TIMELINE

Transplantation tolerance from a historical perspective

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Although transplantation immunology as a distinctive field began with the development of experimental models that showed the feasibility of bone marrow transplantation, organ engraftment was accomplished first in humans, and was thought for many years to occur by drastically different mechanisms. Here, we present our view of the concepts of allograft acceptance and acquired tolerance from a historical perspective, and attempt to amalgamate them into simple and unifying rules that might guide improvements in clinical therapy.

Our paradigm of transplantation immunology (reviewed in REF. 1) had its origin in the nineteenth century. After the cellular, humoral and complement constituents of the immune response were discovered (see TIMELINE), evidence emerged that an immune reaction was responsible for the failure of transplanted tissue and most tumour allografts to survive indefinitely[2]. When transplantation research declined during and after the First World War, these early accomplishments faded. Similarly, the significance of the NEONATAL TOLERANCE shown in tumour and viral-infection models was not fully appreciated until Burnet's formulation of the tolerance and CLONAL-SELECTION hypothesis[3]. Finally, the phenomenon of IMMUNE IGNORANCE was first shown in 1934 (REF. 4), but discounted until its rediscovery many years later[5,6].

The immunological basis of rejection

Modern transplantation immunology is often dated to the experiments by Medawar in 1944, which showed that skin allograft rejection is a HOST-VERSUS-GRAFT (HVG) response[7], the cell-mediated features of which were later defined by Mitchison[8]. The term major histocompatibility complex (MHC) was introduced by Gorer, Lyman and Snell[9] for the genetic locus that encodes antigens associated with allograft rejection, tumour surveillance and other expressions of cell-mediated immunity. The MHC-restricted mechanisms of T-cell recognition of, and response to, antigens, viruses and other intracellular microorganisms were elucidated in the 1970s (reviewed in REF. 10).

NON-CYTOPATHIC MICROORGANISMS (for example, lymphocytic choriomeningitis virus (LCMV) in mice) are controlled primarily by cytotoxic T lymphocytes (CTLs) that recognize as 'non-self' host cells which display complexes composed of self-MHC molecules and peptides derived from the infecting microorganism. Allograft rejection was the apparent transplantation equivalent of the host-versus-pathogen adaptive immune response, but the specific mechanisms governing allograft acceptance remained a puzzle.

The avoidance of rejection

Bone marrow transplantation, 1953–1989. In experiments inspired by Owen's description of blood-cell chimerism in FREEMARTIN CATTLE[11], and by the recognition by Burnet and Fenner[3] of the observation's significance, Billingham, Brent and Medawar[12] showed between 1953 and 1956 that allogeneic spleen and bone marrow (BM) cells induce tolerance when they are not rejected by the incompletely developed immune system of neonatal mouse recipients, and that the tolerance extends to donor strain skin

