Developing immunologic tolerance for transplantation at the fetal stage

Given the shortage of human organs for transplantation, the waiting lists are increasing annually and consequently so is the time and deaths during the wait. As most immune suppression therapy is not antigen specific and the risk of infection tends to increase, scientists are looking for new options for immunosuppression or immunotolerance. Tolerance induction would avoid the complications caused by immunosupressive drugs. As such, taking into account the experience with autoimmune diseases, one strategy could be immune modulation-induced changes in T-cell cytokine secretion or antigen therapy; however, most clinical trials have failed. Gene transfer of MHC genes across species may be used to induce tolerance to xenogenic solid organs. Other options are induction of central tolerance by the establishment of mixed chimerism through hematopoietic stem cell transplantation and the induction of 'operational tolerance' through immunodeviation involving dendritic or Tregs. I propose that, as the recognition and tolerance of proteins takes place in the thymus, this organ should be the main target for immunotolerance research protocols even as early as during the fetal development.

KEYWORDS: blood transfusion ■ immunotolerance ■ thymus

Hugo Mendieta-Zerón

Felipe Villanueva sur 1209 Col. Rancho Dolores CP. 50170 Toluca, México
Tel.: +52 722 219 4122
Fax: +52 722 219 4122
mezh_74@yahoo.com

Human leukocyte antigens

The response to a single antigen is diverse and includes many different antibody molecules, each with unique affinity and specificity. The cloning of genes encoding the immunoglobulins demonstrated that the antibody repertoire is generated during B-cell development by rearrangements of DNA. These combine and assemble different gene segments of V regions from a relatively small group of inherited V region sequences in each locus. The diversity is further increased by a process of somatic hypermutation in mature B cells. The human antibody repertoire is of 10^{11} of different antibody molecules or perhaps even more.

The antibody diversity is generated in four fundamental ways. First, there are many different copies of each type of gene segments that form the region V and different combinations of gene segments can be used in different recombination events. It is mainly responsible for the diversity of V regions of heavy and light chains. A second source of combinatorial diversity originates from the pairing of different combinations of V regions of both chains to form the antigen-binding site. These two mechanisms alone in theory could lead to 2.5×10^{26} different antibody molecules. Third, at the junctions between different gene segments, additional diversity by the recombination process is introduced. Finally, somatic hypermutation introduces point mutations in the rearranged V regions.

The MHC genes are a group of highly polymorphic genes located on the short arm of human chromosome 6 at 6p21.3 segment that encode the HLAs, and their biological function is the presentation of antigenic peptides to recipients of T lymphocytes. It spans more than 4 megabases and include more than 200 genes. These genes are related to the cellular and humoral immune response in the recognition of self and foreign, which in organ transplantation results in acceptance or rejection of the graft depending on the degree of genetic compatibility between individuals who express these antigens. The MHC region is divided into three subgroups:

- Class I loci: HLA-A, -B and -C;
- Class II: HLA-DR, -DQ and -DP;
- Class III: genes coding for other immune components, such as complement proteins C2, C4 and factor B, the enzyme 21-hydroxylase, HSP70 and TNF-α [1,2].

Class I molecules present on the cell surface of virtually all nucleated cells of the body, present antigenic peptides produced by the cell and are recognized by CD8 T cells, whereas tissue distribution of class II is restricted to B lymphocytes, macrophages, dendritic cells (DCs), endothelial cells and T lymphocytes that are activated by antigen-presenting cells (APCs), and are endocytosed and recognized by CD4 helper T cells [3,4].

future medicine part of fsg

The advent of molecular techniques in the field of histocompatibility was essential to enable the identification of numerous HLA alleles that differ by one or more nucleotides, and in some cases, by one or more amino acids [5,6].

The peptides presented by HLA molecules may come from external sources, but mostly they derive from endogenous proteins. During normal maturation of the immune system, tolerance develops to these 'self' proteins. Positive selection, which ensures the survival of T cells that carry T-cell receptors (TCRs) capable of recognizing self-MHC molecules, is believed to be driven by MHC-II$^+$ cortical thymic epithelial cells. On the other hand, elimination of autoreactive T cells by negative selection is driven by MHC-II$^+$ thymic DCs and medullary thymic epithelial cells [7].

Owing to the importance of HLA compatibility in the outcome of transplantation, most allogeneic transplants have been between HLA-matched individuals. However, in the context of organ transplantation, polymorphisms in these endogenous proteins serve as sources of minor histocompatibility (minor H) antigens and form the basis of immunological nonidentity between HLA-matched individuals [8]. HLA molecules are fundamental in T-cell activation, as they bind peptides and present them to T cells. The HLA molecules themselves are termed MHC antigens, and T cells react vigorously when confronting nonidentical HLA molecules.

High-dose chemotherapy or systemic radiotherapy needs to be administered to the recipient prior to transplantation to eradicate host T cells sufficiently to prevent graft rejection. Also, posttransplant treatment of the graft recipient with potent immunosuppression contributes significantly to preventing graft rejection. Despite such prophylaxis, significant graft-versus-host disease (GVHD) and host-against-graft reactions continue to grow [9,10].

Memory T cells

After arriving as precursors from the bone marrow, small numbers of CD4$^-$ CD8$^-$ (double-negative [DN]) thymocytes – the most immature cells in the thymus – proliferate and differentiate into CD4$^+$ CD8$^+$ (double-positive [DP]) cells. Proliferation and differentiation of DN cells are driven by a distinct surface receptor, composed of a TCR-β and pre-TCR [11]. The DP thymocytes die by failure to recognize any of the molecules and consequently survival signal. Remaining DP thymocytes expressing TCRs that bind self-MHC/self-peptide molecules with low affinity are rescued through positive selection and differentiate into MHC-restricted, helper and cytotoxic T cells [12]. Ultimately, positive selection produces a customized T-cell repertoire, consisting of mature T cells expressing receptors, which can identify the individual's MHC proteins. It is equally critical that mature T cells are not activated by self-peptide–MHC ligands and negative selection eliminates thymocytes with high-affinity TCRs for self-MHC/self-peptide ligands by inducing apoptosis in those cells. Low-affinity peptide–MHC ligands are recognized by mature T cells, providing survival signals that maintain the peripheral T-cell pool [13]. By contrast, a T cell is activated when confronting high-affinity peptide–MHC ligand, generally consisting of a self-MHC and a foreign peptide.

Upon exposure to a foreign antigen, antigen-specific T cells proliferate and differentiate into effectors that eliminate the foreign intruder. The vast majority of effector T cells, however, undergo apoptosis as the immune response progresses and the few lymphocytes that survive become long-lived memory T cells [14]. Memory T cells that recognize microbial antigens provide the organism with long-lasting protection against potentially fatal infections. Conversely, memory T cells that recognize donor alloantigens jeopardize the survival of life-saving organ transplants by mediating rejection [15]. Therefore, a point of view in the pursuit of transplantation tolerance is how to coerce an immune response determined to generate T-cell memory into a state of antigen-specific unresponsiveness.

Regardless of which differentiation pathway is operational, memory T cells arise from antigen-activated lymphocytes that proliferated during the expansion phase of the immune response and not from naive T cells that received suboptimal antigenic stimulation and failed to proliferate. The same characteristics of memory T cells that make them very efficient at eliminating microbial pathogens also enable them to rapidly reject foreign tissues. Unlike inbred mice, outbred animals including humans harbor a significant number of memory T cells that are alloreactive. These memory T cells generally arise if an individual is exposed to alloantigens via pregnancy, blood transfusion or a previous organ transplant. However, alloreactive memory T cells also exist in individuals who have never been exposed to foreign tissues before. This phenomenon is commonly referred to as heterologous immunity [16].

Several investigators have provided evidence that alloreactive memory T cells indeed contribute to both acute and chronic allograft rejection. Others have observed that the presence of memory phenotype T cells ($CD45RO^+$) in heart and kidney allograft biopsies and in the peripheral blood of transplant recipients correlates with the incidence and severity of rejection [17]. Moreover, T cells with memory phenotype have been detected in patients receiving high levels of immunosuppression [18], demonstrating that current immunosuppressive therapies do not inhibit the generation or maintenance of T-cell memory.

