] The importance of this *semidirect* pathway of antigen presentation to transplant outcomes remains to be clearly established.

T CELL ACTIVATION

T cell activation requires not only engagement of the TCR by a peptide:MHC complex (signal 1), but also engagement of cell surface costimulatory molecules present on APC (signal 2) and T cell stimulation by cytokines (signal 3) (Fig. 2.6).

Signal 1—TCR Activation

The T cell receptor comprises an alpha and a beta chain that recognize and bind to peptide:MHC complexes. The TCR has no catalytic activity of its own, but forms a complex with six CD3 subunits ($\gamma\delta\varepsilon_2\zeta_2$) that contain cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs).[201] Lymphocyte-specific protein tyrosine kinase (LCK) phosphorylates these ITAMs after TCR ligation resulting in association with the ζ-chain-associated protein kinase: zeta-chain-associated protein kinase 70 (ZAP70).[202] ZAP70 recruitment results in phosphorylation of the adapter proteins SH2 domain-containing leukocyte protein of 76 kD (SLP76) and linker of activated T cells (LAT). LAT facilitates the formation of multiprotein complexes that drive multiple activation pathways[203] leading to de novo expression of a wide range of genes encoding cytokines and cell surface proteins. These signaling pathways are the targets of a number of immunosuppressive medications, including calcineurin inhibitors, and are described in detail elsewhere.[201–206]

Signal 2—Costimulation

To generate sustained T cell activation, engagement of surface costimulatory molecules is required in addition to signal 1 (see Fig. 2.6). These costimulatory molecules determine and mediate short-term function and long-term fate during priming, expansion, and death of T cells. There are many families of costimulatory molecules including those of the immunoglobulin superfamily (e.g., B7), tumor necrosis factor family (e.g., CD154), G protein-coupled receptors (e.g., C3a and C5a receptors), and lectin receptors (e.g., DC-SIGN). As increasing numbers of molecules have been described it has become evident that these interactions are highly complex, involving paracrine and cell contact dependent mechanisms.

In the absence of costimulatory signals, T cells may become anergic, that is they are unresponsive even if they subsequently receive an adequate second signal.[207–211] Anergic cells can also inhibit the activation of neighboring T cells.[212–215]

The molecular basis for the costimulatory or second signal for naive T cell activation was defined as that between CD28 and the B7 family molecule now known as CD80.[216] These costimulatory interactions were not only the first to be described but also the first to be manipulated in the setting of clinical transplantation.[217,218] The cell surface protein CD28 is now known to be a member of a family of similar proteins.[219–221] Activation of downstream signaling via CD28 results from ligation with B7 family proteins, CD80 or CD86. These proteins are expressed by APCs such as DCs and engage CD28 during antigen presentation. Signaling through CD28 in the context of TCR ligation results in an increase in glucose metabolism and high levels of cytokine and chemokine expression, particularly IL-2, a cytokine that promotes T cell proliferation and survival.

CD28-deficient mice have impaired immune responses but can reject skin grafts, albeit in a delayed fashion[222]; this is likely because of other costimulatory proteins that can substitute the action of CD28.[219,221,223] Blocking the CD28 pathway in normal animals inhibits the alloimmune response and results in prolonged graft survival or tolerance.[224–226] The most widely used reagent for this purpose has been cytotoxic T lymphocyte-associated protein 4 (CTLA-4)-Ig. This blocks B7 engagement of both CD28 and CTLA-4, the latter of which could be counterproductive because CTLA-4 acts primarily as a coinhibitory molecule, counterbalancing the effects of CD28. This is evident in the severe phenotype of $CTLA\text{-}4^{-/-}$ mice, in which animals die of lymphoproliferative disorder within a few weeks of birth. Similar in structure to CD28, CTLA-4 inhibits the earliest events in T cell activation. CTLA-4 has a higher affinity for CD80 and CD86 than does CD28[227,228] and its engagement with CD80 induces a lattice structure at the cell surface consisting of alternating CTLA-4 and CD80 homodimers. These properties of CTLA-4 may limit the ability of CD80 to interact with and cluster CD28 at the immune synapse, potentially explaining the finding that low levels of CTLA-4 can be effective at inhibiting immune responses. CTLA-4 may

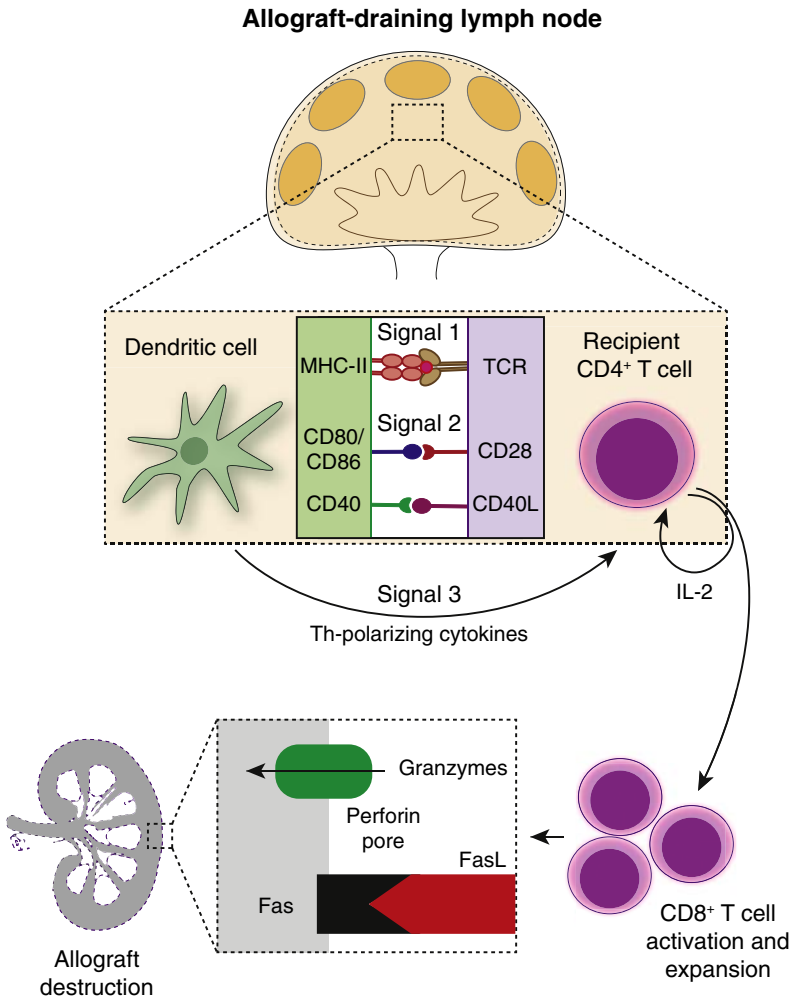

Fig. 2.6 Molecular mechanisms of T cell activation. Within local draining lymph nodes, DC-T cell interaction in the presence of alloantigen results in recipient T cell activation through a variety of molecular mechanisms. Signal 1 is provided by antigen-loaded MHC class II molecules interacting with an antigen-specific TCR. Signal 2 is mediated by costimulatory molecules on antigen-presenting cells, such as CD80/CD86 and CD40, which engage the corresponding receptors on T cells, in this case CD28 and CD40L, respectively. Signal 3 is mediated by APC-derived cytokines, which skew T cells into specific subsets, such as Th1, Th2, or Th17 cells. Activated T cells also secrete IL-2, resulting in autocrine signaling and T cell expansion and survival, and paracrine activation of local T cells, such as $CD8^+$ effector T cells. $CD8^+$ T cell activation can then induce tissue destruction through FasL and perforin/granzyme-mediated mechanisms.

also deliver a negative intracellular signal[229] independent of its effect on CD28 that halts cell cycle progression and IL-2 production[230] or affects TCR engagement.[231] The actions of CTLA-4 may be even more complex at a polyclonal level because CTLA-4 engagement may promote Treg function in vivo.[232,233] A modified CTLA4-Ig fusion protein, belatacept, has undergone robust assessment in two large clinical trials, BENEFIT and BENEFIT-EXT, and these demonstrate that it is as effective as calcineurin-inhibitors (CNIs) in preventing acute TCMR in humans, but avoids CNI-associated nephrotoxicity resulting in better allograft function.[234–236] Belatacept-treated subjects also had a significantly lower rate of development of de novo donor-specific antibody (DSA), and nonhuman primate studies suggest this may be because of inhibition of B cell-T follicular helper (Tfh) cell interactions.[237]

Since the role of CD28 engagement was defined other members of this family of molecules have been identified; bound by various ligands, they generate effects that can broadly be termed costimulatory ICOS, DNAX accessory molecule 1 (DNAM-1) and cytotoxic and regulatory T-cell molecule (CRTAM) or coinhibitory CTLA-4, programmed cell death protein 1 (PD-1), B- and T-lymphocyte attenuator (BTLA), lymphocyte-activation gene 3 (LAG-3), T cell Ig and mucin-domain containing-3 (TIM-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), and leukocyte-associated Ig-like receptor 1 (LAIR-1). A second major family of costimulatory molecules on the T cell surface is the TNF superfamily including CD27, CD134 (OX40), and CD137 (4-1BB), which interact with a range of TNF-receptor family members.[223,238,239] On the T cell surface the TNF receptor family member CD154 (CD40 ligand, gp39) itself interacts with CD40 on B cells, DCs, and monocytes. Larsen and coworkers showed that blocking this interaction could prolong graft survival in a mouse cardiac transplant model.[240] Even more impressive, however, were data that combined CD28 and CD40 blockade induced permanent survival of allogeneic skin grafts in mice with no long-term deterioration of graft integrity.[241] Tolerance to graft antigens could not be shown in these mice, despite the excellent

survival of the transplant itself. In a large animal setting, kidney graft rejection in monkeys can be prevented completely with antibodies to CD154[242]; however, its clinical application is limited by thromboembolic events associated with its expression on platelets. Alternative approaches using nondepleting antibodies to CD40 are therefore now being explored.[243]

An understanding that memory T cells are an important barrier to successful engraftment in the presence of immunosuppression and to tolerance induction, combined with the fact that they may be relatively resistant to CD28 inhibition, has directed interest toward identifying molecular targets in T memory cells. The expression of CD2 on T effector memory cells,[244,245] of and lymphocyte function-associated antigen (LFA)-1 on T memory cells,[246] and even expression of a specific potassium channel on T effector memory cells[247] have been investigated. These approaches require further assessment and are not without potential competing risks.[248,249]

Signal 3—Cytokines

The consequences of TCR engagement and costimulation are proliferation and differentiation of the T cells into effector phenotypes and the concomitant production of cytokines required to stimulate themselves, and other immune cell types, including CD8 T cells, macrophages, DCs, and B cells. IL-2 is a potent activator and proproliferative cytokine for T cells. Its effects are dependent on binding to its cell surface receptor, which has three subunits, α (CD25), β, and γ. During T cell activation the α subunit becomes associated with the other subunits to form a high affinity receptor. Blockade of the IL-2 receptor by targeting the α-chain profoundly inhibits T cell proliferation. CD25 blockade with basiliximab or daclizumab have proven efficacy as induction agents in renal transplantation.[250,251]

A number of other cytokines act to polarize CD4 T cells toward different fates, where they promote different types of immune responses. Th1 polarized cells mainly produce IFNγ and drive cell-mediated inflammation and immunoglobulin class switching to IgG antibodies. Th2 cells produce IL-4, IL-5, and IL-13, and are involved in IgE class switching and eosinophil recruitment. Th17 cells produce IL-17 and IL-22 and play an important role in the clearance of extracellular pathogens and in autoimmune pathology.

