THE COMPLEMENTARY ROLES OF DELETION AND REGULATION IN TRANSPLANTATION TOLERANCE

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Neonatal tolerance of alloantigens was described in mice nearly half a century ago, but unfortunately, the translation of these early findings into the clinical arena proved to be much more challenging than was first anticipated. However, the past decade has seen considerable progress in our understanding of the mechanisms that contribute to transplantation tolerance in experimental models. This review outlines our current understanding of the mechanisms of allograft tolerance, emphasizing the complementary roles of deletion and regulation of alloreactive T cells.

TOLERANCE
Specific immunological unresponsiveness, maintained either by passive mechanisms, such as deletion or functional non-responsiveness (anergy) of antigen-specific T cells, or by active suppression mediated by regulatory T cells.

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Billingham, Brent and Medawar's first description of neonatal tolerance of alloantigens in mice almost half a century ago fuelled high expectations for the clinical application of this phenomenon in transplantation medicine[1]. Crucial to any discussion of the TOLERANCE of ALLOANTIGENS is an understanding of the two pathways by which alloantigens can be recognized by recipient T cells. Although the vigorous nature of T-cell responses to allogeneic MHC molecules was discovered more than 60 years ago[2], the details of the mechanisms by which these antigens are recognized remain controversial. The following section discusses each of these pathways in turn, before directing attention to their temporal relationships during rejection.

Direct allorecognition

The direct pathway involves the stimulation of recipient T cells by donor antigen-presenting cells (APCs)[3], and for several decades it has been considered to be the main mechanism of graft rejection (FIG. 1). This interaction is of a direct nature, occurring between the T-cell receptor (TCR) and intact MHC molecules expressed on the surface of APCs. Three main observations have been put forward to underline the importance of this pathway. First, vigorous proliferation of recipient T cells *in vitro* can be observed in the presence of irradiated donor APCs, in the form of the MIXED LEUKOCYTE REACTION (MLR). The strength of the MLR might reflect the vigour of similar interactions between these cells *in vivo*. Second, various strategies to deplete organ grafts of APCs have allowed prolonged survival of the grafts in MHC-mismatched recipients[4,5]. Furthermore, donor-strain dendritic cells (DCs) have been shown to restore the immunogenicity of MHC-incompatible renal allografts from which APCs have been depleted[6]. Finally, matching of donor and recipient for MHC antigens improves graft survival, both in experimental models and patients. This observation might be reconciled with the high frequency of precursor T cells that show direct alloreactivity, which is estimated to be between 1% and 7% (REF. 7).

Acceptance of the phenomenon of direct allorecognition requires an explanation of its apparent transgression from the concept of self-MHC restriction. Put another way, how do T cells that recognize foreign MHC molecules undergo positive selection in the absence of thymic expression of alloantigens? Recent structural analysis of an alloreactive TCR has indicated that this dilemma might be resolved by the crossreactivity of T cells that are specific for self-MHC with allogeneic MHC[8]. This observation supports previous studies showing that a large proportion of the T cells that participate in a direct alloresponse have a memory phenotype, owing to priming by foreign antigens in the context of self-MHC[9,10].

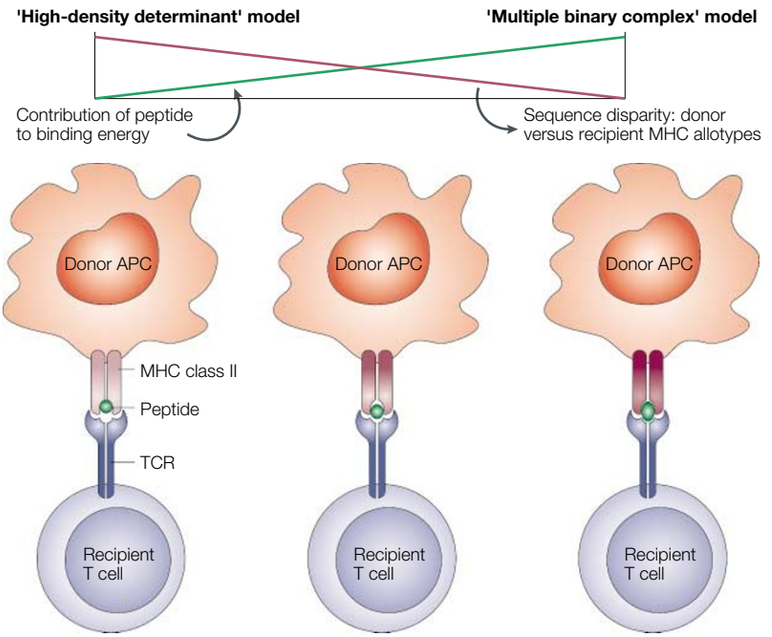

Figure 1 | **Mechanisms of direct allorecognition.** The interactions between donor MHC molecules and recipient T-cell receptors (TCRs) can be viewed as lying along a spectrum, in which the associated peptide contributes increasingly more energy to the overall binding affinity as the donor and recipient allotypes converge. At one end of the spectrum, the peptide has minimal input ('high-density determinant' model), whereas at the other end of the spectrum, the peptide has a crucial role ('multiple binary complex' model). Most interactions between donor antigen-presenting cells (APCs) and recipient T cells are thought to lie somewhere in the middle of the spectrum.

Two complementary hypotheses have been proposed to explain TCR–MHC interactions in the course of direct allorecognition. The first hypothesis, the 'high determinant density' model, proposes that the ligand of the alloreactive T cell is the allogeneic MHC molecule itself, irrespective of the nature of the bound peptide[11]. The second hypothesis, the 'multiple binary complex' model, proposes that the alloreactive T cell is specific for individual peptide–allo-MHC complexes, resembling more closely the pattern of self-MHC restriction[12]. In reality, these two models probably represent two extremes lying at either end of a spectrum in which MHC and peptide contribute, to different degrees, to the overall binding energy between the TCR and its ligand[13].

Indirect allorecognition

In recent years, there has been a surge of interest in the indirect pathway of allorecognition, in which processed peptides of allo-MHC molecules are presented by recipient APCs to effector T cells in the context of self-MHC (FIG. 2). This mechanism of graft rejection was first proposed more than 20 years ago, following the observation that AUG-strain rat kidneys were rejected slowly when transplanted into AS recipients, even after depletion of donor APCs by a 'parking' strategy involving pre-transplantation into first-round AS recipients[6]. Since then, the ability of the indirect alloresponse to effect graft rejection has been shown by studies in which skin grafts from MHC class-II-deficient mice were rejected by MHC class-I-deficient recipients[14]. The recipient mice lacked $CD8^+$ T cells that would recognize donor MHC class I molecules by the direct route, and their $CD4^+$ T cells could be stimulated only by recognition of donor MHC class I molecules presented indirectly in the context of recipient MHC class II molecules.

The indirect route of allorecognition also accounts for transplant rejection mediated by minor histocompatibility antigens — polymorphic peptides derived from non-MHC proteins that were characterized originally by their weaker potential to effect rejection than MHC molecules[15] (BOX 1). However, subsequent studies have shown that multiple differences in minor histocompatibility antigens between MHC-matched donors and recipients might elicit graft rejection with a speed that is comparable to that of MHC-mismatched tissues, underlining the potential importance of minor histocompatibility antigens in graft rejection.

Graft rejection: many effector pathways

Although the relative contributions of direct and indirect allorecognition to the rejection response have been debated for the past two decades, a clearer picture is now beginning to emerge. There are three phases of rejection — hyperacute, acute and chronic. The hyperacute response, which often occurs within 48 hours of engraftment, is induced by pre-formed recipient antibodies that mediate graft rejection by binding to antigens that are expressed by the vascular endothelium of the graft — predominantly blood-group antigens and MHC class I molecules. By contrast, the acute and chronic phases of rejection evolve over the days, weeks, months and years after engraftment. A sequence of events can be envisioned, beginning with the egress of donor APCs — predominantly DCs — from the graft to the draining lymph nodes, where the APCs evoke a direct alloresponse involving both $CD4^+$ and $CD8^+$ effector T cells. This response, which dominates acute rejection, is thought to recruit many cross-reactive $CD4^+$ memory T cells, which are primed against various environmental antigens in the context of self-MHC[9,10] (FIG. 3).

Dying donor APCs in the draining lymph nodes of the recipient provide a vehicle for the supply of donor histocompatibility antigens to recipient APCs. Migrant recipient APCs that traffic through the graft also have the opportunity to capture alloantigens, before egress back to the lymph nodes. Such alloantigens can then evoke an indirect alloresponse, involving both $CD4^+$ T cells, which interact with APCs through MHC class II molecules, and, to a lesser extent, $CD8^+$ T cells, which interact with CROSS-PRESENTING APCs through MHC class I molecules (FIG. 3). The unique ability of DCs both to prime naive T cells and to mediate cross-presentation places them at the centre-stage of the indirect alloresponse, which is thought to contribute predominantly to chronic rejection[16]. However, recent work has indicated the potential of the indirect pathway to mediate acute rejection in circumstances in which the number of T cells that respond to alloantigens in an indirect fashion has been increased by sensitization before transplantation[17,18]. Attention is also focusing now on the potential

ALLOANTIGEN
An antigen with the potential to elicit an immunogenic response on transfer from one individual to another of the same species, by virtue of allelic variance of the gene encoding the antigen between individuals. Allogeneic is a term that refers to genetic differences between individuals of the same species, whereas syngeneic is a term implying genetic identity — for example, in strains of mice that have been inbred.

MIXED LEUKOCYTE REACTION (MLR). A T-cell proliferative response to alloantigen observed *in vitro*. Primary MLRs are directed against gene products of the MHC and, in mice, to Mls determinants — endogenous superantigens that are encoded by genes of mouse mammary tumour virus.

CROSS-PRESENTATION
The presentation of peptides derived from exogenous antigens on MHC class I molecules.