Memory T cells not only endanger allograft survival by causing both acute and chronic rejection, but also impede the induction of transplantation tolerance. Strategies to induce immunologic tolerance to a foreign antigen by exploiting the same principles that underlie tolerance to self-antigens are: deletion, anergy, suppression and immune deviation [19]. Deletion is mediated by the apoptosis of antigen-specific naive or recently activated T cells leading to antigen-specific unresponsiveness. Alternatively, a tolerance-inducing strategy could inactivate T cells without causing their death (anergy), generate regulatory cells that block T-cell activation and function (suppression) or induce the differentiation of naive T cells into a nonharmful phenotype (immune deviation). Moreover, immunomodulatory agents that do not consistently induce donor-specific tolerance invariably fail to suppress immunologic memory in experimental animals [20,21]. $CD8^+$ memory T cells seem to be as susceptible to tolerance as their naive counterparts [22]. Although memory T cells are less susceptible to apoptosis than naive T cells, they can still be coerced to die. Memory $CD8^+$ T cells that migrate to immune-privileged sites undergo apoptosis mediated by the TNF receptor family. Memory T cells are subject to suppression by Tregs and are not rigidly differentiated and may be amenable to immune deviation [23]. Therefore, memory T cells can be potentially controlled via the same mechanisms that mediate naive T-cell tolerance. An alternative approach to targeting memory is to block activation pathways unique to the recall of memory T cells [24].

Controlling T-cell memory responses entail not only suppressing existing memory cells but also inhibiting the generation of new ones. Of course, the greatest challenge that remains is how to achieve all this in an antigen-specific manner: selective deletion or suppression of alloreactive or self-reactive memory T cells without globally compromising the host's immune system.

Immunological tolerance

The lymphocyte response between tolerance and no tolerance is regulated at different levels. One such tolerance is the state of maturation of APCs. In fact, there is a consistent model whereby T-cell interactions with immature APCs lead to the induction of various tolerogenic mechanisms. Antigen targeted to immature DCs *in vivo* leads to the induction of tolerance via abortive expansion, deletion and anergy of the remaining antigen-specific T cells [25]. Interactions between other members of the TNF receptor family OX-40 ligand (OX-40L) and OX40, which are expressed on APCs and T cells, respectively, also provide a decisive signal that can influence tolerance versus autoimmunity. In general, the majority of the literature is consistent with a model where signals that promote APC maturation, such as adjuvants and pathogens, are crucial for converting tolerogenic to activating signals.

The immune system responds to substances that cause damage (danger signals), rather than to those that are simply foreign. Danger signals consist of molecules or molecular structures, released or produced by cells undergoing stress or abnormal cell death, which are perceived by resting APCs and which induce the APCs to become activated, to offer costimulatory signals and, thus, to initiate immune responses. Some endogenous danger signals that recently have been discovered are HSPs, nucleotides, reactive oxygen intermediates, extracellular-matrix breakdown products, neuromediators and cytokines such as interferons [26].

Whereas direct recognition of pathogens by Toll-like receptors and other innate immune receptors is an important mechanism by which APCs are stimulated to promote activation of T cell, other situations, such as organ transplants and tumors, may trigger an immune response [27].

Detection of self-antigens is an important event that contributes to the induction of tolerance or autoimmunity. Evidence from many models demonstrates that self-antigens are cross-presented by bone marrow-derived cells and then induce peripheral T-cell tolerance [28]. DCs can induce peripheral tolerance and cross-present tissue-specific antigens by reconstituting MHC class I expression only on DCs *in vivo*.

Alterations in cell surface molecules that positively act on antigen receptor signaling can lead to breakdown in tolerance [29]. Cell surface negative regulators participate in restraining autoantibody production. For example, CD5 appears to

be a negative regulator of at least some B cells as well as T cells. In fact, in the lysozyme system, CD5 seems to be important for maintaining B-cell anergy. On the contrary, mutations that enhance B-cell receptor signaling by compromising feedback inhibitory pathways enhance B-cell activation during an immune response and lead to increased number of activated B cells, plasma cells and elevated serum immunoglobulin levels.

Although progress has been made in understanding the mechanisms that lead to immunological tolerance, it remains a challenge to induce selective and drug-independent tolerance.

Transplantation

Tissue matching involves identifying MHC antigens on both donor and recipient cells and using donor cells with as many MHC alleles identical to those of the recipient as possible. Matching MHC class I, especially HLA-B, and class II HLA-DR alleles is more important for successful transplantation than matching other MHC antigens; and matching MHC is more important than matching minor H antigens. Transplants must always be matched for blood type antigens, which are found on other body tissues.

HLA matching improves graft survival, but does not prevent rejection, even in MHC-identical siblings (except for identical twins). One reason is that MHC typing using anti-HLA antibodies is imprecise; available antibodies do not detect all MHC alleles and the same antibody may bind two similar, but nonidentical alleles. Another reason is the presence of minor H antigens; these antigens stimulate slow but eventual graft rejection. Finally, time for full typing is limited for cadaver grafts because the organs can survive for only a limited time; heart transplants are matched only for ABO and rhesus (Rh) blood types.

The cause of transplant rejection is the recognition of foreign MHC antigens by T cells and activation of those T cells to become effector cytotoxic or helper T cells. T-cell activation occurs in the case of vascularized grafts of nucleated cells expressing MHC. Autografts and grafts from an identical twin do not have foreign MHC antigens and are usually accepted without medication to prevent rejection. Allografts may have identical or nonidentical alleles at the MHC loci. Xenografts may have MHC antigens so foreign that it is not recognized by T cells and does not activate them, but other antigens (e.g., adhesion molecules and cell surface carbohydrates) can cause very rapid graft rejection.

Allogeneic MHC is recognized by either CD8 T cells (class I) or CD4 T cells (class II); up to 10% of T cells can recognize a given allogeneic MHC because it resembles self-MHC$^+$ foreign peptide. Minor H antigens are usually recognized by CD8 T cells and the number of responding T cells more closely resembles that for a foreign antigen (0.01–0.001% of T cells). Minor H differences are owing to polymorphism in proteins between members of the same species. An example is proteins encoded only on the Y chromosome (H-Y antigens). Since females do not make these proteins, Y antigen peptides are foreign antigens and elicit immune (antimale) responses. Some minor H antigens may actually be foreign peptides presented on self-MHC, so that two people with the same MHC alleles could differ because the donor cells were presenting foreign peptide that could be recognized by the recipient and induce an immune response that would kill the donor cells. Most minor H antigens have not been identified. All cells on the graft express minor H antigens and can be recognized and destroyed by recipient T cells.

Hyperacute graft rejection occurs immediately upon transplantation. It is owing to preformed antibodies, either natural antibodies to blood type antigens or anti-MHC antibodies formed in response to blood transfusions or previous transplants or developed during pregnancy to the baby's paternal MHC antigens. Antibodies react with antigens on vascular endothelial cells and activate complement. Resulting damage blocks blood vessels and starves the organ for oxygen. Hyperacute rejection fatally damages the organ and cannot be reversed; the only treatment is immediate removal of the graft. It can be prevented by careful cross-matching of donor and recipient blood.

Grafts contain passenger leukocytes, APCs bearing both MHC and costimulatory molecules. Passenger leukocytes travel to the draining lymph nodes and activate recipient T cells (direct alloreactivity). Direct activation of recipient T cells is responsible for acute graft rejection that occurs in the first weeks following transplantation; effectors are primarily cytotoxic T lymphocytes. Symptoms of acute rejection include fever, skin rash, impaired organ function and a mononuclear (T-cell) infiltrate into the graft visible on biopsy. Indirect alloreactivity comes from uptake of graft antigens by recipient APC and presentation on self-MHC. Peptides from both MHC and minor H antigens are presented by recipient APCs. Effectors are usually Th1 cells that activate macrophages to cause tissue injury and scarring that can cause chronic rejection or

organ failure.

Chronic rejection usually results in arteriosclerosis of graft vessels; in kidney grafts fibrosis and atrophy of the glomerulus and tubules occurs. Chronic rejection and organ failure are usually owing to alloreactivity, ischemic-reperfusion injury during transplantation, chronic toxicity of antirejection drugs and infection with citomegalovirus. T cells infiltrate the graft and produce cytokines that upregulate CAM expression on vascular endothelium and attract macrophages. Macrophages secrete IL-1, TNF-α and MCP to cause chronic inflammation.

