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REVIEW ARTICLE

Allorecognition and the alloresponse: clinical implications

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Abstract

The artificial transfer of tissues or cells between genetically diverse individuals elicits an immune response that is adaptive and specific. This response is orchestrated by T lymphocytes that are recognizing, amongst others, major histocompatibility complex (MHC) molecules expressed on the surface of the transferred cells. Three pathways of recognition are described: direct, indirect and semi-direct. The sets of antigens that are recognized in this setting are also discussed, namely, MHC protein products, the MHC class I-related chain (MIC) system, minor histocompatibility antigens and natural killer cell receptor ligands. The end product of the effector responses are hyperacute, acute and chronic rejection. Special circumstances surround the situation of pregnancy and bone marrow transplantation because in the latter, the transferred cells are the ones originating the immune response, not the host. As the understanding of these processes improves, the ability to generate clinically viable immunotherapies will increase.

Introduction

The complexity of multicellular organisms requires the ability to recognize multiple different tissues each expressing many dissimilar genes as components of self while maintaining the ability to eliminate foreign proteins such as invading microorganisms. 'Self-compatible' tissues are recognized by the expression of a series of 'histocompatibility' antigens (major and minor) that engage a specific receptor complex found on cells of the immune system [e.g. the mammalian T-cell receptor (TCR)]. That this mechanism provides adequate self-non-self discrimination in even very primitive life forms (1) but is adapted in higher organisms to also provide immunity against environmental pathogens is an example of how the immune system has evolved under pressure from external challenges (2). The primacy of recognizing tissues of disparate individuals within the same species is supported by the much higher frequency of T-cell reactivity against alloantigens (3) than other conventional antigens (in this case keyhole limpet haemocyanin) (4).

Transplantation is the artificial transfer of cells, tissues or organs from one individual to another. Where the graft is syngeneic (genetically identical to the host, e.g. transplantation between identical twins) or autologous (a transplant from one individual into itself such as using stored blood for autotransfusion), there is perfect histocompatibility and no significant immune response is elicited. In this situation, the recipient is fully tolerant to the transplant and accepts it without a rejection phenomenon. Where there is histo*in*compatibility, however, in general an immune response is elicited against the foreign antigens, the magnitude of which determines acceptance or rejection of the transplanted tissues. The reaction can be either an 'alloresponse' (if the transfer occurs between two genetically disparate individuals of the same species) or a 'xenoresponse' (if the transplant is cross-species) depending on the nature of the donor and recipient, the target antigens being referred to as alloantigens and xenoantigens respectively. T lymphocytes occupy a central role in the rejection response to allogeneic

tissues, with depletion or suppression of their function being instrumental in the prolongation of transplant survival. Immunological memory and specificity, hallmarks of T-cell involvement, are both features of allograft rejection as re-exposure to the same alloantigens (re-transplant from the same or genetically identical donor) elicits an accelerated and heightened immune response (second set rejection) than on first encounter (first set rejection), whereas re-transplantation from a third party (unrelated) donor shows only first set rejection.

The response to transplanted tissues follows a two-step process which will be the subject of this review. 'Allorecognition' is the term used to describe the recognition of transplanted allogeneic tissues by the host, while 'alloresponse' denotes the effector mechanisms recruited in the reaction to the foreign tissue and the outcome of those effects. These definitions should be accepted with one caveat, namely that experimental systems often use a clinical endpoint (graft rejection or survival) as the readout and do not dissect out the relative contributions of allorecognition and alloresponse to the endpoint. Nevertheless, an understanding of these mechanisms may be critical to the development of targets for therapy and translation from the laboratory to the bedside. In addition, another important concept needs to be borne in mind, namely that there is a significant difference between 'antigenicity' (i.e. a foreign peptide capable of eliciting immunological recognition) and 'immunogenicity' (i.e. a foreign peptide capable of eliciting an immune response). This distinction is the basis of the clinically applicable therapies that are enabled by an understanding of allorecognition and alloresponses.

Allorecognition

Alloantigens can be divided into major histocompatibility complex (MHC) and minor histocompatibility antigens (mHAg), the former, divided into class I and class II molecules, responsible for eliciting the strongest immune responses to allogeneic tissues. Thymic development of T lymphocytes involves selective survival of thymocytes (mediated by survival signals) capable of recognizing self-MHC molecules. As a result, the mature T-cell repertoire is biased towards recognition of foreign peptides associated with self-MHC molecules as opposed to those associated with non-self MHC, while the response to allogeneic MHC is likely to be as a result of cross-reactivity with allogeneic peptide-bound MHC molecules (i.e. Allo + X = Self + Y) (5).

Allorecognition can proceed via several mechanisms (Figure 1): *direct* allorecognition, whereby T cells recognize determinants on the intact donor MHC molecules displayed on the surface of transplanted cells (6), *indirect* allorecognition in which donor MHC molecules are processed and presented as peptides by self-MHC molecules (in a similar fashion to conventional antigen processing) (7) and a recently described third mechanism termed *semi-direct* allorecognition where trafficking recipient dendritic cells (DC) acquire intact donor MHC:peptide complexes from cells of the graft enabling them to then be able to stimulate antigen-specific immune responses (8).