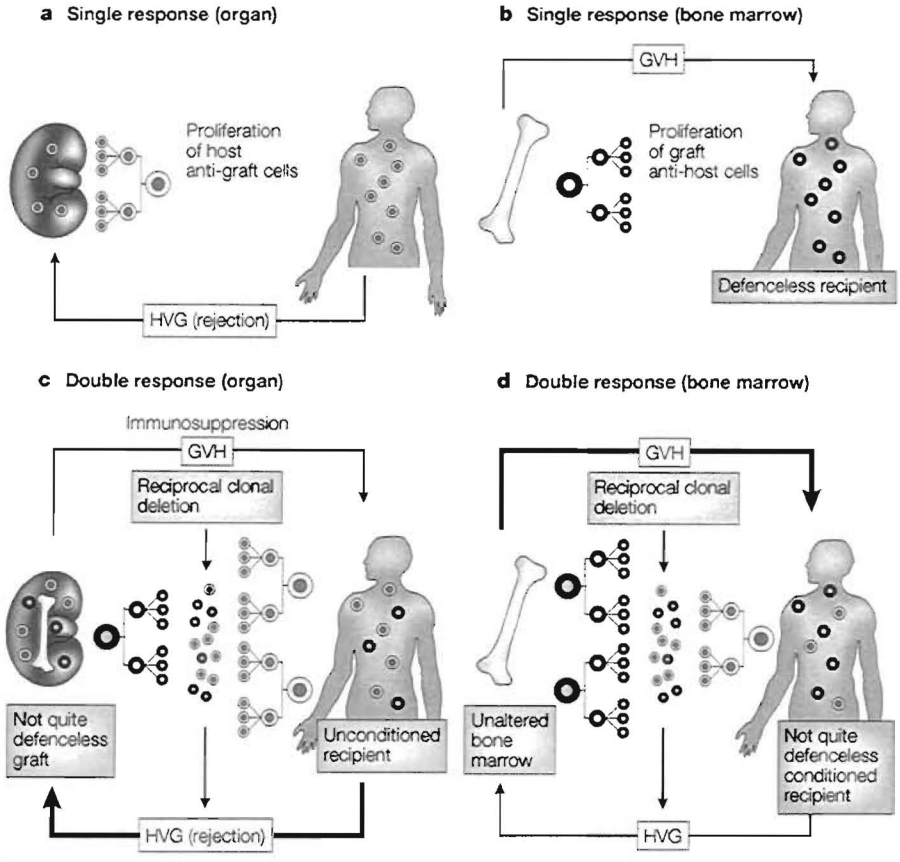


Figure 1 | **Old and new views of transplantation immunology. a** | Illustrates the early conceptualization of immune mechanisms in organ transplantation in terms of a unidirectional host-versus-graft (HVG) response. Although this readily explained organ rejection, it limited possible explanations of organ engraftment. **b** | A mirror image of (**a**) and depicts the early understanding of successful bone marrow (BM) transplantation as a complete replacement of the recipient immune system by that of the donor, with the potential complication of an unopposed lethal unidirectional graft-versus-host (GVH) response: that is, rejection of the recipient by the graft. **c** | Shows the current view of bidirectional and reciprocally modulating immune responses of coexisting immune-competent cell populations that lead to organ engraftment, despite a usually dominant HVG reaction. The transplanted organ, which initially loses most of its passenger leukocytes, apparently remains an important site for donor precursor and stem cells (bone silhouette)[47]. **d** | Represents the current conceived mirror image of (**c**) and shows the reversal of the size proportions of the reciprocally modulating donor and recipient populations of immune cells after successful BM transplantation.

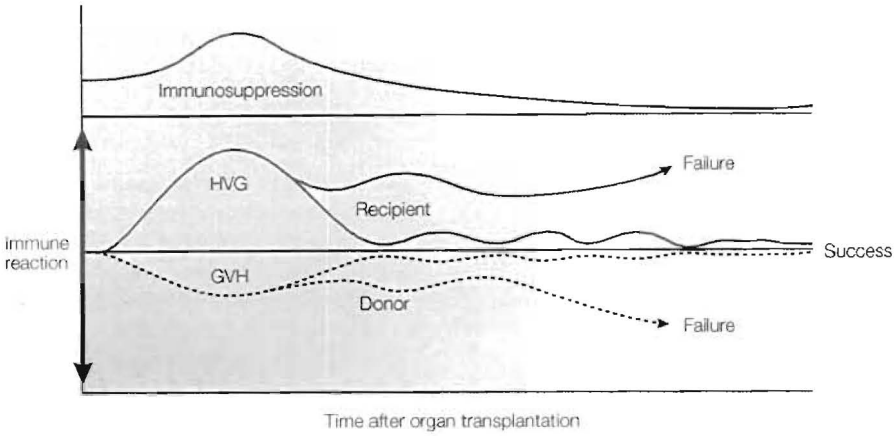


Figure 2 | **Contemporaneous HVG (upright curves) and GVH (inverted curves) responses following organ transplantation.** If some degree of reciprocal clonal exhaustion is not induced and maintained (usually requiring protective immune suppression), one cell population will destroy the other. In contrast to the usually dominant host-versus-graft (HVG) reaction of organ transplantation (shown here), the graft-versus-host (GVH) reaction usually is dominant in the cytoablated bone marrow recipient. Therapeutic failure with either type of transplantation implies the inability to control one, the other, or both of the responses.

allografts. This model is analogous to successful BM-cell transplantation in humans whose immune-deficiency diseases make host cytoablation unnecessary[13]. During 1955–1956, similar tolerance was induced in adult mice whose mature immune system was cytoablated with supralethal total body irradiation (TBI)[14]. The mouse model evolved into clinical BM transplantation for a wide range of indications[15].

