

27. Transplantation immunology: Organ and bone marrow

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The discovery of the human MHC in 1967 launched the field of organ and tissue transplantation. More than 800,000 such transplants have been performed during this time. Although matching of donor and recipient for MHC antigens was shown to be of great importance and continues to be so, the development of pharmacologic agents and antilymphocyte antibodies that interfere with the process of graft rejection has had a crucial role in the success of organ transplantation during the past 2 decades. Enormous progress has been made in understanding the immunologic mechanisms of graft rejection and of graft-versus-host disease. The roles of antibodies, antigen-presenting cells, helper and cytotoxic T cells, immune cell surface molecules, and signaling mechanisms and the cytokines they release have been clarified. This understanding is leading to the development of newer immunosuppressive agents targeting various components of the rejection process. Combinations of these agents work synergistically, leading to lower doses and reduced toxicity. Similarly, the development of effective T-cell depletion techniques has been of great importance for bone marrow transplantation when an HLA-identical sibling is not available. The major obstacle to the performance of solid organ transplantation currently is the shortage of donor organs. (*J Allergy Clin Immunol* 2003;111:S733-44.)

Key words: MHC, HLA, haplotype, mechanisms of graft rejection, immunosuppressive agents, organ transplantation, bone marrow transplantation, immune reconstitution, nonmyeloablative, gene therapy

Although transplantation of organs or tissues from one human being to another had been tried for many centuries, it was not accomplished until the past 3 and a half decades. Success in this endeavor followed the discovery of the human MHC in 1967.¹ Identification of this genetic region launched the field of clinical organ and tissue transplantation. In 1968, the World Health Organization Nomenclature Committee designated that the leukocyte antigens controlled by the closely linked genes of the human MHC be named HLA (for *human leukocyte antigen*).

TRANSPLANTATION ANTIGENS

The MHC

Histocompatibility antigens are tissue cell surface antigens capable of inducing an immune response in a genetically dissimilar (allogeneic) recipient, resulting in the rejection

Abbreviations used

ALG:	Antilymphocyte globulin
APC:	Antigen-presenting cell
ATG:	Antithymocyte globulin
GVHD:	Graft-versus-host disease
IL:	Interleukin
MUD:	Matched unrelated donor
SCID:	Severe combined immunodeficiency

tion of the tissues or cells bearing those antigens. The genes that encode these antigens reside in the MHC region on the short arm of human chromosome 6 (Fig 1). The HLA complex contains more than 200 genes, more than 40 of which encode leukocyte antigens.^{2,3} They and their cell surface and soluble protein products are divided into three classes (I, II, and III) on the basis of their tissue distribution, structure, and function.³⁻⁵ MHC class I and II genes encode codominantly expressed HLA cell surface antigens, and class III genes encode several components of the complement system; all share important roles in immune function.

Class I MHC antigens are present on all nucleated cells and are each composed of a 45-kd α heavy chain encoded by genes of the HLA-A, HLA-B, or HLA-C loci on chromosome 6 and associated noncovalently with a 12-kd protein, β_2 -microglobulin encoded by a gene on chromosome 15 (Fig 2).³ MHC class II antigens have a more limited tissue distribution and are expressed only on B lymphocytes, activated T lymphocytes, monocytes, macrophages, Langerhans cells, dendritic cells, endothelium, and epithelial cells.⁵ Each is a heterodimer composed of noncovalently associated α and β chains of approximately 230 amino acids encoded by genes of the HLA-D region (Fig 2). On cells expressing both class I and class II HLA antigens, there are three class I antigens and three or more (usually four) class II heterodimers. Class III genes are located between the HLA-B and HLA-D loci and determine the structure of three components of the complement system: C2, C4, and factor B.^{3,4}

HLA antigens are inherited in a Mendelian dominant manner. Because of the closeness of the different loci of the MHC and the resultant low crossover frequency, however, HLA genes are almost always inherited together. This fixed combination of genetic determinants is referred to as a *haplotype*. Because chromosome 6 is an autosome, all individuals have two HLA haplotypes (one for each chromosome), and there are only four possible combinations of haplotypes among the offspring of any two parents.

The ABO system

ABO incompatibility does not cause stimulation in mixed leukocyte cultures, indicating that ABO compatibility

