It has been commonly recognized since the earliest days of clinical skin grafting\(^1,2\) that transplanted nonautologous tissues fail unless the recipient’s immune system is modified in some sustained manner.\(^3,4\) In the past century, the underlying immune mechanisms of allograft rejection have become increasingly defined and exploited through immunosuppressive drugs, permitting organ transplantation to become a successful therapy for most end-stage organ diseases. Experimentally, certain modifications of the recipient have also been shown to permit “actively acquired tolerance”\(^4\) of allografted organs—that is, graft acceptance without an ongoing requirement for therapy or apparent immune compromise. These methods have been studied with the intent of facilitating clinical transplantation without immunosuppression-associated adverse effects.

Experimental allograft tolerance is now commonly achieved in rodents and has been demonstrated in higher animals, including pigs,\(^5,6\) dogs\(^7-9\) and non-human primates,\(^10,11\) with increasing regularity. Thus, rejection is not a biologic inevitability. Indeed, in humans, tolerance to transplanted allografts has been achieved, although its occurrence remains uncommon and unpredictable.

In this article, we review the clinical allograft tolerance literature and survey the general mechanisms that are applicable to kidney transplantation. We discuss the anecdotal reports of human tolerance and recent attempts to induce prospectively tolerance clinically and outline efforts to make tolerance a more common and predictable clinical outcome.

DEFINING TOLERANCE

Although there are many definitions of tolerance, clinically it is the maintenance of stable allograft function without clinically evident immunosuppression. This can include true tolerance, the absence of any detectable detrimental immune response and no immunocompromise, and operational tolerance, the gross phenotype of tolerance with an immune response or deficit that has no significant clinical impact. These scenarios manifest through a variety of immunologic processes that are simultaneously at play, including modulations in effector cell precursor frequency, antigen presentation efficiency, effector cell activation threshold, regulation, and altered cell trafficking. Thus, tolerance should be considered a mosaic phenotype—the result of multiple competing variables with the end outcome achieved via many potential combinations.

Tolerance is also a product of one’s environment. Most immunocompetent individuals are capable of mounting an alloimmune response under facilitating conditions, just as apparently healthy
people can develop autoimmunity independent of their genotype.12,13 Similarly, given the cross-reactive nature of alloimmunity, responses to environmental pathogens can evoke an alloimmune response,14 and an inability to foster alloimmunity suggests some element of immunocompromise, although perhaps inconsequential. Thus, tolerance strategies and assessments require knowledge not only of an individual’s immune competency but also of the environmental threats to homeostasis.

Tolerance also varies as a matter of time and susceptibility. Time may reveal that a patient with operational tolerance, established by an inefficient immune response, is actually rejecting slowly. An organ’s functional resilience may also alter a clinician’s view such that an organ with regenerative capacity, such as the liver, may silently endure an immune attack that would lead to loss of a more sensitive allograft, such as a coronary artery. In all, the ultimate definition of tolerance rests with the sensitivity and accuracy of monitoring strategies used and the investigator’s persistence.

TOLERANCE IS, IN GENERAL, POSSIBLE

During pregnancy, a mother’s immune system tolerates an allogeneic fetus. Indeed, the propagation of mammalian species is to some extent predicated on an inability to muster alloimmunity under certain conditions. Consistent with this, clinical cases have been reported whereby graft function has been maintained indefinitely after the cessation of immunosuppression, demonstrating that operational tolerance can be achieved (Table 1). However, these spontaneous phenomena have typically occurred by way of drug nonadherence or withdrawal mandated by complications, and these conditions more commonly lead to rejection. Thus, tolerance is a stochastic event under current treatment regimens.15,16

Physicians likely underestimate the true number of operationally tolerant individuals, in part because of their inability to detect tolerance prospectively and

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<td>Roussey-Kesler et al.15</td>
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*After bone marrow transplant.

*Combined with bone marrow transplant.

Table 1. Tolerant kidney transplant recipients

the assumption that all people need immunosuppression. Clinical graft survival and rejection rates17 suggest that the liver is more tolerated than heart, kidney, and pancreas, whereas the intestine, lung, and skin almost uniformly provoke immunity. A recent review of the published liver transplant experience suggests that elective immunosuppression withdrawal is successful in up to 20% of highly selected liver transplant recipients.18 The rate in unselected cases is likely lower. The reasons for differential tolerability among organs relates at least in part to different degrees of tissue immunogenicity, such as differential densities of cells with high MHC antigen expression and professional antigen-presenting capabilities.19,20 It is attractive to adopt a teleologic view in which organs with primary barrier function such as the skin and gut are skewed toward an immune posture compared with organs with largely internal antigenic exposure. Nevertheless, even the intestine, which is thought to require comparatively high-dosage immunosuppression, has been transplanted under minimal immunosuppressive regimens.21

SPONTANEOUS TOLERANCE AFTER KIDNEY TRANSPLANTATION

The number of kidney transplant recipients reported as tolerant is small (<1%) compared with the total number of kidney transplants performed (Table 1). Studies of these rare individuals allow few generalizations. Tolerance typically appears years after transplantation, suggesting that it was achieved via a process rather than through a sudden induction, with some exceptional cases after posttransplantation lymphoproliferative disease. There is no relationship to the recipient’s underlying renal disease. Donor age tends to be lower in tolerant patients compared with the general population, suggesting that organ resilience, among other things, may be supplementing any immune properties of the recipient. Donor mismatch also tends to be lower, suggesting the relevance of a lower donor-specific precursor frequency. Most cases of tolerance are the result of noncompliance, although the typical outcome of drug discontinuation is graft loss within 8 mo.22 Similarly, modern surveys suggest a graft loss rate of 20 to 40% in patients who are identified as operationally tolerant with relatively short follow-up.15,16 Nonetheless, these reports have shown that some patients do not experience rejection within the follow-up period after drug cessation. The rate of success simply fails to meet the success that is seen with continued immunosuppression, but it does demonstrate that some circumstances lead to tolerance.