The role of different cytokines in allograft rejection has been approached using neutralizing antibodies and mice deficient in specific cytokines. However, interpretation of these experiments is complex, because the absence of the cytokines can influence immune system development, there is redundancy in the action of cytokines, and cytokines may have opposing actions depending on the context. This is illustrated by studies of IL-$2^{-/-}$ or IFN$\gamma^{-/-}$ mice, which demonstrated that neither were required for rejection.[252,253] IL-15 can for example substitute many of the actions of IL-2, and IL-15 transcripts are found in grafts placed in IL-$2^{-/-}$ mice. Subsequent studies demonstrated that whereas neither IL-2 or IFNγ were required for rejection, both were required for tolerance induction.[254] This suggested nonredundant functions for both IL-2[255,256] and IFNγ[257–259] in the function of regulatory T cells (see section on Transplant Tolerance).

T Cell-Mediated Rejection

TCMR, previously known as acute cellular rejection, is the most common type of rejection observed in the current era. TCMR occurs in around 20% of kidney transplant recipients and most frequently occurs in the first 6 months posttransplant (see Fig. 2.1). TCMR is characterized by immune cell infiltration into the graft and may involve epithelial cells, for example a tubulitis in kidney transplants, or in more severe cases, an arteritis. This infiltrate is composed of CD8 T cells, CD4 T cells, monocytes, macrophages, and B cells. The sequence of events that culminate in TCMR include the migration of immune cells into the graft, which is dependent on the production of chemokines by graft-resident cells and on the interaction of adhesion molecules on endothelial cell and immune cells. Once in the graft, cellular effector functions are activated causing damage to the transplant, and the cytotoxic functions of CD8 T cells in particular play a key role in this process.

MIGRATION OF ACTIVATED CELLS INTO THE GRAFT

To enter a site of inflammation, leukocytes must migrate across the vascular endothelium. This migration process is controlled by chemokines, and by cell-cell interactions between the leukocyte and the endothelium.[42] Activated and memory cells bear adhesion proteins, chemokine receptors, and addressins, which allow homing to and migration into peripheral tissues.[260,261] In transplantation, this activation is thought to predominantly take place in secondary lymphoid organs, because aly mice lacking lymphoid organs have reduced graft rejection[262,263] as do splenectomized mice deficient in LTα or LTβ (and therefore lacking in lymph nodes and Peyer's patches).[264]

In small bowel transplantation, recipient-derived leukocytes migrate in large numbers into the mesenteric lymph nodes and Peyer's patches of the graft.[265–268] This likely results from normal homing of immune cells to the large volume of lymphoid tissue within the graft. During chronic rejection, lymphoid neogenesis or ectopic accumulations of lymphoid cells may develop within transplants in mouse models and in humans, enabling local activation of immune cells within the organ.[269] The movement of lymphocytes out of secondary lymphoid organs requires sphenogosine-1-phosphate receptor, a G protein–coupled protein, and this has been targeted using FTY720 (fingolimod). FTY720 acts as a high-affinity agonist for S1P1R, inducing internalization of the receptor. This renders the cells unresponsive to the S1P1, depriving them of an obligatory signal required for egress from lymphoid organs. Lymphocytes are therefore unable to access the peripheral circulation and allograft. Although FTY720 was used in a number of clinical trials in transplantation, its use was associated with macula edema and bradycardia, and therefore it has not entered routine clinical use.[270–272]

The processes underpinning the movement of lymphocytes into the graft are similar to those described for neutrophils in the section on Activation of the Immune System Peritransplant. Of note, antigen-activated lymphocytes migrate into nonlymphoid tissues[260,273,274] and may show

tissue-selective homing and preference for sites where they are most likely to reencounter their specific antigen.[275] This process may be facilitated further by cognate recognition by the T cell of MHC class II/peptide complexes on the vascular endothelium.[276] Blocking adhesion molecule interactions has been attempted in experimental and clinical transplantation.[78,277–279] In general, cocktails of antibodies blocking multiple adhesion molecules are more potent than single antibodies[280] but the results vary. Indeed, in one study a combination of antibodies blocking both ICAM-1 and LFA-1 led to accelerated rejection of rat cardiac allografts.[281] Antisense oligonucleotides have also been used to prevent the expression of ICAM-1 and were effective in prolonging graft survival in experimental models.[282] Small molecule inhibitors also may effectively interrupt the interactions required for leukocyte adhesion and extravasation,[283] and these reagents may simultaneously be effective in blocking IRI and rejection.[284]

Chemokines play a crucial role in leukocyte trafficking under both normal conditions and in the setting of sterile inflammation and rejection. There are more than 40 different chemokines belonging to two major structural families that are recognized by a range of chemokine receptors expressed on immune cells: CC or β chemokines (e.g., MIP-1α/β, [CCL3/4] regulated on activation, normal T cell expressed and secreted [RANTES; also known as CCL5], and monocyte chemoattractant protein [MCP-1; also known as CCL2]) which attract T cells, monocyte/macrophages, DCs, NK cells, and some polymorphs and CXC or α chemokines (e.g., IL-8 [CXCL8] and IFNγ-inducible protein) which primarily attract neutrophils and T cells.[285–289]

A number of experimental transplant models have demonstrated the importance of chemokines in mediating immune cell infiltration in IRI and in acute and chronic rejection.[285,286,290–293] For example, CCR1-deficient mice accept MHC class II mismatched grafts without immunosuppression and MHC class I and II mismatched grafts with only low-dose immunosuppression.[294] CXCL10$^{-/-}$ recipients show normal rejection kinetics of a wildtype graft, but CXCL10$^{-/-}$ grafts placed into wildtype recipients show prolonged survival.[295] These murine studies have sparked interest in therapeutic targeting of chemokine networks in human transplantation, but as yet, no agents are currently used in clinical practice.[296]

MECHANISMS OF CYTOTOXICITY

CD8 cytotoxic T cells damage and destroy target cells by the production of lytic molecules such as perforin and granzyme, and through the induction of Fas-Fas-Ligand-mediated apoptosis (Fig. 2.6). In cell culture systems, MHC-mismatched lymphocytes proliferate and produce cytokines in response to one another in the mixed lymphocyte reaction. This results in the differentiation of CD8 T cells into effectors that lyse target cells bearing the mismatched MHC antigens.[297,298] There is considerable evidence that CD8 T cells are involved in TCMR. First, they make up a significant proportion of infiltrating leukocytes in rejection allografts, in contrast to the low number observed in grafts of animals treated with cyclosporine to prevent rejection.[92,299] Second, cloned populations of CD8 T cells are capable of causing the type of tissue damage associated with rejection. Third, graft rejection may be delayed by CD8 T cell depletion.[130,160,300–302]

The importance of the individual effector mechanisms of CD8 T cells in mediating rejection is debated. Mice deficient in perforin are still able to reject skin[303] and organ grafts,[304] even when the grafts are resistant to Fas-mediated and TNFα-mediated killing,[305] but grafts mismatched only at the MHC class I were found to be rejected more slowly in perforin knockout mice.[304]

A number of elegant experiments in animal transplant models demonstrate that both antigen-specific and nonantigen-specific effector mechanisms because of collateral damage of bystander cells may be involved in graft destruction.[306–310]

COMPLEMENT AND TCMR

Murine models suggest that complement may play a role in acute TCMR. In a fully MHC-mismatched model (C57BL/6 to B10.BR), kidneys from C3-deficient donors survived for long periods without immunosuppressive treatment, in contrast to wildtype C57BL/6 donor grafts that were rejected within 2 weeks. Recipient C3 had only a minor effect in this model.[311] Similarly, in a cardiac allograft model, deficiency of the complement regulator CD55 (DAF) in transplanted hearts resulted in aggressive TCMR.[312] The mechanism by which complement augments TCMR may be a result of the effects of C3a and C5a on antigen-presenting cells[313] or a result of a direct effect on T cells via ligation of C3aR and C5aR.[314]

B Cell Activation and Antibody-Mediated Rejection

B CELL ACTIVATION

B cells are immune cells of the lymphoid lineage that develop in the bone marrow (Fig. 2.7). They are characterized by the expression of antibody as their surface antigen receptor, the B cell receptor (BCR), and other markers such as CD20 and CD19. Antibodies comprise two heavy and two light chains, and are classified according to the heavy chain they express; IgM antibodies have a μ heavy chain, IgG antibodies a γ heavy chain, etc. When B cells emerge from bone marrow, they express an IgM antibody and pass through a transitional phase (expressing high levels of CD24 and CD38 on their surface) before becoming follicular B cells that reside within secondary lymphoid organs (the lymph node and spleen).

When B cells encounter an antigen that binds their surface BCR they may either develop into short-lived plasmablasts that produce early, germ-line encoded antibody[315] or alternatively, become a germinal center B cell. Here, they divide and mutate their variable region genes (somatic hypermutation) in an attempt to produce antibody with a higher affinity for antigen.[316] They also undergo class switching, so that the heavy chain present in antibody changes from μ to one of the other isotypes. During many rounds of division and hypermutation, B cells with a high affinity BCR are positively selected and differentiate into

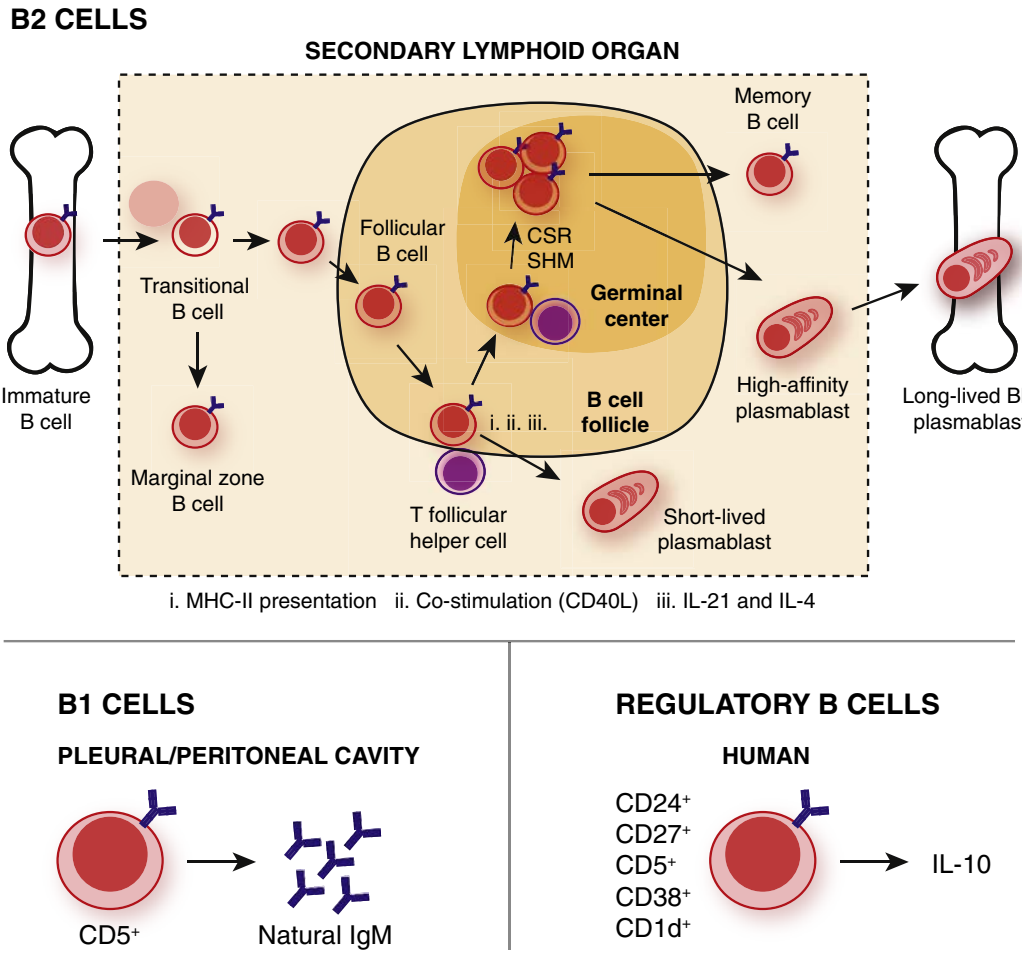

Fig. 2.7 Molecular mechanisms of B cell activation. B cells can be divided into two major populations: B1 and B2. B2 cells contribute to the follicular B cell population within lymphoid organs and to marginal zone B cells in the spleen. After stimulation of the BCR, follicular B cells receive help signals at the interface of the T cell zone through antigen presentation to T follicular helper cells and CD40- and cytokine-mediated signals. Activated follicular B cells undergo rounds to somatic hypermutation (SHM) and class-switch recombination (CSR), resulting in the generation of short-lived plasmablasts, high-affinity long-lived plasma cells, and memory B cells that contribute to the high-affinity class-switched antibody pool. B1 cells are enriched in the pleural and peritoneal cavities and are a major source of natural polyreactive IgM. Regulatory B cells also exist with the capacity to secrete antiinflammatory molecules, such as IL-10, that are essential for the suppression of damaging allo- and autoimmunity.