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.142

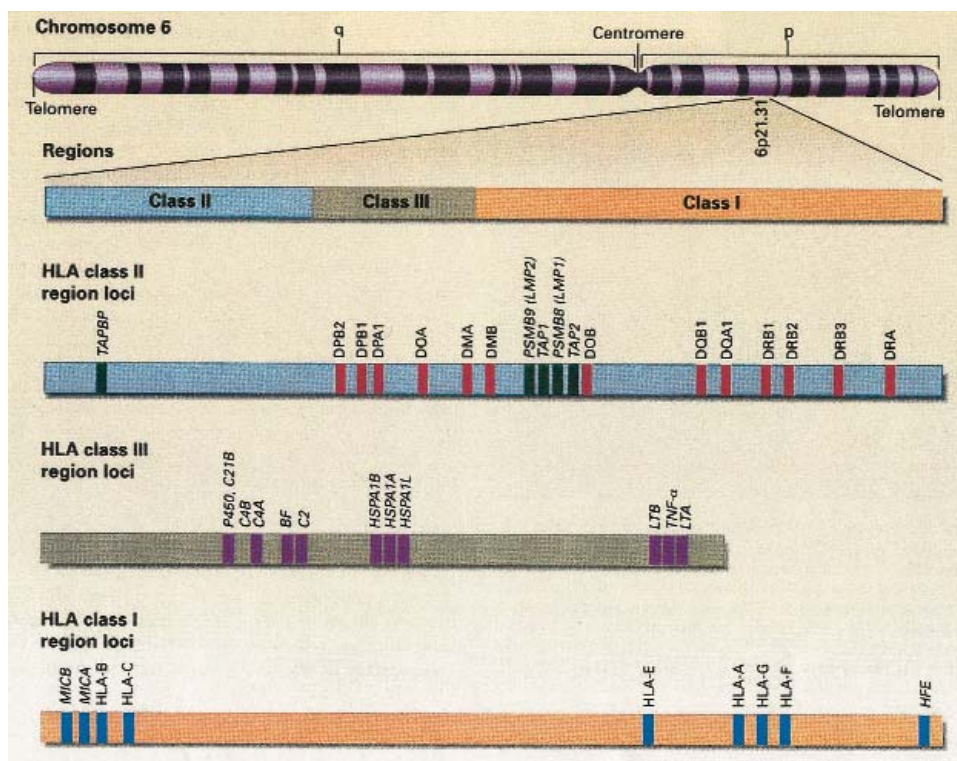


FIG 1. Location and organization of the HLA complex on chromosome 6. *BF*, Complement factor B; *C2*, complement component 2; *C4A*, complement component 4A; *C4B*, complement component 4B; *TAP1*, transporter of antigenic peptides 1; *TAP2*, transporter of antigenic peptides 2; *LTA*, lymphotoxin A; *LTB*, lymphotoxin B. From Klein J, Sato A. The HLA system: first of two parts. *N Engl J Med* 2000;343:703. Reprinted with permission.

bility is of much less importance than HLA compatibility in graft survival. However, ABO incompatibility can result in hyperacute rejection of primarily vascularized grafts, such as kidney and heart.⁶ This occurs because (1) ABO blood group antigens are present on kidney and cardiac grafts, particularly those from A or B secretors, and (2) preformed naturally occurring antibodies to blood group substances are present in mismatched recipients.

Donor-recipient matching

Two general methods are used to pair donors and recipients for transplantation. The first involves the detection of HLA antigens on donor and recipient leukocytes by either serologic or DNA typing methods; the second involves the measurement of the response of immunocompetent cells from the recipient to antigens present on donor cells (and vice versa for bone marrow transplantation). Disparities that are serologically detected are referred to as *antigen mismatches*, whereas differences that can be identified only by DNA-based typing are called *allele mismatches*.

Cross-matching

Serologic cross-matching is of particular importance to the success of primarily vascularized grafts, such as

kidney and heart. Serum from the prospective recipient is tested against cells from the potential donor for the presence of antibodies to red blood cell or HLA antigens. The presence of such antibodies correlates with hyperacute renal graft rejection.⁶ For this reason, a positive serologic cross-match has been considered an absolute contraindication to renal transplantation.

Usefulness of HLA typing in clinical organ and tissue transplantation

Although typing for intrafamilial transplants of all types is clearly of great value, the usefulness of HLA typing in cadaveric kidney grafting has been a point of controversy since cyclosporine became available.⁷ Although short-term survival rates did not appear to be that different for closely or poorly matched cadaveric kidneys, the degree of HLA matching is correlated with long-term survival. Until 1980, usually only HLA-identical siblings could be used as bone marrow donors, because both graft rejection and lethal graft-versus-host disease (GVHD) are common complications if this is not the case.⁸ Fortunately, the development during the past 2 decades of techniques to rigorously deplete post-thymic T cells from donor marrow has permitted numerous successful half-matched marrow transplants with no or minimal GVHD.^{9,10}

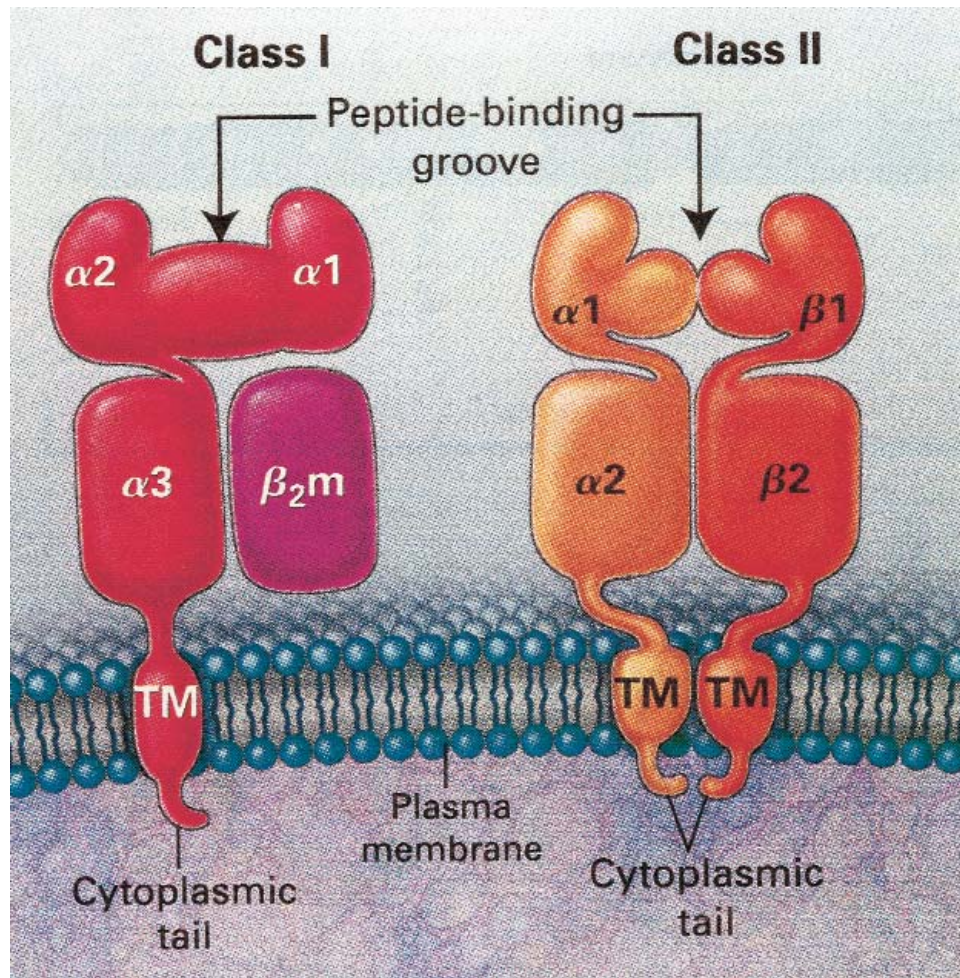


FIG 2. Structures of HLA class I and class II molecules. β_2 -Microglobulin (β_2m) is the light chain of the class I molecule. TM, Transmembrane component. From Klein J, Sato A. The HLA system: first of two parts. N Engl J Med 2000;343:704. Reprinted with permission.