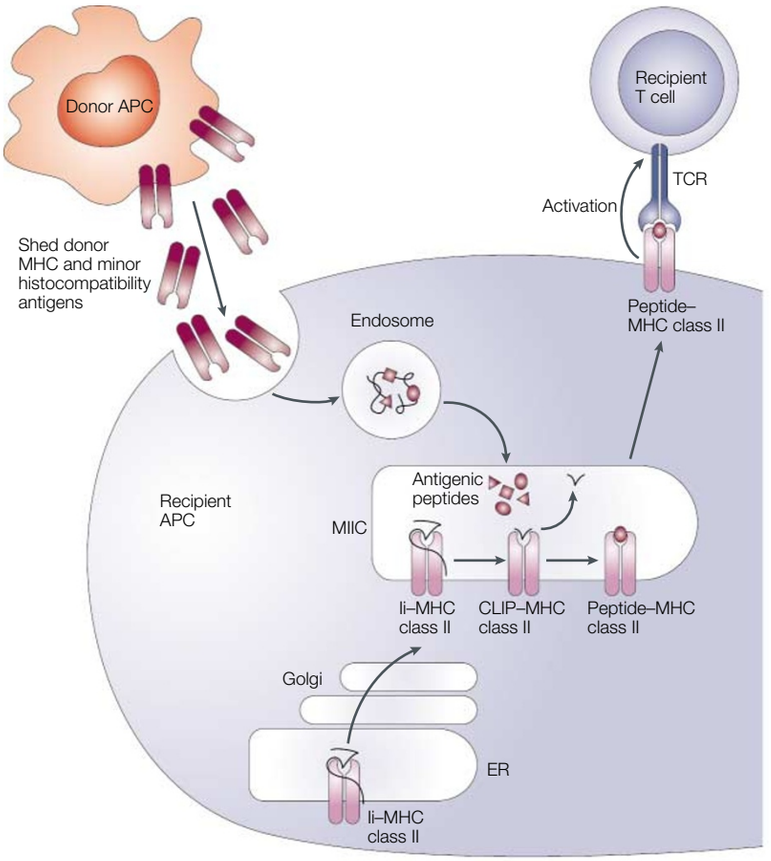

Figure 2 | **Mechanisms of indirect allorecognition.** Alloantigens are shed from the surface of donor antigen-presenting cells (APCs) or are taken up as dying allogeneic cells by host APCs. Recipient MHC class II molecules complexed with invariant chain (Ii) are assembled in the endoplasmic reticulum (ER) before being transported through the Golgi complex to the trans-Golgi network. From there, they are routed to a late endosomal vesicle with lysosomal characteristics, known as the MHC class-II-enriched compartment (MIIC). Partially degraded donor MHC molecules are loaded onto recipient MHC molecules. The assembled complex of donor MHC peptide and recipient MHC molecule is then expressed at the cell surface. Both donor MHC class I and class II molecules can be presented on recipient MHC class II molecules in this manner; cross-presentation of donor MHC peptides on recipient MHC class I molecules might occur also. CLIP, class-II-associated invariant chain peptide; TCR, T-cell receptor.

EPITOPE SPREADING
Intramolecular epitope spreading describes the progressive generation of immune responses to multiple epitopes in any one antigen, driven by the sequential cooperation of T helper (T_H) cells and B cells recognizing successive, new epitopes. Intermolecular epitope spreading refers to the recruitment of T_H cells and B cells that recognize successive epitopes in different antigens, driven by the non-specific, bystander activation of antigen-presenting cells by the inflammatory reaction occurring in the graft bed. In practice, both responses might occur concurrently — for example, in the context of allograft rejection or autoimmune disease.

importance of EPITOPE SPREADING during the course of the indirect alloresponse, with the sequential recruitment of immunogenic responses to self-peptides[19] and CRYPTIC DETERMINANTS[20], both of which could amplify the damage that is mediated by the initial immunological insult.

The influence of the direct pathway seems to diminish with time after transplantation, as shown by several clinical studies[21–24]. By contrast, the indirect anti-donor response is often sustained — increased frequencies of T cells with indirect specificities have been reported in patients undergoing chronic rejection of cardiac[25], renal[24,26] and pulmonary[27] transplants. The perpetual trafficking of recipient DCs through the transplanted tissue is likely to provide a continuous stimulus for the indirect alloresponse in draining lymphoid tissue. Taken together, these observations indicate that the indirect alloresponse is the most important threat to long-term transplant survival, underpinning the importance of mechanisms that regulate the activity of alloreactive cells on a chronic basis after the initial induction of allograft tolerance. The basis of this regulated silencing of alloreactive T cells is explored in the following section, before attention is directed to the events that must occur during the induction of tolerance to enable regulatory mechanisms to gain dominance.

Transplantation tolerance: regulatory T cells

Established tolerance is characterized by regulation. A characteristic feature of many models of transplantation tolerance is the transferable nature of the phenomenon of tolerance to a naive animal by T cells[28]. The transferred T cells can prevent the rejection of tissues that express the antigens to which the T-cell donor was tolerant[29–31]. LINKED SUPPRESSION might be observed also, in the form of tolerance to third-party antigens introduced with a graft bearing the tolerated antigens[32,33]. Recent studies have characterized further the phenotypic and functional nature of the cells that mediate this transferable tolerance[34–36]. The following three sections provide an overview of these cells in the context of transplantation tolerance, beginning with a historical perspective.

The history of suppressor T cells. Following the pioneering work of Gershon, who was the first to describe the phenomenon of T-cell-mediated suppression[37], many groups have shown that allograft tolerance can be transferred between hosts by T cells. By the mid-to-late 1980s, a large literature had accumulated based on studies of suppressor T-cell hybridomas carried out *in vitro*. These immortalized cells were claimed both to secrete suppressor factors that bind antigen in the absence of MHC molecules and to express the I–J determinant[38]. I–J was thought to be encoded in the *MHC* locus between the *I–A* and *I–E* loci, and was investigated using antisera raised between two mouse strains that were MHC recombinants. Elaborate networks of suppressor T (T_S) cells were described, involving T_S1, T_S2 and T_S3 cells, all acting in cascades[39]. Many of these original ideas were questioned with the advent of molecular genetics. Molecular characterization of the mouse MHC class II region established that no *I–J* locus exists, and cloning of the TCR α- and β-chain genes showed a lack of rearrangement in many of these 'suppressor hybridomas'[40]. These observations cast doubt on much of the literature on suppressor T cells, particularly those papers invoking I–J-expressing T cells and antigen-binding suppressor factors.

Owing to this unsatisfactory chapter in modern immunology, suppressor T cells became a taboo subject[41]. Nevertheless, transferable tolerance remained a robust phenomenon, underscoring the inability of deletion alone to account for allograft tolerance. Some progress has been made in defining the cell types that are instrumental in mediating regulation and their specificity, despite the fact that their mechanisms of action remain unknown.

Characterization of regulatory T cells. An important advance in our understanding of suppressor T cells came with the work of Sakaguchi and others, who

Box 1 | **Minor histocompatibility antigens**

Minor histocompatibility antigens are polymorphic proteins that present barriers to the transplantation of organs between genetically non-identical individuals who are matched for proteins encoded by the MHC, which are known as H-2 proteins in mice and HLA proteins in humans[136]. Of importance are those proteins that are encoded by sex chromosomes, autosomes and mitochondrial DNA. Among the best characterized of the minor histocompatibility antigens are those encoded by the mouse Y chromosome, which are known collectively as H-Y. Up to five *H-Y* loci have been described — for example, the gene *Smcy*, which encodes the minor histocompatibility antigen H-Y/K^k, and the gene *Uty*, which encodes H-Y/D^b (REF. 137). Respective examples of mouse autosomal and mitochondrial minor histocompatibility antigens include β2-microglobulin and MTFα (maternally transmitted factor-α), which is encoded by the gene *mt-Nd1* (mitocondrial NADH dehydrogenase 1). Although many minor histocompatibility differences might exist between donor and recipient, a hierarchy of immunodominance is observed in practice such that only a small number of minor histocompatibility antigens ultimately seem to matter[138].

investigated the mechanisms that are responsible for autoimmune disease in mice thymectomized at an age of three days[42]. Thymectomized animals lacked a population of $CD4^+CD25^+$ cells that form 5–10% of splenic T cells in unmanipulated mice (BOX 2). The regulatory potential of these cells was shown by their ability to prevent autoimmune disease when adoptively transferred from normal to thymectomized mice. $CD4^+$ T cells were first implicated in transferable tolerance by the work of Hall and others in the 1980s (REFS 29–31), pre-dating by several years the realization that $CD4^+CD25^+$ T cells constitute a distinct and specialized regulatory population. The relevance of $CD4^+CD25^+$ T cells to transplantation was illustrated by the finding that these cells can attenuate mouse GRAFT-VERSUS-HOST DISEASE (GVHD)[34] and can transfer tolerance of mouse islet allografts between recipients[43]. However, regulatory T cells of other phenotypes have been shown also, contributing to the maintenance of tolerance in other models. For example, Waldmann and others have recently described a $CD4^+CD25^-$ regulatory T-cell population, with a potency that is lower by a factor of ten on a cellular basis than that of the $CD4^+CD25^+$ population[44]. Furthermore, NATURAL KILLER T (NKT) CELLS have also been ascribed regulatory properties in the context of allotransplantation[45].

The importance of $CD4^+CD25^+$ T cells is undisputed; however, the mechanisms by which they suppress other T cells remain obscure. Studies of mouse and human T cells carried out *in vitro* have indicated that their suppressive effects require cell–cell contact[46,47]. However, apparently discordant results have been derived from experiments carried out *in vivo*, in which suppression could be reversed by the use of a neutralizing interleukin-10 (IL-10)-specific monoclonal antibody[48,49]. One way to reconcile these data was to propose that the $CD4^+CD25^+$ T cells induce the release of IL-10 by a second cell population — not present *in vitro* — to amplify the tolerogenic signal *in vivo* (as shown recently by Dieckmann and others[50]). This theme of 'catalytic' cascades of regulatory T cells was developed further by Jonuleit and colleagues, who showed that INFECTIOUS TOLERANCE could be mediated by $CD4^+CD25^+$ T cells interacting with $CD4^+$ T helper (T_H) cells in a contact-dependent manner[51]; transforming growth factor-β (TGF-β) released by the secondary population of regulatory cells was responsible, in part, for their suppressive influence. Furthermore, a circulating $CD8^+CD28^-$ suppressor T-cell population — present in human recipients of cardiac transplants who were tolerant of their grafts — was shown recently to induce the upregulation of expression of immunglobulin-like transcript 3 (ILT3) and ILT4 on donor monocytes and DCs, rendering the APCs capable of anergizing $CD4^+$ T_H cells[52]. The upregulation of expression of ILT3 and ILT4 was thought to represent a more generic mechanism enabling regulatory T cells of various types to generate tolerogenic APCs, with potentially widespread effects on alloreactive T cells.

The regulatory T cells have indirect allospecificity. Soon after the phenomenon of self-restricted mediation of graft tolerance by regulatory T cells was proposed[53], several studies emerged in support of this concept. The early work of Lagaaij and others[54,55] showed that the survival of human cardiac and renal allografts could be improved by the prior transfusion of whole blood from a donor sharing at least one HLA-DR antigen with the recipient, in contrast to the deleterious effects of completely MHC-mismatched blood. These findings were explained by postulating the presentation of MHC class I allopeptides of the graft by blood-donor APCs through the shared MHC class II molecules that are recognized by self-restricted regulatory T cells. Complementary studies showing that tolerance of allopeptides can be induced by their oral administration[56] were explained by a mechanism involving indirect presentation of the allopeptides to regulatory T cells by intestinal APCs. However, confirmation of these ideas awaited the work of Niimi and others[33], who showed that oral exposure to a single donor alloantigen was sufficient to induce unresponsiveness to a fully allogeneic cardiac allograft, mediated by linked suppression through the indirect pathway. The ability of killed or sonicated allogeneic cells to induce oral tolerance of mouse corneal allografts added further credence to the involvement of the indirect alloresponse[57].