Transplantation challenges

Improved success in transplantation depends on increasing technical expertise, the availability of transplant centers to do HLA matching and minimize organ delivery time and the availability of immunosuppressive drugs that block T-cell activation to alloantigens. Still problems still exist in the form of shortages of organs, previous existing diseases that destroy the transplanted organs (e.g., diabetes), side effects of immunosuppressive drugs and high cost.

Given the shortage of human organs for transplantation, there has been an increasing interest in using animal organs, mainly pigs. Transgenic pigs have been created that have five human genes: *CD46*, *CD55*, *CD59*, *DAF* and H-transferase. The first four of these encode human complement-inhibitory proteins that block human complement from damaging the pig organs, but much more work needs to be done before xenografting from pigs to humans is practical. One potential problem that must be avoided, even if the transplantation rejection can be dealt with, is the transfer of potentially lethal viruses from animals to humans via xenografts.

Donor marrow can be treated before transplantation with antibodies to markers on mature T cells (anti-CD3 [Figure 1], anti-CD4 [Figure 2] and CD8) to reduce the possibility of GVHD. Cord blood from the placentas of newborns contains high frequencies of stem cells and low frequencies of mature T cells and can replace bone marrow as a source of hematopoietic cell transplants.

Despite the improvement of early-graft survival, late-graft loss caused by chronic rejection and the lethal consequences of long-term immunosuppression, such as infection and cancer, remain major concerns for the transplantation community. Tolerance induction would avoid these complications [30]. The options are induction of central tolerance by establishment of mixed chimerism through hematopoietic stem cell (HSC) transplantation [31] and the induction of 'operational tolerance' through immunodeviation involving dendritic or Tregs.

The biological concept of microchimerism, the bidirectional trafficking and stable long-term persistence of small numbers of allogeneic (fetal and maternal) cells in a genetically different organ, has gained considerable attention.

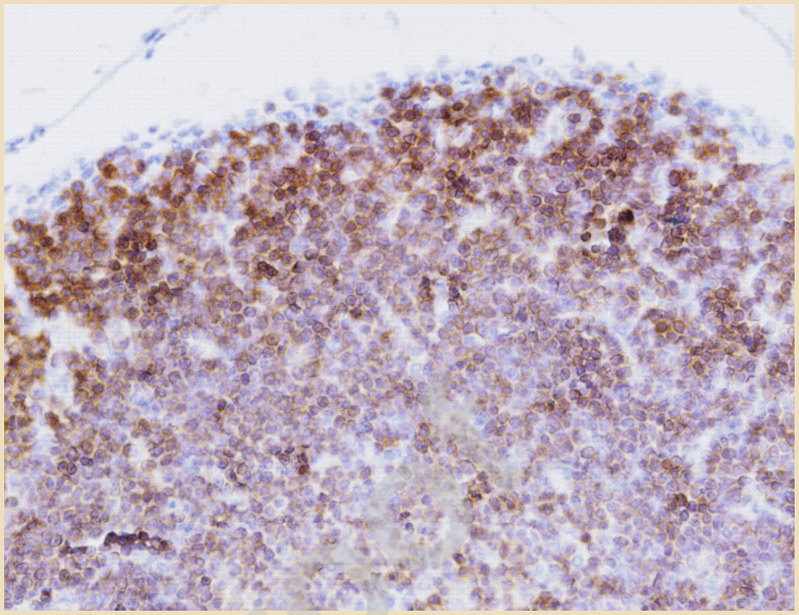

Figure 1. CD3⁺ lymphocytes marked on thymus. Markers used for diagnosis purposes, not for immunomodulation (magnification ×40).

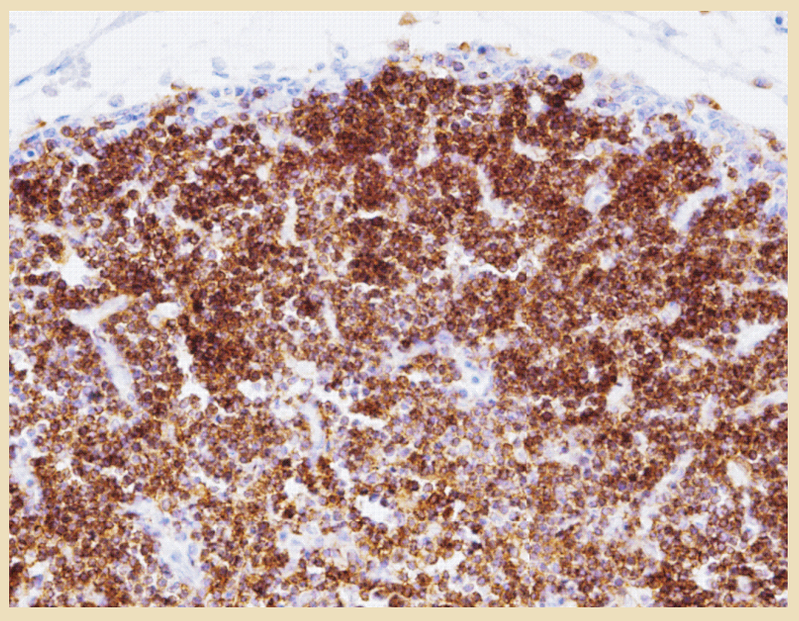

Figure 2. CD4⁺ lymphocytes marked on thymus. Markers are used for diagnosis purposes, not for immunomodulation (magnification ×40).

Microchimeric cells can modify immunological recognition or tolerance, affect the course and outcome of various diseases and demonstrate stem cell-like or regenerative potential [32,33]. The presence of semiallogeneic cells in a host can have significant immunological effects on transplantation tolerance and rejection [34]. Consistent with these observations, T-cell-replete HSC transplantation between mutually microchimeric mothers and their HLA-haploidentical offspring has been shown to be feasible, although the degree of microchimerism-associated tolerance appears to substantially differ among the cases [35].

Mixed chimerism is not an absolute recipe for induction of transplantation tolerance to organ allografts; microchimerism may not be sufficient for induction of tolerance to highly immunogenic organs, whereas full donor chimerism can induce robust transplantation tolerance to all organs.

There are different ways to induce chimerism: the classical stem cell transplantation with myeloablative conditioning and the more acceptable reduced-intensity conditioning or even nonmyeloablative stem cell transplantation based on selective deletion of alloreactive T cells *in vitro* prior to cell infusion or *in vivo* by administration of cyclophosphamide [36].

Allogeneic HSC transplantation has been a curative therapeutic option for a wide range of immune hematologic malignant and nonmalignant disorders including genetic diseases and inborn errors [37]. Stem cell therapy has emerged in the last years as a promising strategy for the induction of tolerance after organ transplantation. Mesenchymal stromal cells, neuronal stem/progenitor cells, HSCs and embryonic stem cells can modulate the immune response and induce peripheral or central tolerance [38,39]. However, the major limitation in broad utilization of hematopoietic cellular transplantation/organ transplant therapy has been the toxicity of conditioning regimens used to generate a favorable environment to allow for bone marrow engraftment [40].

Central clonal deletion of donor-specific alloreactive cells associated with mixed chimerism reliably produced long-term graft tolerance [41]. In this setting, depletion of recipient T cells and antilymphocyte antibodies and subsequent repopulation by donor and recipient hematopoietic cells are prerequisites for tolerance induction. The dose of donor HSCs is a critical factor influencing the efficacy of this tolerance-inducing regimen. Other important parameters include MHC class II expression by donor cells and engraftment of donor T cells.

A substantial amount of work has focused on T-cell depletion (TCD) of the donor stem-cell graft as a method for preventing GVHD [42]. However, TCD is associated with an increased rate of severe and often fatal infections, a higher incidence of graft rejection and an increased risk of leukemia recurrence. As an alternative to TCD, other techniques capable of inducing antigen-specific tolerance are conceptually appealing in that they would prevent GVHD without resulting in profound post-grafting immunosuppression [43].

One approach to the development of antigen-specific tolerance builds on the observation in murine models whereby exposure of antigen-activated T cells to antibodies against the invariant CD3 domain of the TCR can induce apoptosis specifically in activated cells, thereby preventing GVHD [44]. A second approach for the development of antigen-specific tolerance is based on the 'two-signal model' of T-cell activation. T-cell activation requires not only stimulation of the TCR with its appropriate antigen in the context of MHC, but also a second 'costimulatory' signal provided by CD28. A blocking anti-CD28 antibody caused inactivation of the *L-2* gene [45]; and transplantation using *CD28*-knockout mice as donors resulted in partial protection of recipients from lethal GVHD [46].