The avoidance of lethal GRAFT-VERSUS-HOST DISEASE (GVHD) in the experimental tolerance models and in humans requires a close tissue match. Until human-leukocyte-antigen (HLA) matching became available in 1968, a decade after the discovery by Dausset and van Rood of the first HLA antigens, prolonged survival after clinical BM transplantation was limited to a single case[16]. GVHD seemed to be a mirror-image version of tissue and organ rejection (HVG) in that the host (FIG. 1b), rather than the graft (FIG. 1a), was the immune target.

The unidirectional MIXED-LYMPHOCYTE-REACTION assays, introduced in 1964, became widely accepted 'minitransplant models', reinforcing the idea that one-way immune reactions — GVH or HVG — were induced after BM and organ transplantation, respectively. Accordingly, successful BM transplantation was generally viewed as total replacement of the recipient immune apparatus, even after the discovery in 1989 that recipient leukocytes could be found in the blood of essentially all human "complete donor BM chimeras"[17]. The early hypothesis that donor and recipient immune-competent cells might coexist, become mutually non-reactive and even function collaboratively (for example, in a joint response to a new infection)[18,19] lacked experimental support and was abandoned.

Clinical organ transplantation, 1959–1991. The strategy of co-transplanting BM and skin allografts to supralethally irradiated mice was extended in the late 1950s by John Mannick and David Hume to kidney/BM transplantation in irradiated beagle and outbred dogs, but yielded only a single survival exceeding 1 month (73 days). Although the survival of dog kidney transplants was even worse when the adjunct BM was omitted, six humans conditioned with sublethal doses of TBI (4.5 Gy) achieved renal allograft function for at least 1 year (the first in Boston and the next five in Paris)[20–22]. The era of drug immunosuppression then began after Schwartz and Dameshek[23] showed that the anti-leukaemic drug 6-mercaptopurine was immunosuppressive in rabbits. When about 5% of dog kidney recipients treated by Calne and Zukoski with 6-mercaptopurine, or its analogue azathioprine, survived for ≥100 days, human trials were undertaken by Murray and colleagues[24]. In the sixth case of kidney transplantation, a renal allograft from a non-related donor functioned for 17 months after its transplantation under azathioprine-based therapy[24].

At first, the results with drug therapy were no better than with TBI. However, when large doses of prednisone were added to azathioprine in response to clinically diagnosed rejections, two key observations were made, as described in the title of a report of ten cases: "The reversal of rejection in human renal homografts with subsequent development of homograft tolerance"[25]. The partial tolerance referred to the time-related diminution of dependence on immunosuppression, which eventually was stopped[26] in two of the patients whose grafts still function after 39 years.

Timeline | **Major developments in transplantation**

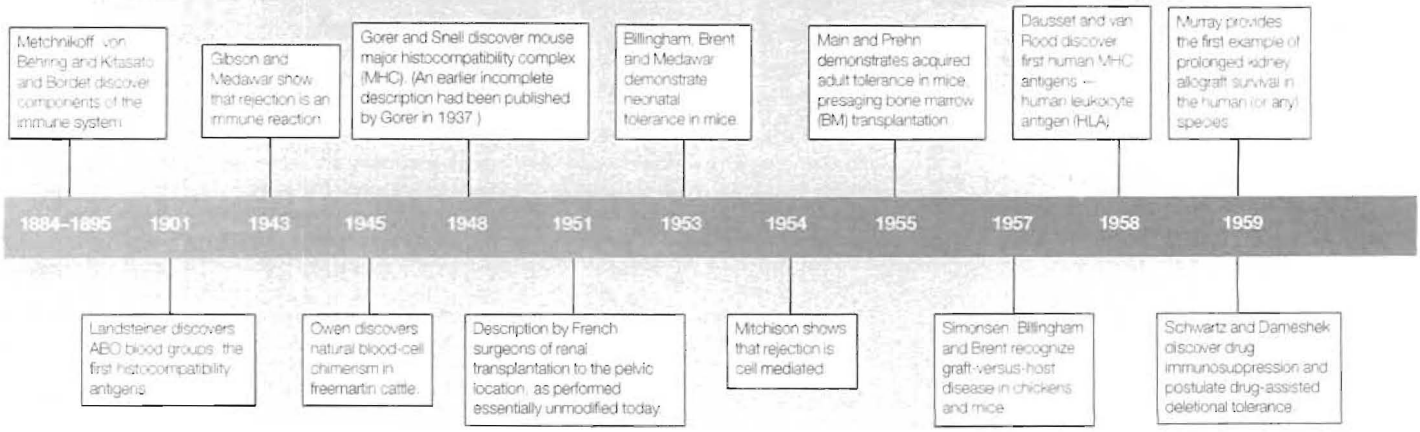


Table 1 | **Differences of organ and bone marrow transplantation**

Feature	Organ	Bone marrow
Host cytoablation	No	Yes*
HLA matching	Not essential	Critical
Principal complication	Rejection	GVHD
Immunosuppression free	Uncommon	Common
Term for success	Acceptance	Tolerance

*This therapeutic step allows a relatively unopposed graft-versus-host reaction and accounts for the other differences. GVHD, graft-versus-host disease; HLA, human leukocyte antigen.