Direct allorecognition

Direct allorecognition was long believed to be the only mechanism by which allogeneic antigens could be recognized in the donor graft (Figure 1A). When measured, this response to allogeneic MHC molecules is of high frequency (9). Two non-mutually exclusive theories regarding the molecular mechanisms of this high frequency have been proposed, namely the 'high determinant density' and 'multiple binary complex' models which differ in the importance they allot to the presence of peptide in the allogeneic MHC–peptide complex.

In the former, it has been proposed that alloreactive T cells are directly able to recognize the exposed polymorphic residues on allogeneic MHC, thus consigning the bound peptide to secondary importance. This model predicts that if every MHC molecule on a cell surface can serve as a ligand for an allospecific T cell then the antigen density on the cell surface would be extremely high, in marked contrast with the density of a specific peptide plus MHC. The high ligand density available for stimulating alloreactive T cells implies that receptors of much lower affinities would be able to respond to the foreign MHC, leading to a high frequency of alloreactivity. This hypothesis is supported by demonstration that blocking the TCR-contacting regions of allo-MHC using synthetic peptides (10) or site-specific mutations inhibits specific alloresponses (11), presumably through inhibition of TCR–MHC contact (12). Additionally, alloreactivity in the absence of peptide has previously been shown (13).

The multiple binary complex model proposes that recognition of peptide bound by allogeneic MHC is of primary importance to direct allorecognition in a manner akin to conventional self-restricted responses (14). Multiple different bound peptides, in combination with one allogeneic MHC gene product, may produce determinants recognized by different cross-reactive T cells. Although the peptide is likely to be naturally processed and derived from a serum or cellular protein, the set of peptides bound by an allogeneic MHC molecule is often substantially different from that bound by the self-MHC homologue because of sequence variation in the peptide-binding groove. This model predicts that if each bound peptide is an essential component of the determinant recognized by alloreactive T cells, each peptide–allo-MHC complex will be recognized by a different alloreactive T cell and a single MHC incompatibility can stimulate a wide diversity of T cells. This hypothesis is supported by the mutant-transfected T2-I-A^b