either memory B cells or plasma cells.[317] A subset of CD4 T cells, known as Tfh cells, are engaged by germinal center (GC) B cells presenting antigen to them. This Tfh-B cell interaction is essential for the progress of the GC reaction and for the development of memory B cells (that express CD27) and plasma cells.[318,319] Only a small proportion of plasma cells arising from the GC become established as long-lived plasma cells in the bone marrow. They reside within a number of limited niches, do not proliferate, but act as long-term antibody factories, producing IgG.[320] Plasma cells have also been described in inflamed tissues in autoimmunity and within allografts.[321–324]

B CELL FUNCTION

B lymphocytes are best known for their ability to produce antibodies—the soluble (humoral) mediators of an adaptive immune response. In addition, B cells can act as important antigen-presenting cells for CD4 T cells, thereby initiating cellular immunity, and may produce cytokines that can both activate and regulate a range of other immune cells. B cells also contribute to the development of secondary lymphoid organs (lymph node and spleen), and can therefore have broad effects on immune responses far beyond their most obvious function of antibody generation.

Alloantibodies

Antibodies are the only soluble components of the adaptive immune system and have wider tissue distribution than their cellular immune counterparts. Alloantibodies, particularly those that recognize donor HLA, can mediate hyperacute rejection, acute ABMR, and chronic ABMR. Early efforts to assess whether recipient antibodies could potentially recognize donor cells were based on the cytotoxic crossmatch assay, incubating recipient serum with donor cells in the presence of complement.[325] Currently, HLA genotyping of the donor and recipient and the use of single HLA antigen beads (SAB) allow a more precise assessment of the titer of DSA, assisting in the assessment of pretransplant immunologic risk.[326,327] Currently, around 30% of patients on the kidney transplant waiting list are sensitized and have varying levels of HLA antibodies that may preclude transplantation or require an antibody-reduction strategy to allow transplantation to proceed.[328] A meta-analysis of published

data suggests that even low titers of pretransplant DSA (detectable by SAB but with a negative flow cytometric crossmatch), doubles the risk of ABMR and increases the risk of graft failure by 76%.[329] In addition, the development of de novo DSA posttransplant in nonsensitized transplant recipients is associated with an increased frequency of acute ABMR and worse graft survival.[330] The pathogenicity of HLA DSA also varies according to its specificity, with MHC class II DSA having a worse effect on the allograft than class I DSA.[330,331] In addition, non-HLA antibodies such as those binding MICA or angiotensin II type 1 receptors may also have a deleterious effect on grafts.[332–334] Sigdel and colleagues used high-density protein arrays to analyze serum samples obtained from 172 renal transplant recipients and identified 38 de novo non-HLA antibodies that significantly associated with the development of chronic allograft injury on protocol biopsies.[335] Other non-HLA binding antibodies that may negatively affect the allograft include antibodies binding apoptotic cells.[336,337]

Antigen Presentation to CD4 T Cells

In order for CD4 T cells to orchestrate adaptive immune responses, they must be activated by antigen presented to them in the context of an MHC class II molecule, together with costimulatory signals. B cells are very effective APCs and have several advantages over DCs, traditionally considered to be the principle APC. First, they may have a high-affinity, specific receptor for antigen (the BCR), and can therefore efficiently and rapidly acquire large amounts of antigen for presentation.[338,339] Second, they can clonally proliferate, and therefore may rapidly become the numerically dominant APC. Murine models have shown the importance of B cells for T cell activation in vivo,[340,341] including in the context of autoimmunity[342] and alloimmunity.[343] In human kidney transplant recipients, transcriptomic analysis of kidney biopsies with TCMR supports the notion that B cells contribute to worse outcomes in TCMR. Sarwal and colleagues identified a B cell transcriptomic signature (CD20, CD74, immunoglobulin heavy and light chains) in allografts with steroid-resistant TCMR and a poorer outcome.[344] Furthermore, the 11 genes found to comprise a "common rejection module," present in samples obtained from a variety of organs during rejection, include the chemokines CXCL9 and CXCL10[345] that are produced by B cells after interactions with cytotoxic T cells.[346] Two B cell genes (CD72 and BTLA) are also among those most differentially expressed in biopsies with TCMR.[347] Together, these data emphasize the potential importance of B cells as APCs in transplantation, and in this context, it is notable that B cell depletion has proven to be an effective treatment for autoimmune diseases considered to be mediated by T cells, including rheumatoid arthritis, multiple sclerosis, and type 1 diabetes mellitus.[348–350]

Formation and Maintenance of Secondary Lymphoid Tissue

B cells produce lymphotoxins (LTs) and thereby initiate the formation and shape the architecture of lymph nodes and spleen.[351–353] Their ongoing production of LTα1β2 is also required for the maintenance and integrity of subcapsular sinus macrophages that form a protective screen around the perimeter of lymph nodes.[354] B cell production of VEGF-A may also promote intranodal lymphangiogenesis, increasing antigen and DC distribution in the lymph nodes.[355] They also may be involved in the generation of tertiary lymphoid structures that emerge in inflamed organs affected by autoimmunity, for example in Sjogren disease, rheumatoid arthritis, or type 1 diabetes mellitus,[356,357] that may be the source of some autoantibodies.[321,358]

Production of Proinflammatory Cytokines

B cells have the capacity to produce a number of cytokines after stimulation and can thereby affect a variety of immune cells.[359] IL6 is a cytokine required for T-dependent antibody responses,[360] but B cells can themselves produce this cytokine,[361] and this may contribute to autoimmune pathology, for example, via Th17 cell activation leading to exacerbation of experimental autoimmune encephalomyelitis.[362] Indeed, B cells from patients with multiple sclerosis also demonstrate increased IL-6 production compared with healthy controls.[362] B cell production of TNFα and IFNγ can also promote Th1 T cells[361,363] and macrophage activation.[364]

Recently so-called innate response activator (IRA) B cells have been described. These splenic IgM+ B cells can produce granulocyte macrophage-colony stimulating factor (GM-CSF) in response to LPS, a TLR4 ligand, facilitating the mobilization of neutrophils.[365] This makes an important contribution to pathogen containment in mouse models of gram-negative sepsis and in pleural responses to gram-positive bacteria[366] but may exacerbate atherosclerosis via Th1 cell activation.[367] These B cells are thought to originate from B1 B cells, a subset enriched in the peritoneal and pleural cavities.[368,369]

B Cells as Regulators of the Immune Response

There is increasing evidence that B cells can not only act as effectors of the immune response but can also play an immunoregulatory role, particularly via the production of IL-10[370] but also by contact-dependent mechanisms (e.g., via PD-L1 expression[371]) and perhaps by direct granzyme-B-dependent cytotoxicity.[372]

IL-10-producing B cells have been shown to be important in limiting autoimmunity in mouse models. In mice, several groups have identified IL-10-producing B cells within a number of B cell populations including B1, transitional, and marginal zone subsets.[373–375] IL-10-producing B cells have also been identified in humans and comprise around 5% of circulating B cells, although cells with the potential to produce IL-10 may be found at higher frequencies. As in mice, human IL-10-producing B cells are present within different subsets, including transitional B cells. Several markers, such as CD5, CD1d, Tim-1, CD9, and CD80, have also been reported to localize to these "regulatory cells."[376–381] Unlike regulatory T cells that can be identified by expression of Foxp3, a subset-specific transcription factor has not been identified in regulatory B cells. Some recent data suggest that plasma cells may be the main source of IL-10 in vivo.[382]

The potential importance of B cells with a regulatory capacity has now been identified in a number of disease contexts, including systemic lupus erythematosus (SLE),[376,377] allergy,[379,383] transplant tolerance,[384,385] and protection from allograft rejection.[386] In particular, the balance

between the production of IL-10 and proinflammatory cytokines such as IL-6 and TNFα may be important in regulating immune responses. For example, a lower number of CD24/CD38^{high} transitional B cells that produce high levels of IL-10 versus TNFα is associated with worse allograft outcome in renal transplant recipients.[387] In contrast, in patients with drug-free long-term graft function that are deemed "operationally tolerant" there is a significant increase in the number of total B cells, particularly memory B cells and B cells expressing CD1d and CD5. In vitro, B cells purified from these subjects had a relative increase in FcγRIIB expression and an increase in B cell scaffold protein with ankyrin repeats 1 (BANK1), a negative regulator of CD40 signaling.[388] Other studies show a higher proportion of CD24/CD38^{high} transitional B cells associated with long-term transplant tolerance and B cells from tolerant subjects produce more IL-10 after in vitro stimulation.[384,385]

ANTIBODY-EFFECTOR FUNCTION IN ABMR

Acute ABMR is uncommon in nonsensitized transplant recipients and is difficult to treat. The diagnosis of ABMR has been facilitated by more sensitive methods of alloantibody detection and by the identification of complement activation in allograft biopsies via C4d staining. Clinical features of ABMR include a decline in allograft function, the presence of donor-specific HLA antibodies, C4d deposition in peritubular capillaries, and evidence of acute vascular injury (e.g., capillaritis, with neutrophils in capillaries). ABMR, particularly in its chronic form, may occur in the absence of C4d deposition on biopsy.[83,389,390] Analysis of transcripts may also assist in making a more accurate assessment of the type of rejection, particularly in diagnosing antibody-mediated rejection, compared with standard histopathological analysis, even in the absence of C4d staining.[391,392]

A gradual decline in allograft function with time is almost universally observed. This was previously termed *chronic allograft nephropathy* but the more recent Banff Classifications have sought to distinguish nonimmunologic insults, for example, CNI toxicity, from chronic rejection, particularly chronic ABMR. Chronic ABMR is characterized by vasculopathy and in the kidney is evidenced by glomerulopathy (double contouring in peripheral capillary loops) and peritubular capillary basement. There may also be peritubular capillary C4d staining, although this is not universal. Clinically, this usually occurs in patients with detectable HLA DSA, often in the context of noncompliance.

The histologic features of ABMR result from well-established pathways of alloantibody effector function, namely direct activation of endothelial cells via binding to MHC[393] complement activation (via the classical pathway) or recruitment and activation of immune cells that express receptors for the Fc portion of IgG or complement receptors (including neutrophils, macrophages, monocytes, DCs, and NK cells[394]; Fig. 2.8).