In 1966, heterologous anti-lymphoid globulin (ALG) was added in a triple-agent protocol[27] that was used for the first successful transplantation of the human liver in July 1967 and heart in January 1968.

The repeated demonstration that organ transplantation was feasible without adjunct BM cells, together with the striking differences between organ and BM transplantation (TABLE 1), led to a consensus by the early 1960s that organ engraftment did not involve donor leukocyte chimerism. This conclusion ostensibly was congruent with the identification of the 'sessile' and/or recirculating passenger leukocytes of BM origin contained in all organs, as the immunogenic component of allografts[28–30]. When it was subsequently learned that most of these 'passenger leukocytes' are replaced in the engrafted organ by comparable recipient cells, it was assumed that the donor leukocytes had undergone immune destruction either within the graft or after their migration to host lymphoid organs, with selective preservation of the specialized parenchymal cells of the organ.

The resulting explanation of organ engraftment by means other than the chimerism-associated mechanisms of BM transplantation was not challenged until small numbers of multilineage donor haematopoietic cells (that is, microchimerism) were shown in 1992 in the tissues or blood of 30 long-surviving human liver or kidney allograft recipients[31,32]. Organ engraftment (FIG. 1c) and BM-cell engraftment (FIG. 1d) seemed to be mirror images with reversed proportions of donor–recipient haematolymphopoietic cells, placing both in a continuum of leukocyte chimerism-associated tolerance models that could be related to the neonatal mouse experiments of Billingham, Brent and Medawar[12], and to Owen's original observation of blood-cell chimerism in freemartin cattle[11].