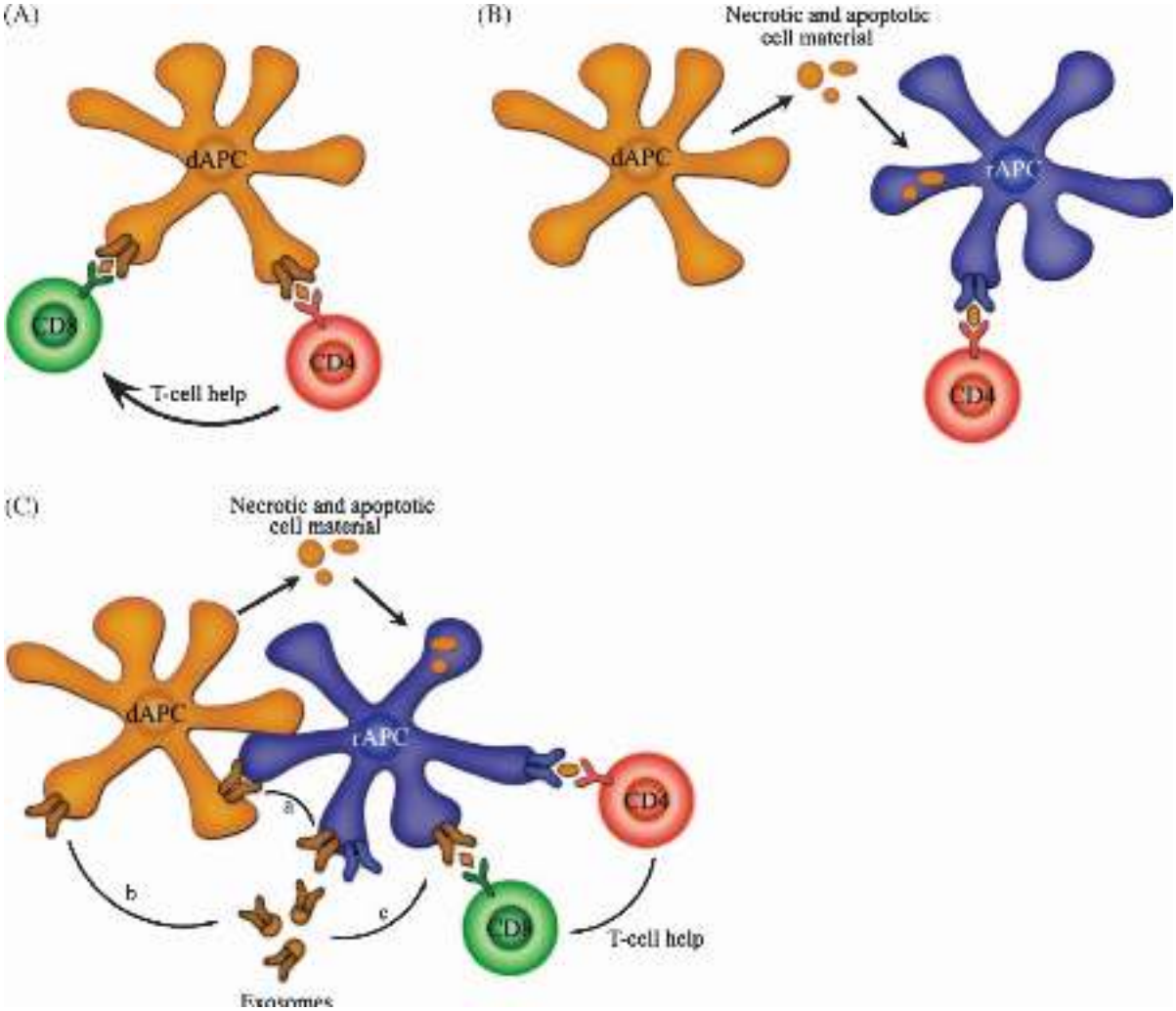

Figure 1 Direct, indirect and semi-direct pathways of allorecognition. (A) Direct pathway. Recognition of intact foreign major histocompatibility complex (MHC) on donor antigen-presenting cell (APC) primes CD4 and $CD8^+$ recipient T cells. $CD4^+$ cells then provide T-cell help for the effector function of $CD8^+$ cells. (B) Indirect pathway. The indirect pathway involves presentation of processed allogeneic MHC shed from foreign cells through cell necrosis and apoptosis. Recipient APCs present the processed peptides in the context of self-MHC class II to MHC class II restricted $CD4^+$ T cells. (C) Semi-direct pathway. Cell-to-cell contact between donor and recipient APC may transfer intact membrane components including intact allo-MHC (a). Likewise, donor APC can release small vesicles, known as 'endosomes' containing intact MHC (b), which fuse with the membrane of recipient APCs (c). The recipient APC, now chimaeric for MHC, stimulate direct pathway CD4 and CD8 responses through intact foreign MHC and indirect responses through processing and presentation of peptides of foreign MHC acquired from necrotic and apoptotic cell material. Given that the same APC stimulates both CD4 and CD8 cells, linked help can occur.

cell line that is unable to process antigen and is incapable of stimulating allospecific responses and in which transfection with stable peptide–MHC class II complex restores the ability to stimulate alloresponses (15). Similarly, alloreactive $CD8^+$ T cells have been shown to be specific for a self-peptide presented by foreign class I molecules, with no evidence of peptide-independent components (3). Furthermore, displacement of endogenous peptides from allogeneic antigen-presenting cells (APCs) by incubation with exogenous peptides leads to loss of allorecognition by allospecific T cells (16). Another study looked at the peptide-complex recognition ability of 12 cytotoxic allogeneic T-cell clones and for all of them the allorecognition was peptide specific whether the allogeneic MHC molecules were expressed on normal cells or antigen-processing-deficient cells (13).

The vigorous nature of the direct alloresponse and its immediacy in comparison with the indirect pathway (see below) is the result of direct recognition of intact MHC by T cells without the need for processing and presentation by self-MHC. Mechanistically, it is likely that direct allorecognition can proceed via both mechanisms discussed above, the overall contribution of each being related to the site and magnitude of the differences in MHC molecules between responder and stimulator cells. Specifically, where

the allogeneic MHC is structurally very disparate from responder MHC, the alloresponse may be directed against residues on the MHC itself (high determinant density pattern of recognition), whereas where self and foreign MHC are closely matched, the focus of the alloreactivity may be directed towards epitopes of endogenous peptides that are displayed by stimulator but not by responder MHC molecules (multiple binary complex pattern) (17).

Indirect allorecognition

The indirect pathway refers to the recognition of processed peptides of allogeneic histocompatibility antigen presented by self-MHC (7) and therefore differs from the direct pathway by the requirement for antigen processing (Figure 1B). There is considerable evidence for the involvement of this pathway in graft rejection (18) including studies of human recipients of heart, kidney and liver allografts with *in vitro* detection of indirect response showing a strong correlation with episodes of clinical rejection (19).

Alloantigens shed from a graft are, in general, processed as exogenous antigens and therefore presented by APCs in association with self-MHC class II. Therefore, the response to alloantigen presented by the indirect pathway is dominated by $CD4^+$ T cells. While there is considerable amplification of the rejection response through the generation of multiple epitopes via processing of alloantigens, the natural corollary is that responses to the indirect pathway are comparatively slower compared with those to the direct pathway. It is also likely that the indirect response is responsible for long-term responses to engrafted tissues once passenger (donor) APC, and by inference direct responses, are exhausted.

The importance of the indirect pathway is suggested by demonstrations that immunization of animals with peptides of allogeneic MHC (by definition able to elicit only indirect rather than direct responses) results in vigorous allograft rejection (20), whereas intrathymic injection of similar peptides down-modulates the indirect response sufficiently to prolonged survival of subsequent allografts of the same MHC type (21). Similarly, in the antibody response to transplanted tissues, B-cell function is dependent on T-cell help from $CD4^+$ T cells stimulated through the indirect, rather than the direct response (22).

Semi-direct allorecognition

Recently, a number of publications have shown that intact cell surface molecules, including MHC, can be transferred between cells of the immune system and that MHC-recipient cells become able to stimulate T-cell responses as a result (8) (Figure 1C). Although the mechanism of this transfer is likely to involve cell-to-cell contact (23), other mechanisms such as release and uptake of small vesicles (exosomes) have also been implicated (24).