MECHANISMS OF GRAFT REJECTION

Role of antibody

The strongest evidence for a role for antibody in graft rejection is the hyperacute rejection of primarily vascularized organs, such as the kidney and heart. Antidonor antibodies can be demonstrated in recipients undergoing these reactions.⁶ These antibodies combine with HLA antigens on endothelial cells, with subsequent complement fixation and accumulation of polymorphonuclear cells. Endothelial damage then occurs, probably as a result of enzymes released from polymorphonuclear leukocytes; platelets then accumulate, thrombi develop, and the result is renal cortical necrosis or myocardial infarction.

Leukocytes and cytokines in graft rejection

Allograft rejection results from the coordinated activation of alloreactive T cells and antigen-presenting cells (APCs). Although acute rejection is a T-cell-dependent process, the destruction of the allograft results from a

broad array of effector mechanisms. Cell-cell interactions and the release by primed helper T cells of multiple types of cytokines (interleukin [IL]-2, IL-4, IL-5, IL-7, IL-10, IL-15, tumor necrosis factor- α , and interferon- γ) recruit not only immunocompetent donor-specific CD4⁺ T cells, CD8⁺ cytotoxic T cells, and antibody-forming B cells but also nonspecific inflammatory cells, which constitute the majority of cells infiltrating an allograft.¹¹

Stimulation of CD4⁺ T cells through their antigen receptors is not sufficient to initiate T-cell activation, unless costimulation is provided by interaction of other ligand-receptor pairs present on the surfaces of T cells and APCs during the encounter. Some of these interactive pairs include the T-cell surface molecule CD2 and its ligand CD58 on APCs; CD11a/CD18:CD54; CD5:CD72; CD40 ligand:CD40; and CD28:CD80 or CD86. Unless signals are provided through one or more of these receptor-ligand interactions (particularly through CD40L: CD40 and CD28:CD80 or CD86) or by cytokines (such as IL-1 and IL-6 from the APC), CD4⁺ T-cell anergy or tolerance induction occurs when the T-cell receptor inter-

acts with the APC.¹² Thus, T-cell accessory proteins and their ligands on APCs are target molecules for antirejection therapy. If costimulation does occur, the CD4⁺ T cell becomes activated, which leads to stable transcription of genes important in T-cell activation.

CD8⁺ T cells recognize antigenic peptides displayed on MHC class I molecules and represent a major cytotoxic effector lymphocyte population in graft rejection. Donor class I molecules on donor APCs in the graft directly activate cytotoxic effector lymphocytes. However, CD8 activation also requires a costimulatory second signal, as well as an IL-2 signal. It has been shown that activation of CD8⁺ cytotoxic effector lymphocytes is highly dependent on signaling through the γ chain of multiple cytokine receptors, whereas this is not true for activation of CD4⁺ T cells.¹² Activated CD8⁺ T cells proliferate and mature into specific alloreactive clones capable of releasing granzyme (serine esterase), perforin, and toxic cytokines, such as tumor necrosis factor- α .

Cyclosporine and tacrolimus interfere significantly with the activation process initiated by interaction of APCs with the T-cell receptor and many of the costimulatory molecules. An exception, however, is the CD28:CD80 or CD86 costimulatory pathway, which is independent of protein kinase C and calcium but can also lead to stable transcription of the IL-2 gene and other activation genes. That pathway is resistant to inhibition by cyclosporine and tacrolimus. Stimulation of the B cell by antigen occurs through its antigen receptor (surface immunoglobulin), but costimulation is also required for B-cell activation. This costimulation can be provided by cytokines released by T cells or through many of the same T-cell protein-ligand pairs important in T-cell-APC costimulation, because these ligands are also present on B cells.

Once T-cell activation has occurred, autocrine T-cell proliferation continues as a consequence of the expression of the IL-2 receptor (IL-2R). Interaction of IL-2 with its receptor triggers the activation of protein tyrosine kinases and phosphatidylinositol-3-kinase, resulting in translocation into the cytosol of an IL-2R-bound serine-threonine kinase, Raf-1. This in turn leads to the expression of several DNA-binding proteins, such as c-Jun, c-Fos, and c-Myc, and to progression of the cell cycle.¹¹ The consequence of all of these events is the development of graft-specific, infiltrating cytotoxic T cells. Cytokines from the T cells also activate macrophages and other inflammatory leukocytes and cause upregulation of HLA molecules on graft cells. The activated T cells also stimulate B cells to produce anti-graft antibodies. Ultimately, all these cellular and humoral factors destroy the graft.

IMMUNOSUPPRESSION

Because there is no method that will suppress the host's immune response to antigens of the graft and at the same time maintain other immune responses, rejection must be prevented by nonspecific immunosuppressive agents. The development of immunosuppressive strate-

gies during the past 4 decades reflects enormous progress in understanding the cellular and molecular mechanisms that mediate allograft rejection.¹³ The success of transplantation between unrelated donors and recipients can be attributed to implementation of these strategies. Because these agents depress both specific and nonspecific immunity, however, they render the recipient more susceptible to both infection and malignancy. Indeed, infection is the most important cause of transplant recipient death. Thus, all patients must have the immunosuppressive regimen fine-tuned to prevent rejection yet minimize the risk of infection—too high a dose, and infection supervenes; too small a dose, and the graft is rejected.

The immunosuppressive agents used in most centers for nearly 2 decades were corticosteroids, azathioprine and cyclosporine. Several new agents have been introduced during the past few years: mycophenolate mofetil, which has a similar but more effective mode of action to that of azathioprine; tacrolimus, which has a mode of action and side effects similar to those of cyclosporine, and sirolimus; which blocks IL-2-induced T-cell cycle progression.