Two additional approaches have provided support for the theory of indirect allospecificity of transferable graft tolerance. First, the depletion of donor APCs from rat cardiac allografts by a 'parking' strategy in primary recipients — to preclude involvement of the direct pathway of antigen presentation — enabled the permanent acceptance of grafts by naive secondary hosts when syngeneic splenocytes from tolerant rats were engrafted at the same time[58]. Second, linked suppression of mouse skin allograft rejection across MHC class II barriers was shown to operate through indirect recognition of minor histocompatibility antigens[59]. So, recipient CBC/Ca × BALB/c ($H\text{–}2^k \times H\text{–}2^d$) F_1 mice rendered tolerant of B10.D2 ($H\text{–}2^d$) skin grafts under the cover of monoclonal antibodies specific for CD4 and CD8 were also tolerant of B10.BR ($H\text{–}2^k$) skin, which is compatible with the re-processing of B10 minor histocompatibility antigens on both $H\text{–}2^d$ and $H\text{–}2^k$ class II MHC molecules. Furthermore, (CBC/Ca × BALB/c) F_1 mice tolerant of

CRYPTIC DETERMINANTS
T-cell responses to proteins are directed consistently towards a single or a limited number of epitopes, a phenomenon that is known as immunodominance. Certain epitopes — known as cryptic epitopes — are represented poorly on self-MHC molecules, by virtue of inefficient processing or presentation by antigen-presenting cells. Although these epitopes (or clusters of epitopes — determinants) are normally 'concealed' from the immune system, they might become important in pathological states, such as in allograft rejection.

LINKED SUPPRESSION
Induction of tolerance to antigen 'A' can induce linked suppression of responses to a third-party antigen 'B', if 'B' is processed and presented by the same antigen-presenting cell as 'A'. This phenomenon depends on the presence of regulatory $CD4^+$ T cells.

GRAFT-VERSUS-HOST DISEASE
(GVHD). A disease that results from the recognition of host tissues by T cells that are present in the graft. GVHD is often monitored by its impact on the gastrointestinal system, where it has the potential to cause villous atrophy with attendant diarrhoea. If controlled, certain forms of GVH reaction (such as graft-versus-leukaemia effects) might have therapeutic potential.

NATURAL KILLER T CELLS (NKT cells). NK1.1$^+$ lymphoid cells, the morphology and function of which are intermediate between those of T cells and natural killer (NK) cells. NKT cells produce interleukin-4, might be CD4$^-$ CD8$^-$ or CD4$^+$CD8$^-$, and express low levels of αβ T-cell receptor (TCR) with an invariant α-chain and restricted β-chain specificity. Many of these TCRs recognize antigens that are presented by the non-classical MHC-like molecule CD1.

INFECTIOUS TOLERANCE The phenomenon by which 'professional' regulatory T cells confer suppressive properties on secondary subsets of T cells *in vivo*, acting in 'infectious' cascades. For example, CD4$^+$CD25$^+$ T cells can confer regulatory properties on CD4$^+$ T helper cells through contact-dependent interactions; these secondary regulatory T cells mediate their suppressive influence, in part, by the production of transforming growth factor-β.

B10.D2 skin showed linked suppression of (B10.BR × AKR) F_1 skin allograft rejection (FIG. 4).

More recent studies have provided increasingly convincing evidence for the indirect recognition of alloantigens as a mechanism driving tolerance. Experiments carried out *in vitro* showed that the regulatory function of CBA CD4$^+$CD45RB^{low} T cells from mice tolerant of B10 cardiac allografts could be disclosed only in the presence of (CBA × B10) F_1 APCs, such that indirect allorecognition could occur[49]. Complementary studies carried out *in vivo*, in which donor and recipient mice were genetically engineered to prevent direct or indirect alloresponses, showed that loss of the indirect pathway rendered co-stimulatory blockade ineffective

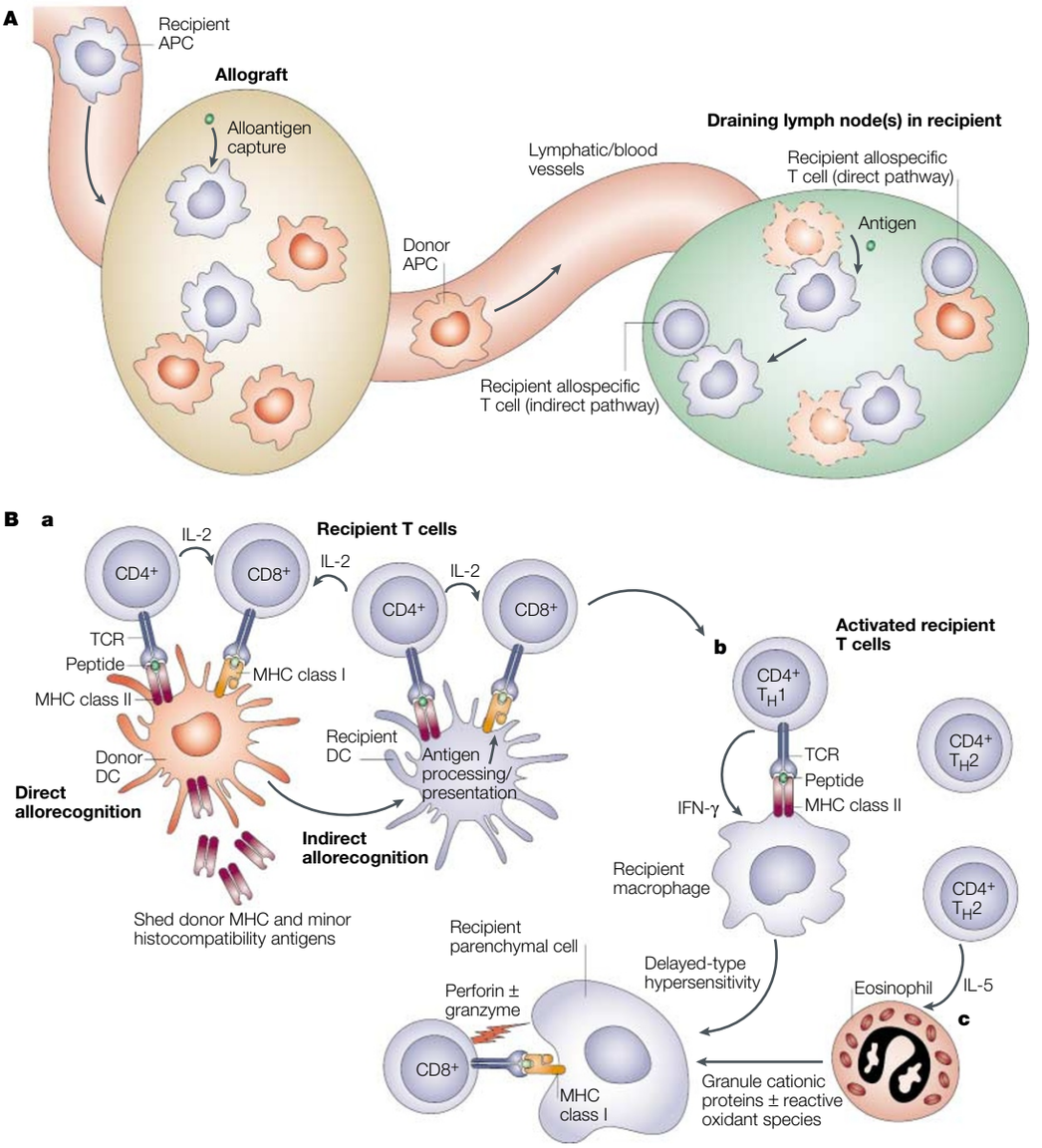

Figure 3 | **Cellular reactions characterizing the alloresponse. A** | The role of antigen-presenting cells (APCs) in the alloresponse. Donor APCs trafficking through the draining lymph nodes of the recipient elicit a direct alloresponse. Furthermore, these APCs provide a vehicle for the supply of donor histocompatibility antigens to recipient APCs. Migrant recipient APCs trafficking through the graft also have the opportunity to capture alloantigens, before egress back to the lymph nodes. Such alloantigens are then able to evoke an indirect alloresponse, involving CD4$^+$ T cells interacting with APCs through MHC class II molecules and, to a lesser extent, CD8$^+$ T cells interacting with cross-presenting APCs through MHC class I molecules. The unique ability of dendritic cells (DCs) to prime naive T cells and to mediate cross-presentation places them at the centre-stage of the indirect alloresponse, which is thought to contribute mainly to chronic rejection[16]. **B** | Integration of the alloresponse. Interaction of the direct and indirect pathways of allorecognition mediates graft rejection. Apart from the help that is given by CD4$^+$ T cells to CD8$^+$ cytotoxic T cells (**a**), helper T cells might also elicit a delayed-type hypersensitivity response mediated by macrophages activated by the secretion of interferon-γ (IFN-γ) (**b**). Furthermore, there is evidence to indicate that CD4$^+$ T cells might mediate direct cytotoxicity through their expression of FAS ligand (CD95L; CD178). Eosinophilic inflammation elicited by T helper 2 (T_H2) CD4$^+$ T cells through interleukin-5 (IL-5) might also damage the graft (**c**). Note the central role of recipient CD4$^+$ T cells.

Box 2 | **CD4⁺CD25⁺ regulatory T cells**

Defining characteristics[139]

- **Constitutive expression of CD25 and CD152 (cytotoxic T lymphocyte antigen 4, CTLA4)**[140]
- **High-level expression of CD44, and low-level expression of CD45RB and CD62L: activated or memory phenotype**[42]
- **Anergy, which can be broken by interleukin-2 (IL-2)**[42]
- **Ability to suppress the proliferation of and secretion of IL-2 by CD4⁺CD25⁻ T cells *in vitro*, in a contact-dependent, but non-antigen-specific, manner, requiring ligation of the T-cell receptor**[46,47]
- **Ability to regulate the function of CD8⁺ T cells**[141]
- **Dependence on IL-10 and CD152 for suppression of intestinal inflammation *in vivo***[48,142]
- **Development in the thymus, involving high-avidity interactions with thymic cortical epithelium**[143]
- **Unknown identity of cell-surface molecules mediating suppression**
- **Expression of glucocorticoid-induced tumour-necrosis factor receptor superfamily member 18 (GITR), the activation of which abrogates suppression**[144,145]
- **Ability to mediate infectious tolerance by contact-dependent interactions with CD4⁺ T helper cells, the acquired suppressor function of which is mediated, in part, by transforming growth factor-β** (REF. 51)

at prolonging the survival of skin and cardiac allografts, whereas loss of the direct pathway promoted graft survival[60].