Traditional descriptions of cross-talk between the direct and indirect pathways (e.g. that indirect pathway $CD4^+$ T cells can both amplify and diminish direct pathway $CD8^+$ T-cell responses) (25, 26) have relied on a four-cell, unlinked model, whereby $CD8^+$ T cells are stimulated through the direct pathway by donor cells, while helper or regulatory $CD4^+$ T cells are recruited through interaction with recipient DC presenting allogeneic MHC through the indirect pathway.

The description of MHC transfer helps to resolve the paradox that the four-cell hypothesis is non-compliant with the dogma that CD4 and CD8 T cells are recruited (and linked) by the same APC by proposing an alternative method of alloantigen presentation. This 'semi-direct' pathway of allorecognition (27), whereby recipient APCs acquire allogeneic MHC:peptide complex through MHC transfer (and stimulate $CD8^+$ T cells through the direct pathway) as well as peptides of allogeneic histocompatibility antigens (which are processed and recruit $CD4^+$ T cells through the indirect pathway) links direct and indirect allorecognition through a single APC and also provides a mechanism for the observed cross-talk between them.

It is also a possibility that molecular transfer of MHC in comparison with antigen processing may lead to a more faithful delivery of allogeneic antigens to lymph node resident T cells. Although there is no direct evidence in support of this, $CD4^+$ T cells acquiring MHC by membrane transfer are capable of both stimulating and inhibiting autologous CD4 cells responses in the same manner as 'professional' APCs (28). Therefore. the semi-direct pathway of allorecognition may have implications for the regulation of responses to allogeneic tissues.

Relative contribution of the direct, indirect and semi-direct pathways to allorecognition

The presence of passenger APC in donor tissues at the time of transplantation dictates that the direct anti-donor alloresponse is vigorous in the early period post-engraftment and diminishes with the death and removal of these APCs over time. The indirect alloresponse, on the contrary, requiring antigen capture and processing, is less rapid than the direct pathway but continues for the life of the graft as graft-derived antigens are continuously acquired and processed. As a ratio of alloresponsiveness, therefore, direct allorecognition predominates in the early post-transplant period, while the indirect pathway becomes more prominent with time. Of clinical relevance is the observation that rejection of transplanted tissues is more commonly observed in the early post-engraftment period (usually the first 6 months), while tolerance to grafts develop at a later time point. This correlates with demonstrations that regulatory $CD4^+$ $CD25^{hi}$ T cells that can mediate transplant tolerance

have indirect rather than direct pathway alloreactivity (29). The relative contribution of the semi-direct pathway to clinical rejection is as yet unknown.

Histocompatibility antigens

MHC protein products

The protein products of MHC molecules, expressed on the surface of all nucleated cells, are responsible for the immune response to allogeneic tissues. Of all the genes included in this region, two highly variable groups are central in allorecognition. These are the class I and class II molecules. Class I molecules are known as human leukocyte antigens (HLA)-A, -B and -C in humans and H2-K, -D, -L in mice and are constitutively expressed on most nucleated cells. Class II molecules are known as HLA-DR, -DP and -DQ in humans and H2-A and -E in mice and are constitutively expressed only by bone marrow-derived APCs, such as macrophages, DC, B lymphocytes and by thymic epithelial cells. The convention is to identify the genes in Roman letters (e.g. HLA-DRB or H2-D) and the encoded proteins in corresponding Greek symbols (e.g. HLA-DR βor H2-Aβ) (Figure 2).

The MHC contains the most variable functional genes described in vertebrates. At three of the more variable human MHC loci, HLA-A, HLA-B and HLA-DRB1, 243, 499 and 321 alleles have been resolved worldwide, respectively, and nucleotide diversity in the human MHC is up to two orders of magnitude higher than the genomic average (30). This polymorphism underlies the extreme difficulty in finding perfectly matched organs or unrelated bone marrow donors that will not induce a strong anti-MHC alloresponse. MHC genes are inherited from the parents as a whole set or haplotype, and because each individual has two sets of chromosomes, one haplotype will come from the mother and the other from the father.

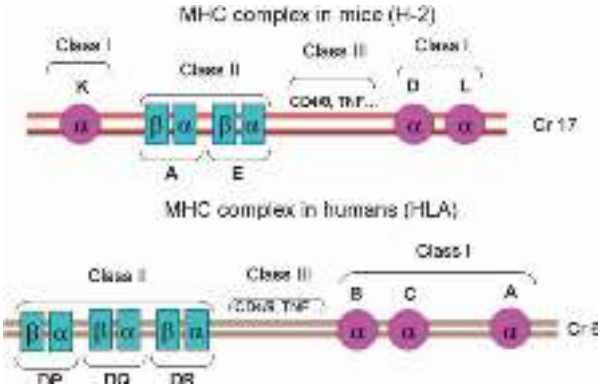

Figure 2 The major histocompatibility complex (MHC) in mice and humans. MHC genes are encoded on chromosomes 6 and 17 in humans in humans and mice, respectively.
Adapted from http://pathmicro.med.sc.edu/ghaffar/mhc2000.htm