Gene transfer of MHC genes across species may be used to induce tolerance to xenogenic solid organs, should stem cells from another species be used as a source of engineered tissue [47]. Other studies have focused on the role of inflammatory cytokines and host APCs in the pathogenesis of GVHD. During an innate immune response, the capacity of NK cells to preferentially kill targets lacking MHC class I is directly influenced by their expression of inhibitory MHC-recognizing receptors [48]. Each subpopulation of lymphocytes employs activating multisubunit immune recognition receptors to initiate cellular functions.

Blood

The glycosphingolipids present on the surface of cells are involved in cell–cell recognition and are antigenic, accounting for certain blood-group substances. Before any transfusion is started, it is necessary to draw blood for serologic typing. Specific antigens (A, B, AB and O) are found in each of the major blood groups. Whenever the red cell membrane is devoid of the A or B antigen, the serum contains antibodies (anti-A or anti-B, respectively). ABO blood group antigens

are expressed throughout the whole body. The presence of glycosphingolipid and glycoprotein antigens on the surfaces of cells make it necessary to match blood or tissue types before carrying out either a blood transfusion or a tissue tansplantation. The explanation for cases of hemolytic disease of the newborn as a result of ABO incompatibility is that natural anti-A and anti-B of mothers with blood group O are of the IgG class and can cross the placenta, whereas those of mothers with blood type A or B are predominantly IgM and cannot cross the placenta [49].

The highly immunogenic Rh antigens are a group of polypeptides closely linked to the red blood cell membranes. The Rh antigen system is polymorphic, consisting of D, C, c, E and e antigens [50]. The genes encoding the Rh blood group system have been mapped to chromosome 1p34 [51]. A total of 85% of the population also has the RhD antigen present on their erythrocytes. Patients whose red blood cells (RBCs) contain the D antigen are classified as Rh positive. Patients who are Rh-negative do not, however, possess RhD antibodies. A total of 55% of the Rh-positive white population is heterozygous for the presence of the *RhD* gene. If a Rh-negative woman is pregnant from a heterozygous Rh-positive partner, her fetus has a 50% chance of being heterozygous Rh-positive like the father and a 50% chance of being Rh-negative like the mother. In the latter case, the fetus will be unaffected by RhD alloimmunization. When an Rh-negative pregnant woman has anti-D alloantibodies directed against the RhD antigen on the erythrocyte membrane of her Rh-positive fetus, red blood cell destruction leading to fetal hemolytic anemia can be the cause of fetal hydrops and intrauterine fetal death (erythroblastosis fetalis) [52]. In spite of current recommendations for anti-D immune globulin administration during and after pregnancy in Rh-negative women, only a low percentage become immunized [53]. Currently, intrauterine therapy consisting of the intravascular injection of RBC into the umbilical vessels is the mainstay of therapy for severe fetal anemia [54]. This treatment may require repeated cordocenteses [55], but some pregnancies are so severely affected that fetal anemia cannot be corrected by intrauterine transfusions even if they are initiated at an early gestational age.

It is possible to determine the RhD blood type on single human cells, including blastomeres obtained from a human embryo [56]. This will allow avoidance of RhD-positive pregnancies in selected cases of severe anti-RhD alloimmunization.

Packed RBCs are the most frequently administered blood component. Since it lacks A, B and Rh surface antigens, group O, Rh-negative blood can be given to most potential recipients. Other less common antigens on the O-negative cells may cause a transfusion reaction if the recipient serum contains antibodies to these less common antigens. Taking into account that most of the population lacks antibodies to these less-common antigens, transfusion of O, Rh-negative peripheral RBCs is relatively safe. Group O, Rh-negative whole blood should not be given because the donor serum may contain high anti-A or anti-B titers, which could cause hemolysis of the recipient RBCs.

Risks & complications of transfusion therapy

Transfusion reactions with an immunologic basis include hemolytic and nonhemolytic (allergic) reactions. Acute hemolytic transfusion reactions are the most dangerous and feared complication of blood transfusion and must be immediately and rapidly recognized and treated. Major hemolytic reactions (0.04%) result from the interaction of antibodies in the recipient plasma with antigens on the RBC of the donor. Hemolysis resulting from the transfusion reaction produces free hemoglobin and RBC stromal debris, which results in hemoglobinuria and renal tubular damage. Classic signs and symptoms of chest pain, back pain, chills, fever and dyspnea may become apparent after the transfusion of small volumes (10–30 ml) of incompatible blood. Gross hemoglobinuria may be present; however, oozing and progressive unexplained hypotension may be the only clues leading to this diagnosis in anesthetized patients.

The mortality rate from hemolytic transfusion reactions varies between 17 and 53%. Nonhemolytic transfusion reactions (febrile, anaphylactoid and noncardiogenic pulmonary edema) are more common than are hemolytic reactions, with an incidence of 2–10%. These reactions are secondary to leukocytes or plasma proteins and can be reduced by using of leukocyte-depleted blood products or premedication with antihistamines. Treatment of these reactions includes discontinuing the blood transfusion and treatment with an antihistamine, epinephrine and steroids.

The alloimmunization is a condition caused primarily by the receptor antibodies against HLA of a foreign individual, present in a blood product transfusion. The presence of antibodies

directed against transfused platelets can cause removal or their destruction by the immune system of the recipient. This implies a considerable reduction in the total number of platelets transfused. When this occurs in repeated transfusions, the individual is said to have refractive platelet transfusion.

The fetus

The fetus is an allograft normally not rejected in pregnancy even though half of the baby's antigens are foreign to the mother. Cells from the fetus come into contact with the mother's cells in the placenta and may even enter the mother's circulation during pregnancy. The mother does make antibodies to the father's HLA antigens; women who have had several pregnancies are the best source of anti-HLA antibodies for serological typing.

The placenta, particularly the trophoblast, plays a major role in preventing rejection. Trophoblast cells do not express classical class I or class II MHC that would activate maternal CD8 and CD4 T cells. Instead, trophoblast cells express HLA-G that binds killer cell immunoglobulin-like receptors on NK cells and blocks their recognition (NK cells kill cells not expressing MHC in the absence of killer cell immunoglobulin-like receptor binding). Cells in the placenta also express the enzyme indoleamine 2,3-dioxygenase that rapidly catabolizes tryptophan, which starves maternal T cells for this amino acid and reduces their ability to respond. Pregnant mice given inhibitors of indoleamine 2,3-dioxygenase reject allogeneic but not syngeneic fetuses. Evidence also exists for specific T-cell tolerance to paternal MHC during the pregnancy. Finally, trophoblast cells secrete IL-1, IL-10 and TNF-α, which suppress Th1 responses.

Classic and contemporary anatomic studies of human embryos have revealed that human hematopoiesis begins in the second to third embryonic weeks with formation of mesoderm-derived blood islands in the extraembryonic mesoderm of the developing secondary yolk sac. The hematopoietic output of the yolk sac is gradually replaced by intraembryonic sites in the following sequence: the aorta–gonad–mesonephros, which is generated from the para-aortic splanchnopleure region [57,58]; embryonic liver, which becomes active at approximately 5 weeks of gestation [59] and finally; the fetal bone marrow [60].

Human umbilical cord blood is a rich source of hematopoietic precursor cells [61], which most likely represent a developmental stage intermediate between fetal liver and adult bone marrow.

Microchimerism can occur as a result of blood transfusion, solid organ transplantation or twin–twin transfusion. The most frequent and physiological cause of microchimerism is pregnancy, owing to the bidirectional exchange of cells between the fetus and the mother. The transfer of fetal cells into the maternal circulation begins at 4–6 weeks of gestational age [62] and can persist in maternal blood and tissues after delivery [63].