Immunosuppressive agents can be categorized by whether they (1) interrupt lymphocyte cell division, (2) deplete lymphocytes, (3) interfere with lymphocyte maturational events, (4) interfere with immune cell costimulation, (5) modulate ischemia-reperfusion injury, or (6) facilitate induction of tolerance.¹³ They can also be grouped into those used for induction therapy, for prophylaxis against rejection, for reversal of acute rejection episodes, and for maintenance immunosuppression.

Antilymphocyte and antithymocyte globulins

Antibodies from animals immunized with human lymphoid cells are useful agents for induction therapy, as well as for reversal of acute rejection episodes.¹⁴ They consist of the IgG fraction of serum from horses or rabbits immunized with either lymphocytes (antilymphocyte globulin [ALG]) or thymocytes (antithymocyte globulin [ATG, Thymoglobulin]) or of murine monoclonal antibodies to T-cell surface antigens (CD3, OKT3).¹⁵ In general, ALG, ATG, and OKT3 decrease the onset, severity, and number of rejection episodes.

Monoclonal antibodies to cytokine receptors

Prevention of graft rejection has also been approached by inhibiting cytokines from interacting with their receptors. Chimeric or humanized murine anti-IL-2R α chain antibodies have been developed for clinical use.^{16,17} The advantage of these monoclonal antibodies to the IL-2R α chain is that such molecules are present only on activated T lymphocytes; therefore the main effect is on T cells possibly activated by graft antigens.

Calcineurin inhibitors

The main action of calcineurin inhibitors (cyclosporine and tacrolimus) is that they prevent the synthesis of IL-2 and other cytokines that might be produced by T cells activated by allografts.¹⁸ Through its hydrophobicity, cyclosporine penetrates cell membranes

TABLE I. Solid organ rejection patterns: Renal rejection as an example

Type	Time after transplantation	Signs and symptoms	Rapidity of onset	Immune component	Pathologic findings	Treatment	Success rate (%)
Hyperacute	<24 h	Fever, anuria	Hours	Antibody and complement	Polymorphonuclear neutrophil deposition and thrombosis	None	0
Accelerated	3-5 d	Fever, graft swelling, oliguria, tenderness	1 d	Non-complement-fixing antibody	Vascular disruption, hemorrhage	ALG, ATG, anti-CD3	60
Acute	6-90 d	Oliguria, salt retention, graft swelling, tenderness, sometimes fever	Days to weeks	T cells and antibody	Tubulitis, endovasculitis	Steroids, ALG, ATG, anti-CD3	60-90
Chronic	>60 d	Edema, hypertension, proteinuria, occasional hematuria	Months to years	Antibody	Vascular onion skinning	None	0

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to gain access to and bind to the cytoplasmic isomerase protein cyclophilin. The complex then inhibits calcineurin, an intracellular phosphatase critical for the translocation of signals from the T-cell receptor to the nucleus. In this manner, it blocks transcription of the IL-2 gene. In addition, it also blocks the synthesis of other cytokines and thereby interferes with activated CD4⁺ helper T-lymphocyte function.¹⁸ As a consequence, T-cell proliferation and differentiation of precursor cytotoxic lymphocytes are blocked. Tacrolimus binds to a cytoplasmic isomerase protein in the same way that cyclosporine does, but it binds to a different one, the FK-binding protein.¹⁹ The complex formed inhibits calcineurin to prevent T-cell receptor signal transduction to the nucleus. Tacrolimus thus inhibits synthesis of IL-2, IL-3, interferon- γ , and other cytokines; however, it was found to be 100 times more potent than cyclosporine as an immunosuppressive agent.^{19,20}

Cytokine receptor signal transduction inhibitors

Sirolimus (Rapamune) has a structure similar to tacrolimus, and its activity is dependent on its binding to the FK-binding protein. However, the complex formed does not inhibit calcineurin but instead prevents the phosphorylation of p70^{S6} kinase. This blocks signal transduction from many cell-surface cytokine receptors, including IL-2R, IL-4R, IL-15R, and IL-10R.²¹ Both in vitro and in vivo studies have shown a synergistic effect of sirolimus with cyclosporine, as would be expected because sirolimus prevents cytokine receptor signaling and cyclosporine inhibits cytokine production.^{22,23}

Combination therapy

No agent is the perfect nonspecific immunosuppressive drug. Antilymphocyte antibodies, nucleoside synthesis inhibitors, steroids, cyclosporine (or tacrolimus), anti-IL-2R α chain, and sirolimus all affect allorecognition

and antigen-driven T-cell proliferation at different points in the T-cell activation process. Thus the combined use of several of these types of agents provides a synergistic effect, rather than a merely additive effect.¹³

SOLID ORGAN TRANSPLANTATION

The explosive growth of transplantation since the discovery of HLA in 1967 is attested by the fact that by 2001, a total of 535,075 kidney transplants had been reported from 588 transplant centers worldwide, along with 117,984 stem cell transplants from 249 centers, 61,195 heart transplants from 236 centers, 100,179 liver transplants from 235 centers, 17,002 pancreas or pancreas-kidney transplants from 163 centers, 297 intestinal transplants from 10 centers, and 16,432 lung or heart-lung transplants from 120 centers, for a total of 848,164 transplants in the last half century.²⁴

Kidney transplantation

Despite major improvements in dialysis techniques, renal transplantation remains the treatment of choice for end-stage renal disease in patients of nearly all ages.^{25,26} There is no lack of recipients for such transplants. Estimates of new cases of end-stage renal disease range from 1.5 to 3 cases/million people annually.

For adults and most children, the renal transplant operation has become standardized.²⁷ The earlier practice of removing the patient's diseased kidneys 2 to 3 weeks before transplantation has not been carried out routinely in recent years, except for patients with hypertension or infection, and nephrectomy is now performed at the time of transplantation.