These molecules play a critical role in the normal immune system, namely the presentation of peptides in a form that can be recognized by T cells. In particular, $CD8^+$ T cells recognize peptides presented by class I molecules and $CD4^+$ T cells recognize those presented by class II molecules and this is valid both for the self-MHC molecules as well as the allo-MHC molecules. From the crystal structures of the extracellular portions of human class I and class II molecules, it is now clear that the MHC molecules form a 'groove' where the peptide to be presented is bound (31). The peptides presented are the result of the natural processing of cellular and serum proteins. The peptide-binding groove of the MHC molecules on each cell is thus occupied by a very diverse (several hundreds) set of different peptides. Class I molecules are mainly occupied by peptides originating from intracellular proteins, whereas those presented by class II molecules have mainly an extracellular origin (32); although cross-presentation of peptides of extracellular origin has been widely demonstrated in class I molecules (33). It has been confirmed that the TCR recognizes a complex of two MHC helixes and a bound peptide. In the allorecognition setting in a direct pathway response, these MHC–peptide complexes recognized are from the allogeneic tissue. Recently, it has been shown that complementarity-determining region (CDR) 3α could undergo rearrangements to adapt to structurally different peptide residues. This CDR3 loop flexibility helps to explain TCR binding cross-reactivity and thus supports the conundrum of T cells responding to MHC molecules that they have not been selected to recognize (34).

The MHC class I-related chain (MIC) system

In 1994, two new polymorphic families of MHC class I-related genes, termed MHC class I-related chain A (MICA) and B (MICB), were described (35). These genes are located near the HLA-B locus on chromosome 6 and encode cell surface glycoproteins that do not associate with β-2 microglobulin. These molecules function as restriction elements for intestinal γ/δT cells and they behave as cell stress molecules. MICA is expressed in endothelial cells, keratinocytes and monocytes, but not in $CD4^+$, $CD8^+$ or $CD19^+$ lymphocytes (36). It is, therefore, likely that the polymorphic MICA molecule may be a target for specific antibodies and T cells in solid organ grafts or in graft *vs* host disease (GvHD) (37). The anti-MICA antibodies induce a prothrombotic state, characterized by a loss of surface heparan sulphate and thrombomodulin from cultivated endothelial cells (38). In fact, in kidney transplants, two prospective trials, after 1 and 4 years, have provided strong evidence that HLA and MICA antibodies are associated with graft failure (39).

Minor histocompatibility antigens

A different set of polymorphic non-MHC proteins have been identified that are important in provoking transplant rejection, they were defined by Snell and colleagues as mHAg, as the rejection reactions they induced in mice were slower (40). In principle, any protein that has polymorphisms within a species can become mHAg. Peptides from these proteins are presented to T cells in an MHC class I or class II restricted manner (41). The number of possible mHAgs in transplants performed between genetically unrelated, MHC-matched individuals, is very large. However, the reactions seem to be restricted to a few epitopes, thus dubbed immunodominant (41). The molecular basis for this phenomenon is incompletely understood, although it has recently been shown that both the duration of individual mHAg presentation and the avidity of T-cell antigen recognition influence the magnitude of the cytotoxic response that ensues (42).

The frequency of T cells responding to these antigens in non-transplanted individuals is very small and can only be measured *in vitro* after *in vivo* immunization or repeated stimulations, as opposed to direct pathway responses. When alloresponses of mHAgs have been measured, the cells that respond to these antigens are generally $CD8^+$ T cells, implying that most mHAgs are peptides bound to self-MHC class I molecules. However, peptides bound to self-MHC class II molecules can also participate in the response to MHC-identical grafts (43). The *in vivo* correlate of an immune response to an mHAg is transplant rejection, or in MHC-matched individuals, GvHD (44). GvHD is a series of manifestations and symptoms that appear after bone marrow transplantation (BMT) and results from an immune response of the immunocompetent cells of the donor against the tissues of the recipient. The effector immune responses are specifically described later on. Notably, even though mHAgs are named minor, and the frequency of responders to these antigens is very low, after transplantation, a single immunodominant mHAg can induce GvHD. Apart from gene polymorphisms, homozygous gene deletions can also serve as mHAgs as it has recently been described for an autosomal gene in the UDP-glycosyltransferase 2 family (45).

Minor HLA antigens important in transplantation have been described from different cellular origins.

(a) Encoded by sex chromosomes: The most thoroughly studied are a set of proteins encoded on the male-specific Y chromosome that are known collectively as H-Y antigens. The absence of Y-chromosome-specific gene products in females induces responses to male antigens. In fact, these responses are very frequent (37–50%) in women with previous male pregnancies (46), whereas male anti-female responses are not seen (because both males and females express X-chromosome-derived genes). To date, the number of H-Y epitopes described in humans that are important in transplantation is 10 (47). These are restricted by either class I or class II molecules and originate in six different loci of the Y chromosome (DFFRY, SMCY, TMSB4Y, UTY, DBY and RPS4Y1).

(b) Encoded by autosomes: Non-Y-linked mHAgs have also been shown by T cells from patients with GvHD after BMT between HLA identical individuals. The first example identified in humans was named 'HA' (48) after the patient. Recognition of this peptide was restricted by class I molecules. In the interim, other antigens have been identified for humans (HA-1, -2, -3, -8, HB-1, ACC-1, etc.); their cellular origin is varied: Mysoin 1G, LBC oncogen, BCL2A1, and some not yet identified genes (47) are examples.