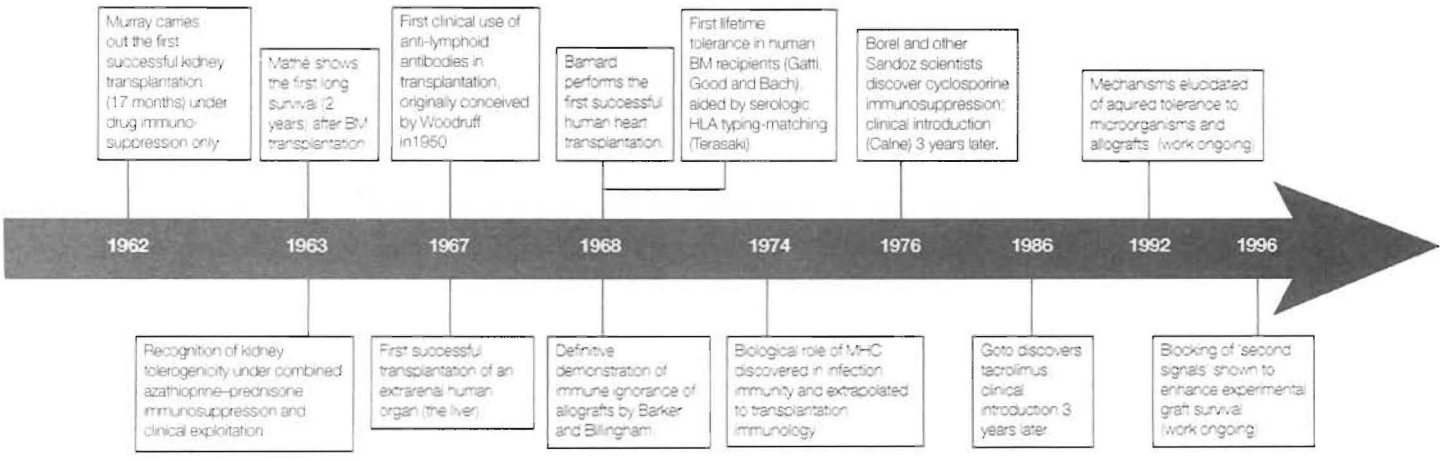
Mechanisms of non-reactivity

Chimerism and clonal deletion. CLONAL EXHAUSTION was postulated between 1959 and 1968 to explain acquired non-reactivity to a variety of antigens[23,33,34], including allogeneic cells[18] and, in 1969, as the basis of organ tolerance[35]. However, the existence and importance of this mechanism have only been formally established since 1990 (REFS 36,37). With the finding in 1992 of microchimerism years after transplantation in surviving organ recipients, it was deduced that the crucial period for allo-engraftment was immediately post-transplantation and consisted of "... [acute] responses of coexisting donor and recipient [immune] cells, each to the other, causing reciprocal clonal exhaustion, followed by peripheral clonal deletion" (FIG. 2)[31]. Although this hypothesis has been validated experimentally[1,26,38], the molecular pathways of deletion-associated apoptosis observed in transplant models are not yet fully understood. Moreover, the role of the late microchimerism has been controversial[39].

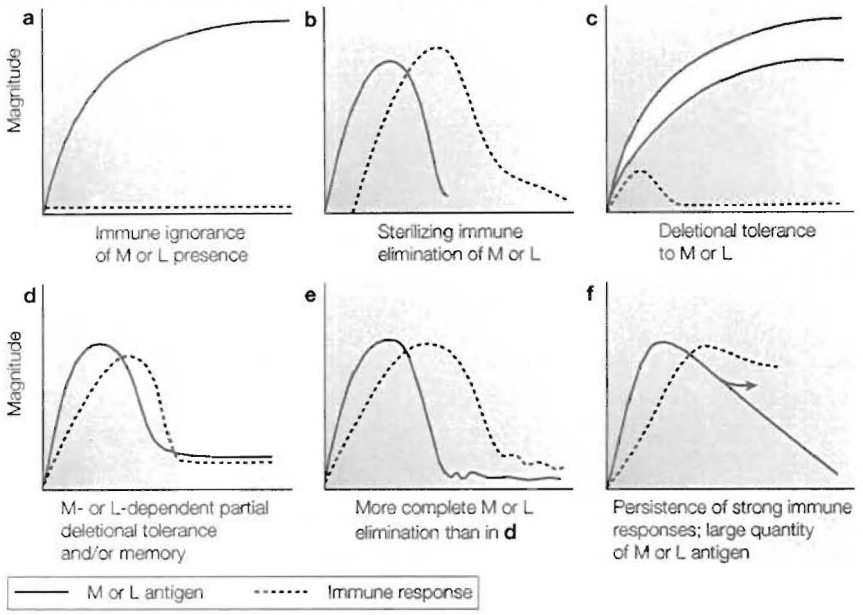
Immune ignorance

We believe that antigen that fails to reach organized lymphoid collections is ignored by the immune system[40]. In an early example, Stone, Owings and Gey[4] reported that parathyroid tissue, which had been cultured for 2 weeks and transplanted into loose areolar subcutaneous tissue, functioned for prolonged periods in dog and human recipients. Long regarded as not credible, their findings are explained, in retrospect, by two features of the procedure. The first was the choice of a subcutaneous implantation site, which, like other privileged sites (for example, hamster cheek pouch and brain), has limited lymphatic drainage. Illustrating this principle in 1957, Frey and Wenk showed that no immune response is induced by otherwise sensitizing chemicals if the antigen is prevented from migrating to draining lymph nodes or spleen, an observation subsequently extended to skin allografts by Barker and Billingham[5].

The second feature of the Stone procedure became apparent with the demonstration by Lafferty and co-workers[6] that tissue culture comparable with that used 4 decades earlier in the parathyroid experiments depletes endocrine tissue of passenger leukocytes that are capable of migrating to host lymphoid organs. Similarly, allografts lose immunogenicity when their passenger leukocytes are removed from tissues or organs in 'parking experiments'[28–30] or by other means.