Hypotheses: developing tolerance *in uterus*

The induction of immunologic tolerance is an important clinical goal in transplantation and autoimmunity. Immunologic tolerance is traditionally defined as specific unresponsiveness to a self or foreign antigen while maintaining reactivity to other antigens [64]. In the context of transplantation, a tolerant patient is someone who is capable of mounting an effective immune response to vaccines and microbial pathogens, but is incapable of rejecting the transplanted organ. Although several immunomodulatory strategies have been used successfully to induce immunologic tolerance in rodents, the same strategies have failed in larger animals and in humans. Examples of induced tolerance in organ transplant recipients or in patients with autoimmune disease have been rare and often unintentional [36,39]. The answer to this question most likely lies in the immunologic barriers that preclude the induction of antigen-specific unresponsiveness. These barriers include the limitations of peripheral (extrathymic) immunoregulatory mechanisms that are commonly exploited to induce tolerance (T-cell deletion, suppression, deviation and anergy), the large repertoire of alloreactive T cells in the case of transplantation and the unavoidable fact that the adaptive immune response, by virtue of evolutionary design, is destined to generate immunologic memory. It is the last barrier that is perhaps the most important obstacle to immunologic tolerance [65].

Although the thymus provides an important mechanism to eliminate initial self-reactive T cells, many tissue-specific proteins are not expressed in sufficient quantities to induce tolerance. One objective would be the development of HLA receptor desensitization during the embryonic stage to tolerate future transplants. The thymus arises bilaterally from the third and fourth branchial pouches and contains elements derived from all three germinal

layers [66,67]. Development begins in gestational week 6. Migration of tissue occurs during the week 8, leading to a fusion of the bilateral lobes, with the thymus occupying its final position in the anterosuperior mediastinum. In the course of its development, until gestational week 9, the thymus remains purely epithelial. By week 10, small lymphoid cells migrate from fetal liver and bone marrow, leading to lobulation of the gland. Further differentiation into cortex and medulla is completed by 14–16 weeks [68].

To complement central tolerance events, several mechanisms of peripheral tolerance exist. One key mediator of peripheral tolerance is the role of APC. APCs, such as DCs, capture self-antigens from other cells and present them to autoreactive T cells (cross-presentation) to induce T-cell tolerance by deletion or anergy [28]. The induction of peripheral T-cell tolerance is dependent upon the concentration of self-antigen [69]. If the self-antigen is expressed at low levels, then minimal cross-presentation occurs and the self-reactive 'ignorant' T cells remain in the T-cell repertoire. Events that modulate self-antigen expression, such as tissue necrosis or tumor development, will influence the level of cross-presentation. The fate of the autoreactive T cells will then be determined by whether or not the event that leads to increased cross-presentation is coupled with events that lead to APC maturation. If increased levels of self-antigen expression occur in the absence of signals that promote full APC maturation, then tolerance will occur; by contrast, if self-antigen is detected in the presence of proinflammatory signals or other events that promote APC maturation, then tolerance will be broken and autoimmunity will arise by activating ignorant T cells.

A thymocyte's TCR must bind strong enough to at least one type of self-peptide–MHC complex to receive survival signals and emigrate from the thymus (a process called positive selection). With a view on the recent data, it seems more appropiate to regard the activation and downregulation of T cells as a process of 'multisignal integration', with the TCR mainly responsible for the specificity of the response [70]. Recent studies have suggested that subsets of cells including regulatory cells, suppressor cells, NK cells and NK T cells can all influence tolerance and autoimmunity via APC/DC-dependent or -independent mechanisms [71,72].

The thymic microenvironment is exceptional in its ability to sustain production of T cells but it is a longstanding question as to which bone marrow-derived cell seeds the thymus and to what level this cell is committed to the T-cell lineage. Furthermore, many tissue-specific proteins are not expressed at sufficient levels to induce tolerance in the thymus. Understanding the basis for CD4/CD8 lineage choice in the thymus is central to our understanding of thymocyte development [73].

Although there are multiple theoretical solutions to the challenge of self/nonself discrimination, Burnet and Fenner proposed as a corollary to the clonal selection theory that the problem can be solved on the basis of time considerations; any antigen to which the immune system is exposed to in the fetal or early neonatal stages is flagged as self and induces long-term tolerance [74]. Lederberg extended this notion by postulating that immature lymphocytes have a heightened sensitivity to tolerance induction [75]. This 'newborn privilege' was tested by introducing foreign molecules as surrogates for self and, indeed, it did prove possible to induce tolerance by administration of allogeneic cells, viruses or proteins to neonates but not in adults [76–78]. However, neonatal exposure was not always tolerogenic and the outcome was clearly dose dependent [79,80]. By genetic engineering, cDNAs encoding allergens for most disease-eliciting epitopes can be used as templates before modification by several different strategies (e.g., fragmentation, mutation, deletion, oligomerization and production of hybrids). These derivatives can be designed to maintain T-cell epitopes and structures required for the induction of protective antibody responses. Furthermore, it is possible to directly modulate the immunogenic/tolerogenic properties of these molecules by genetic modification, which in principle would even allow the use of adjuvants to be bypassed [81].

DNA vaccination consists of purified plasmid DNA containing an antigen's coding sequences introduced into the tissue via intramuscular injection or particle bombardment. This method is providing new insights into some of the basic immunological mechanisms of vaccination such as antigen presentation, the role of effector cells and immunoregulatory factors [82].

DNA vaccination for transplantation has been less investigated compared with DNA vaccines for infectious disease, cancer and pathological autoimmunity, but this technique could likely reduce the need for systemic immunosuppressants and be applied to the prevention of chronic rejection, which remains a major barrier to successful allotransplantation [83]. For example, immunization with nonpolymorphic

Table 1. Embryo–fetal hematopoiesis.

Embryonic age	Organ of hematopoiesis	Thymus development
16–18.5 days	Yolk sac	
27–40 days	Aorta–gonad–mesonephros	
5–6 weeks of gestation to end of second trimester	Embryonic liver	Week 6: beginning Week 8: fusion of the bilateral lobes Week 10: lymphoid cells migrate from fetal liver and bone marrow
Start of second trimester to end of gestation	Fetal bone marrow	Weeks 14–16: differentiation into cortex and medulla

antigens found in both donor allograft and recipient would be an attractive means to prevent long-term graft rejection. Some approaches have been reached in animal models [84].

In addition, DNA vaccines may enable us to manipulate the immune system in situations where the response to agents is inappropriate or ineffective. In this regard, DNA vaccines have a potential application for the study of neonatal tolerance and autoimmunity [85]. If the most common HLA antigens were introduced by purified plasmid DNA in an early embriogenic stage, the baby would be tolerant to those antigens.

At the heart of the knowledge, it is clear that tolerance will depend on the time and dose of the foreign antigen to be exposed in the host. We suggest that to generate T-cell memory into a state of permanent antigen-specific unresponsiveness, it is necessary to expose the specific antigens into the thymus between weeks 4 and 8 of embriogenesis (Table 1). As hematopoiesis begins between the second and third embryonic weeks, it could be possible to get a tolerance to nonself antigens if exposed during this period, for example, inducing tolerance to ABO blood group antigens to produce an embryo capable of being a receptor from any kind of blood type (Table 2). Therefore, the natural candidates to breed the first multitolerant human being should be a mother with group A and father with groups B or AB, a mother with group B and father with groups A or AB, or a mother with group AB and father with groups A, B or AB (Box 1).

The window to get a multitolerant human being might be from the 6th to the 16th week of gestation in accordance with the thymus development (Table 1). We should first try with cats as animal models, as they only have three blood types: A, B and AB. Repetitive low-dose intravascular injection of RBC or specific carbohydrates from erythrocyte membranes into the umbilical vessels should be the most achievable technique to be used, in fact, this is a usual intrauterine therapy for severe fetal anemia [54], so after the first success with animals, humans can be the next step.

Table 2. Blood type compatibility chart

Blood type of recipient	Red cells donor can be from
O^+	O^+or O^-
O^-	O^-
A^+	Any A^+; A^-; O^+or O^-
A^-	Any A^-or O^-
B^+	Any B^+; B^-; O^+or O^-
B^-	Any B^-or O^-
AB^+	Any AB^+; AB^-; A^+; A^-; B^+; B^-; O^+; or O^-
AB^-	Any AB^-; A^-; B^-or O^-

Future perspective

Despite progress in the clinical setting and in the laboratory with respect to blood transfusion [86], several aspects deserve special attention. First, the steady increase in blood product consumption and shortage of some phenotypes [87]. Second, a decline in regular blood donations over the next decades in many countries as a result of the aging of populations. Third, emerging alternatives such as artificial blood substitutes or *in vitro* stem cell-derived blood components are still in early stages of development and are not expected to be put in practice within the next few years [88]. Taken together, a declining donation rate and an increase in the consumption of blood components require novel approaches to solve this problem.