(c) Encoded by mitochondrial DNA (mtDNA): Tracking of an mHAg to the small mitochondrial genome from the studies of a maternally transmitted transplantation antigen informed that such peptides could become histocompatibility antigens (49). Cytotoxic T lymphocytes (CTL) were used to test candidate peptides derived from polymorphic regions of the enzyme mt-ND1. A simple amino acid difference in the peptide was found to account for immunogenicity. Subsequently, additional mitochondrial genes in mouse and rat have been found to encode mH peptides, and several are presented to T cells by non-classical, MHC class I molecules (50). In the humans, however, no effect was observed on cumulative disease-free survival or incidence rate of GvHD when the clinical effect of mtDNA mismatches was studied in a Japanese cohort (51).

Natural killer-cell-mediated allorecognition

Recent genetic studies have established that the killer cell immunoglobulin-like receptor (KIR) genomic region displays extensive diversity through variation in gene content and allelic polymorphism within individual KIR genes. It is shown by family segregation analysis, genomic sequencing and gene order determination that genomic diversity by gene content alone gives rise to more than 20 different KIR haplotypes and at least 40–50 KIR genotypes (52). The importance of this recognition stems from the fact that in the clinical setting of mismatched hematopoietic stem cell transplantation, donor *vs* recipient natural killer (NK) cell alloreactivity has been associated with better outcome (53). This alloreactivity derives from a mismatch between inhibitory receptors for self-MHC class I molecules on donor NK clones and the MHC class I ligands on recipient cells. NK-cell function is regulated by clonally distributed inhibitory receptors that are specific for self-MHC class I molecules. Lack of engagement of these receptors results in target cell lysis (missing self-recognition), which has the potential to eliminate the remaining malignant recipient-originated cells (54). The role of NK-cell alloreactivity in

solid organ transplantation is less known. Results in animal models show that NK cells are neither necessary nor sufficient for acute immune rejection – which does not exclude an NK-cell contribution to the rejection process (55).

The alloresponse

When an alloantigen that is both antigenic and immunogenic is recognized by any of the pathways mentioned above, the resulting effector arm of the immune system is termed the alloresponse. In such a response, the innate and adaptive immune systems function synergistically to reject the allograft through non-exclusive pathways: including contact-dependent T-cell cytotoxicity, granulocyte activation by either T helper 1 (Th1)- or Th2-derived cytokines, NK-cell activation, alloantibody production and complement activation.

Grafted tissue destruction is achieved through different mechanisms: (i) direct cytotoxicity exerted by $CD4^+$ or $CD8^+$ T cells that are recognizing donor MHC molecules through the direct pathway, (ii) macrophage-mediated delayed type hypersensitivity stimulated by $CD4^+$ and $CD8^+$ T cells that have been activated through the direct or the indirect pathway and (iii) complement activation or antibody-dependent cytotoxicity of grafted cells opsonized by allogeneic antibodies (56). The important role of these alloantibodies in mediating rejection has been emphasized by Terasaki (57). Of note, the presence of anti-donor antibodies implies that B-cell to T-cell cross-talk in response to alloantigens has occurred; this by definition should have occurred via the indirect pathway. Namely, B cells recognizing antigen via their B-cell receptor have internalized it, processed it to peptides that have then been presented in the context of self-MHC to T cells, which have provided help for B-cell effector function and antibody class switching.

In terms of T-cell polarized effector responses, both Th1 and Th2 responses can result in rejection responses, particularly in the human setting (56). The role of interleukin 17 (IL-17) and transplant rejection is yet to be elucidated (58).

This combined cellular and molecular response is reflected *in vivo* by different manifestations. The following descriptions have been extensively studied for kidney grafts because of availability of biopsy material from engrafted tissue, but the features apply to all solid organ transplants.

Hyperacute rejection

This is the term applied to very early graft loss, usually within the first 48 h. It occurs when preformed antibodies are present in the recipient's serum, specific for donor antigens expressed on graft vascular endothelial cells. Such antibodies fall into two main categories: low affinity immunoglobulin M (IgM) antibodies, which are specific for ABO blood group antigens and high affinity IgG antibodies directed against HLA antigens. The binding of these antibodies to their targets triggers activation of clotting, complement and kinin cascades leading to intravascular thrombosis, ischaemia and subsequent necrosis. Previously, ABO blood matching in solid organ transplantation was mandatory between donor and recipient. However, recently, protocols to overcome the humoral response have been developed (including pretransplantation plasmaphoresis) and experience in transplanting into presensitized recipients is being obtained (59). Natural antibodies exist in humans against the Galactose-α-1-3-galactose epitope present in all other mammals and constitute one of the major impediments to successful xenotransplantation (60). The generation of Gal-deficient pigs has overcome hyperacute anti-Gal-mediated xenograft rejection in nonhuman primates. However, non-Gal anti-porcine natural antibodies still represent a potentially relevant immunological hurdle in a subgroup of individuals by inducing endothelial damage in xenografts (61). The second group of antibodies consists of high affinity IgG antibodies directed against donor HLA antigens. As already mentioned, the existence of anti-MHC alloantibodies indicates indirect pathway T-cell sensitization. These usually occur as a result of previous immunization, by blood transfusions, pregnancies or failed allografts. They also occur in 1% of the population for no obvious reason (62). A full immunological evaluation with ABO blood group determination, HLA typing, screening for antibody to HLA phenotypes and cross-matching need to be gathered before transplantation to avoid antibody-mediated hyperacute rejection or to proceed with specific protocols in highly sensitized or in positive T-cell cross-match patients (63).