Box 1 | **Analogies between immune responses to infections or transplants**



The analogies between the adaptive immune response or non-response (dashed lines) induced by the antigen of non-cytopathic microorganisms (M) and the analogous migratory leukocytes (L) of allografts (solid lines) have been obscured by the presence of contemporaneous host-versus-graft (HVG) and graft-versus-host (GVH) responses after transplantation and the additional factor of therapeutic immunosuppression. Under circumstances of both infection and transplantation, the immune response or non-response is regulated mainly by the migration and localization of the antigen. **a** | If the antigen fails to reach organized host lymphoid tissue, as occurs with the extralymphatic spread of an infection (for example, the human papilloma (wart) virus), there is no immune response (immune ignorance). Similarly, avoidance of host lymphoid organs by transplanting tissue to privileged sites or by depleting the allograft of passenger leukocytes might allow prolonged survival of 'Stone–Lafferty' transplants, without evolution of donor-specific non-reactivity providing the mobile antigen (L) remains extralymphatic. Rejection of the graft can often be precipitated by leakage of graft cells into the blood or lymph circulation (for example, after an infection or trauma[5] or an immunizing injection of donor cells[6,29]. **b** | Illustrates the immune elimination of a spreading non-cytopathic microorganism that has reached host lymphoid organs and induced an antigen-specific response. The outcome is comparable with the rejection of an outlying organ after its passenger leukocytes migrate to host lymphoid organs and induce an anti-donor response. After complete removal of the antigen, the immune response subsides. **c** | Depicts how the persistence of mobile live antigen that has access to host lymphoid organs can exhaust and delete the antigen-specific immune response. With infection, this might result in a stable carrier state (for example, the viral hepatitides, or lymphocytic choriomeningitis virus infestation). The transplant analogy is 'complete' repopulation of a recipient by donor bone marrow (BM)-derived cells after pure stem-cell transplantation to a severe combined immune deficiency (SCID) mouse, or after the less complete replacement (macrochimerism) with clinical BM transplantation to a host whose immune response is weakened in advance by cytoablation or cytoreduction. This kind of tolerance in organ recipients is most often associated with macrochimerism[30]. **d** | Represents the acute control of an infection by incomplete elimination of microorganisms, resulting in cellular plus antibody 'memory' sustained by the residual live antigen (for example, herpesvirus). In the transplantation analogy, partial deletional tolerance induced at the outset under immunosuppression might be sustained by the residual donor cells (microchimerism) with, or sometimes without, the aid of immunosuppression. **e** | Shows more complete elimination of microorganisms than in (**d**) with 'memory' (for example, tuberculosis or measles). In the transplantation analogy, survival of the minority population of leukocytes requires continuous immunosuppression. **f** | Illustrates the survival of a large quantity of microorganisms despite a strong persistent immune response, resulting in acute–chronic infection and a spectrum of immunopathology (for example, chronic aggressive hepatitis). The transplantation analogy is chronic rejection or GVHD, which might be refractory to treatment with immunosuppression.

The reduced immunogenicity has been explained by the elimination of leukocyte subsets expressing MHC class II antigens or co-stimulatory molecules, such as B7 (CD80/86) —for example, donor dendritic cells[41]. However, passenger leukocytes might be immunogenic primarily because they can migrate to lymphoid organs, whereas the fixed parenchymal cells generally cannot. The results of experiments with fractionated liver cell suspensions[42] and with tumour cells lacking MHC class II or so-called 'second signals'[43] are consistent with this alternative hypothesis.

Immune ignorance of heart allografts has been definitively studied during the past 5 years in mutant (*aly/aly*) mice that have normal T lymphocytes and a spleen, but lack Peyer's patches and lymph nodes. Cardiac allografts drained into the circulation by vascular anastomosis are indolently rejected, but when splenectomy is also performed, the hearts are permanently accepted[44]. In addition to exemplifying immune ignorance, the results contradicted the historical dogmas that the immune response to primarily vascularized organs does not require the presence of host lymphoid organs, and that intragraft responses are generated 'directly' in the transplanted organ.

Collaborative mechanisms

With the recognition in the 1990s that the key event in allograft acceptance is cell migration and relocation[31] and that the adaptive immune response to non-cytopathic organisms is determined by the migration patterns of the pathogen[40,45], the analogies between infection and transplantation shown in BOX 1 were obvious. In essence, the dose, kinetics and localization of antigen in or outside lymphatic tissues regulate immune responsiveness or unresponsiveness not only against infection and allografts, but also against tumours and self. An adaptive immune response could then be viewed as "a balance between potentially reactive lymphocytes versus the composition, quantity, kinetics, and distribution of antigen (foreign or extralymphatic self) within the host"[1].

In this context, the presumably rare Stone-Lafferty graft (BOX 1a) is ignored because it contains so few leukocytes capable of migrating to organized host lymphoid collections. By contrast, the transplant outcomes (including irreversible acute rejection; BOX 1b) are analogous with those following spreading blood-borne infections by non-cytopathic microorganisms, in which variable combinations of clonal exhaustion-deletion and immune ignorance might result in degrees of responsiveness and non-responsiveness.