Immunosuppressive regimens. Until cyclosporine became available in the early 1980s, most centers used a combination of azathioprine (Imuran) and prednisone to prevent graft rejection. Beginning in 1983, many centers began to use cyclosporine (in lieu of azathioprine) with

lower doses of prednisone for immunosuppression.²⁸ Cyclosporine has been given in varying doses at different centers but has generally been given intravenously during or just after transplantation and on the day after. It is then subsequently administered orally and gradually tapered, depending on signs of toxicity or rejection and blood levels. Trough blood levels are monitored by a radioimmunoassay, and doses are adjusted to maintain them above 200 ng/mL. Prednisone is given on the day of transplantation and gradually reduced during the course of 12 weeks. In many centers the induction agents consist of one of the anti-IL-2R α chain antibodies, Dacluzimab or Basileximab, along with steroids, mycophenolate mofetil (instead of azathioprine), and tacrolimus (instead of cyclosporine). Some transplant surgeons are combining tacrolimus with steroids and sirolimus.²⁹ Acute rejection episodes are treated with intravenous pulses of high-dose methylprednisolone.^{30,31} Among the most useful agents have been ALG for 5 days, ATG for 5 days, and OKT3 for 1 to 14 days.

Rejection. Rejection is the most common problem during the 3 months immediately after kidney grafting.^{32,33} Except for hyperacute rejection, most such episodes can be partially or completely reversed by one of the previously described immunosuppressive agents. Rejection episodes are classified as follows (Table I):

1. Hyperacute rejection occurs within the first 48 hours after the anastomosis takes place in recipients with preformed antileukocyte antibodies. It is characterized by fever and anuria. The binding of cytotoxic antibodies to the vascular endothelium activates complement, with subsequent aggregation of neutrophils and platelets, resulting in thrombosis. This is an irreversible event, and the only treatment option is immediate graft removal.
2. Accelerated rejection occurs on the third to the fifth day after transplantation. It is accompanied by fever, graft swelling, oliguria, and tenderness. It is thought to be mediated by non-complement-fixing antibodies to antigens present in the donor kidney. Histopathologically, it is characterized by vascular disruption with hemorrhage. The most effective treatments are antilymphocyte reagents, with or without plasmapheresis; these have a success rate of about 60% in reversing this process.
3. Acute rejection, the most common form, is due to a primary allogeneic response occurring within the first 6 to 90 days after transplantation. It is mediated by both T cells and antibodies, which cause tubulitis and vasculitis, respectively. High-dose pulses of steroids and antilymphocyte reagents are effective in reversing the T-cell response about 80% to 90% of the time, but antilymphocyte antibodies only reverse the vasculitis about 60% of the time.
4. Chronic rejection occurs when the tenuous graft tolerance is disturbed 2 or more months after transplantation. It is characterized by marked proteinuria, occasional hematuria, hypertension, and the nephrotic syndrome. The primary mediator of this type of

rejection is antibody. A kidney biopsy is usually necessary to distinguish rejection from cyclosporine or tacrolimus nephrotoxicity. There is no effective treatment.

Efficacy. Renal grafts from HLA-identical sibling donors have a 10-year survival of about 74%. Those from "6 antigen-matched" cadavers have a survival of 57% (65% if HLA identical by DNA typing) at 10 years. Grafts from family members sharing one haplotype have a 10-year survival of about 54%. Those from HLA-mismatched cadaveric donors have a survival of 40% at 10 years.²⁶

Liver transplantation

Liver transplantation had its inception in 1963, when the diseased liver of a 3-year-old child with extrahepatic biliary atresia was replaced.³⁴ Although that patient died, subsequent successes have established liver transplantation as standard therapy for advanced chronic liver disease.³⁵ Since 1983, the 1-year survival rates have increased from 25% to 78%, depending on the age and health of the recipient, the underlying condition, and various clinical considerations.

Liver transplantation is indicated for chronic end-stage liver disease, fulminant acute liver failure, and cancer limited to the liver.³⁶ As with renal transplantation, combined therapy attacking several facets of the potential rejection process is used for liver transplantation. Anti-IL-2R α chain antibodies are given intravenously on the day of transplantation, followed by tacrolimus, which is given intravenously initially and orally thereafter, and then by mycophenolate mofetil and steroids tapered slowly over a year. Survival has increased by 20% in the last 12 years with tacrolimus-based immunosuppression. Although this improvement may be the result of several factors, retransplantation as a result of acute or chronic rejection has been completely eliminated in patients treated with tacrolimus.

As is the case for all solid organ transplantation, lack of suitable donors is a major problem for liver transplantation. Since 1988 this organ shortage has been approached by partial hepatectomies of living related donors.³⁷ Also, single cadaveric livers have been used for two patients. Donor safety is much greater with use of the left lateral segment; the recipients undergo total hepatectomies.

Heart, heart-lung, and lung transplantation

The various forms of cardiomyopathy are the most common indications for heart transplantation, followed by congenital heart disease. Immunosuppressive regimens for heart transplantation are similar in many respects to those already described for renal and hepatic grafts. Usually an anti-IL-2R α chain monoclonal antibody is given for induction therapy on the day of transplantation, along with high-dose intravenous methylprednisolone. Prednisone is given after the operation and maintained orally until it is discontinued after the first normal findings from an endomyocardial biopsy. Tacrolimus is then begun as the primary immunosuppressive agent.³⁸

Since the introduction of cyclosporine 20 years ago, the results of cardiac transplantation have improved greatly. The International Heart Transplantation Registry has shown a 4-year survival of 71% for patients receiving cyclosporine- or tacrolimus-based triple immunosuppression therapy.³⁹ Survival is influenced by the age of the recipient; patients younger than 40 years have a better survival.

Heart-lung transplantation has been used most successfully in the treatment of cystic fibrosis.⁴⁰ Lung transplantation has been performed for the following major diagnostic categories: cystic fibrosis, pulmonary vascular disease, bronchiolitis obliterans, pulmonary alveolar proteinosis, and pulmonary fibrosis.

BONE MARROW TRANSPLANTATION

Since 1955, more than 117,984 bone marrow transplantations have been performed worldwide at 249 centers in the treatment of more than 50 different fatal diseases.²⁴ Most of these transplants have been autologous, with 25,000 to 35,000 such transplants performed annually, compared with approximately 15,000 allogeneic transplants done annually.