Direct Stimulation of Endothelial MHC

Independently of FcγR engagement, IgG can directly activate allograft endothelium. Binding of DSA to HLA induced intracellular signaling and endothelial cell survival and proliferation, mediated by upregulation of the fibroblast growth factor (FGF) receptor and increased FGF ligand binding. Furthermore, DSA deposition could induce expression of endothelial P-selectin for adherence of monocytes. Reed and colleagues have produced an elegant body of work demonstrating that HLA antibodies can have direct effects on allograft endothelial cells via variable region binding.[393]

Complement Activation

IgG immune complexes can activate complement via the classical pathway and this process is evident in ABMR by the detection of C4d on peritubular capillaries.[83] Further evidence that complement fixation may contribute to the pathogenicity of alloantibodies is provided by the studies investigating C1q or C3d binding. Loupy et al. investigated 1016 antibody-compatible renal transplant recipients and demonstrated that patients that developed C1q-binding DSA after transplantation had the lowest 5-year rate of graft survival (54%), compared with those with noncomplement-DSA (93%).[395] A more recent study suggests that the detection of HLA antibodies that bind to C3d may have an even greater prognostic significance in patients with acute ABMR.[396] A number of complement inhibitors, including eculizumab and C1 esterase inhibitors, are currently being trialed for both the prevention of ABMR in antibody-incompatible transplant recipients,[397] and for the treatment of ABMR.[398,399]

FcγR Activation

The absence of C4d staining in more than half of biopsies with late ABMR highlights the importance of complement-independent mechanisms in mediating the deleterious effects of DSAs.[83,391] Furthermore, some IgG isotypes (IgG4) cannot fix complement, whereas IgG2 has a limited complement-activating capacity compared with IgG1 and IgG3.[400] FcγRs bind to the Fc portion of IgG and mediate activation of the immune cells that express them (Fig. 2.8). In humans, there are several activating receptors (FcγRIIA, FcγRIIC, FcγRIIIA, and FcγRIIIB) and a single inhibitory receptor, FcγRIIB, which plays a critical role in suppressing IgG-mediated inflammation.[401,402] FcγRs are widely expressed on immune cells, including neutrophils, monocytes, macrophages, DCs, mast cells, NK cells, and B cells but the effect of FcγR ligation varies between cells. For example, neutrophils express FcγRIIA and FcγRIIIB, and cross-linking leading to phagocytosis, cytokine and superoxide production, neutrophil adhesion, and extracellular trap formation (NETosis),[403–410] whereas on macrophages and dendritic cells, FcγR ligation induces proinflammatory cytokine production, including TNFα and IL-1β, phagocytosis, CCR7-dependent migration, and antigen presentation.[411–414]

There are several lines of evidence implicating FcγR activity in rejection severity and graft survival in ABMR. NK cells, in particular, have received significant attention as an immune cell subset mediating FcγR-dependent inflammation in AMBR. Hirohashi et al. demonstrated that chronic allograft vasculopathy in murine heat allografts was NK cell and FcγR dependent, inflammation being attenuated after anti-NK1.1 IgG-treatment or in the presence of $F(ab')_2$ fragments of IgG2a DSA.[415] Consistent with a role in human ABMR, *FCGR3A* and other NK cell-associated transcripts correlate with the presence of DSA and ABMR.[416,417] Furthermore, several groups have examined

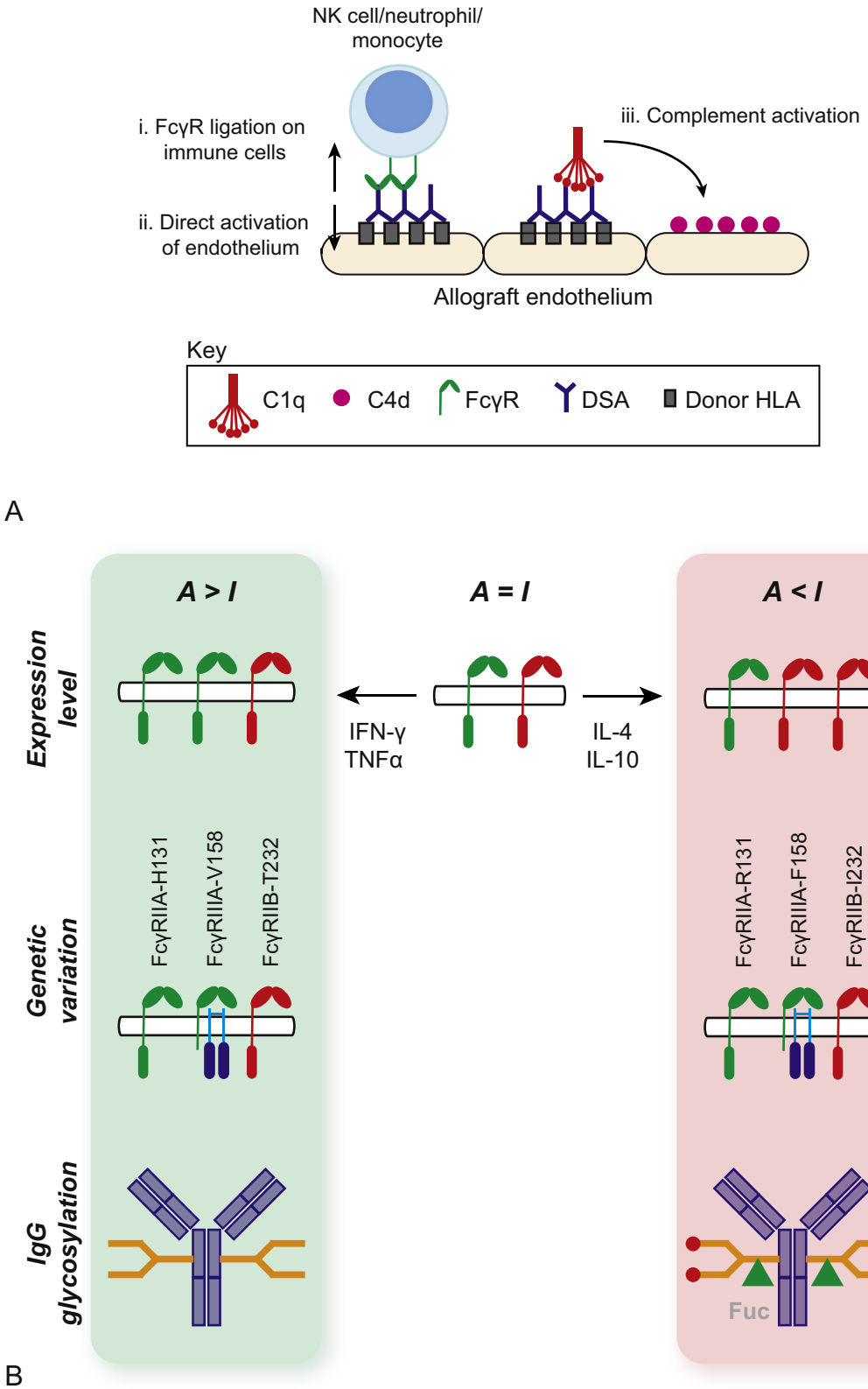

Fig. 2.8 Mechanisms of antibody-mediated rejection. (A) Donor-specific antibody (DSA) binding to allograft endothelium can activate a variety of inflammatory mechanisms: (i) engagement of FcγRs on local and circulating immune cells, such as NK cells; (ii) direct activation of allograft endothelium through cross-linking of endothelial cell surface molecules; (iii) activation complement via the classical pathway. (B) Numerous factors influence the ability of IgG-FcγR engagement to direct inflammation and tissue damage. FcγR cellular expression levels are controlled by the cytokine milieu: inflammatory mediators, such as IFNγ and TNFα increase expression levels of activating FcγR, increasing the activating-to-inhibitory (A:I) ratio. FcγR polymorphisms can influence FcγR expression level or signaling capacity. Finally, the IgG glycosylation state alters the capacity of antibodies to interact with different FcγRs: sialylated IgG exhibits reduced binding to activating type I FcγRs, whereas defucosylated IgG increases FcγRIIIA binding affinity. *Fuc,* ucose; *SA,* sialic acid. Green receptor, activating FcγR (A). Red receptor, inhibitory FcγR (I).

activating FcγR single nucleotide polymorphisms (SNPs) in kidney transplant recipients. A recent study of a cohort of 85 DSA-positive kidney allograft recipients by Arnold et al. demonstrated that individuals with the high-affinity FcγRIIIA-V158 variant showed higher rate of peritubular capillaritis, an effect independent of C1q-binding or capillary C4d.[418] Functionally, a model NK cell line expressing FcγRIIIA-V158 produced over two fold more IFNγ upon incubation with HLA antibody-coated cells compared with those expressing low-affinity FcγRIIIA-F158, consistent with the potential role of NK cells in driving ABMR.

FcγR associations have also been identified independently of NK cells and FcγRIIIA. Whereas allograft survival was increased in patients with the FcγRIIA-R/R131 genotype in one study,[419] in subsequent studies the same genotype was associated with acute rejection.[420] The FcγRIIA-R131 variant exhibits reduced binding affinity for IgG1 and IgG2 subclasses and has been postulated to contribute to pathology through inefficient clearance of deposited IgG within allografts.[421,422] This is consistent with the high levels FcγRIIA expression on professional phagocytes. Indeed, monocytes expressing the high-affinity FcγRIIA-H/H131 variant were found to adhere more strongly to HLA antibody-activated endothelium.[423] In a larger study of 200 kidney transplant recipients who had lost their grafts, the FcγRIIA-R/R131 genotype was associated with early graft loss, particularly in those patients who were DSA positive.[424]

In mouse models, FcγRIIB-deficient mice develop alloantibody-driven chronic vasculopathy analogous to human chronic rejection in a cardiac allograft model (BM12 organs into C57BL/6 mice).[425] This study is consistent with the known role of FcγRIIB in suppressing humoral immunity, although the exact mechanism of IgG-mediated pathology was not dissected. Indeed, in a model of antibody-mediated nephritis, myeloid-specific FcγRIIB deficiency is sufficient to exacerbate tissue inflammation.[426] A number of nonsynonymous SNPs have been identified in the *FCGR2B* gene in humans, with one occurring at a notable frequency (rs1050501). This SNP encodes an isoleucine-to-threonine substitution at position 232, located within the transmembrane domain.[402] This substitution results in receptor loss of function as a result on impaired lateral mobility and recruitment to signaling domains.[427–429] Surprisingly, despite being a major risk factor in SLE and the heightened inflammatory responses of monocytes from FcγRIIB-T232 individuals to IgG, no association was observed between FcγRIIB-T232 and graft or patient survival in a large study of more than 2800 renal transplant recipients.[430] However, patients were not stratified, for example based on DSA or ABMR status, possibly masking effects.

Curiously, human cultured aortic endothelial cells also express FcγRI and FcγRII and mediated IgG internalization, cytokine production, and the upregulation of adhesion molecules directly.[431,432] This expression can be further enhanced with IFNγ and TNFα in vitro. However, whether this occurs in vivo and whether it contributes to IgG-mediated phenomena in allografts remains to be addressed.

Transplant Tolerance

Given the adverse effect of long-term immunosuppression, one of the key goals in transplantation is the generation of immunologic nonresponsiveness (tolerance) to the allograft. Animal models have suggested that costimulatory blockade may provide, in theory, a mechanism of achieving this (as discussed in the section on T Cell-Mediated Rejection), although this has not directly translated to clinical practice in humans.