Similarly, the demand for transplants will keep increasing [89]. In support of this notion is the growing number of patients with end-stage renal disease [90], the use of stem cells for cardiac ischemia or liver progenitor cells as the organ procurement is limited [91,92].

It is clear from some studies that the new immunosuppressive drugs control acute rejection better and have potentially short-term economic advantages. However, their long-term

cost–effectiveness has not been determined [93]. In other words, the immunosuppresive treatment is expensive and not easily affordable for all patients. Herein, reducing or eliminating the necessity of immunosuppresive drugs would be one of the greatest scientific successes of all times.

Of particular concern is the disparity between patients awaiting transplantation and available organs that has forced many of them to go overseas to receive a transplant [94]. It is, therefore, imperative to reinforce the organ donation culture while further work is developed to get new options for immunotolerance beyond the drugs that we have in our clinical practice nowadays.

Overall, the worldwide tendency of chronic diseases that produce organ failure has been accompanied by challenges that need to be faced as specialists look to the future. Emerging issues such as the aging of the population bring new pressures on the availability of an affordable organ supply. These serious difficulties require innovative strategies and commitment of resources.

There have been described innovative approaches for induction of transplantation tolerance. Among these, the use of antigen-specific tolerogenic DCs that target autoreactive T cells is an attractive strategy, with the aim of reprogramming the immune system [95,96].

New results establish a role in immune tolerance for short-course immune induction therapy, defined as a specific, short-term immune modulation using a therapeutic agent to induce T-cell nonresponsiveness, limiting the need for long-term maintenance immune suppression [40]. In the clinical setting, this kind of tolerance has been observed as an unexpected phenomenom in many patients that weaned themselves off immune therapies over time without transplant rejection.

Research concerning HLA-G, a natural tolerogenic molecule involved in the maternal–fetal tolerance has made this molecule a critical key in the tolerance of allogeneic transplants. Indeed, HLA-G inhibits NK cell and cytotoxic T-lymphocyte cytolytic activity, $CD4^+$ T-cell alloproliferative responses, T cell and NK cell ongoing proliferation and DC maturation [97,98].

Another strategy for immune tolerance is the implantation of tissue obtained early during embryogenesis as a way to reduce immunogenicity of transplants. Adaptation of this methodology for transplanting organs so as to induce organogenetic tolerance would revolutionize transplantation therapy [99].

It is recognized that inhibiting endogenous danger signals may prevent APCs to become activated [100]. By using this approach, HSPs, nucleotides, reactive oxygen intermediates, extracellular-matrix breakdown products, neuromediators, cytokines, among others, must all be considered for potential design of blocking drugs to improve immune tolerance to foreign antigens. Should the early exposition in uterus of alloantigens be reached, we may begin to see easier tolerance to organ transplantation as the activation of APCs by danger signals would be diminished.

In summary, the surgical techniques for transplantation have evolved faster than the medical treatments to induce immunosuppression. In the coming years, the side effects of drugs will likely be reduced. It is likely that xenografts will be improved even with the development of chimeras, but researchers will still be thinking in 100% HLA compatibility transplantation with new alternatives such as artificial organ developments, genetically modified mouse or pig models, therapeutic HLA-G molecules and tolerogenic dendritic cells [101,102] and perhaps in two or three generations we will be talking about multitolerant human beings.

Box 1. Parents' blood types to have a baby group AB.

Mother group A and father is:
- Group B: baby group A, B, AB or O
- Group AB: baby group A, B or AB

Mother group B and father is:
- Group A: baby group A, B, AB or O
- Group AB: baby group A, B or AB

Mother group AB and father is:
- Group A: baby group A, B or AB
- Group B: baby group A, B or AB
- Group AB: baby group A, B or AB

Acknowledgements

The author thanks Martha Liliana Romero Prieto, Hospital Universitario Fundación Santa Fe, Colombia for her excellent help with the immunostaining. The author would also like to thank Luis Cejudo Espinosa, Autonomous University of the State of Mexico, for his excellent assistance with editing the manuscript.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Despite improved success in transplantation, problems regarding shortages of organs, side effects of immunosuppressive drugs and high cost remain.
- There has been an increasing interest in using animal organs, particularly pigs, but one potential problem is the transfer of potentially lethal viruses from animals to humans via xenografts.
- More recent therapeutic strategies are based on induction therapies, which concentrate on profound immune cell depletion at the time of transplant, when immune activation is most intense.
- Current therapeutic strategies to manipulate the immune response are certainly capable of reducing autoimmunity and reducing short-term rejection rates; however, they are associated with significant adverse events and, in the case of transplantation, have yielded little reduction in long-term rejection rates. Recently, in the transplant setting, there has been a shift to include concurrent cell infusions, broadly defined as hematopietic cell transplant, at the time of organ transplantation as a means of inducing tolerance.
- Therapeutic HLA-G molecules and tolerogenic dendritic cells are actively investigated for tolerance induction in transplantation.
- It could be possible to get a tolerance to nonself antigens if exposed between the second and third embryonic weeks, for example inducing tolerance to ABO blood group antigens to get an embryo capable of being a receptor from any kind of blood type.
- The window to get a multitolerant individual might be from weeks 6 to 16 of gestation in accordance with the thymus development.

Bibliography

Papers of special note have been highlighted as:
▪ of interest
▪▪ of considerable interest

1 Apostolopoulos V, Lazoura E, Yu M. MHC and MHC-like molecules: structural perspectives on the design of molecular vaccines. *Adv. Exp. Med Biol.* 640, 252–267 (2008).

2 Kruskall MS. The major histocompatibility complex: the value of extended haplotypes in the analysis of associated immune diseases and disorders. *Yale J. Biol. Med.* 63(5), 477–486 (1990).

3 Rocha N, Neefjes J. MHC class II molecules on the move for successful antigen presentation. *EMBO J.* 27(1), 1–5 (2008).

4 Blanchard N, Shastri N. Coping with loss of perfection in the MHC class I peptide repertoire. *Curr. Opin Immunol.* 20(1), 82–88 (2008).

5 Blasczyk R, Wehling J, Kotsch K *et al.* The diversity of the HLA class I introns reflects the serological relationship of the coding regions. *Beitr. Infusionsther. Transfusionsmed.* 34, 231–235 (1997).

6 Solberg OD, Mack SJ, Lancaster AK *et al.* Balancing selection and heterogeneity across the classical human leukocyte antigen loci: a meta-analytic review of 497 population studies. *Hum. Immunol.* 69(7), 443–464 (2008).

7 Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu. Rev. Immunol.* 21, 139–176 (2003).

8 Krichen H, Sfar I, Jendoubi-Ayed S *et al.* Genetic polymorphisms of immunoregulatory proteins in acute renal allograft rejection. *Transplant. Proc.* 41(8), 3305–3307 (2009).

9 Ferrara JL, Levine JE, Reddy P *et al.* Graft-versus-host disease. *Lancet* 373(9674), 1550–1561 (2009).

▪ **Excellent review of this phenomenon.**

10 Riddell SR, Appelbaum FR. Graft-versus-host disease: a surge of developments. *PLoS. Med.* 4(7), E198 (2007).

11 Drakopoulou E, Outram SV, Rowbotham NJ *et al.* Non-redundant role for the transcription factor Gli1 at multiple stages of thymocyte development. *Cell Cycle* 9(20), 4144–4152 (2010).

12 Huseby ES, Kappler JW, Marrack P. Thymic selection stifles TCR reactivity with the main chain structure of MHC and forces interactions with the peptide side chains. *Mol. Immunol.* 45(3), 599–606 (2008).

13 Tanchot C, Lemonnier FA, Perarnau B *et al.* Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science* 276(5321), 2057–2062 (1997).

14 Arens R, Schoenberger SP. Plasticity in programming of effector and memory CD8 T-cell formation. *Immunol. Rev.* 235(1), 190–205 (2010).

▪ **Complete explanation of the CD8 T-cell formation.**

15 Lakkis FG. Transplantation tolerance: a journey from ignorance to memory. *Nephrol. Dial. Transplant.* 18(10), 1979–1982 (2003).