The limitations of regulation. Although they are pivotal to the maintenance of transplantation tolerance, various experimental models have shown that regulatory mechanisms alone cannot induce tolerance across MHC-mismatched barriers. Deletional contraction or functional 'silencing' of the pre-existing peripheral alloreactive T-cell pool is required during the induction of tolerance, to enable the balance between the competing influences of alloreactive and regulatory T cells to be tipped towards regulation[61]. The earliest evidence for regulatory T cells came from studies of cardiac allografts transplanted from PVG (RT1^c) to DA (RT1^a) strain rats, under cover of treatment with either cyclosporin A[29,31,62,63] or anti-PVG hyperimmune serum[30,64]. Generally, tolerance of graft alloantigens was observed from five to seven weeks after transplantation, progressively 'maturing' with time from the date of engraftment[65]. The phenomenon of tolerance was shown to be transferable by CD4⁺ T cells derived from tolerant recipients, which were thought to be allospecific suppressor T cells that had undergone clonal expansion in the weeks after transplantation[31,63]. More recent studies have specifically implicated CD4⁺CD25⁺ T cells in the maintenance of transplantation tolerance[66,67].

Further work by Scully and others[68] confirmed that transferable tolerance in CBA/Ca mice receiving B10.BR skin grafts — involving many mismatches of minor histocompatibility antigens — showed progressive development with time, first being apparent from five weeks after engraftment. Subsequent studies examining the functional characteristics of regulatory T cells used a depleting CD4-specific monoclonal antibody to foster the induction of tolerance, providing a direct means of contracting the alloreactive T-cell pool to enable regulation to gain the 'upper hand'[58,67,69]. Alternative conditioning therapies — for example, the use of non-depleting CD154 (CD40 ligand)-specific monoclonal antibody — have been associated also with the deletion of potentially aggressive T cells, alongside the development of infectious tolerance[70].

Further insights into the competing influences of alloreactive and regulatory T cells have come from studies comparing the ability of immune deviation to induce tolerance across minor and major histocompatibility complex barriers. Although immune deviation alone was able to prevent the rejection of allografts mismatched for minor histocompatibility antigens, it could not prevent the rejection of allografts that differed in terms of MHC molecules, for which the number of alloreactive T cells to be controlled by regulatory mechanisms would have been at least tenfold higher[71,72].

Taken together, these studies indicate that maintenance of allograft tolerance is a state that requires the functional dominance of regulatory T cells over pathogenic alloreactive T cells. Such dominance is only likely to occur *in vivo* as a result of the contraction or 'silencing' of the pre-existing alloreactive T-cell pool, combined with strategies that foster the amplification of regulation[61,73]. What role regulatory T cells have in the induction of tolerance remains unclear, although a recent study prompts consideration of the influence of CD4⁺CD25⁺ T cells in both the induction and maintenance phases of graft tolerance[34].

The next section considers the pivotal role of deletion of alloreactive T cells in the induction of allograft tolerance, drawing evidence from experiments in both rodent and large-animal models.

Transplantation tolerance: the need for deletion

Induction of tolerance: the pivotal role of deletion. The induction of tolerance in experimental models of transplantation can be divided into two broad strategies — central and peripheral — the names being derived from the site at which T cells are targeted[61,74] (FIG. 5). Although there are fundamental differences between these strategies, they both require a two-pronged approach, because two distinct barriers to tolerance exist in the T-cell compartment. Mature T cells in the peripheral lymphoid compartment at the time of transplantation present the first barrier. Otherwise unmanipulated, thymectomized animals reject allografts with the same kinetics as control recipients, which shows that mature T cells that are present in the animal at the time of transplantation are sufficient to mediate graft rejection, without the need for new thymic emigrants. Hence, central-tolerance strategies that are designed to prevent alloreactive T cells from maturing in the thymus must also consider the problem of pre-existing, mature T cells. Conversely, peripheral approaches to the induction of tolerance must incorporate a mechanism to inactivate the pool of new, maturing T cells that are produced continuously in the thymus.

CHIMERISM/MACROCHIMERISM
The phenomenon of generating a composite of genetically distinct individuals — for example, after an allogeneic bone-marrow graft. Macrochimerism as applied to bone-marrow transplantation is a state characterized by the persistence of >5% circulating donor-derived cells.

LETHAL IRRADIATION
A dose of irradiation that is sufficient to induce complete myeloablation, which would be lethal without exogenous re-constitution of the bone marrow. This dose varies from 900 to 1200 rad in mice, depending on the strain.

Vβ TRACKING
The mouse T-cell receptor (TCR) β-locus contains approximately 20 Vβ segments. By molecular analysis of the Vβ segments that are expressed by T cells in the peripheral lymphoid tissues — known as Vβ tracking — an impression of the diversity of the TCR repertoire can be gained. Thus, Vβ tracking has been used to show the absence of host T cells responding to superantigens associated with donor MHC molecules, which is consistent with deletion of the reactive T cells in the thymus.

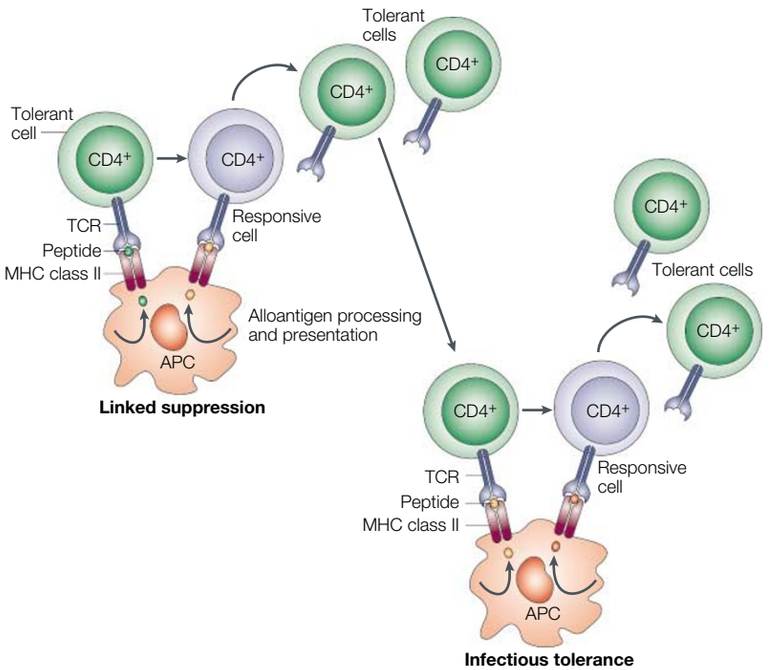

Figure 4 | **The phenomena of linked suppression and infectious tolerance.** Peripheral tolerance *in vivo* is associated with the related phenomena of linked suppression and infectious tolerance, both of which are dependent on regulatory CD4+ T cells. Induction of tolerance to antigen 'A' (green) — for example, by treatment with non-depleting CD4- and/or CD8-specific monoclonal antibody — can induce linked suppression of responses to a third-party antigen 'B' (orange), if 'B' is processed and presented by the same antigen-presenting cell (APC). Furthermore, these secondary, tolerant cells might confer tolerance on a third population of T cells that recognize a third-party antigen 'C' (red), as long as 'C' is processed and presented by the same APC as 'B'. The molecular mechanisms mediating both linked suppression and infectious tolerance remain unclear, but they might involve cytokines, surface molecules and competition for the APC.

Induction of central tolerance. The most important event in the induction of central tolerance is the deletion of alloreactive T cells in the thymus before they can be exported to the periphery[74]. The goal of this approach is to harness the mechanisms that mediate tolerance of self-antigens as a means of inducing tolerance of alloantigens, a strategy that requires the delivery of antigen to the thymic microenvironment.

The oldest and best studied method of inducing central tolerance, known as mixed haematopoietic CHIMERISM, is also the one that is closest to clinical development. Owen's studies in 1945 noted that genetically diverse Freemartin cattle sharing a placental circulation *in utero* were tolerant of each other's tissues[75]. Capitalizing on this observation, Billingham, Brent and Medawar induced neonatal tolerance by injecting newborn mice with allogeneic cells, before their development of a fully competent immune system[1]. In both cases, tolerance was thought to be achieved by the central deletion of alloreactive T cells, fostered by access of circulating alloantigens to the neonatal thymus. Ildstad and Sachs extended this concept to adult animals by the reconstitution of LETHALLY IRRADIATED mice with a mixture of T-cell-depleted, allogeneic donor- and self-type bone marrow — T-cell depletion of donor bone marrow being necessary to prevent GVHD, and T-cell depletion of syngeneic bone marrow being necessary to prevent rejection of donor bone marrow[76]. These animals developed multi-lineage MACROCHIMERISM and long-term tolerance of all donor tissues, a state that was maintained by donor APCs populating the recipient thymus[74]. TCR-transgenic models and Vβ TRACKING of T cells responding to endogenous SUPERANTIGENS expressed on donor cells have both been used to confirm the process of central deletion[77]. The depletion of donor antigen from mixed chimeras using monoclonal antibodies specific for donor MHC molecules resulted in loss of tolerance and the emergence of donor-reactive T cells from the recipient thymus[78]. Thymectomy of mice before depletion of donor cells enabled the preservation of tolerance, thereby showing both the role of intrathymic deletion and the necessity of donor cells for the induction of tolerance.

Direct evidence of the tolerogenic potential of central alloantigen presentation awaited experiments carried out by Barker and others, who showed that intrathymic injection of antigens could induce tolerance in adult animals; either donor splenocytes[79] or the donor tissue itself[80] could be used to induce tolerance of subsequent allografts. The success of engraftment in these studies required the treatment of recipients with a single dose of anti-lymphocyte serum (ALS) at the time of intrathymic injection of antigens — a strategy designed to deplete pre-existing mature alloreactive T cells in the periphery.

Another approach that has been studied widely — although it is not understood completely — was pioneered by Monaco, who pre-treated mouse allograft recipients with ALS and donor bone marrow 14 days before transplantation[81]. Treatment with ALS depleted the recipients of mature alloreactive lymphocytes, which was thought to enable the injected donor bone-marrow cells to home to the thymus to mediate central deletion of developing anti-donor thymocytes. Furthermore, stem cells present in the donor bone marrow were believed to enable the establishment of multilineage macrochimerism. However, direct evidence of a role for the thymus was lacking; indeed, prior thymectomy improved the induction of tolerance in some cases, perhaps by potentiating the phase of lymphocyte depletion (A. Monaco, personal communication). A general theme that underlies all of these various models is the apparent ability to maintain tolerance independently of macrochimerism, despite the importance of chimerism for its induction[82].

Induction of peripheral tolerance. Strategies to induce peripheral tolerance target mature T cells, by blocking either surface molecules that transduce activating signals, or related downstream intracellular signalling events. One of the first successes in this area was treatment with antibodies specific for the T-cell co-receptor CD4, used alone[83] or in combination with monoclonal antibodies specific for CD8 (REFS 84,85). This approach was used initially with depleting antibodies, under the assumption that T-cell depletion was necessary for the

SUPERANTIGEN
An antigen that reacts with all of the T cells belonging to a particular T-cell receptor (TCR) Vβ-region family, by virtue of its ability to crosslink MHC class II and specific Vβ molecules in a manner that is independent of direct interaction between the MHC and TCR. Superantigens stimulate a much greater number of T cells than do conventional antigens.