Certain unique problems distinguish bone marrow transplantation from grafting of solid organs, such as the kidney, liver, and heart. The first problem is that immunocompetent cells both in the recipient and in the donor marrow or blood have the potential to reject each other, resulting in graft rejection on the one hand and GVHD on the other.⁴¹ The second concern is that successful unfractionated marrow grafting usually requires strict donor and recipient MHC class II antigen compatibility to minimize such reactions.⁴¹ Finally, except for patients with severe combined immunodeficiency (SCID), complete DiGeorge anomaly, or identical twin donors, even HLA-identical recipients have to be treated in advance with cytotoxic and myeloablative agents to prevent graft rejection.⁴¹

Diseases treated successfully by allogeneic bone marrow transplantation include radiation injury, primary immunodeficiencies, hemoglobinopathies, aplastic anemia, multiple myeloma, leukemia, neuroblastoma, non-Hodgkin lymphoma, inborn errors of metabolism, and certain autoimmune diseases.⁴² In addition, autologous marrow transplantation has been used with lethal irradiation or chemotherapy in the treatment of patients with some hematologic malignancies, solid tumors, or breast cancer and is the most common type of bone marrow transplant currently being performed.⁴³

Clinical features of GVHD

Acute GVHD begins 6 or more days after transplantation (or after transfusion in the case of nonirradiated blood products).⁴⁴ Signs of GVHD include fever, a morbilliform erythematous rash, and severe diarrhea.⁴⁵ The rash becomes progressively confluent and may involve the entire body surface; it is both pruritic and painful and eventually leads to marked exfoliation. Eosinophilia and

lymphocytosis develop, followed shortly by hepatosplenomegaly, exfoliative dermatitis, protein-losing enteropathy, bone marrow aplasia, generalized edema, susceptibility to infection, and death.⁴⁶ Skin biopsy specimens reveal basal vacuolar degeneration or necrosis, spongiosis, single-cell dyskeratosis, eosinophilic necrosis of epidermal cells, and a dermal perivascular round cell infiltration. Similar necrotic changes occur in the liver, intestinal tract, and eventually most other tissues.

Treatment of GVHD

Many regimens have been used to mitigate GVHD in both HLA-incompatible and HLA-compatible bone marrow transplants. In MHC-compatible bone marrow transplants into patients with SCID or complete DiGeorge anomaly, it is not usually necessary to give immunosuppressive agents to prevent or mitigate the mild GVHD that may occur, although occasionally steroids are used to treat more severe forms of the condition. For unfractionated HLA-identical marrow transplants into all patients for whom pretransplantation chemotherapy is given to prevent rejection, however, it is necessary to use prophylaxis against GVHD. Patients usually are given methotrexate on the first, third, sixth, and 11th days after transplantation and weekly thereafter until day 100 and receive cyclosporine daily for 6 months.⁴⁷ Newer immunosuppressive regimens are being explored, including mycophenolate mofetil with methotrexate and cyclosporine.⁴⁸

Once GVHD has become established, it is extremely difficult to treat. Antithymocyte serum, steroids, cyclosporine, tacrolimus, anti-IL-2R α chain antibodies, mycophenolate mofetil, and murine monoclonal antibodies to human T-cell surface antigens have ameliorated some cases, but the course has been inexorably fatal in many patients similarly treated.^{49,50} The best approach to GVHD reactions is preventive, and by far the best preventive approach is the removal of all post-thymic T cells from the donor marrow or blood.

HLA-identical bone marrow transplantation for severe T-cell immunodeficiency

The only adequate therapy for patients with severe forms of cellular immunodeficiency is immunologic reconstitution by transplantation of immunocompetent tissue. Until 1980, only HLA-identical unfractionated bone marrow could be used for this purpose because of the lethal GVHD that ensued if mismatched donors were used.⁵¹ In most cases, both T-cell and B-cell immunity have been reconstituted by such fully matched transplants, with evidence of function detected very soon after unfractionated marrow transplantation.⁵² Analysis of the genetic origins of the immune cells in the engrafted patients has revealed that, although the T cells are all of donor origin, the B cells are often those of the recipient.⁹ Initially, it was considered that bone marrow was effective in conferring immunity in patients with SCID, because it provided normal stem cells, but it is apparent from later experience with T-cell-depleted marrow⁵³ that the early restoration of immune function after unfrac-

tionated HLA-identical marrow transplantation is by adoptive transfer of mature T and B cells in the donor marrow.⁵² Unfortunately, unfractionated bone marrow transplantation has not been possible for more than 85% of the immunodeficient patients who could have benefited because of the lack of HLA-identical donors.¹⁰ As a consequence, most such patients died before 1982.⁵¹

HLA-haploidentical bone marrow transplantation for severe T-cell deficiency

The fact that totally HLA-disparate fetal liver cells could correct the immune defect in a few such patients without causing GVHD gave hope that HLA-disparate marrow stem cells could do the same if all donor post-thymic T cells could be removed. Early success in T-cell depletion was achieved in experimental animals by treating donor marrow or spleen cells with anti-T-cell antiserum or agglutinating the unwanted cells with plant lectins.⁵⁴ The remaining immature marrow or splenic non-T cells restored lymphohematopoietic function to lethally irradiated MHC-disparate recipients without lethal GVHD. This approach was applied to human beings in the early 1980s and has been highly successful in infants with SCID.

The time to development of immune function after haploidentical stem cell grafts is quite different from that after unfractionated HLA-identical marrow. Lymphocytes with mature T-cell phenotypes and functions fail to rise significantly until 3 to 4 months after transplantation; normal T-cell function is reached between 4 and 7 months.¹⁰ B-cell function develops much more slowly, averaging 2 to 2.5 years for normalization; many do not have B-cell function develop, despite normal T-cell function.¹⁰ Genetic analyses of the lymphocytes from such chimeric patients have revealed all T cells to be genetically donor, whereas the B cells and APCs almost always remain those of the recipient.¹⁰

These observations indicate that the thymic microenvironment of most infants with SCID is capable of differentiating half-matched normal stem cells to mature and functioning T lymphocytes that can cooperate effectively with host B cells for antibody production.⁵⁶ Thus, the genetic defect in most cases does not involve the thymus.