▪▪ **High-quality paper explaining the transplantation tolerance.**

16 Selin LK, Brehm MA. Frontiers in nephrology. heterologous immunity, T cell cross-reactivity, and alloreactivity. *J. Am. Soc. Nephrol.* 18(8), 2268–2277 (2007).

17 Ibrahim S, Dawson DV, Sanfilippo F. Predominant infiltration of rejecting human renal allografts with T cells expressing CD8 and CD45RO. *Transplantation* 59(5), 724–728 (1995).

18 Gallon L, Gagliardini E, Benigni A *et al.* Immunophenotypic analysis of cellular infiltrate of renal allograft biopsies in patients with acute rejection after induction with alemtuzumab (Campath-1H). *Clin. J. Am. Soc. Nephrol.* 1(3), 539–545 (2006).

19 Daniel C, Nolting J, von BH. Mechanisms of self-nonself discrimination and possible clinical relevance. *Immunotherapy* 1(4), 631–644 (2009).

20 Moore DJ, Markmann JF, Deng S. Avenues for immunomodulation and graft protection by gene therapy in transplantation. *Transpl. Int.* 19(6), 435–445 (2006).

21 Akst LM, Siemionow M, Dan O *et al.* Induction of tolerance in a rat model of laryngeal transplantation. *Transplantation* 76(12), 1763–1770 (2003).

22 Jones ND, Brook MO, Carvalho-Gaspar M *et al.* Regulatory T cells can prevent memory CD8- T-cell-mediated rejection following polymorphonuclear cell depletion. *Eur. J. Immunol.* 40(11), 3107–3116 (2010).

23 Carvalho-Gaspar M, Jones ND, Luo S *et al.* Location and time-dependent control of rejection by regulatory T cells culminates in a failure to generate memory T cells. *J. Immunol.* 180(10), 6640–6648 (2008).

24 Watson AR, Lee WT. Defective T cell receptor-mediated signal transduction in memory CD4 T lymphocytes exposed to superantigen or anti-T cell receptor antibodies. *Cell. Immunol.* 242(2), 80–90 (2006).

25 Hawiger D, Masilamani RF, Bettelli E *et al.* Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells *in vivo*. *Immunity* 20(6), 695–705 (2004).

26 Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr. Opin Immunol.* 13(1), 114–119 (2001).

27 Marincek BC, Kuhnle MC, Srokowski C *et al.* Heat shock protein-antigen fusions lose their enhanced immunostimulatory capacity after endotoxin depletion. *Mol. Immunol.* 46(1), 181–191 (2008).

28 Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. *Annu. Rev. Immunol.* 19, 47–64 (2001).

29 Hermiston ML, Xu Z, Majeti R *et al.* Reciprocal regulation of lymphocyte activation by tyrosine kinases and phosphatases. *J. Clin. Invest.* 109(1), 9–14 (2002).

30 Toungouz M, Donckier V, Goldman M. Tolerance induction in clinical transplantation. the pending questions. *Transplantation* 75(Suppl. 9), S58–S60 (2003).

31 Ophir E, Reisner Y. Induction of tolerance in organ recipients by hematopoietic stem cell transplantation. *Int. Immunopharmacol.* 9(6), 694–700 (2009).

32 Klonisch T, Drouin R. Fetal-maternal exchange of multipotent stem/progenitor cells: microchimerism in diagnosis and disease. *Trends Mol. Med.* 15(11), 510–518 (2009).

33 Zhang C, Wang M, Racine JJ *et al.* Induction of chimerism permits low-dose islet grafts in the liver or pancreas to reverse refractory autoimmune diabetes. *Diabetes* 59(9), 2228–2236 (2010).

34 Dutta P, Burlingham WJ. Microchimerism. tolerance vs. sensitization. *Curr. Opin Organ Transplant.* 16(4), 359–365 (2011).

35 Ichinohe T. Long-term feto-maternal microchimerism revisited. Microchimerism and tolerance in hematopoietic stem cell transplantation, *Chimerism.* 1(1), 39–43 (2010).

▪▪ **Crucial paper to understand the microchimerism and its relation to tolerance.**

36 Prigozhina T, Slavin S. Transplantation of hematopoietic stem cells for induction of unresponsiveness to organ allografts. *Springer Semin. Immunopathol.* 26(1–2), 169–185 (2004).

37 Roncarolo MG, Gregori S, Lucarelli B *et al.* Clinical tolerance in allogeneic hematopoietic stem cell transplantation. *Immunol. Rev.* 241(1), 145–163 (2011).

38 Sordi V, Piemonti L. Therapeutic plasticity of stem cells and allograft tolerance. *Cytotherapy* 13(6), 647–660 (2011).

39 Sayegh MH, Fine NA, Smith JL *et al.* Immunologic tolerance to renal allografts after bone marrow transplants from the same donors, *Ann. Intern. Med.* 114(11), 954–955 (1991).

40 Getts DR, Shankar S, Chastain EM *et al.* Current landscape for T-cell targeting in autoimmunity and transplantation. *Immunotherapy* 3(7), 853–870 (2011).

▪▪ **Previous article published in *Immunotherapy* reinforcing the notions of transplantation and T cells.**

41 Sykes M. Mixed chimerism and transplant tolerance. *Immunity* 14(4), 417–424 (2001).

42 Andre-Schmutz I, Dal CL, Fischer A *et al.* Improving immune reconstitution while preventing GvHD in allogeneic stem cell transplantation. *Cytotherapy* 7(2), 102–108 (2005).

43 Auletta JJ, Cooke KR. Bone marrow transplantation: new approaches to immunosuppression and management of acute graft-versus-host disease. *Curr. Opin Pediatr.* 21(1), 30–38 (2009).

44 Kabelitz D, Janssen O. Antigen-induced death of T-lymphocytes. *Front. Biosci.* 2, D61–D77 (1997).

45 Guillonneau C, Seveno C, Dugast AS *et al.* Anti-CD28 antibodies modify regulatory mechanisms and reinforce tolerance in CD40Ig-treated heart allograft recipients. *J. Immunol.* 179(12), 8164–8171 (2007).

46 Ogawa S, Nitta K, Hara Y *et al.* CD28 knockout mice as a useful clue to examine the pathogenesis of chronic graft-versus-host reaction. *Kidney Int.* 58(5), 2215–2220 (2000).

47 Preston SL, Alison MR, Forbes SJ *et al.* The new stem cell biology: something for everyone. *Mol. Pathol.* 56(2), 86–96 (2003).

48 Leibson PJ. The regulation of lymphocyte activation by inhibitory receptors. *Curr. Opin Immunol.* 16(3), 328–336 (2004).

49 Murray NA, Roberts IA. Haemolytic disease of the newborn. *Arch. Dis. Child Fetal Neonatal Ed.* 92(2), F83–F88 (2007).

50 Reid ME. Transfusion in the age of molecular diagnostics. *Hematology Am. Soc. Hematol. Educ. Program* 171–177 (2009).

51 Avent ND, Ridgwell K, Tanner MJ *et al.* cDNA cloning of a 30 kDa erythrocyte membrane protein associated with Rh (rhesus)-blood-group-antigen expression. *Biochem. J.* 271(3), 821–825 (1990).

52 Liumbruno GM, D'Alessandro A, Rea F *et al.* The role of antenatal immunoprophylaxis in the prevention of maternal–foetal anti-Rh(D) alloimmunisation. *Blood Transfus.* 8(1), 8–16 (2010).

53 Fung Kee FK, Eason E, Crane J *et al.* Prevention of Rh alloimmunization. *J. Obstet. Gynaecol. Can.* 25(9), 765–773 (2003).

54 Moise KJ Jr. Management of rhesus alloimmunization in pregnancy. *Obstet. Gynecol.* 112(1), 164–176 (2008).

55 Wylie BJ, D'Alton ME. Fetomaternal hemorrhage. *Obstet. Gynecol.* 115(5), 1039–1051 (2010).

56 Van DV, I, Chong SS, Cota J *et al.* Single-cell analysis of the RhD blood type for use in preimplantation diagnosis in the prevention of severe hemolytic disease of the newborn. *Am. J. Obstet. Gynecol.* 172(2 Pt 1), 533–540 (1995).