A number of immune cell subsets have immunoregulatory capacity, and may therefore contribute to tolerance induction in transplantation, including CD4 T cells, B cells, DCs, and macrophages. These cells broadly act via the production of antiinflammatory cytokines such as IL-10 and TGFβ, and contact-dependent inhibition. The best-characterized regulatory cell subset are $CD4^+CD25^+$ Tregs cells that modulate immune responses via IL-10, TGFβ, and IL-35,[433] adenosine production,[434] down-regulation of DC costimulation, and up-regulation of indoleamine-pyrrole 2,3-dioxygenase (IDO).[215] Tregs also may have more direct actions on effector cell viability through mechanisms that involve granzyme B.[435] The phenotype of $CD4^+CD25^+$ Tregs cells is highly dependent on the expression of the transcription factor Foxp3, which is required to mediate regulatory activity including suppression of IL-2 and IFNγ production, and expression of CTLA-4 and glucocorticoid-induced TNFR-related protein (GITR). Foxp3 is therefore an important marker of Treg activity, although the phenotype of peripherally generated Tregs may not be stable in all circumstances.[436] CTLA-4 appears to play an important role in Treg function,[233,437] engaging CD80/86 on DCs and inducing IDO expression.[215,438] There is ample evidence for the importance of Tregs in many models of experimental transplantation tolerance,[439–441] well-illustrated in models using donor-specific transfusion, and nondepleting anti-CD4 antibody in which Treg function seems to play a crucial mechanistic role.[442] Perhaps more importantly, recent evidence in humanized animal models suggests that the infusion of nonspecifically expanded Tregs can abrogate the acquisition of transplant arteriosclerosis, opening potential translation into the clinical setting.[443–446] In this context, a clinical trial in kidney transplant is underway, the ONE Study, which expands regulatory cells ex vivo (including Tregs) and aims to initially assess safety (https://clinicaltrials.gov/ct2/show/NCT02129881).

Perhaps one of the most surprising findings on the nature of allograft tolerance in humans has been gained by studying the handful of human subjects who fail to reject their allografts in spite of little or no immunosuppression. These observations have arisen in a tiny fraction of noncompliant patients or in cases where immunosuppression has been stopped in the context of malignancy. Analysis of the peripheral blood mononuclear cell (PBMC) transcriptome in these tolerant transplant recipients identified an excess of B cell gene transcripts and an increase in CD20 mRNA in the urine of tolerant subjects.[388,447] In addition, a significant increase in the number of total B cells, particularly memory B cells and B cells expressing CD1d and CD5, has been observed in these subjects,[388] whereas other groups have shown a higher proportion of IL-10-producing $CD24/CD38^{high}$ transitional B cells.[384,385] In contrast, a lower number of IL-10-producing $CD24/CD38^{high}$ transitional B cells has been associated with worse allograft outcome in renal transplant recipients.[387] Together, these data suggest that manipulation of the regulatory fraction of the B cell compartment may also hold utility in efforts to induce transplant tolerance.

Factors Influencing Rejection Beyond the Graft—The Microbiome

The human body is a complex ecosystem of host cells and commensal microorganisms and it is increasingly appreciated that the composition of the microbiome has significant consequences for host homeostasis and inflammation. Indeed, it is clear that the microbiome has a significant influence on the activity of host immune cells, not only within mucosal barrier sites, such as the gastrointestinal tract, but also systemically.[448–450]

With respect to transplant rejection, environment-interfacing organs, such as the gut, are the most likely to be influenced by the composition of the microbiome. For example, monocolonization of germ-free mice with segmented filamentous bacteria is sufficient to induce local Th17 cells that contribute to intestinal inflammation.[451] Conversely, tolerogenic DCs and microbiota-derived short-chain fatty acids have direct roles in the priming of local Treg responses to suppress damaging inflammatory responses.[452] Therefore it is possible that a transplanted organ's microbial flora may dictate subsequent alloimmunity and rejection, opening the way for targeted manipulation of the microbiome in these individuals.[453]

In addition to the importance of the donor organ microbiome, organ failure and transplantation have secondary effects on the recipient microbiome, characterized by a loss of diversity and species richness. Ongoing immunosuppression, prophylactic antibiotic administration, dietary restrictions, and IRI have effects on commensal dysbiosis and the emergence of pathobionts, increasing the risk of enteric infections and inflammation.[454] For example, advanced renal failure is associated with urea and uric acid influx into the gastrointestinal tract and alterations in the intestinal microbiota and increases in microbial families, such as Proteobacteria. A similar dysbiosis is observed in individuals with acute and chronic liver disease and in patients with small bowel transplant rejection. In renal allografts, the magnitude of IR-induced acute kidney injury may be influenced by the gut microbiota, via the production of short-chain fatty acids.[455] In a mouse model of minor antigen-mismatched skin grafts, pretreatment of donor and recipient mice with broad-spectrum antibiotics or the use of germ-free mice significantly prolonged the survival of allografts, attributed to a reduction in the ability of antigen-presenting cells to induce alloreactive T cells.[456] In renal transplant recipients, changes in the urine microbiome have been associated with adverse features on biopsy, including interstitial fibrosis and tubular atrophy.[457] Therefore further research is required to delineate the contribution of the microbiota to allograft rejection.

Conclusion

The immunologic basis of transplant rejection was proposed by Gorer[458] and defined by Medawar, who demonstrated an immune response that is specific for donor tissue, is consequent upon infiltration by leukocytes, and displays memory.[459–462] In the 70 years since these discoveries, our knowledge of the detail and complexity of both innate and adaptive immune systems has been transformed by basic research. After the initial intense focus on T cell response that has been accompanied by increasing success in targeting this pathway to prevent TCMR, attention has now turned to innate and humoral immunity. Innate immune responses mediate sterile inflammation in IRI and compromise immediate and long-term graft function. Organ shortage has necessitated the use of allografts from suboptimal donors that are more susceptible to the effects of IRI. Therefore developing treatments that can ameliorate IRI is of significant clinical interest. With prolonged patient and allograft survival and an increasing number of patients being listed for retransplantation, the issue of allosensitization is also a major problem. The presence of DSA is still a significant barrier to transplantation, and there is need for an increased understanding of the mechanisms of B cell and antibody effector function to identify immunosuppressants that can deal with this arm of the immune system. These studies will need to move away from the historical reliance on mouse models, in part because of significant differences in HLA expression between mouse and human endothelium, and because our increasing genomic and informatic capabilities will enable experimental medicine studies using human subjects, with all their complexity and diversity.

References

1. Naesens M, Sarwal MM. Molecular diagnostics in transplantation. Nat Rev Nephrol 2010;6(10):614–28.
2. Obhrai J, Goldstein DR. The role of toll-like receptors in solid organ transplantation. Transplantation 2006;81(4):497–502.
3. Nakhaei P, et al. RIG-I-like receptors: sensing and responding to RNA virus infection. Semin Immunol 2009;21(4):215–22.
4. Philpott DJ, Girardin SE. Nod-like receptors: sentinels at host membranes. Curr Opin Immunol 2010;22(4):428–34.
5. Ricklin D, et al. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 2010;11(9):785–97.
6. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991–1045.
7. Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol 2008;8(4):279–89.
8. Mariathasan S, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 2006;440(7081):228–32.
9. Yamasaki K, et al. NLRP3/cryopyrin is necessary for interleukin-1beta (IL-1beta) release in response to hyaluronan, an endogenous trigger of inflammation in response to injury. J Biol Chem 2009;284(19):12762–71.
10. Murphy SP, Porrett PM, Turka LA. Innate immunity in transplant tolerance and rejection. Immunol Rev 2011;241(1):39–48.
11. Land WG, et al. Transplantation and damage-associated molecular patterns (DAMPs). Am J Transplant 2016;16(12):3338–61.
12. Jin C, Flavell RA. Molecular mechanism of NLRP3 inflammasome activation. J Clin Immunol 2010;30(5):628–31.
13. Zhou R, et al. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010;11(2):136–40.
14. Steri M, et al. Overexpression of the cytokine BAFF and autoimmunity risk. N Engl J Med 2017;376(17):1615–26.
15. Walport MJ. Complement. First of two parts. N Engl J Med 2001;344(14):1058–66.
16. Walport MJ. Complement. Second of two parts. N Engl J Med 2001;344(15):1140–4.
17. Zuber J, et al. New insights into postrenal transplant hemolytic uremic syndrome. Nat Rev Nephrol 2011;7(1):23–35.
18. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turning offensive. Nat Rev Nephrol 2016;12(7):383–401.
19. Pickering MC, et al. C3 glomerulopathy: consensus report. Kidney Int 2013;84(6):1079–89.
20. Servais A, et al. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. Kidney Int 2012;82(4):454–64.

21. Zand L, et al. Clinical findings, pathology, and outcomes of C3GN after kidney transplantation. J Am Soc Nephrol 2014;25(5): 1110–7.
22. McCaughan JA, O'Rourke DM, Courtney AE. Recurrent dense deposit disease after renal transplantation: an emerging role for complementary therapies. Am J Transplant 2012;12(4):1046–51.
23. Thurman JM, et al. Lack of a functional alternative complement pathway ameliorates ischemic acute renal failure in mice. J Immunol 2003;170(3):1517–23.
24. Thurman JM, et al. Acute tubular necrosis is characterized by activation of the alternative pathway of complement. Kidney Int 2005;67(2):524–30.
25. Thurman JM, et al. Altered renal tubular expression of the complement inhibitor Crry permits complement activation after ischemia/reperfusion. J Clin Invest 2006;116(2):357–68.
26. de Vries B, et al. The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. Am J Pathol 2004;165(5):1677–88.
27. Moller-Kristensen M, et al. Mannan-binding lectin recognizes structures on ischemic reperfused mouse kidneys and is implicated in tissue injury. Scand J Immunol 2005;61(5):426–34.
28. Farrar CA, et al. Local extravascular pool of C3 is a determinant of postischemic acute renal failure. FASEB J 2006;20(2):217–26.
29. Parker MD, et al. Ischemia-reperfusion injury and its influence on the epigenetic modification of the donor kidney genome. Transplantation 2008;86(12):1818–23.
30. Damman J, et al. Local renal complement C3 induction by donor brain death is associated with reduced renal allograft function after transplantation. Nephrol Dial Transplant 2011;26(7):2345–54.
31. Zheng X, et al. Protection of renal ischemia injury using combination gene silencing of complement 3 and caspase 3 genes. Transplantation 2006;82(12):1781–6.
32. Zhou W, et al. Predominant role for C5b-9 in renal ischemia/reperfusion injury. J Clin Invest 2000;105(10):1363–71.
33. de Vries B, et al. Complement factor C5a mediates renal ischemia-reperfusion injury independent from neutrophils. J Immunol 2003;170(7):3883–9.
34. de Vries B, et al. Inhibition of complement factor C5 protects against renal ischemia-reperfusion injury: inhibition of late apoptosis and inflammation. Transplantation 2003;75(3):375–82.
35. Arumugam TV, et al. A small molecule C5a receptor antagonist protects kidneys from ischemia/reperfusion injury in rats. Kidney Int 2003;63(1):134–42.
36. Zheng X, et al. Gene silencing of complement C5a receptor using siRNA for preventing ischemia/reperfusion injury. Am J Pathol 2008;173(4):973–80.
37. Ichim TE, et al. RNA interference: a potent tool for gene-specific therapeutics. Am J Transplant 2004;4(8):1227–36.
38. Patel H, et al. Therapeutic strategy with a membrane-localizing complement regulator to increase the number of usable donor organs after prolonged cold storage. J Am Soc Nephrol 2006;17(4):1102–11.
39. Sacks S, et al. Targeting complement at the time of transplantation. Adv Exp Med Biol 2013;735:247–55.
40. Kaabak M, et al. A prospective randomized, controlled trial of eculizumab to prevent ischemia-reperfusion injury in pediatric kidney transplantation. Pediatr Transplant 2018;22(2).
41. Kirby JA. Function of leukcocyte adhesion molecules during allograft rejection. In: Tilney NL, Strom TB, Paul LC, editors. *Transplantation Biology: Cellular and Molecular Aspects*. Philadelphia, PA: Lippincott-Raven Publishers; 1996.
42. Stepkowski SM. Therapeutic potential for adhesion antagonists in organ transplantation. Curr Opin Organ Transplant 2002;7:366–72.
43. McEver RP, et al. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 1989;84(1):92–9.
44. Pober JS, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumour necrosis factor and immune interferon. J Immunol 1986;137:1893–6.
45. Cose S, et al. Evidence that a significant number of naive T cells enter non-lymphoid organs as part of a normal migratory pathway. Eur J Immunol 2006;36(6):1423–33.
46. Laskowski I, et al. Molecular and cellular events associated with ischemia/reperfusion injury. Ann Transplant 2000;5(4):29–35.
47. McDonald B, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. Science 2010;330(6002):362–6.
48. Karmakar M, et al. Neutrophil P2X7 receptors mediate NLRP3 inflammasome-dependent IL-1beta secretion in response to ATP. Nat Commun 2016;7:10555.
49. Jones ND, et al. Regulatory T cells can prevent memory CD8+ T-cell-mediated rejection following polymorphonuclear cell depletion. Eur J Immunol 2010;40(11):3107–16.
50. Kish DD, et al. Neutrophil expression of Fas ligand and perforin directs effector CD8 T cell infiltration into antigen-challenged skin. J Immunol 2012;189(5):2191–202.
51. Kreisel D, et al. Emergency granulopoiesis promotes neutrophil-dendritic cell encounters that prevent mouse lung allograft acceptance. Blood 2011;118(23):6172–82.
52. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013;13(3):159–75.
53. Sayah DM, et al. Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation. Am J Respir Crit Care Med 2015;191(4):455–63.
54. Fournier BM, Parkos CA. The role of neutrophils during intestinal inflammation. Mucosal Immunol 2012;5(4):354–66.
55. Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. Annu Rev Immunol 2015;33:643–75.
56. Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. Immunity 2014;41(1):21–35.
57. Dannappel M, et al. RIPK1 maintains epithelial homeostasis by inhibiting apoptosis and necroptosis. Nature 2014;513(7516): 90–4.
58. Takahashi N, et al. RIPK1 ensures intestinal homeostasis by protecting the epithelium against apoptosis. Nature 2014;513(7516): 95–9.
59. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol 2016;12(1):49–62.
60. Nauseef WM, Borregaard N. Neutrophils at work. Nat Immunol 2014;15(7):602–11.
61. Glass CK, Natoli G. Molecular control of activation and priming in macrophages. Nat Immunol 2016;17(1):26–33.
62. Chen CJ, et al. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. Nat Med 2007;13(7):851–6.
63. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140(6):821–32.
64. Tsung A, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005; 201(7):1135–43.
65. Armstrong DA, et al. Neutrophil chemoattractant genes KC and MIP-2 are expressed in different cell populations at sites of surgical injury. J Leukoc Biol 2004;75(4):641–8.
66. Chen Y, et al. ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors. Science 2006;314(5806):1792–5.
67. Springer TA. Adhesion receptors of the immune system. Nature 1990;346(6283):425–34.
68. Wu H, et al. HMGB1 contributes to kidney ischemia reperfusion injury. J Am Soc Nephrol 2010;21(11):1878–90.
69. Leemans JC, et al. Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney. J Clin Invest 2005;115(10):2894–903.
70. Wu H, et al. TLR4 activation mediates kidney ischemia/reperfusion injury. J Clin Invest 2007;117(10):2847–59.
71. Iyer SS, et al. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. Proc Natl Acad Sci USA 2009;106(48):20388–93.
72. Melnikov VY, et al. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. J Clin Invest 2002;110(8):1083–91.
73. Faubel S, et al. Caspase-1-deficient mice are protected against cisplatin-induced apoptosis and acute tubular necrosis. Kidney Int 2004;66(6):2202–13.
74. Liu HF, et al. Effects of specific interleukin-1beta-converting enzyme inhibitor on ischemic acute renal failure in murine models. Acta Pharmacol Sin 2005;26(11):1345–51.
75. Kelly KJ, et al. Antibody to intercellular adhesion molecule 1 protects the kidney against ischemic injury. Proc Natl Acad Sci USA 1994;91(2):812–6.
76. Kelly KJ, et al. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. J Clin Invest 1996; 97(4):1056–63.