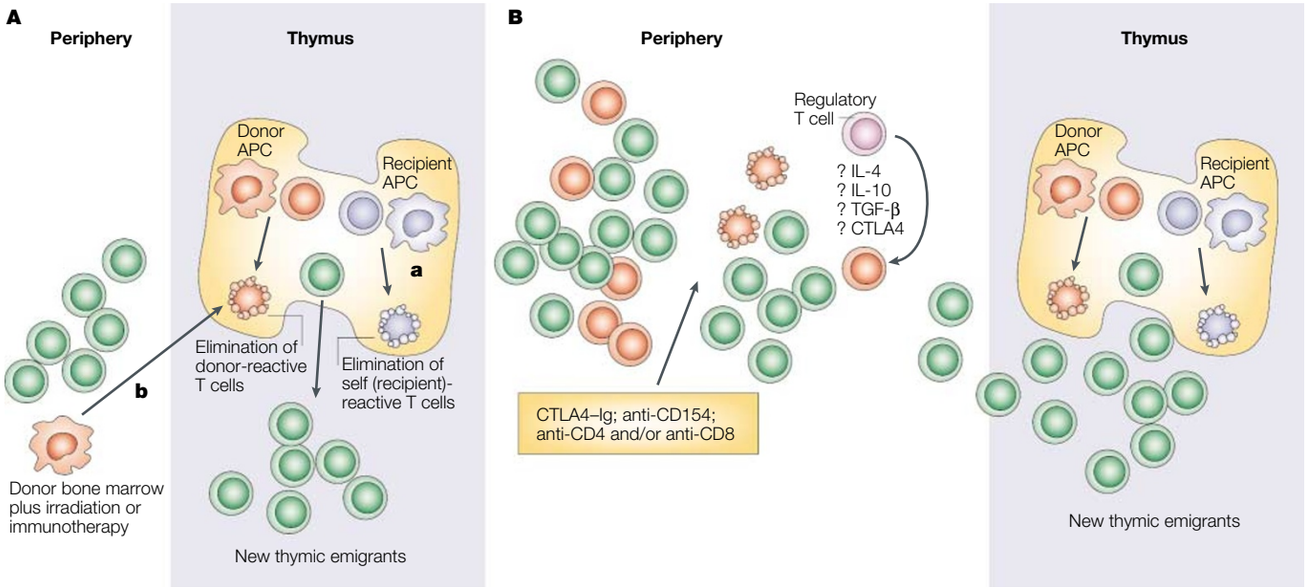

Figure 5 | **Strategies to induce central and peripheral allograft tolerance. A** | Central allograft tolerance. Negative selection of recipient T cells with high avidity for peptide-self-MHC complexes expressed by antigen-presenting cells (APCs) in the thymic medulla helps to shape the peripheral repertoire of T cells, enabling the deletion of the majority of cells with autoaggressive potential (**a**). Adoptive transfer of donor bone marrow to a recipient conditioned by irradiation or immunotherapy harnesses the phenomenon of negative selection to eliminate donor-reactive T cells, by their central deletion after interaction with donor APCs that have accessed the recipient thymus (**b**). Mature T cells exiting the thymus into the periphery (new thymic emigrants) are devoid of both self-reactive and donor-reactive cells. **B** | Peripheral allograft tolerance. Peripheral tolerance targets pre-existing, mature donor-reactive T cells, by blocking either the transduction of activation signals through surface molecules or the related downstream intracellular signalling events. All of the strategies that are illustrated have been shown to foster the development of regulatory T cells, which in most cases are $CD4^+$, with evidence for $CD4^+CD45RB^{low}$, $CD4^+CD25^+$ and $CD4^+CD25^-$ phenotypes. Although speculative, peripheral strategies might also show a component of central tolerance, as shown in part **A** — APCs from the allograft can take up residence in the thymus and induce central deletion of newly formed alloreactive T cells. CTLA4, cytotoxic T lymphocyte antigen 4; Ig, immunoglobulin; IL, interleukin; TGF-β, transforming growth factor-β.

induction of tolerance. However, subsequent studies have shown that non-depleting CD4-specific monoclonal antibodies — both with and without non-depleting CD8-specific monoclonal antibodies — are effective also[86]. The mechanism of this approach remains unclear, although the induction of apoptosis owing to alterations in T-cell signalling induced by ligation of CD4 has been proposed[87].

The discovery that co-stimulation is important for the activation of naive T cells[88] prompted consideration of co-stimulatory blockade as a means of manipulating T-cell-mediated immune responses *in vivo*. The most widely studied co-stimulatory pathway is that of the CD28 receptor, which is expressed by most T cells[89]. Activated APCs express its ligands, CD80 and CD86 (formerly known as B7.1 and B7.2)[90–92]. Co-stimulation through CD28 is required for optimal cytokine production, and the proliferation and survival of activated T cells[93]; blocking this pathway with either B7-specific monoclonal antibodies or the fusion protein cytotoxic T lymphocyte antigen-4–immunoglobulin (CTLA4–Ig)[94,95] prevents allograft rejection, enabling the induction of transplant tolerance in certain models[96].

CD154 (CD40 ligand) is expressed by activated T cells, and it is another target for co-stimulatory blockade[97,98]. Binding of CD154 to its ligand CD40 induces the expression of other co-stimulatory molecules (for example, CD80 and CD86), adhesion molecules and pro-inflammatory cytokines by APCs and endothelial cells[98–100]. Monoclonal antibodies specific for CD154 have proven to be effective in several animal models[101,102], having an additive effect when used with CD28-specific monoclonal antibodies[103]. A similar benefit of dual therapy with monoclonal antibodies specific for CD28 and CD45RB[104,105] indicates the presence of many, non-overlapping co-stimulatory signals, each offering the potential to enhance treatment regimens by a combined approach.

The immunosuppressive drug rapamycin is a complementary therapy, blocking pro-mitotic signals transduced through the common cytokine-receptor γ-chain (γc), which forms an integral part of the receptors for the cytokines IL-2, IL-4, IL-7 and IL-15 (REF. 106). So, rather than blocking the induction of expression of cytokines, rapamycin blocks the delivery of signals by the cytokines themselves, synergizing with co-stimulatory blockade to induce transplantation tolerance[107].

How do these various strategies promote tolerance? Emerging new data indicate a role for deletion during the induction of peripheral tolerance, even when the reagent that is administered to promote tolerance is not

inherently T-cell depleting. This phenomenon has been studied in the context of co-stimulatory blockade — both of the CD28–B7 and CD40–CD154 pathways — and with the use of rapamycin. There are two distinct ways in which activated T cells can undergo apoptosis. The first, known as death by neglect or passive cell death, occurs when activated T cells are deprived of growth factors[108,109]. This form of cell death can be inhibited by the constitutive expression of survival genes, such as those encoding BCL-2 and BCL-X_L[109,110]. By contrast, activation-induced cell death (AICD) occurs as a consequence of persistent antigenic stimulation. The main mechanism of AICD is autoligation of FAS (CD95) on the surface of T cells by FAS ligand (CD95L; CD178) expressed by the T cells themselves[111]. The induction of AICD by the FAS–FASL pathway requires previous priming of T cells by IL-2 (REF. 112). Thus, T cells from IL-2-deficient mice fail to undergo apoptosis during the terminal phase of the immune response[111]. Recent work has shown that the induction of tolerance across MHC barriers by co-stimulatory blockade requires intact pathways for both passive cell death and AICD. Mice carrying a transgene encoding Bcl-X_L in the T-cell lineage and mice deficient for IL-2 each resist the induction of tolerance by co-stimulatory blockade[107,113,114]. This requirement for apoptosis of alloreactive T cells might be attributable to the high frequency of cells responding to allogeneic MHC molecules[115,116]. This model is supported by the observation that transplantation tolerance can be induced across minor histocompatibility barriers — in which alloreactive T cells are less frequent by a factor of at least ten — by skewing the immune response towards T_H2 cytokines, without apoptosis of alloreactive T cells[72]. The ability of co-stimulatory blockade to render Bcl-X_L-transgenic or IL-2-deficient mice tolerant of minor histocompatibility antigen-mismatched allografts further supports this hypothesis[117].

Taken together, these data highlight the importance of deletional contraction of the alloreactive, anti-donor T-cell repertoire for stable transplantation tolerance. Once the frequency of anti-donor T cells has been reduced by central or peripheral deletion, or both, regulatory mechanisms can gain control of the remaining peripheral alloreactive T cells and newly emigrant alloreactive T cells from the thymus.

Clinical tolerance: how far from our grasp?

When the induction of neonatal tolerance in mice was first described more than 40 years ago, the application of this phenomenon to the clinical arena was anticipated to be a matter of only years away[1]. However, attempts to achieve tolerance in large-animal models have proven to be more challenging than was envisioned initially.

First, the translation of tolerance protocols that have been developed in rodents to large animals has been frustrated by crucial differences in both the genetic make-up of these animals and their systems of husbandry. Most rodent models have used highly inbred strains, often chosen for their ease of manipulation. By contrast, large animals that are used in experimental settings have generally not been inbred, with the exception of miniature pigs[118]. Although genetic heterogeneity mimics the clinical scenario more closely, it adds another dimension to the allogeneic barrier to transplantation. The use of young, highly inbred rodents housed in clean facilities involves a largely naive peripheral T-cell repertoire, in contrast to the greater proportion of memory T cells that are present in the typical transplant recipient, owing to age and encounter with environmental antigens. The higher proportion of memory T cells presents another barrier to transplantation, because these cells have greater resistance to tolerance than their naive counterparts[119,120]. A further problem that is inherent in the translation of results from small to large animals, and then to humans, lies in the differences between the models and the clinical situations that they are taken to mimic. A popular model in mice and rats is the heterotopic cardiac allograft, in which the transplanted heart is anastomosed to the main blood vessels of the recipient, without perturbation of the 'native' heart. The function of the transplanted organ is assessed generally by surface palpation of its contractions, a strategy that precludes the detection of rejection responses that are of insufficient magnitude to mediate cessation of contractile function. Marked histopathological damage could precede frank rejection, skewing the impression of 'tolerance' to include covert responses of potentially pathogenic impact. The ideal rodent model would be one in which the allograft is orthotopic, replacing completely the native organ; however, at present, other than the engraftment of islets of Langerhans into diabetic recipients, orthotopic transplantation remains impracticable. Further difficulties lie in the lack of appropriate reagents or in intrinsically different toxicities of reagents between rodents and large animals; a procedure that is effective and safe in a rodent might not be so in a pig or primate. All of the preceding considerations prompt caution in the extrapolation of observations in rodents to larger animals and humans; a phenomenon that is observed in a rodent merely offers promise for the success of a similar strategy in a larger animal, without any guarantees.