57 Tavian M, Hallais MF, Peault B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. *Development* 126(4), 793–803 (1999).

58 Zambidis ET, Peault B, Park TS *et al.* Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive, and definitive stages resembling human yolk sac development. *Blood* 106(3), 860–870 (2005).

59 Tavian M, Peault B. Embryonic development of the human hematopoietic system. *Int. J. Dev. Biol.* 49(2–3), 243–250 (2005).

60 De Miguel MP, Arnalich MF, Lopez IP *et al.* Epiblast-derived stem cells in embryonic and adult tissues. *Int. J. Dev. Biol.* 53(8–10), 1529–1540 (2009).

61 Malgieri A, Kantzari E, Patrizi MP *et al.* Bone marrow and umbilical cord blood human mesenchymal stem cells. state of the art. *Int. J. Clin. Exp. Med.* 3(4), 248–269 (2010).

62 Ariga H, Ohto H, Busch MP *et al.* Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy. implications for noninvasive prenatal diagnosis. *Transfusion* 41(12), 1524–1530 (2001).

63 Leduc M, Aractingi S, Khosrotehrani K. Fetal-cell microchimerism, lymphopoiesis, and autoimmunity. *Arch. Immunol. Ther. Exp. (Warsz.)* 57(5), 325–329 (2009).

64 Lleo A, Invernizzi P, Gao B *et al.* Definition of human autoimmunity – autoantibodies versus autoimmune disease. *Autoimmun. Rev.* 9(5), A259–A266 (2010).

65 Golshayan D. Pascual M. Tolerance-inducing immunosuppressive strategies in clinical transplantation: an overview. *Drugs* 68(15), 2113–2130 (2008).

66 Lele SM, Lele MS, Anderson VM. The thymus in infancy and childhood. Embryologic, anatomic, and pathologic considerations. *Chest Surg. Clin. N. Am.* 11(2), 233–253 (2001).

▪▪ **Very complete description of the thymus development.**

67 Suster S. Rosai J. Histology of the normal thymus. *Am. J. Surg. Pathol.* 14(3), 284–303 (1990).

68 Nishino M, Ashiku SK, Kocher ON *et al.* The thymus. a comprehensive review. *Radiographics* 26(2), 335–348 (2006).

69 Parish IA, Heath WR. Too dangerous to ignore: self-tolerance and the control of ignorant autoreactive T cells. *Immunol. Cell Biol.* 86(2), 146–152 (2008).

70 Kroczek RA, Mages HW, Hutloff A. Emerging paradigms of T-cell co-stimulation. *Curr. Opin Immunol.* 16(3), 321–327 (2004).

71 Coquerelle C, Moser M. DC subsets in positive and negative regulation of immunity. *Immunol. Rev.* 234(1), 317–334 (2010).

72 Ralainirina N, Poli A, Michel T *et al.* Control of NK cell functions by $CD4^+CD25^+$ regulatory T cells. *J. Leukoc. Biol.* 81(1), 144–153 (2007).

73 Collins A, Littman DR, Taniuchi I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. *Nat. Rev. Immunol.* 9(2), 106–115 (2009).

74 Burnet FM, Fenner F. The production of antibodies. *J. Immunol*. 66, 485–486 (1951).

75 Lederberg S. Genes and antibodies. *Science* 129(3364), 1649–1653 (1959).

76 Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature* 172(4379), 603–606 (1953).

77 Dixon FJ, Mauer PH. Immunologic unresponsiveness induced by protein antigens. *J. Exp. Med.* 101(3), 245–257 (1955).

78 Guerau-de-Arellano M, Martinic M, Benoist C *et al.* Neonatal tolerance revisited: a perinatal window for Aire control of autoimmunity. *J. Exp. Med.* 206(6), 1245–1252 (2009).

79 Nossal GJ. The immunological response of foetal mice to influenza virus, *Aust. J. Exp. Biol. Med. Sci.* 35(6), 549–557 (1957).

80 Smith RN, Howard JC. Heterogeneity of the tolerant state in rats with long established skin grafts. *J. Immunol.* 125(5), 2289–2294 (1980).

81 Egger M, Hauser M, Himly M *et al.* Development of recombinant allergens for diagnosis and therapy. *Front. Biosci. (Elite Ed.)* 1, 77–90 (2009).

82 Li GP, Liu ZG, Qiu J *et al.* DNA vaccine encoding Der p 2 allergen generates immunologic protection in recombinant Der p 2 allergen-induced allergic airway inflammation mice model. *Chin. Med. J. (Engl.)* 118(7), 534–540 (2005).

83 Li AF, Escher A. DNA vaccines for transplantation. *Expert Opin. Biol. Ther.* 10(6), 903–915 (2010).

84 Li A, Chen J, Hattori M *et al.* A therapeutic DNA vaccination strategy for autoimmunity and transplantation, *Vaccine* 28(8), 1897–1904 (2010).

85 Mor G, Eliza M. Plasmid DNA vaccines. Immunology, tolerance, and autoimmunity. *Mol. Biotechnol.* 19(3), 245–250 (2001).

86 Manner PA, Rubash HE, Herndon JH. Prospectus. Future trends in transfusion. *Clin. Orthop. Relat. Res.* (357), 101–115 (1998).

87 Bagnis C, Chiaroni J, Bailly P. Elimination of blood group antigens: hope and reality. *Br. J. Haematol.* 152(4), 392–400 (2011).

88 Seifried E, Klueter H, Weidmann C *et al.* How much blood is needed? *Vox Sang.* 100(1), 10–21 (2011).

89 Evans RW, Manninen DL, Dong FB. An economic analysis of pancreas transplantation: costs, insurance coverage, and reimbursement. *Clin. Transplant.* 7(2), 166–174 (1993).

90 Elsharif ME, Elsharif EG, Gadour WH. Costs of hemodialysis and kidney transplantation in Sudan: a single center experience. *Iran. J. Kidney Dis.* 4(4), 282–284 (2010).

91 Mayr M, Fukuda K. Cardiovascular stem cells revisited. *J. Mol. Cell. Cardiol.* 50(2), 257 (2011).

92 Sokal EM. From hepatocytes to stem and progenitor cells for liver regenerative medicine: advances and clinical perspectives. *Cell Prolif.* 44(Suppl. 1), 39–43 (2011).

93 Gentil MA, Cantarell AC, González Roncero FM *et al.* Impact of the new drugs in the cost of maintenance immunosuppression of renal transplantation. Is it justified? *Nephrol. Dial. Transplant.* 19(Suppl. 3), III77–III82 (2004).

94 Kwon CH, Lee SK, Ha J. Trend and outcome of Korean patients receiving overseas solid organ transplantation between 1999 and 2005. *J. Korean Med. Sci.* 26(1), 17–21 (2011).

95 Torres-Aguilar H, Aguilar-Ruiz SR, González-Pérez G *et al.* Tolerogenic dendritic cells generated with different immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory $CD4^+$ T cells. *J. Immunol.* 184(4), 1765–1775 (2010).

96 Harry RA, Anderson AE, Isaacs JD *et al.* Generation and characterisation of therapeutic tolerogenic dendritic cells for rheumatoid arthritis. *Ann. Rheum. Dis.* 69(11), 2042–2050 (2010).

97 Liang S, Ristich V, Arase H *et al.* Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6–STAT3 signaling pathway. *Proc. Natl Acad. Sci. USA* 105(24), 8357–8362 (2008).

98 Ristich V, Liang S, Zhang W *et al.* Tolerization of dendritic cells by HLA-G. *Eur. J. Immunol.* 35(4), 1133–1142 (2005).

99 Hammerman MR. Organogenetic tolerance *Organogenesis* 6(4), 270–275 (2010).

100 Shin OS, Harris JB. Innate immunity and transplantation tolerance. the potential role of TLRs/NLRs in GVHD. *Korean J. Hematol.* 46(2), 69–79 (2011).

101 Favier B, Howangyin KY, Wu J *et al.* Tolerogenic function of dimeric forms of HLA-G recombinant proteins. A comparative study *in vivo. PLoS ONE* 6(7), E21011 (2011).

102 Naranjo-Gómez M, Raich-Regue D, Onate C *et al.* Comparative study of clinical grade human tolerogenic dendritic cells. *J. Transl Med.* 9, 89 (2011).