77. Rabb H, et al. Role of CD11a and CD11b in ischemic acute renal failure in rats. Am J Physiol 1994;267(6 Pt 2):F1052–8.
78. Haug CE, et al. A phase I trial of immunosuppression with anti-ICAM-1 (CD54) mAb in renal allograft recipients. Transplantation 1993;55(4):766–72. Discussion 772-3.
79. Salmela K, et al. A randomized multicenter trial of the anti-ICAM-1 monoclonal antibody (enlimomab) for the prevention of acute rejection and delayed onset of graft function in cadaveric renal transplantation: a report of the European Anti-ICAM-1 Renal Transplant Study Group. Transplantation 1999;67(5):729–36.
80. Takada M, et al. Early cellular and molecular changes in ischemia/reperfusion injury: inhibition by a selectin antagonist, P-selectin glycoprotein ligand-1. Transplant Proc 1997;29(1-2):1324–5.
81. Singbartl K, Green SA, Ley K. Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. FASEB J 2000;14(1):48–54.
82. Osama Gaber A, et al. YSPSL (rPSGL-Ig) for improvement of early renal allograft function: a double-blind, placebo-controlled, multicenter Phase IIa study(1,2,3). Clin Transplant 2011;25(4):523–33.
83. Haas M, et al. Banff. 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014;14(2):272–83.
84. Sund S, et al. Glomerular monocyte/macrophage influx correlates strongly with complement activation in 1-week protocol kidney allograft biopsies. Clin Nephrol 2004;62(2):121–30.
85. Pilmore HL, et al. Early up-regulation of macrophages and myofibroblasts: a new marker for development of chronic renal allograft rejection. Transplantation 2000;69(12):2658–62.
86. Hanvesakul R, et al. KIR and HLA-C interactions promote differential dendritic cell maturation and is a major determinant of graft failure following kidney transplantation. PLoS One 2011;6(8):e23631.
87. Hanvesakul R, et al. Donor HLA-C genotype has a profound impact on the clinical outcome following liver transplantation. Am J Transplant 2008;8(9):1931–41.
88. Uehara S, et al. NK cells can trigger allograft vasculopathy: the role of hybrid resistance in solid organ allografts. J Immunol 2005;175(5):3424–30.
89. Murphy WJ, Kumar V, Bennett M. Rejection of bone marrow allografts by mice with severe combined immune deficiency (SCID): evidence that NK cells can mediate the specificity of marrow graft rejection. J Exp Med 1987;165:1212–7.
90. Murphy WJ, Kumar V, Bennett M. Acute rejection of murine bone marrow allografts by natural killer cells and T cells: differences in kinetics and target antigens recognized. J Exp Med 1987;166(5):1499–509.
91. Armstrong HE, et al. Prolonged survival of actively enhanced rat renal allografts despite accelerated cellular infiltration and rapid induction of both class I and class II MHC antigens. J Exp Med 1987;165(3):891–907.
92. Bradley JA, Mason DW, Morris PJ. Evidence that rat renal allografts are rejected by cytotoxic T cells and not by nonspecific effectors. Transplantation 1985;39(2):169–75.
93. Kim J, et al. The activating immunoreceptor NKG2D and its ligands are involved in allograft transplant rejection. J Immunol 2007;179(10):6416–20.
94. Kirwan SE, Burshtyn DN. Regulation of natural killer cell activity. Curr Opin Immunol 2007;19(1):46–54.
95. van Bergen J, et al. KIR-ligand mismatches are associated with reduced long-term graft survival in HLA-compatible kidney transplantation. Am J Transplant 2011;11(9):1959–64.
96. Karre K. NK cells, MHC class I molecules and the missing self. Scand J Immunol 2002;55(3):221–8.
97. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today 1990;11(7):237–44.
98. Herberman RB, et al. Natural killer cells: characteristics and regulation of activity. Imm Rev 1979;44:43–70.
99. Bix M, et al. Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice. Nature 1991;349:329–31.
100. Eberl G, et al. Innate lymphoid cells: a new paradigm in immunology. Science 2015;348(6237):aaa6566.
101. Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity 2012;37(4):601–10.
102. Sonnenberg GF, et al. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. Immunity 2011;34(1):122–34.
103. Zaiss DM, et al. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. Immunity 2015;42(2):216–26.
104. Hanash AM, et al. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. Immunity 2012;37(2):339–50.
105. Lindemans CA, et al. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. Nature 2015;528(7583):560–4.
106. Bernink JH, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol 2013;14(3):221–9.
107. Vely F, et al. Evidence of innate lymphoid cell redundancy in humans. Nat Immunol 2016;17(11):1291–9.
108. Sallusto F, Lanzavecchia A. Heterogeneity of CD4+ memory T cells: functional modules for tailored immunity. Eur J Immunol 2009;39(8):2076–82.
109. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. Cell 2001;106:263–6.
110. Worbs T. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. J Exp Med 2006;203(3):519–27.
111. Esterházy D, et al. Classical dendritic cells are required for dietary antigen–mediated induction of peripheral Treg cells and tolerance. Nat Immunol 2016;17(5):545–55.
112. Coombes JL, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 2007;204(8):1757–64.
113. Denning TL, et al. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. J Immunol (Baltimore, MD:1950) 2011;187(2):733–47.
114. den Dunnen J, et al. IgG opsonization of bacteria promotes Th17 responses via synergy between TLRs and FcγRIIa in human dendritic cells. Blood 2012;120(1):112–21.
115. Coccia M, et al. IL-1β mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. J Experiment Med 2012;209(9):1595–609.
116. Revu S, et al. IL-23 and IL-1β drive human Th17 cell differentiation and metabolic reprogramming in absence of CD28 costimulation. Cell Rep 2018;22(10):2642–53.
117. Kullberg MC, et al. IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. J Exp Med 2006;203(11):2485–94.
118. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime Rep. 2014;6:13.
119. Hutchinson JA, et al. Cutting edge: immunological consequences and trafficking of human regulatory macrophages administered to renal transplant recipients. J Immunol 2011;187(5):2072–8.
120. Puga I, et al. B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. Nat Immunol 2011;13(2):170–80.
121. Leliefeld PH, Koenderman L, Pillay J. How neutrophils shape adaptive immune responses. Front Immunol 2015;6:471.
122. Chu VT, et al. Eosinophils promote generation and maintenance of immunoglobulin-A-expressing plasma cells and contribute to gut immune homeostasis. Immunity 2014;40(4):582–93.
123. Chu VT, et al. Eosinophils are required for the maintenance of plasma cells in the bone marrow. Nat Immunol 2011;12(2):151–9.
124. Wong TW, et al. Eosinophils regulate peripheral B cell numbers in both mice and humans. J Immunol (Baltimore, MD: 1950) 2014;192(8):3548–58.
125. Hepworth MR, et al. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4+ T cells. Science 2015;348(6238):1031–5.
126. Hepworth MR, et al. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. Nature 2013;498(7452):113–7.
127. Oliphant CJ, et al. MHCII-Mediated dialog between group 2 innate lymphoid cells and CD4+ T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. Immunity 2014;41(2):283–95.