Efficacy of bone marrow transplantation in immunodeficiency diseases

Although precise figures are not available, during the past 34 years, more than 1200 patients worldwide with different forms of genetically determined immunodeficiency have been given bone marrow transplants in attempts to correct their underlying immune defects. Possibly because of earlier diagnosis before untreatable opportunistic infections develop, the results have improved considerably during the last 2 decades.⁵⁵ A worldwide survey conducted by me from 1994 through 1997, with subsequent additions of published cases from the literature, revealed that 239 of 302 or 79% of patients with primary immunodeficiency transplanted with HLA-identical marrow during a period of 34 years were surviving.

SCID. Bone marrow transplantation has been more widely applied and more successful in infants with SCID than any other primary immunodeficiency. The use of pretransplant myelosuppressive or myeloablative conditioning is advocated by some investigators, but since infants with SCID lack T cells, there is no need to give pretransplantation chemotherapy. From 1968 to 1977, only 14 of 48 infants with SCID (29%) were long-term survivors of successful HLA class II compatible bone marrow transplants.⁵¹ In the same survey and literature review, only 126 infants with SCID were reported as having received HLA-identical marrow, and 106 or 84% were surviving. By contrast, 477 infants with SCID had received haploidentical marrow, and 301 or 63% survived worldwide; however, the haploidentical transplants were not T-cell depleted until after 1980. Nevertheless, this is a major accomplishment, because SCID is 100% fatal without marrow transplantation or, in the case of adenosine deaminase-deficient SCIDs, enzyme-replacement therapy.⁵⁷ A longitudinal study of 193 European patients with SCID who had received haploidentical T-cell-depleted bone marrow transplants, most of whom had received pretransplantation chemotherapy, revealed that only 92, or 48%, were long-term survivors.⁵⁸ During the past 2 decades, my associates and I have performed transplants in 128 infants with SCID, and 100 of these (78%) are currently surviving from 1 month to more than 20 years after transplantation. No pretransplantation conditioning was given except to 3 infants who also received cord blood transplants. Only 15 had HLA-identical donors; all 15 are surviving with functioning grafts. One hundred thirteen patients with SCID received half-matched related donor stem cells prepared with the soy lectin sheep red blood cell rosetting T-cell depletion technique and 85, or 75%, of these patients are currently surviving. These studies have shown that such transplants can provide normal numbers of T cells and normalize T-cell function in all known molecular types of SCID. Most impressive is the fact that 34 of 35 infants (97%) undergoing transplantation in the first 3.5 months of life are currently surviving.^{9,10} Thus, there appears to be no advantage in performing such transplants in utero^{59,60} as opposed to performing them soon after birth.¹⁰ In utero transplants also carry the risks associated with injecting the fetus and the inability to detect GVHD during gestation.

The lack of complete B-cell function in some SCID patients receiving bone marrow transplants has led some investigators to advocate for pretransplant chemotherapy in all subjects. Such suggestions have to be balanced against the possible toxic effects of such treatments.

Other primary immunodeficiencies. The second largest group of patients with immunodeficiency given bone marrow transplants since 1968 are those with the Wiskott-Aldrich syndrome.⁶¹ In a recent report from the International Bone Marrow Transplant Registry, 170 patients with Wiskott-Aldrich syndrome had undergone transplantation, and the 5-year probability of survival for all subjects was 70% (95% confidence interval 63%-77%).⁶¹ Probabilities differed according to donor type:

87% (95% confidence interval 74%-93%) with HLA-identical sibling donors, 52% (95% confidence interval 37%-65%) with other related donors, and 71% (95% confidence interval 58%-80%) with matched unrelated donors (MUDs, $P = .0006$). Boys who had received a MUD transplant before 5 years of age had survivals similar to those receiving HLA-identical sibling transplants.

Patients with combined immunodeficiencies characterized by less severe T-cell defects than in SCID constitute the third largest group of patients given bone marrow transplants since 1968.⁶² Thirty-three of 65 patients so treated (51%) were reported as surviving.⁵⁵ Forty-five patients with Omenn syndrome were reported as having received marrow transplants since 1968, and 23 or 51% were alive at the time.⁵⁵ Twenty-five of 33 patients with leukocyte adhesion deficiency (76%) were alive after bone marrow transplantation.⁵⁵ Fourteen of 26 patients with the bare lymphocyte syndrome (54%) were alive after having been given marrow transplants.^{63,64} Eighteen of 23 patients with Chediak-Higashi syndrome who received bone marrow transplants (78%) were reported to be alive.⁵⁵ Twenty-one of 29 patients with chronic granulomatous disease who had received bone marrow transplants (72%) were reported as surviving.⁶⁵ Patients with the complete DiGeorge syndrome have undergone both marrow and thymic transplantations. Two of 3 who received unfractionated HLA-identical sibling marrow survived.⁶⁶ Recently, 16 infants with complete DiGeorge syndrome underwent transplantation with cultured unrelated thymic tissue, and 10 of these are surviving.⁶⁷

Other disorders treated successfully by bone marrow transplantation include X-linked hyper-IgM (13 of 21 surviving),⁶⁸ reticular dysgenesis (9 of 11 surviving),⁶⁹ purine nucleoside phosphorylase deficiency (4 of 9 surviving),⁷⁰ cartilage hair hypoplasia (4 of 7 surviving), X-linked lymphoproliferative syndrome (6 of 9 surviving),⁷¹ pigmentary dilution (Griscelli) syndrome (3 of 7 surviving),⁷² IL-2 deficiency (2 of 2 surviving),⁶² common variable immunodeficiency (0 of 4 surviving), chronic mucocutaneous candidiasis (0 of 1 surviving); ataxia-telangiectasia (0 of 4 surviving), and Fas (CD95) deficiency (1 of 1 surviving).⁷³