Acute rejection

In the absence of any preformed antibodies, solid organ grafts can still be rejected after a few days. In the clinical setting, with the presence of pharmacological immunosuppression, this form of rejection usually occurs between 5 days and 3 months after transplantation. Histological findings in acute rejection (AR) generally show a diffuse interstitial cellular infiltrate composed of both $CD4^+$ and $CD8^+$ T cells, where the picture is dominated by $CD8^+$ T cells with an activated or memory, $CD45RO^+$, phenotype (64). Whereas, for other forms of AR, such as vascular rejection, the infiltrating cells found in the intimal arteritis lesions of the biopsies are predominantly macrophages and T cells are in the minority (65). Recently, the specific transcriptional activity of the infiltrating cells has been associated with clinically significant acute cellular rejection to differentiate it from other forms of lymphocytic infiltrates (66). In some animal models, it is notable that both $CD4^+$ T-cell and $CD8^+$ T-cell populations can reject solid organ allografts independently, while in others there is some

evidence that $CD4^+$ cells are an absolute requirement (67). In the clinical setting, it is likely that both cell subtypes are involved in the rejection process. In summary, it appears that the AR process is a complex event composed of many effector cells including $CD4^+$ T cells, $CD8^+$ T cells and macrophages.

Chronic rejection

This term was used in the initial years to describe slow late deterioration of graft function. Recently, the term is limited to mean late graft loss caused by a host-anti-graft immune response (68). Several factors contribute to the pathogenesis of late graft loss. To better understand the mechanisms involved in this process, the terminology is being redefined. The array of changes found in biopsies of grafts with progressive dysfunction is referred to as chronic allograft nephropathy. This is characterized by chronic interstitial fibrosis, tubular atrophy, vascular occlusive changes and glomerulopathy (69).

Despite limiting the term chronic rejection (CR) to describe the immune-mediated chronic changes present in late graft dysfunction, there are disagreements about the histological changes that constitute CR. The Banff criteria include extension of interstitial fibrosis, tubular atrophy, mesangial matrix increase, chronic glomerular changes (presence of 'double contours' in capillary loops thought to be secondary to basement membrane duplication) and chronic vascular changes. Several authors agree that the vascular features of CR are disruption of the elastic lamina, the presence of inflammatory cells in the intima (endothelialitis) and fibrous intimal thickening because of proliferation of myofibroblasts (70). Other authors claim that the specific changes related to immune-related responses are endothelialitis, tubulitis and complement (C4d) deposition in peritubular capillaries (68). Others argue that the vascular changes are the primary immunological insult and the parenchymal fibrosis changes are secondary to the ischaemia (71). There is common consent that the detection of C4d deposits in the presence of donor-specific alloantibodies in the circulation imply a B-cell involvement in CR (68). These and other findings suggest that antibody-mediated rejection is important in graft failure (57). In fact, in recipients of renal, cardiac and lung allografts, the development of anti-HLA antibodies is linked to the development of CR (72). Although common consent has not yet been reached concerning the histology of CR, it is clear that there are risk factors linking chronic transplant dysfunction and the anti-donor immune response.

The damage sustained to an allograft is therefore the result of a complex process; it usually does not represent a single entity but the summated effects of tissue injury from several pathogenic insults and the graft's healing response, modified by alloimmunity and immunosuppression. A mixed histological picture is thus common with several drivers of tissue damage and fibrosis often operating simultaneously (73). Additionally, the processes underlying CR can develop very early, in fact in kidney transplants it has been described to appear as early as 3 months post-transplantation (70).

Special considerations

There are several circumstances that pose specific problems for alloresponsiveness. Pregnancy, for instance, carries a significant alloantigen challenge as 50% of foetal histocompatibility antigens are paternally derived (they are antigenic) and should elicit a rejection response. Nevertheless, in contrast to partially matched transplanted allografts, tolerance rather than rejection develops to the foetal tissues (i.e. these tissues are not fully immunogenic) (74). Foetal attempts to evade the maternal immune response [by diversion of expression of histocompatibility antigens from classical forms (e.g. HLA-A and HLA-B) towards non-classical forms (such as HLA-E, HLA-F and HLA-G) on cells of the trophoblast (75)] are only partially successful as T cells alloreactive to paternal antigens persist throughout pregnancy (74). The relative immunological privilege that is afforded to the developing foetus in spite of this is the result of cooperation between the maternal and foetal immune systems and are characterized by a number of mechanisms which are reviewed in Hunt (76). Briefly, production of inhibitory factors, either soluble [e.g. IL-10 (77), transforming growth factor-β (TGF-β) (78) and indoleamine 2,3-dioxygenase (IDO) (79)] or cell-bound [e.g. programmed death ligand-1 (PDL-1) (80) and FasL (81)] as well as a significant increase in the proportion of $CD4^+$ $CD25^+$ Tregs, both locally (uterine) and systemically (spleen and lymph node) (82), enhance tolerance towards paternally derived alloantigens during pregnancy. Indeed, the role of Tregs cannot be underestimated as Treg deficiency leads to termination of pregnancy between genetically disparate but not genetically identical parents (82). In fact, the cumulative effects of these mechanism may underlie and explain the frequent amelioration of human (maternal) autoimmune diseases during pregnancy (83) and the detectable persistence of foetal cells in the maternal circulation many years following parturition (84).