Nevertheless, after several decades of disappointment, there are now real grounds for optimism on the basis of principles that have been derived from small-animal models. The need for a wave of peripheral T-cell deletion to shrink the repertoire of T cells with direct anti-donor allospecificity is now recognized clearly[113]. This finding in mice was borne out by the work of Thomas and others in a primate transplantation model[121]. In combination with drugs such as deoxyspergualin, an immunotoxin conjugated to a CD3-specific monoclonal antibody enabled long-term survival of renal allografts[122,123]. The theoretical basis of this approach, which is being tested in human clinical trials, is to induce a phase of wholesale T-cell deletion, from which reconstitution is engineered to occur under cover of a drug fostering the development of allograft tolerance. So, a monoclonal antibody specific for CD52 — known as CamPath1 — has been used in humans to deplete T cells immediately after renal transplantation,

CALCINEURIN
A calcium/calmodulin-dependent serine phosphatase that is activated by the release of intracellular calcium from storage vesicles into the cytosol, mediated by the second messenger inositol-1,4,5-triphosphate. Calcineurin dephosphorylates nuclear factor of activated T cells (NFAT), which then translocates to the nucleus before binding to the enhancer of the gene encoding interleukin-2. Calcineurin is the target of both cyclosporin and tacrolimus.

ANERGY
A state of specific immunological tolerance in which the lymphocyte becomes functionally non-responsive — for example, by ligation of the T-cell receptor by peptide–MHC in the absence of effective co-stimulation. The anergic phenotype can be broken by exogenous interleukin-2.

in combination with low-dose cyclosporin[124]. A more targeted form of deletion is also being evaluated, using donor haematopoietic stem cells and partial peripheral T-cell depletion achieved using varying combinations of irradiation, monoclonal antibodies and cytotoxic drugs[74,118,125,126]. Although the chimerism that is achieved by such strategies is transient, it seems to be sufficient to induce a wave of T-cell depletion that enables other mechanisms of tolerance to gain dominance. The main barrier to widespread trials of this approach is the toxicity of the conditioning regimen, although the recent use of high-dose donor bone marrow in conjunction with co-stimulatory blockade might be a breakthrough in this area[127].

A second insight derived from experimental models, which is now being translated to human clinical trials, concerns the interplay between immunosuppressive drugs and mechanisms of tolerance. CALCINEURIN inhibitors, such as cyclosporin, have revolutionized clinical transplantation, but they lack the finesse required to enable the optimal development of allograft tolerance, because calcium-dependent signals seem to be necessary for the induction of crucial regulatory mechanisms, as shown *in vitro* and *in vivo*[113]. For example, the development of both mouse and human T-cell ANERGY involves calcium signalling[128], which might also prove to be pivotal for the activation of dedicated regulatory T cells[129]. Furthermore, calcineurin inhibitors prevent the optimal secretion of IL-2, which has a crucial role in T-cell deletion by AICD[130] and is likely to be implicated in the development of $CD4^+CD25^+$ T cells[131]. By contrast, rapamycin enables the induction of T-cell anergy and does not inhibit IL-2 secretion, thereby allowing IL-2-induced deletion of T cells while preventing IL-2-induced clonal expansion[132]. These advantages have led to increasing interest in the use of immunosuppressive protocols that include rapamycin[133]. The finding that rapamycin enables T-cell deletion in mouse models — in contrast to calcineurin inhibitors — has fuelled the next generation of clinical trials of CamPath1, which combine this reagent with rapamycin[107]. Similar clinical trials are planned for CD3-specific immunotoxins[134].

Although these findings offer hope for the generation of new approaches to the induction of allograft tolerance in the clinic, the history of this field is peppered with examples of strategies that have succeeded in small animals, yet have shown minimal utility in primates. Some of these examples are recent, including the failure of CD28 blockade to induce long-term graft survival and tolerance in non-human primates, despite efficacy in mice[135]. Although blockade of the CD154 co-stimulatory pathway has proved to be more efficacious in primates and humans[135], the unique association of this reagent with venous thromboses in humans — but not in rodents — has forced the early termination of these trials. Modifications of these approaches and the use of combined modalities are being explored at present. Only time will tell whether such protocols will favour the induction of robust clinical allograft tolerance.

Conclusion

Much has been learned about the mechanisms and regulation of transplantation immunity since the enlightened observations of Snell almost half a century ago[3]. Two pathways of allorecognition have been characterized, and their relative contributions to the rejection response are being evaluated. The overlapping mechanisms of transferable allograft tolerance are being dissected rapidly, with convincing new evidence for the role of $CD4^+CD25^+$ regulatory T cells. New strategies are being harnessed to promote allograft tolerance in an attempt to fine-tune the deficiencies of conventional immunosuppression. This field is poised to welcome yet further breakthroughs in the near future, which are anticipated not only to increase our knowledge of transplantation, but also to bring better quality of life to the many patients who are faced with the prospect of this daunting therapeutic modality.

1. Billingham, R. E., Brent, L. & Medawar, P. B. Actively acquired tolerance of foreign cells. *Nature* **172**, 603–606 (1953). **The first description of neonatal tolerance of alloantigens in mice.**
2. Gorer, P. The genetic and antigenic basis of tumor transplantation. *J. Pathol. Bacteriol.* **44**, 691–697 (1937).
3. Snell, G. The homograft reaction. *Annu. Rev. Microbiol.* **11**, 439 (1957).
4. Lafferty, K. A., Bootes, A., Dart, G. & Talmage, D. Effect of organ culture on the survival of thyroid allografts in mice. *Transplantation* **22**, 138 (1976).
5. Batchelor, J. R., Welsh, K. I., Maynard, A. & Burgos, H. Failure of long surviving, passively enhanced kidney allografts to provoke T-dependent alloimmunity. I. Retransplantation of AS X AUGF1 kidneys into secondary AS recipients. *J. Exp. Med.* **150**, 455–464 (1979).
6. Lechler, R. I. & Batchelor, J. R. Restoration of immunogenicity to passenger-cell-depleted kidney allografts by the addition of donor-strain dendritic cells. *J. Exp. Med.* **155**, 31–41 (1982).
7. Suchin, E., Wells, A. D. & Turka, L. A. Quantifying the frequency of alloreactive cells *in vivo*: new answers to an old question. *J. Immunol.* **166**, 973–981 (2001).
8. Daniel, C., Horvath, S. & Allen, P. M. A basis for alloreactivity: MHC helical residues broaden peptide recognition by the TCR. *Immunity* **8**, 543–552 (1998).
9. Lombardi, G. *et al.* Are primary alloresponses truly primary? *Int. Immunol.* **2**, 9–13 (1990). **An elegant study showing that a large proportion of the T cells that participate in the direct alloresponse have a memory phenotype, owing to priming by foreign antigens in the context of self-MHC.**
10. Merkenschlager, M. *et al.* Allorecognition of HLA-DR and -DQ transfectants by human CD45RA and CD45RO CD4 T cells: repertoire analysis and activation requirements. *Eur. J. Immunol.* **21**, 79–88 (1991).
11. Bevan, M. J. High determinant density may explain the phenomenon of alloreactivity. *Immunol. Today* **5**, 128–129 (1984).
12. Matzinger, P. & Bevan, M. J. Hypothesis: why do so many lymphocytes respond to major histocompatibility antigens? *Cell. Immunol.* **29**, 1–5 (1977).
13. Obst, R., Netuschil, N., Klopfer, K., Stevanovic, S. & Rammensee, H.-G. The role of peptides in T-cell alloreactivity is determined by self-major histocompatibility complex molecules. *J. Exp. Med.* **191**, 805–812 (2000).
14. Auchincloss, H. Jr *et al.* The role of 'indirect' recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. *Proc. Natl Acad. Sci. USA* **90**, 3373–3377 (1993).
15. Counce, S., Smith, P., Barter, R. & Snell, G. D. Strong and weak histocompatibility fine differences in mice and their role in the rejection of homografts of tumors and skin. *Ann. Surg.* **144**, 1988–2204 (1956).
16. Benichou, G., Valujskikh, A. & Heeger, P. S. Contributions of direct and indirect T-cell alloreactivity during allograft rejection in mice. *J. Immunol.* **162**, 352–358 (1999).
17. Benham, A. M., Sawyer, G. J. & Fabre, J. W. Indirect T-cell allorecognition of donor antigens contributes to the rejection of vascularized kidney allografts. *Transplantation* **59**, 1028–1032 (1995).
18. Braun, M. Y. *et al.* Acute rejection in the absence of cognate recognition of allograft by T cells. *J. Immunol.* **166**, 4879–4883 (2001).
19. Fedoseyeva, E. V. *et al. De novo* autoimmunity to cardiac myosin after heart transplantation and its contribution to the rejection process. *J. Immunol.* **162**, 6836–6842 (1999).
20. Boisgerault, F. *et al.* Induction of T-cell response to cryptic MHC determinants during allograft rejection. *Hum. Immunol.* **61**, 1352–1362 (2000).
21. Chow, D., Saper, V. & Strober, S. Renal transplant patients treated with total lymphoid irradiation show specific unresponsiveness to donor antigens in the mixed leukocyte reaction (MLR). *J. Immunol.* **138**, 3746–3750 (1987).
22. DeBruyne, L. A., Renlund, D. G. & Bishop, D. K. Evidence that human cardiac allograft acceptance is associated with a decrease in donor-reactive helper T lymphocytes. *Transplantation* **59**, 778–783 (1995).
23. Hornick, P. I. *et al.* Assessment of the contribution that direct allorecognition makes to the progression of chronic cardiac transplant rejection in humans. *Circulation* **97**, 1257–1263 (1998).
24. Baker, R. J. *et al.* Loss of direct and maintenance of indirect alloresponses in renal allograft recipients: implications for the pathogenesis of chronic allograft nephropathy. *J. Immunol.* **167**, 7199–7206 (2001).