Glossary

CLONAL EXHAUSTION
A state of non-reactivity when all precursor lymphocytes are induced by persistent antigen(s) to become effector cells, purging the immune-response repertoire of this specificity(s).

CLONAL SELECTION
In Burnet's original hypothesis (1949), antibody synthesis occurred after an antigen locked onto a membrane-bound receptor (a version of the antibody) at the surface of an immunocyte. In the mid-to-late 1950s, this event was postulated to be clonal by Jerne, Talmage and Burnet.

FREEMARTIN CATTLE
'Fraternal twins' whose two placentas fuse allowing fetal cross circulation, with induction of mutual specific nonreactivity.

GRAFT-VERSUS-HOST DISEASE
(GVHD). The immune reaction against a graft recipient mounted by immune-competent cells of a graft.

HOST VERSUS GRAFT
(HVG). The immune reaction mounted by the host against grafted tissue or an organ from the same species (alloresponse) or a different species (xenoresponse).

IMMUNE IGNORANCE
Failure of the immune response to recognize the presence of antigen that does not reach organized lymphoid tissue.

NEONATAL TOLERANCE
The development of specific immune non-reactivity to antigen introduced during fetal or early postnatal life, before maturation of the immune system.

NON-CYTOPATHIC MICROORGANISM
A virus, bacteria, protozoa, fungus or microparasite that does not kill host cells and can be accommodated in ways that allow the coexistence of host and pathogen.

MIXED LYMPHOCYTE REACTION
(MLR). A tissue-culture technique introduced by Barbara Bain and by Fritz Bach and Kurt Hirschorn in 1964 for *in vitro* testing of T-cell reactivity.

At one extreme, clonal exhaustion induced by overwhelming numbers of leukocytes (BOX 1c) might allow unrestricted subsequent passage of donor cells between lymphoid and non-lymphoid compartments.

With less-complete deletion, cells that survive primary exposure to lymphoid organs leave the blood and lymphoid tissues after 30–60 days and move to host non-parenchymal tissues and organs (for example, skin and heart[32,46]) or back to the transplanted organ[47]. If sufficient numbers of these cells steadily emigrate from the extralymphatic sites to host lymphoid organs, the clonal exhaustion induced at the outset might be perpetuated with or without maintenance immunosuppression (BOX 1d,e). If this traffic is minimal or irregular, however, the donor-specific responses might lead instead to acute (or chronic) organ rejection or permanent recipient sensitization; experimental variables that might tip the balance towards tolerance or rejection have been shown by Anderson and Matzinger[48] in mouse experiments. Even with the sustained presence of chimerism, the persistence of CTL and antibody responses might result in chronic rejection or GVHD (BOX 1f).

What regulates alloimmune responses?

We have not discussed here the large body of historical[2] and recent work indicating the potential importance of immunoregulatory T cells and other changes in the host immune response for the mediation of immunity or tolerance. Particularly intense interest has focused on antigen-non-specific[49] and antigen-specific T cells[50] that can downregulate both autoimmune and alloimmune responses. In addition, immature donor dendritic cells might prolong organ allograft survival[51] in contrast to mature dendritic cells that efficiently elicit rejection[41].

Immunoregulation by such subsets of special recipient or donor immune cells, alone or in concert[52], could be important in future strategies of clinical transplantation. The same might be true of controlled changes in the host cytokine profile, or of the deliberate production of idiotypic or 'enhancing' antibodies. Elucidation of these frequently reported, but still poorly understood, regulation mechanisms will be necessary for their efficient exploitation. For now, we argue that the clinical and experimental observations in transplantation are most comprehensible in terms of antigen dose, localization and time during which the antigen is in organized lymphoid organs.

Clinical implications

From the historical perspective reviewed here, it is possible to analyse what has been, and what might be, accomplished in clinical transplantation. Except for Stone–Lafferty grafts, the persistence of donor haematopoietic cells (that is, chimerism) above some threshold required to maintain the clonal exhaustion induced at the outset is a necessary condition for long allograft survival. Reciprocal deletion of the characteristic double immune response of transplantation evolves spontaneously in some experimental organ transplant models (especially with leukocyte-rich liver allografts)[26], but immunosuppression is usually required to prevent one immune cell population from destroying the other (that is, rejection or GVHD) long enough for the deletion to occur (FIG. 2).