Efficacy of bone marrow transplantation in malignancy

The success of marrow transplantation in curing malignancy depends on a number of factors, the most important of which are the type of malignant disease, the stage of that disease, and the age of the recipient.⁷⁴ For patients with acute myelogenous leukemia during first complete remission, the projected disease-free survivals at 4 years were 55% for allogeneic transplantation, 48% for autologous transplantation, and 30% for intensive chemotherapy.⁷⁵ For patients with chronic myelogenous leukemia, the 3-year leukemia-free survivals were 57% for transplantations done in the first chronic phase, 41% in accelerated or second chronic phase, and 18% in more advanced disease.⁷⁴ The best survival rates (50% to 70%) with the lowest probability of relapse (20%) occurred in patients

younger than 20 years who had acute nonlymphocytic leukemia and underwent transplantation in first remission and in patients with chronic myelogenous leukemia who underwent transplantation in the chronic phase.⁷⁶

The rationale for allogeneic bone marrow transplantation in leukemia is the hope that the leukemic cells can be reduced or eliminated by irradiation or chemotherapy and that the grafted allogeneic normal T cells can then reject any remaining leukemic cells.⁷⁷ Supporting a need for T cells in the graft is the fact that T-cell-depleted bone marrow transplants have been associated with a higher degree of leukemia recurrence.⁷⁸

Efficacy of bone marrow transplantation in hemoglobinopathies, osteopetrosis, and metabolic storage diseases

Bone marrow transplantation has been highly effective for the treatment of homozygous β -thalassemia, with survivals reaching 70% to 80% for marrow transplants from HLA-identical siblings.⁷⁹ Likewise, HLA-identical bone marrow transplantation has also been successful for patients with sickle cell disease, with 59 patients known to have been treated, 55 of whom were surviving with 50 free of sickle cell disease.⁸⁰ The European Bone Marrow Transplantation Group reported on 69 patients with autosomal recessive osteopetrosis who were given HLA-identical or haploidentical bone marrow transplants between 1976 and 1994.⁸¹ Recipients of genotypically HLA-identical marrow had an actuarial probability for 5-year survival with osteoclast function of 79%.

Nonmyeloablative bone marrow transplantation

For patients with preexisting organ damage, there is significant morbidity and mortality from traditional conditioning regimens with busulfan and cyclophosphamide or irradiation. Because of this, there has been increasing interest in developing conditioning regimens that are less toxic.⁸² This has been accomplished by either total lymphoid irradiation or a combination of nucleoside analogs and anti-lymphocyte antibody preparations. Although these regimens are significantly less cytotoxic than high-dose alkylating agents and total-body irradiation, they are profoundly immunosuppressive. Opportunistic infections, such as the reactivation of cytomegalovirus, remain clinical obstacles when nonmyeloablative stem cell transplants are performed with these agents, especially in elderly and previously immunosuppressed patients. GVHD prophylaxis with cyclosporine and methotrexate, with added mycophenolate mofetil in some cases, has been necessary, because GVHD is common after nonmyeloablative transplantation.

The conditioning regimens used for patients with chronic granulomatous disease have been fludarabine, busulfan, and ATG in 1 case or cyclophosphamide, fludarabine, and ATG;⁸³ 7 of the 11 were surviving with mixed chimerism and good health. Gaspar et al⁸⁴ at Great Ormond Street treated 21 patients with a variety of different immunodeficiencies with four different nonmyeloablative conditioning

regimes.⁸⁴ At a median follow-up of 13 months, 19 of 21 patients (90%) were still alive after transplantation.

Placental blood transplantation

Unfractionated cord blood, which is a rich source of stem cells, is an alternative source of hematopoietic stem cells for transplantation.⁸⁵ More than 1500 cord blood transplants have been performed for a variety of malignant and genetic diseases.⁸⁶ Part of the rationale for using cord blood is that the incidence and severity of GVHD appears to be lower than with unfractionated adult MUD transplants, even when there is only partial matching.⁸⁷ Relative to adult MUD transplants, however, the graft failure rate is higher with cord blood transplantation. There is also a longer period before neutrophil engraftment and a much longer period of thrombocytopenia (100 days) than after HLA-identical related or MUD unfractionated marrow transplants.⁸⁶

Gene therapy

The reports by Cavazzana-Calvo et al⁸⁸ and Hacein-Bey-Abina et al⁸⁹ of successful gene therapy in infants with X-linked SCID represented a major step forward, because repeated efforts to achieve gene correction of adenosine deaminase-deficient SCID had failed during the decade before 2000. The group at the Hôpital Necker in Paris has thus far treated 11 patients with X-linked SCID with gene-corrected autologous bone marrow cells, and all 11 are surviving. Nine infants had normal T- and B-cell functions develop after the treatments. Two did not and were given allogeneic bone marrow transplants. The 9 who did acquire normal immune function did not require intravenous immunoglobulin infusions and were at home without any medication.

However, a serious adverse event occurred in two patients treated by this group. Shortly before varicella developed, the first patient was discovered to have a high white blood cell count as a result of an expanded clonal population of circulating $\gamma\delta$ -positive T cells. The white blood cell count became much higher and became a leukemia-like process that was treated with chemotherapy. The clone has been shown to carry the inserted gene.⁹⁰ The position of insertion is in an intron in a gene on chromosome 11 called *LMO2*. The product of the *LMO2* gene is crucial for normal hematopoiesis and serves a regulatory function.⁹¹ However, *LMO2* is also an oncogene that is aberrantly expressed in acute lymphoblastic leukemia of childhood. The second patient developed an α/β T-cell proliferation.

Insertional oncogenesis has long been known to be a potential complication of retroviral vector gene transfer attempts, because the integration occurs at random in the genome. This complication has been thought to be unlikely with such vectors, because they cannot reproduce themselves and cannot repeatedly insert into the cell's chromosomes to increase the likelihood of malignant change. Before this case, malignant changes had not been seen in any human beings given retroviral vectors for gene transfer. Whether the leukemic process seen in

these children indicates that the risk of insertional mutagenesis is higher than previously estimated is unknown. Whatever the case, it will be necessary to weigh this potential risk against the risks and benefits of other methods of treatment and determine which is best for patients with SCID.⁹²

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