25. Suciu-Foca, N., Ciubotariu, R., Itescu, S., Rose, E. A. & Cortesini, R. Indirect allorecognition of donor HLA-DR peptides in chronic rejection of heart allografts. *Transplant Proc.* **30**, 3999–4000 (1998).
26. Sayegh, M. H. Why do we reject a graft? Role of indirect allorecognition in graft rejection. *Kidney Int.* **56**, 1967–1979 (1999).
27. Reznik, S. I. *et al.* Indirect allorecognition of mismatched donor HLA class II peptides in lung transplant recipients with bronchiolitis obliterans syndrome. *Am. J. Transplant.* **1**, 228–235 (2001).
28. Hall, B. M. Mechanisms of induction of tolerance to organ allografts. *Crit. Rev. Immunol.* **20**, 267–324 (2000).
29. Hall, B. M., Jelbart, M. E. & Dorsch, S. E. Suppressor T cells in rats with prolonged cardiac allograft survival after treatment with cyclosporine. *Transplantation* **37**, 595–600 (1984).
30. Hall, B. M. Mechanisms maintaining enhancement of allografts. *J. Exp. Med.* **161**, 123–133 (1985).
31. Hall, B. M., Pearce, N. W., Gurley, K. E. & Dorsch, S. E. Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. III. Further characterisation of the $CD4^+$ suppressor cell and its mechanism of action. *J. Exp. Med.* **171**, 141–157 (1990).
32. Wise, M. P., Bemelman, F., Cobbold, S. P. & Waldmann, H. Cutting edge: linked suppression of skin graft rejection can operate through indirect recognition. *J. Immunol.* **161**, 5813–5816 (1998).
33. Niimi, M., Shirasugi, N., Ikeda, Y. & Wood, K. J. Oral antigen induces allograft survival by linked suppression via the indirect pathway. *Transplant. Proc.* **33**, 81 (2001).
34. Taylor, P. A., Noelle, R. J. & Blazar, B. R. $CD4^+CD25^+$ immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J. Exp. Med.* **193**, 1311–1317 (2001).
35. Graca, L., Cobbold, S. P. & Waldmann, H. Identification of regulatory T cells in tolerated allografts. *J. Exp. Med.* **195**, 1641–1646 (2002).
36. Karim, K., Bushell, A. & Wood, K. J. Regulatory T cells in transplantation. *Curr. Opin. Immunol.* **14**, 584–591 (2002).
37. Gershon, R. K., Cohen, P., Hencin, R. & Liebhaber, S. A. Suppressor T cells. *J. Immunol.* **108**, 586–590 (1972).
The first paper to articulate the concept of regulatory T cells.
38. Eardley, D. D. *et al.* Ly-1 inducer and Ly-1,2 acceptor T cells in the feedback suppression circuit bear an I-J subregion controlled determinant. *Immunogenetics* **11**, 549–557 (1980).
39. Cantor, H. & Gershon, R. K. Immunological circuits. *Fed. Proc.* **38**, 2058–2064 (1979).
40. Dorf, M. E., Kuchroo, V. K. & Collins, M. Suppressor T cells: some answers but more questions. *Immunol. Today* **13**, 241–243 (1992).
41. Green, D. R. & Webb, D. R. Saying the 'S' word in public. *Immunol. Today* **14**, 523–525 (1993).
42. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. & Toda, M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**, 1151–1164 (1995).
An elegant study documenting the role of $CD4^+CD25^+$ regulatory T cells in the maintenance of self-tolerance.
43. Gregori, S. *et al.* Regulatory T cells induced by 1α,25-dihydroxyvitamin D_3 and mycophenolate mofetil treatment mediate transplantation tolerance. *J. Immunol.* **167**, 1945–1953 (2001).
44. Graca, L. *et al.* Both $CD4^+CD25^+$ and $CD4^+CD25^-$ regulatory cells mediate dominant transplantation tolerance. *J. Immunol.* **168**, 5558–5565 (2002).
A provocative study showing the potential complexity of regulatory T-cell networks.
45. Seino, K. *et al.* Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proc. Natl Acad. Sci. USA* **98**, 2577–2581 (2001).
46. Thornton, A. M. & Shevach, E. M. $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T-cell activation *in vitro* by inhibiting interleukin-2 production. *J. Exp. Med.* **188**, 287–296 (1998).
A study outlining many of the pivotal characteristics of $CD4^+CD25^+$ regulatory T cells.
47. Thornton, A. M. & Shevach, E. M. Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific. *J. Immunol.* **164**, 183–190 (2000).
48. Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L. & Powrie, F. An essential role for interleukin-10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* **190**, 995–1004 (1999).
49. Hara, M. *et al.* IL-10 is required for regulatory T cells to mediate tolerance to alloantigens *in vivo*. *J. Immunol.* **166**, 3789–3796 (2001).
50. Dieckmann, D., Bruett, C. H., Ploettner, H., Lutz, M. B. & Schuler, G. Human $CD4^+CD25^+$ regulatory, contact-dependent T cells induce interleukin-1-producing, contact-independent type-1-like regulatory T cells. *J. Exp. Med.* **196**, 247–253 (2002).
51. Jonuleit, H. *et al.* Infectious tolerance: human $CD25^+$ regulatory T cells convey suppressor activity to conventional $CD4^+$ T helper cells. *J. Exp. Med.* **196**, 255–260 (2002).
52. Chang, C. C. *et al.* Tolerization of dendritic cells by T_S cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nature Immunol.* **3**, 237–243 (2002).
53. Butcher, G. W. & Howard, J. C. Genetic control of transplant rejection. *Transplantation* **34**, 161–166 (1982).
54. Lagaaij, E. L. *et al.* Effect of one-HLA-DR-antigen-matched and completely HLA-DR-mismatched blood transfusions on survival of heart and kidney allografts. *N. Engl. J. Med.* **321**, 701–705 (1989).
55. Lagaaij, E. L. *et al.* Blood transfusion-induced changes in cell-mediated lympholysis: to immunize or not to immunize? *J. Immunol.* **147**, 3348–3352 (1991).
56. Sayegh, M. H., Khoury, S. J., Hancock, W. W., Weiner, H. L. & Carpenter, C. B. Induction of immunity and oral tolerance with polymorphic class II major histocompatibility complex allopeptides in the rat. *Proc. Natl Acad. Sci. USA* **89**, 7762–7766 (1992).
57. Niederkorn, J. Y. & Mayhew, E. Phenotypic analysis or oral tolerance to alloantigens: evidence that the indirect pathway of antigen presentation is involved. *Transplantation* **73**, 1493–1500 (2002).
58. Yin, D. & Fathman, C. G. CD4-positive suppressor cells block allotransplant rejection. *J. Immunol.* **154**, 6339–6345 (1995).
59. Wise, M. P., Bemelman, F., Cobbold, S. P. & Waldmann, H. Linked suppression of skin graft rejection can operate through indirect recognition. *J. Immunol.* **161**, 5813–5816 (1998).
60. Yamada, A. *et al.* Cutting edge: recipient MHC class II expression is required to achieve long-term survival of murine cardiac allografts after costimulatory blockade. *J. Immunol.* **167**, 5522–5526 (2001).
61. Li, X. C., Strom, T. B., Turka, L. A. & Wells, A. D. T-cell death and transplantation tolerance. *Immunity* **14**, 407–416 (2001).
A succinct review outlining the importance of deletional mechanisms for the induction of allograft tolerance.
62. Hall, B. M., Jelbart, M. E., Gurley, K. E. & Dorsch, S. E. Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. *J. Exp. Med*. **162**, 1683–1694 (1985).
63. Hall, B. M., Gurley, K. E. & Dorsch, S. E. Specific unresponsiveness in rats with prolonged allograft survival is dependent upon the graft and suppressor T cells. *Transplant. Proc.* **19**, 495–496 (1987).
64. Pearce, N. W., Spinelli, A., Gurley, K. E., Dorsch, S. E. & Hall, B. M. Mechanisms maintaining antibody-induced enhancement of allografts. II. Mediation of specific suppression by short lived $CD4^+$ T cells. *J. Immunol.* **143**, 499–506 (1989).
65. Bordes-Aznar, J., Lear, P. A., Strom, T. B., Tilney, N. L. & Kupiec-Weglinski, J. W. Kinetics of cyclosporin-A-induced unresponsiveness to cardiac allografts in rats. *Transplant. Proc.* **15**, 500 (1983).
66. Chen, J. *et al.* $CD4^+CD25^+$ T cells as regulators of alloimmune responses. *Transplant. Proc.* **33**, 163–164 (2001).
67. Hara, M. *et al.* IL-10 is required for regulatory T cells to mediate tolerance to alloantigens *in vivo*. *J. Immunol.* **166**, 3789–3796 (2001).
68. Scully, R., Qin, S., Cobbold, S. & Waldmann, H. Mechanisms in CD4 antibody-mediated transplantation tolerance: kinetics of induction, antigen dependency and role of regulatory T cells. *Eur. J. Immunol.* **24**, 2383–2392 (1994).
69. Bushell, A., Niimi, M., Morris, P. J. & Wood, K. J. Evidence for immune regulation in the induction of transplantation tolerance: a conditional but limited role for IL-4. *J. Immunol.* **162**, 1359–1366 (1999).
70. Graca, L., Honey, K., Adams, E., Cobbold, S. P. & Waldmann, H. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. *J. Immunol.* **165**, 4783–4786 (2000).
71. Mueller, R., Davies, J. D., Krahl, T. & Sarvetnick, N. IL-4 expression by grafts from transgenic mice fails to prevent allograft rejection. *J. Immunol.* **159**, 1599–1603 (1997).
72. Li, X. C., Zand, M. S., Li, Y., Zheng, X. X. & Strom, T. B. On histocompatibility barriers, T_H1 to T_H2 immune deviation, and the nature of the allograft responses. *J. Immunol.* **161**, 2241–2247 (1998).
73. Sho, M. *et al.* Physiological mechanisms of regulating alloimmunity: cytokines, CTLA-4, $CD25^+$ cells, and the alloreactive T-cell clone size. *J. Immunol.* **69**, 3744–3751 (2002).
74. Sykes, M. Mixed chimerism and transplant tolerance. *Immunity* **14**, 417–424 (2001).
75. Owen, R. D. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* **102**, 400–401 (1945).
76. Ildstad, S. T. & Sachs, D. H. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature* **307**, 168–170 (1984).
77. Wekerle, T. & Sykes, M. Mixed chimerism as an approach for the induction of transplantation tolerance. *Transplantation* **68**, 459–467 (1999).
78. Khan, A., Tomita, Y. & Sykes, M. Thymic dependence of loss of tolerance in mixed allogeneic bone marrow chimeras after depletion of donor antigen. Peripheral mechanisms do not contribute to maintenance of tolerance. *Transplantation* **62**, 380–387 (1996).
79. Odorico, J. S., Posselt, A. M., Naji, A., Markmann, J. F. & Barker, C. F. Promotion of rat cardiac allograft survival by intrathymic inoculation of donor splenocytes. *Transplantation* **55**, 1107 (1993).
80. Posselt, A. M. *et al.* Induction of donor-specific unresponsiveness by intrathymic islet transplantation. *Science* **249**, 1293–1295 (1990).
81. Gozzo, J. J., Wood, M. L. & Monaco, A. P. Use of allogenic, homozygous bone marrow cells for the induction of specific immunologic tolerance in mice treated with antilymphocyte serum. *Surg. Forum* **21**, 281–284 (1970).
82. Umemura, A. *et al.* Dissociation of hemopoietic chimerism and allograft tolerance after allogeneic bone marrow transplantation. *J. Immunol.* **167**, 3043–3048 (2001).
83. Benjamin, R. J. & Waldmann, H. Induction of tolerance by monoclonal antibody therapy. *Nature* **320**, 449–451 (1986).
84. Cobbold, S. P., Jayasuriya, A., Nash, A., Prospero, T. D. & Waldmann, H. Therapy with monoclonal antibodies by elimination of T-cell subsets *in vivo*. *Nature* **312**, 548–551 (1984).
85. Cobbold, S. P., Martin, G., Qin, S. & Waldmann, H. Monoclonal antibodies to promote marrow engraftment and tissue graft tolerance. *Nature* **323**, 164–166 (1986).
86. Chen, Z., Cobbold, S., Metcalfe, S. & Waldmann, H. Tolerance in the mouse to major histocompatibility complex-mismatched heart allografts, and to rat heart xenografts, using monoclonal antibodies to CD4 and CD8. *Eur. J. Immunol.* **22**, 805–810 (1992).
87. Newell, M. K., Haughn, L. J., Maroun, C. R. & Julius, M. H. Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen. *Nature* **347**, 286–289 (1990).
88. Jenkins, M. K., Ashwell, J. D. & Schwartz, R. H. Allogeneic non-T spleen cells restore the responsiveness of normal T-cell clones stimulated with antigen and chemically modified antigen-presenting cells. *J. Immunol.* **140**, 3324–3330 (1987).
89. Tan, P. *et al.* Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. *J. Exp. Med.* **177**, 165 (1993).
90. Freedman, A. S., Freeman, G., Horowitz, J. C., Daley, J. & Nadler, L. M. B7, a B-cell-restricted antigen that identifies preactivated B cells. *J. Immunol.* **139**, 3260–3267 (1987).
91. Freeman, G. J. *et al.* Murine B7-2, an alternative CTLA4 counter-receptor that costimulates T-cell proliferation and interleukin-2 production. *J. Exp. Med.* **178**, 2185–2192 (1993).
92. Boussiotis, V. A. *et al.* Activated human B lymphocytes express three CTLA-4 counter-receptors that costimulate T-cell activation. *Proc. Natl Acad. Sci. USA* **90**, 11059–11063 (1993).
93. June, C. H., Bluestone, J. A., Nadler, L. M. & Thompson, C. B. The B7 and CD28 receptor families. *Immunol. Today* **15**, 321–331 (1994).
94. Lenschow, D. *et al.* Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4–Ig. *Science* **257**, 789–792 (1992).
95. Turka, L. A. *et al.* T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection *in vivo*. *Proc. Natl Acad. Sci. USA* **89**, 11102–11105 (1992).
96. Lin, H. *et al.* Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4–Ig plus donor-specific transfusion. *J. Exp. Med.* **178**, 1801–1806 (1993).
97. Noelle, R. J. *et al.* A 39-kDa protein in activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. *Proc. Natl Acad. Sci. USA* **89**, 6550–6554 (1992).
98. Durie, F. H., Foy, T. M., Masters, S. R., Laman, J. D. & Noelle, R. J. The role of CD40 in the regulation of humoral and cell-mediated immunity. *Immunol. Today* **15**, 406–411 (1994).
99. Hollenbaugh, D. *et al.* Expression of functional CD40 by vascular endothelial cells. *J. Exp. Med.* **182**, 33–40 (1995).
100. Tian, L., Noelle, R. J. & Lawrence, D. A. Activated T cells enhance nitric oxide production by murine splenic macrophages through gp39 and LFA-1. *Eur. J. Immunol.* **25**, 306–309 (1995).