However, the chimerism-dependent clonal exhaustion, might be subverted by inappropriate post-transplant immunosuppression. Although over-immunosuppression can shut down clonal activation and prevent organ allograft rejection, which allows many of the donor leukocytes to survive and migrate to non-lymphoid sites, the further movement and pleiotropic immunological effects of these cells is unpredictable. Consequently, neither the presence nor quantity of microchimerism can be used to accurately guide management[31,32,39].

By contrast, the donor-specific clonal expansion of the conventional BM recipient is reduced enough by prior cytoablation to be efficiently deleted by the donor leukocytes before these infused cells are rejected, with minimal dependence on immunosuppression. The widespread use of combined BM-organ transplantation in cytoablated recipients has been barred so far by the many parameters involved, of which the most restrictive is the need for a histocompatible donor for avoidance of GVHD.

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Compromise strategies between the radically different regimens of BM and organ transplantation have been extensively tested. The prototype compromise consists of donor BM-cell infusion after weakening the recipient's immune responsiveness in advance by non-myelotoxic cytoreduction (for example, with sublethal irradiation or anti-lymphoid antibody preparations), and then the use of low doses of immunosuppression after transplantation. Production of 'mixed macrochimerism' with acceptably few GVHD complications has been reported in rodents and inbred miniature pig organ-transplant models, and in a small series of patients with haematological disorders given BM cells from one HLA haplotype-matched familial donors[53]. In simpler non-conditioning protocols first used clinically in 1976 by Monaco and co-workers[54], donor BM cells

have been administered to human organ recipients treated with heavy (that is, potentially anti-tolerogenic) conventional immunosuppression. Despite a manifold increase in microchimerism in several trials, drug freedom has not been achieved except, apparently, in recent recipients of familial kidneys who were given megadoses of donor BM cells in Ahmedabad, India[55].

The value of donor pretreatment combined with minimal post-transplant immunosuppression has been suggested by the permanent donor-specific tolerance achieved without adjunct BM cells in 12 monkey kidney recipients that were conditioned with a depleting dose of immunotoxin and then treated with a 14-day course of deoxyspergualine afterward[56]. Although such short-term protocols regularly allow the induction of tolerance in rodents, they have not been used clinically. In the historical clinical experience, tolerance after human kidney transplantation has been rarely observed, and almost exclusively when the 'weak' immunosuppressant azathioprine was administered before and after transplantation, adding prednisone only for overt rejection[26]. Drug independence has been observed far more frequently after transplantation of the liver, but in large numbers only when the original immunosuppression was with azathioprine-based regimens that included pretreatment with ALG, or in patients who were weaned from tacrolimus[57,58].

It might be possible to achieve drug-free tolerance with organs less well-endowed with leukocytes than the liver, using a clinical protocol of host conditioning with ALG and minimal post-transplant immunosuppression that was introduced in 1966 (REF. 27), but ultimately abandoned. Armed with modern drugs, including powerful anti-lymphoid antibody agents, a markedly reduced need for early and maintenance immunosuppression (including nearly complete elimination of prednisone) has been reported from several centres. In Cambridge (United Kingdom), Calne *et al.*[59] have treated cadaver kidney recipients with a few perioperative doses of a humanized depleting anti-CD52 monoclonal antibody (T and B cell plus monocyte reactive), followed by low-maintenance doses of cyclosporine alone.

Conclusion

Although much of the progress in transplantation has depended on the development of increasingly potent immunosuppressants, elucidation of the mechanisms of allograft acceptance has set the stage for more discriminating use of these agents. Theoretically, it should be possible in the future to apply immunosuppression and/or immunostimulation in just the right balanced way to either achieve immunological indifference (ignorance) in some cases, particularly with hormone-producing cells and small organs, or to reliably achieve the perfect equilibrium of mutual immune reactivity and T-cell exhaustion or to obtain stable drug-free and antigen-dependent T-cell exhaustion and chimerism. A universally applicable protocol for organ transplantation will probably be some modernized version of the empirically derived flexible formulas that originally made organ transplantation a practical patient service. The key therapeutic principles are: first, recipient pretreatment using antibodies or other modalities for conditioning; and second, minimal short-term immunosuppression after transplantation. The value of adjunct donor haematolymphopoietic cells should then become apparent.

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