128. von Burg N, et al. Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. Proc Nat Acad Sci USA 2014;111(35):12835–40.
129. Magri G, et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. Nat Immunol 2014;15(4):354–64.
130. Peugh WN, et al. The role of H-2 and non-H-2 antigens and genes in the rejection of murine cardiac allografts. Immunogenetics 1986;23(1):30–7.
131. Flowers ME, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood 2011;117(11):3214–9.
132. Dallman MJ, Mason DW, Webb M. Induction of Ia antigens on murine epidermal cells during the rejection of skin allografts. Eur J Immunol 1982;12:511–8.
133. de Waal RMW, et al. Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. Nature (Lond) 1983;303:426–9.
134. Fellous M, et al. Interferon-dependent induction of mRNA for the major histocompatibility antigens in human fibroblasts and lymphoblastoid cells. Proc Natl Acad Sci USA 1982;79:3082–6.
135. Fuggle SV, McWhinnie D, Morris PJ. Precise specificity of induced tubular class II antigens in renal allografts. Transplantation 1987;44:214–20.
136. Mason DW, Dallman MJ, Barclay AN. Graft-versus-host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium. Nature 1981;293:150–1.
137. Tewari MK, et al. A cytosolic pathway for MHC class II-restricted antigen processing that is proteasome and TAP dependent. Nat Immunol 2005;6(3):287–94.
138. Bevan MJ. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J Exp Med 1976;143(5):1283–8.
139. Chicz RM, et al. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. Nature 1992;358(6389):764–8.
140. O'Brien SJ, et al. Genetic basis for species vulnerability in the cheetah. Science 1985;227:1428–34.
141. Felix NJ, et al. Alloreactive T cells respond specifically to multiple distinct peptide-MHC complexes. Nat Immunol 2007;8(4):388–97.
142. Ely LK, et al. T-cells behaving badly: structural insights into alloreactivity and autoimmunity. Curr Opin Immunol 2008;20(5):575–80.
143. Carosella ED, Gregori S, LeMaoult J. The tolerogenic interplay(s) among HLA-G, myeloid APCs, and regulatory cells. Blood 2011;118(25):6499–505.
144. Deschaseaux F, et al. HLA-G in organ transplantation: towards clinical applications. Cell Mol Life Sci 2011;68(3):397–404.
145. Milner CM, Campbell RD. Genetic organization of the human MHC class III region. Front Biosci 2001;6:D914–26.
146. Turner D, et al. Cytokine gene polymorphism and heart transplant rejection. Transplantation 1997;64(5):776–9.
147. Scott DM, et al. Identification of a mouse male-specific transplantation antigen H-Y. Nature 1995;376:695–8.
148. Wang W, et al. Human H-Y: a male-specific histocompatibility antigen derived from the SMCY protein. Science 1995;269:1588–90.
149. Greenfield A, et al. An H-YDb epitope is encoded by a novel mouse Y chromosome gene. Nat Genet 1996;14. 474-48.
150. Scott DM, et al. Why do some females reject males? The molecular basis for male-specific graft rejection. J Mol Med 1997;75(2):103–14.
151. Superina RA, et al. Assessment of primarily vascularized cardiac allografts in mice. Transplantation 1986;42:226–7.
152. Fabre JW, Morris PJ. Studies on the specific suppression of renal allograft rejection in presensitised rats. Transplantation 1975;19:121–33.
153. Steinmuller D, Wachtal SS. Passenger leukocytes and induction of allograft immunity. Transplant Proc 1980;12:100–6.
154. Hart DN, Fabre JW. Kidney-specific alloantigen system in the rat: characterisation and role in transplantation. J Exp Med 1980;151:651–66.
155. Roopenian D, Choi EY, Brown A. The immunogenomics of minor histocompatibility antigens. Immunol Rev 2002;190:86–94.
156. Simpson E, Roopenian D, Goulmy E. Much ado about minor histocompatibility antigens. Immunol Today 1998;19:108–12.
157. Goulmy E. Minor histocompatibility antigens: from transplantation problems to therapy of cancer. Hum Immunol 2006;67(6):433–8.
158. Gurley KE, Lowry RP, Clarke-Forbes RD. Immune mechanisms in organ allograft rejection: II. T helper cells, delayed type hypersensitivity and rejection of renal allografts. Transplantation 1983;36:401–5.
159. Hall BM, DeSaxe I, Dorsch SE. The cellular basis of allograft rejection in vivo: restoration of first set rejection of heart grafts by T helper cells in irradiated rats. Transplantation 1983;36:700–5.
160. Lowry RP, Gurley KE, Forbes RD. Immune mechanisms in organ allograft rejection. I. Delayed-type hypersensitivity and lymphocytotoxicity in heart graft rejection. Transplantation 1983;36(4):391–401.
161. Loveland BE, et al. Delayed type hypersensitivity and allograft rejection in the mouse: correlation of effector cell phenotype. J Exp Med 1981;153:1044–57.
162. Steinman RM, Witmer MD. Lymphoid dendritic cells are potent stimulations of the primary mixed leucocyte reaction in mice. Proc Natl Acad Sci USA 1978;75:5132–6.
163. Daar AS, et al. Demonstration and phenotypic characterisation of HLA-DR positive interstitial dendritic cells widely distributed in human connective tissue. Transplant Proc 1983;XV(suppl. 1):311–5.
164. Hart DN, Fabre JW. Demonstration and characterisation of Ia positive dendritic cells in the interstitial connective tissues of the rat heart and other tissues, but not brain. J Exp Med 1981;154:347–61.
165. Berry MR, et al. Renal sodium gradient orchestrates a dynamic antibacterial defense zone. Cell 2017;170(5):860–74.
166. Larsen CP, Morris PJ, Austyn JM. Migration of dendritic leukocytes from cardiac allografts into host spleens: a novel pathway for initiation of rejection. J Exp Med 1990;171(1):307–14.
167. Larsen CP, et al. Migration and maturation of Langerhans cells in skin transplants and explants. J Exp Med 1990;172:1483–93.
168. Reis e Sousa C, Stahl PD, Austyn JM. Phagocytosis of antigens by Langerhans cells in vitro. J Exp Med 1993;178:509–19.
169. Steinman RM, et al. Dendritic cells are the peripheral stimulators of the primary mixed leukocyte reaction in mice. J Exp Med 1983;157:613–27.
170. Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. Curr Top Microbiol Immunol 2006;311:17–58.
171. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol 2003;21:685–711.
172. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. Annu Rev Immunol 2005;23:975–1028.
173. Mahnke K, Enk AH. Dendritic cells: key cells for the induction of regulatory T cells? Curr Top Microbiol Immunol 2005;293:133–50.
174. Rotschke O, et al. On the nature of peptides involved in T cell alloreactivity. J Exp Med 1991;174:1059–71.
175. Golding H, Singer A. Role of accessory cell processing and presentation of shed H-2 alloantigens in allospecific cytotoxic T lymphocyte responses. J Immunol 1984;133:597–605.
176. Parham P, et al. Inhibition of alloreactive cytotoxic T lymphocytes by peptides from the OL2 domain of HLA-A2. Nature 1987;325:625–8.
177. Morris GP, Ni PP, Allen PM. Alloreactivity is limited by the endogenous peptide repertoire. Proc Natl Acad Sci USA 2011;108(9):3695–700.
178. Wise M, et al. CD4 T cells can reject major histocompatibility complex class I-incompatible skin grafts. Eur J Immunol 1999;29(1):156–67.
179. Auchincloss H, et al. The role of "indirect" recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. Proc Natl Acad Sci USA 1993;90(8):3373–7.
180. Lee RS, et al. Indirect recognition by helper cells can induce donor-specific cytotoxic T lymphocytes in vivo. J Exp Med 1994;179(3):865–72.
181. Lee RS, et al. CD8+ effector cells responding to residual class I antigens, with help from CD4+ cells stimulated indirectly, cause rejection of "major histocompatibility complex-deficient" skin grafts. Transplantation 1997;63(8):1123–33.
182. Najafian N, et al. Enzyme-linked immunosorbent spot assay analysis of peripheral blood lymphocyte reactivity to donor HLA-DR peptides: potential novel assay for prediction of outcomes for renal transplant recipients. J Am Soc Nephrol 2002;13(1):252–9.
183. Poggio ED, et al. Alloreactivity in renal transplant recipients with and without chronic allograft nephropathy. J Am Soc Nephrol 2004;15(7):1952–60.

184. Baker RJ, et al. Loss of direct and maintenance of indirect alloresponses in renal allograft recipients: implications for the pathogenesis of chronic allograft nephropathy. J Immunol 2001;167(12):7199–206.
185. Hornick PI, et al. Significant frequencies of T cells with indirect anti-donor specificity in heart graft recipients with chronic rejection. Circulation 2000;101(20):2405–10.
186. Waanders MM, et al. Monitoring of indirect allorecognition: wishful thinking or solid data? Tissue Antigens 2008;71(1):1–15.
187. Smith HJ, et al. T Lymphocyte responses to nonpolymorphic HLA-derived peptides are associated with chronic renal allograft dysfunction. Transplantation 2011;91(3):279–86.
188. Shirwan H. Chronic allograft rejection. Do the Th2 cells preferentially induced by indirect alloantigen recognition play a dominant role? Transplantation 1999;68(6):715–26.
189. Yamada A, et al. Further analysis of the T-cell subsets and pathways of murine cardiac allograft rejection. Am J Transplant 2003;3(1):23–7.
190. Cramer DV, et al. Cardiac transplantation in the rat I. The effect of histocompatibility differences on graft arteriosclerosis. Transplantation 1989;47:414–9.
191. Nadazdin O, et al. Contributions of direct and indirect alloresponses to chronic rejection of kidney allografts in nonhuman primates. J Immunol 2011;187(9):4589–97.
192. Bestard O, et al. Circulating alloreactive T cells correlate with graft function in longstanding renal transplant recipients. J Am Soc Nephrol 2008;19(7):1419–29.
193. Sauve D, et al. Alloantibody production is regulated by CD4+ T cells' alloreactive pathway, rather than precursor frequency or Th1/Th2 differentiation. Am J Transplant 2004;4(8):1237–45.
194. Conlon TM, et al. Germinal center alloantibody responses are mediated exclusively by indirect-pathway CD4 T follicular helper cells. J Immunol 2012;188(6):2643–52.
195. Lachmann N, et al. Antihumoral rejection therapy by proteasome inhibitor bortezomib: a case series. Clin Transpl 2009:351–8.
196. Hourmant M, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol 2005;16(9):2804–12.
197. Herrera OB, et al. A novel pathway of alloantigen presentation by dendritic cells. J Immunol 2004;173(8):4828–37.
198. Bedford P, Garner K, Knight SC. MHC class II molecules transferred between allogeneic dendritic cells stimulate primary mixed leukocyte reactions. Int Immunol 1999;11(11):1739–44.
199. Harshyne LA, et al. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. J Immunol 2001;166(6):3717–23.
200. Russo V, et al. Acquisition of intact allogeneic human leukocyte antigen molecules by human dendritic cells. Blood 2000;95(11):3473–7.
201. Love PE, Hayes SM. ITAM-mediated signaling by the T-cell antigen receptor. Cold Spring Harb Perspect Biol 2010;2(6):a002485.
202. Wang H, et al. ZAP-70: an essential kinase in T-cell signaling. Cold Spring Harb Perspect Biol 2010;2(5):a002279.
203. Balagopalan L, et al. The LAT story: a tale of cooperativity, coordination, and choreography. Cold Spring Harb Perspect Biol 2010;2(8):a005512.
204. Jordan MS, Koretzky GA. Coordination of receptor signaling in multiple hematopoietic cell lineages by the adaptor protein SLP-76. Cold Spring Harb Perspect Biol 2010;2(4):a002501.
205. Wucherpfennig KW, et al. Structural biology of the T-cell receptor: insights into receptor assembly, ligand recognition, and initiation of signaling. Cold Spring Harb Perspect Biol 2010;2(4):a005140.
206. Fernandes RA, et al. What controls T cell receptor phosphorylation? Cell 2010;142(5):668–9.
207. Lamb JR, et al. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. J Exp Med 1983;157:1434–47.
208. Jenkins MK, et al. Molecular events in the induction of a nonresponsive state in interleukin 2-producing helper T-lymphocyte clones. Proc Natl Acad Sci USA 1987;84:5409–13.
209. Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. J Exp Med 1987;165:302–19.
210. Schwartz RH. A cell culture model for T lymphocyte clonal anergy. Science 1990;248:1349–56.
211. Schwartz RH. T cell clonal anergy. Curr Opin Immunol 1997;9:351–7.
212. Carlin LM, et al. Secretion of IFN-gamma and not IL-2 by anergic human T cells correlates with assembly of an immature immune synapse. Blood 2005;106(12):3874–9.
213. Frasca L, et al. Anergic T cells effect linked suppression. Eur J Immunol 1997;27:3191–7.
214. Lombardi G, et al. Anergic T cells as suppressor cells in vitro. Science 1994;264:1587–9.
215. Cobbold SP, et al. Connecting the mechanisms of T-cell regulation: dendritic cells as the missing link. Immunol Rev 2010;236:203–18.
216. Linsley PS, Clark EA, Ledbetter JA. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc Natl Acad Sci USA 1990;87:5031–5.
217. Archdeacon P, et al. Summary of the US FDA approval of belatacept. Am J Transplant 2012;12(3):554–62.
218. Dharnidharka VR. Costimulation blockade with belatacept in renal transplantation. N Engl J Med 2005;353(19):2085–6. Author reply:2085–6.
219. Clarkson MR, Sayegh MH. T-cell costimulatory pathways in allograft rejection and tolerance. Transplantation 2005;80(5):555–63.
220. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol 2005;23:515–48.
221. Riley JL, June CH. The CD28 family: a T-cell rheostat for therapeutic control of T-cell activation. Blood 2005;105(1):13–21.
222. Kawai K, et al. Skin allograft rejection in CD28-deficient mice. Transplantation 1996;61(3):352–5.
223. Sharpe AH, Abbas AK. T-cell costimulation—biology, therapeutic potential, and challenges. N Engl J Med 2006;355(10):973–5.
224. Turka LA, et al. T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. Proc Natl Acad Sci USA 1992;89(22):11102–5.
225. Pearson TC, et al. Transplantation tolerance induced by CTLA-4 Ig. Transplantation 1994;57:1701–6.
226. Lenschow DJ, et al. CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. Immunity 1996;5:285–93.
227. Collins AV, et al. The interaction properties of costimulatory molecules revisited. Immunity 2002;17(2):201–10.
228. Schwartz JC, et al. Structural mechanisms of costimulation. Nat Immunol 2002;3(5):427–34.
229. Fallarino F, Fields PE, Gajewski TF. B7-1 engagement of cytotoxic T lymphocyte antigen 4 inhibits T cell activation in the absence of CD28. J Exp Med 1998;188(1):205–10.
230. Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med 1996;183(6):2533–40.
231. Schneider H, et al. Reversal of the TCR stop signal by CTLA-4. Science 2006;313(5795):1972–5.
232. Wing K, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322(5899):271–5.
233. Ise W, et al. CTLA-4 suppresses the pathogenicity of self antigen-specific T cells by cell-intrinsic and cell-extrinsic mechanisms. Nat Immunol 2010;11(2):129–35.
234. Vincenti F, et al. Three-year outcomes from BENEFIT, a randomized, active-controlled, parallel-group study in adult kidney transplant recipients. Am J Transplant 2012;12(1):210–7.
235. Pestana JO, et al. Three-year outcomes from BENEFIT-EXT: a phase III study of belatacept versus cyclosporine in recipients of extended criteria donor kidneys. Am J Transplant 2012;12(3):630–9.
236. Masson P, et al. Belatacept for kidney transplant recipients. Cochrane Database Syst Rev 2014;11:CD010699.
237. Kim EJ, et al. Costimulation blockade alters germinal center responses and prevents antibody-mediated rejection. Am J Transplant 2014;14(1):59–69.
238. Linterman MA, Vinuesa CG. Signals that influence T follicular helper cell differentiation and function. Semin Immunopathol 2010;32(2):183–96.
239. Zhu Y, Yao S, Chen L. Cell surface signaling molecules in the control of immune responses: a tide model. Immunity 2011;34(4):466–78.
240. Larsen CP, et al. Long term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996;381:434–8.