101. Durie, F. H. *et al.* Antibody to the ligand of CD40, gp39, blocks the occurrence of the acute and chronic forms of graft-vs-host disease. *J. Clin. Invest.* **94**, 1333–1338 (1994).
102. Larsen, C. P. *et al.* CD40–gp39 interactions play a critical role during allograft rejection: suppression of allograft rejection by blockade of the CD40–gp39 pathway. *Transplantation* **61**, 4–9 (1996).
103. Larsen, C. P. *et al.* Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* **381**, 434–438 (1996).
104. Rothstein, D. M. *et al.* Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long-term engraftment and tolerance in stringent transplant models. *J. Immunol.* **166**, 322–329 (2001).
105. Lazarovits, A. I. *et al.* Prevention and reversal of renal allograft rejection by antibody against CD45RB. *Nature* **380**, 717–720 (1996).
106. Gourishankar, S., Turner, P. & Halloran, P. New developments in immunosuppressive therapy in renal transplantation. *Exp. Opin. Biol. Ther.* **2**, 483–501 (2002).
A timely review of immunosuppressive strategies that are used in renal transplantation.
107. Li, Y. *et al.* Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nature Med.* **5**, 1298–1302 (1999).
108. Boise, L. H. *et al.* Bcl-x, a Bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* **74**, 597–608 (1993).
109. Boise, L. H. *et al.* CD28 costimulation can promote T-cell survival by enhancing the expression of Bcl-x_L. *Immunity* **3**, 87–98 (1995).
110. Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R. D. & Korsmeyer, S. J. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* **348**, 334–336 (1990).
111. Van Parijs, L. & Abbas, A. K. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* **280**, 243–248 (1998).
112. Lenardo, M. J. Interleukin-2 programs mouse αβ T lymphocytes for apoptosis. *Nature* **353**, 858–861 (1991).
113. Wells, A. D. *et al.* Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. *Nature Med.* **5**, 1303–1307 (1999).
A key paper outlining the role of deletion in the induction of tolerance of MHC-mismatched allografts.
114. Wekerle, T. *et al.* Peripheral deletion after bone marrow transplantation with costimulatory blockade has features of both activation-induced cell death and passive cell death. *J. Immunol.* **166**, 2311–2316 (2001).
115. Matzinger, P. & Bevan, M. J. Hypothesis: why do so many lymphocytes respond to major histocompatibility antigens? *Cell. Immunol.* **29**, 1–5 (1977).
116. Suchin, E. J. *et al.* Quantifying the frequency of alloreactive T cells *in vivo*: new answers to an old question. *J. Immunol.* **166**, 973–981 (2001).
117. Kishimoto, K. *et al.* T_H1 cytokines, programmed cell death, and alloreactive T-cell clone size in transplant tolerance. *J. Clin. Invest.* **109**, 1471–1479 (2002).
118. Huang, C. A. *et al.* Stable mixed chimerism and tolerance using a nonmyeloablative preparative regimen in a large-animal model. *J. Clin. Invest.* **105**, 173–181 (2000).
119. Chalasani, G., Dai, Z., Konieczny, B. T., Baddoura, F. K. & Lakkis, F. G. Recall and propagation of allospecific memory T cells independent of secondary lymphoid organs. *Proc. Natl Acad. Sci. USA* **99**, 6175–6180 (2002).
120. Valujskikh, A., Pantenburg, B. & Heeger, P. S. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *Am. J. Transplant.* **2**, 501–509 (2002).
121. Thomas, F., Ray, P. & Thomas, J. M. Immunological tolerance as an adjunct to allogeneic tissue grafting. *Microsurgery* **20**, 435–440 (2000).
122. Thomas, J. M. *et al.* Peritransplant tolerance induction in macaques: early events reflecting the unique synergy between immunotoxin and deoxyspergualin. *Transplantation* **68**, 1660–1673 (1999).
123. Thomas, J. M. *et al.* Durable donor-specific T- and B-cell tolerance in rhesus macaques induced with peritransplantation anti-CD3 immunotoxin and deoxyspergualin: absence of chronic allograft nephropathy. *Transplantation* **69**, 2497–2503 (2000).
124. Calne, R. *et al.* Campath IH allows low-dose cyclosporine monotherapy in 31 cadaveric renal allograft recipients. *Transplantation* **68**, 1613–1616 (1999).
125. Manilay, J. O., Pearson, D. A., Sergio, J. J., Swenson, K. G. & Sykes, M. Intrathymic deletion of alloreactive T cells in mixed bone marrow chimeras prepared with a nonmyeloablative conditioning regimen. *Transplantation* **66**, 96–102 (1998).
126. Wekerle, T. *et al.* Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Nature Med.* **6**, 464–469 (2000).
127. Wekerle, T., Kurtz, J., Bigenzahn, S., Takeuchi, Y. & Sykes, M. Mechanisms of transplant tolerance induction using costimulatory blockade. *Curr. Opin. Immunol.* **14**, 592–600 (2002).
A timely review of the various mechanisms — deletion, anergy and dominant suppression — mediating tolerance induced by co-stimulatory blockade.
128. Sloan-Lancaster, J., Steinberg, T. H. & Allen, P. M. Selective activation of the calcium signaling pathway by altered peptide ligands. *J. Exp. Med.* **184**, 1525–1530 (1996).
129. Rafiq, K. *et al.* Regulation of the IL-10 production by human T cells. *Scand. J. Immunol.* **53**, 139–147 (2001).
130. Refaeli, Y., Van Parijs, L., London, C. A., Tschopp, J. & Abbas, A. K. Biochemical mechanisms of IL-2-regulated Fas-mediated T-cell apoptosis. *Immunity* **8**, 615–623 (1998).
131. Wolf, M., Schimpl, A. & Hunig, T. Control of T-cell hyperactivation in IL-2-deficient mice by $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells: evidence for two distinct regulatory mechanisms. *Eur. J. Immunol.* **31**, 1637–1645 (2001).
132. Kahan, B. D. Sirolimus: a comprehensive review. *Exp. Opin. Pharmacother.* **2**, 1903–1917 (2001).
133. Kahan, B. D. & Camardo, J. S. Rapamycin: clinical results and future opportunities. *Transplantation* **72**, 1181–1193 (2001).
134. Rivalan, J. Immunosuppression, ongoing clinical trials. *Presse Med.* **30**, 38–40 (2001).
135. Kirk, A. *et al.* CTLA4–Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc. Natl Acad. Sci. USA* **94**, 8789–8794 (1997).
136. Simpson, E. Minor transplantation antigens: mouse models for human host-versus-graft, graft-versus-host and graft-versus-leukemia reactions. *Arch. Immunol. Ther. Exp.* **46**, 331–339 (1998).
137. King, T. R. *et al.* Deletion mapping by immunoselection against the H-Y histocompatibility antigen further resolves the Sxra region of the mouse Y chromosome and reveals complexity of the Hya locus. *Genomics* **24**, 159–168 (1994).
138. Wettstein, P. J. Immunodominance in the T-cell response to multiple non-H-2 histocompatibility antigens. II. Observation of a hierarchy among dominant antigens. *Immunogenetics* **24**, 24–31 (1986).
139. Shevach, E. M. $CD4^+CD25^+$ suppressor T cells: more questions than answers. *Nature Rev. Immunol.* **2**, 389–400 (2002).
140. Takahashi, T. *et al.* Immunologic self-tolerance maintained by $CD25^+CD4^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* **192**, 303–310 (2000).
141. Piccirillo, C. A. & Shevach, E. M. Cutting edge: control of $CD8^+$ T-cell activation by $CD4^+CD25^+$ immunoregulatory cells. *J. Immunol.* **167**, 1137–1140 (2001).
142. Read, S., Malmstrom, V. & Powrie, F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD4^+CD25^+$ regulatory cells that control intestinal inflammation. *J. Exp. Med.* **192**, 295–302 (2000).
143. Bensinger, S. J., Bandeira, A., Jordan, M. S., Caton, A. J. & Laufer, T. M. Major histocompatibility complex class-II-positive cortical epithelium mediates the selection of $CD4^+CD25^+$ immunoregulatory T cells. *J. Exp. Med.* **194**, 427–438 (2001).
A study providing information about the ontogeny of $CD4^+CD25^+$ regulatory T cells.
144. Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y. & Sakaguchi, S. Stimulation of $CD25^+CD4^+$ regulatory T cells through GITR breaks immunological self-tolerance. *Nature Immunol.* **3**, 135–142 (2002).
145. McHugh, R. S. *et al.* $CD4^+CD25^+$ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* **16**, 311–323 (2002).
This paper describes the first gene-expression approach to the elucidation of $CD4^+CD25^+$ regulatory T-cell function.

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