Another situation that requires special consideration is GvHD. The mechanism of allorecognition during GvHD is similar to host recognition of donor antigens but in reverse, that is to say that donor T cells recognize alloantigens on host APC (direct allorecognition) (85) and on syngeneic (donor) APC (indirect allorecognition) although it is probably the contribution of the host APC, which is of critical importance in GvHD (85). Although a full description of these processes is beyond the scope of this review [the reader is directed towards reference (86)], allorecognition (by donor T cells of recipient 'alloantigens') clearly plays

a central role as donor TCR Vα and Vβ usage appear restricted and skewed in both murine (87) and human (88) GvHD and often show specificity for distinct immunodominant antigens, usually of the mHAg family (87). This latter is of importance as GvHD develops even if the donor–recipient pair are matched for HLA antigens (89). Not surprisingly, perhaps, the risk of GvHD is higher in the setting of gender mismatching, especially if the female donor is multiparous or has had previous blood transfusions (presumably through sensitization to the H-Y antigen) (90).

Although most frequent following allogeneic BMT (91), GvHD does also occur in association with solid organ transplants including liver (92), intestine (93), lung (94), pancreas (95) and kidney (96). The relative risk of developing GvHD (higher in BMT than in solid organ transplants) is thought to be related to the number of donor lymphocytes which are transferred along with the allografted tissue especially because murine models show a dose–response relationship between GvHD severity and T-cell infusate number and depletion of donor T cells reduces the risk and severity of the disease. The pathophysiology of this condition consists of a three-step process (86), namely (i) tissue damage incited by the conditioning regime leading to upregulation of inflammatory mediators such as IL-1, tumour necrosis factor alpha (TNF-α), adhesion molecules and increased expression of MHC and co-stimulatory molecules on recipient APCs, (ii) migration of donor T cells to lymphoid tissues, activation and differentiation through interaction with host (and donor) APC followed by migration to target tissues of GvHD (including mucosal surfaces and skin) and (iii) an effector phase characterized by tissue injury mediated by reactive oxygen species and cytolytic mechanisms including TNF-α, perforin, granzyme and Fas–FasL interactions.

The role of regulatory cells, including Natural Killer T (NKT) cells and $CD4^+$ $CD25^+$ Tregs in GvHD is unclear at present although they are capable of suppressing GvHD (97, 98). The latter may operate through mechanisms including IL-10 production and can be expanded *in vitro* for this purpose (99).

The translational relevance of the basic science

The study of allorecognition and alloresponses is more than merely the study of immunology in the context of an artificial model. The very real immunological emphasis on maintenance of self-integrity through the exclusion of tissues belonging to genetically disparate members of the same species while permitting tolerance to semi-allogeneic foetal tissues argues in favour of the existence of a dynamic structure, which lends itself to modification and which may be manipulated through appropriate interventions. The identification of different pathways of allorecognition and different patterns of clinical alloresponses emphasizes the concept that a number of different targets may exist for monitoring or manipulation to engender clinical tolerance to engrafted transplants without the need for high dose broad-spectrum immunosuppression that currently carries with it the risk of life-threatening infections and malignancy.

In particular, there are two ongoing international studies, the Immune Tolerance Network and the European Union study, which aim to identify indices of tolerance that may distinguish those kidney transplant recipients who are apt to develop tolerance to a transplant from those that are not; and effector mechanisms whose alteration/modification may be critical in engendering tolerance.

With regard to immune manipulation, the most promising cellular therapies that are being tested to inhibit the response in solid organ transplantation are the adoptive transfer of *ex vivo* T cells with regulatory function and DC with specific tolerogenic potential. This illustrates the concept of antigenicity and immnogenicity whereby therapy aims to alter the response to an alloantigen, which is antigenic so as to render it non-immunogenic. In the context of allogeneic stem cell transplantation, the focus is on the ability to control relapses of the original leukaemic disease. In this area, *in vitro* cultured leukaemia-reactive CTL lines selected on their ability to inhibit the proliferation of leukaemic progenitor cells *in vitro* have been successfully applied to treat accelerated phase Chronic Myeloid Leukaemia (CML) (100).

Concluding remarks

In summary, allorecognition and the alloresponse are key components of the immune response that may actually predate the development of immunity. They are, in addition, dynamic entities, which may manifest as either acceptance or rejection of foreign tissues. An understanding of the mechanisms underlying allorecognition and the alloresponse as well as pathways of tolerance development will be essential in the design of clinically viable immunotherapy aimed at preservation of functional allografts without immunosuppression or development of GvHD.

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