241. Larsen CP, et al. CD40-gp39 interactions play a critical role during allograft rejection: suppression of allograft rejection by blockade of the CD40-gp39 pathway. Transplantation 1996;61(1):4–9.
242. Kirk AD, et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat Med 1999;5(6):686–93.
243. Badell IR, et al. Nondepleting anti-CD40-based therapy prolongs allograft survival in nonhuman primates. Am J Transplant 2012;12(1):126–35.
244. Weaver TA, et al. Alefacept promotes co-stimulation blockade-based allograft survival in nonhuman primates. Nat Med 2009;15(7): 746–9.
245. Lo DJ, et al. Selective targeting of human alloresponsive CD8+ effector memory T cells based on CD2 expression. Am J Transplant 2011;11(1):22–33.
246. Badell IR, et al. LFA-1-specific therapy prolongs allograft survival in rhesus macaques. J Clin Invest 2010;120(12):4520–31.
247. Grgic I, et al. Blockade of T-lymphocyte KCa3.1 and Kv1.3 channels as novel immunosuppression strategy to prevent kidney allograft rejection. Transplant Proc 2009;41(6):2601–6.
248. De Jager PL, et al. The role of the CD58 locus in multiple sclerosis. Proc Natl Acad Sci USA 2009;106(13):5264–9.
249. Bloomgren G, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med 2012;366(20):1870–80.
250. Nashan B, et al. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. CHIB. 201 International Study Group. Lancet 1997;350(9086):1193–8.
251. Vincenti F, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. N Engl J Med 1998;338(3):161–5.
252. Steiger J, et al. IL-2 knockout recipient mice reject islet cell allografts. J Immunol 1995;155:489–98.
253. Saleem S, et al. Acute rejection of vascularized heart allografts in the absence of IFNg. Transplantation 1996;62:1908–11.
254. Dai Z, Lakkis FG. The role of cytokines, CTLA-4 and costimulation in transplant tolerance and rejection. Curr Opin Immunol 1999;11:504–8.
255. Sakaguchi S, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8–27.
256. Maloy KJ, Powrie F. Fueling regulation: IL-2 keeps CD4+ Treg cells fit. Nat Immunol 2005;6(11):1071–2.
257. Warnecke G, et al. CD4+ regulatory T cells generated in vitro with IFN-{gamma} and allogeneic APC inhibit transplant arteriosclerosis. Am J Pathol 2010;177(1):464–72.
258. Sawitzki B, et al. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function in vivo. J Exp Med 2005;201(12):1925–35.
259. Wood KJ, Sawitzki B. Interferon gamma: a crucial role in the function of induced regulatory T cells in vivo. Trends Immunol 2006;27(4):183–7.
260. Mackay CR. Homing of naive, memory and effector lymphocytes. Curr Opin Immunol 1993;5:423–7.
261. Mackay CR. Immunological memory. Adv Immunol 1993;53:217–65.
262. Lakkis FG, et al. Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. Nat Med 2000;6(6):686–8.
263. Lakkis FG. Where is the alloimmune response initiated? Am J Transplant 2003;3(3):241–2.
264. Zhou P, et al. Secondary lymphoid organs are important but not absolutely required for allograft responses. Am J Transplant 2003;3(3):259–66.
265. Ingham-Clark CL, et al. Lymphocyte infiltration patterns in rat small-bowel transplants. Transplant Proc 1990;22:2460.
266. Kim PC, et al. Immunologic basis of small intestinal allograft rejection. Transplant Proc 1991;23:830.
267. Toogood GJ, et al. The immune response following small bowel transplantation II: a very early cytokine response in the gut associated lymphoid tissue. Transplantation 1997;63:1118–23.
268. Toogood GJ, et al. The immune response following small bowel transplantation I. An unusual pattern of cytokine expression. Transplantation 1996;62:851–5.
269. Baddoura FK, et al. Lymphoid neogenesis in murine cardiac allografts undergoing chronic rejection. Am J Transplant 2005;5(3):510–6.
270. Salvadori M, et al. FTY720 versus MMF with cyclosporine in de novo renal transplantation: a 1-year, randomized controlled trial in Europe and Australasia. Am J Transplant 2006;6(12):2912–21.
271. Tedesco-Silva H, et al. Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. Transplantation 2006;82(12):1689–97.
272. Hoitsma AJ, et al. FTY720 combined with tacrolimus in de novo renal transplantation: 1-year, multicenter, open-label randomized study. Nephrol Dial Transplant 2011;26(11):3802–5.
273. Butcher EC. The regulation of lymphocyte traffic. Curr Top Microbiol Immunol 1986;128:85–122.
274. Picker LJ, Butcher EC. Physiological and molecular mechanisms of lymphocyte homing. Annu Rev Immunol 1992;10:561–91.
275. Santamaria-Babi LF, et al. Migration od skin-homing T cells across cytokine-activated human endothelial cell layers involves interaction of the cutaneous lymphocyte-associated antigen (CLA), the very late antigen-4 (VLA-4) and the lymphocyte function-associated antigen-1 (LFA-1). J Immunol 1995;154:1543–50.
276. Marelli-Berg FM, et al. Antigen recognition influences transendothelial migration of CD4+ T cells. J Immunol 1999;162:696–703.
277. Cosimi AB, Conti D, e.a. Delmonico FL. In vivo effects of monoclonal antibody to ICAM-1 (CD54) in nonhuman primates with renal allografts. J Immunol 1990;144:4604–12.
278. Dallman MJ, et al. Lymphokine production in allografts-analysis of RNA by northern blotting. Transplant Proc 1989;20:296–8.
279. Hourmant M, et al. A randomized multicenter trial comparing leukocyte function-associated antigen-1 monoclonal antibody with rabbit antithymocyte globulin as induction treatment in first kidney transplantations. Transplantation 1996;62(11):1565–70.
280. Yang HC, et al. In situ expression of platelet-derived growth factor (PDGF-beta) during chronic rejection is abolished by retransplantation. J Surg Res 1995;59:205–10.
281. Morikawa M, et al. Cardiac allografts in rat recipients with simultaneous use of anti-ICAM-1 and anti-LFA-1 monoclonal antibodies leads to accelerated graft loss. Immunopharmacology 1994;28:171–82.
282. Stepkowski SM, et al. An oral formulation for intracellular adhesion molecules-1 antisense oligonucleotides. Transplant Proc 2001;33(7-8):3271.
283. Nemoto T, et al. Small molecule selectin ligand inhibition improves outcome in ischemic acute renal failure. Kidney Int 2001;60(6):2205–14.
284. Subramanian S, et al. Attenuation of renal ischemia-reperfusion injury with selectin inhibition in a rabbit model. Am J Surg 1999;178(6): 573–6.
285. Hancock WW. Chemokines and transplant immunobiology. J Am Soc Nephrol 2002;13(3):821–4.
286. Hancock WW, et al. Chemokines and their receptors as markers of allograft rejection and targets for immunosuppression. Curr Opin Immunol 2003;15(5):479–86.
287. Merani S, et al. Chemokines and their receptors in islet allograft rejection and as targets for tolerance induction. Cell Transplant 2006;15(4):295–309.
288. Oppenheim JJ, et al. The role of chemokines in transplantation. In: Tilney NL, Strom TB, Paul LC, editors. *Transplantation Biology: Cellular and Molecular Aspects*. Philadelphia: Lippincott-Raven Publishers; 1996. p. 187–200.
289. Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annu Rev Immunol 2000;18:217–42.
290. Lentsch AB, et al. Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and KC. Hepatology 1998;27:1172–7. [Corrected and republished article originally printed in Hepatology. 1998;27(2):507–12].
291. DeVries ME, Ran L, Kelvin D. On the edge: the physiological and pathophysiological role of chemokines during inflammatory and immunological responses. Semin Immunol 1999;11:95–104.
292. Fairchild RL, et al. Expression of chemokine genes during rejection and long-term acceptance of cardiac allografts. Transplantation 1997;63(12):1807–12.
293. Grandaliano G, et al. Monocyte chemotactic peptide-1 expression and monocyte infiltration in acute renal transplant rejection. Transplantation 1997;63(3):414–20.
294. Gao JW, et al. Polymorphisms in cytotoxic T lymphocyte associated antigen-4 influence the rate of acute rejection after renal transplantation in 167 Chinese recipients. Transpl Immunol 2012;26(4): 207–11.