The small number of tolerant patients has precluded systematic mechanistic analysis in humans. However, a recently established registry formed by the Immune Tolerance Network has embarked on exploratory mechanistic study.16 Early reports suggest significant differential expression of some candidate genes, particularly those associated with regulatory and effector T cell function, such as IL-10, FoxP3, and CXCR3 in the urinary sediment of tolerant patients. These preliminary findings fit into a general paradigm of regulatory control as one element influencing ultimate graft outcome. Other recent analyses of operationally tolerant liver transplant recipients revealed distinct monocellular cell profiles, including higher frequency of CD4+CD25+ T regulatory cells23,24 and
plasmacytoid dendritic cells\textsuperscript{25} in the peripheral blood compared with patients on maintenance immunosuppression and healthy control subjects. However, in-depth characterization of tolerant patients remains in its infancy, and there is currently no way to predict tolerance in an individual patient. Also, even fully characterized, detailed descriptions of complex circumstances do not equate with control over the outcome.\textsuperscript{26}

Nevertheless, from the clinical experience accumulated so far, it is reasonable to hypothesize that some patients, perhaps most, do not need to take daily immunosuppressive medications at the same dosage for life. In light of the growing recognition that alloimmunity fluctuates with environmental immunity,\textsuperscript{14,27} it is likely that the risk for rejection undulates and may be able to be controlled with variable, risk-related dosing regimens. For example, because the pace of a de novo alloimmune response has some time requirement and rejection is typically slow to develop when noncompliance is late,\textsuperscript{22} intermittent immunosuppression may be completely sufficient in many cases. This is supported by recent results using spaced immunosuppressive therapy after depletional induction.\textsuperscript{28} However, if the need for nonspecific immunosuppression is transient, then perhaps is tolerance.

It is interesting to note that the transition of tolerance was established with its first description by Billingham et al.\textsuperscript{4} Although many have cited the classic experiments of Medawar’s group as a description of robust and enduring tolerance, their original experiment reported that only three of five mice became tolerant of skin grafts after perinatal injection of donor tissue, and one of these three animals lost the skin graft between days 75 and 91 after transplantation. This is paralleled by reports of late allograft rejection\textsuperscript{29,30} and graft function deterioration\textsuperscript{15} in long-term tolerant patients.

The reasons for tolerance’s instability are likely related to the intrinsic nature of the adaptive immune system; namely, it is adaptive. In keeping with the exceptional insight of Medawar, this, too, was anticipated in the original defining studies.\textsuperscript{4} The phenomenon of alloimmunity derived from immunity against cross-reactive environmental pathogens is referred to as heterologous immunity and has been clearly shown to deter or break experimental tolerance\textsuperscript{14} (reviewed by Selin et al.\textsuperscript{27}). It is likely that this process is increasingly at play in the clinic, where transplantation is performed in recipients with mature and varied environmental exposure histories.

**STRATEGIES TO ACHIEVE TOLERANCE IN THE CLINIC**

An understanding of spontaneous tolerance is useful to reaffirm its credibility as a clinical goal. However, observations must transition to interventional studies if tolerance is to have clinical impact. The transplant literature is replete with successful preclinical strategies from which to draw for clinical investigation. Indeed, tolerance has been achieved in many preclinical large animal models, including dog,\textsuperscript{7–9} pig,\textsuperscript{5} and nonhuman primates\textsuperscript{31,32} (reviewed by Kean et al.\textsuperscript{10} and Kirk\textsuperscript{11}). The rodent literature on tolerance is exhaustive and also well reviewed.\textsuperscript{14,33–35} This experience generally shows tolerance to be more readily achieved in small, inbred animals compared with large, outbred animals, and even robust animal models seem less rigorous than adult humans. This likely reflects species-specific differences and differential environmental antigen exposure, T cell repertoire diversity, primed T cell pool size, and rigor in long-term follow-up. Thus, all attempts to achieve tolerance in humans remain decidedly experimental, subject to appropriate oversight, and require informed patient consent.\textsuperscript{36}

Modern clinical tolerance strategies have focused on specific mechanistic principles that primarily target single immune organs or sites of antigen exposure (Figure 1). Each approach has sought control over initial antigen exposure and subsequent immune repertoire development through alterations of the antigen source and route and attenuation of the resultant response. They differ in, among other things, the sustainability of the effect with some approaches, such as depletional induction, dependent on physiologic peripheral maintenance (reviewed by Kirk\textsuperscript{37}) and others, such as chimerism, actively shaping the effector repertoire over time.\textsuperscript{38} This highlights the role of antigen exposure as a necessary step to activate the immune system for both tolerance and rejection and has been called a “window of opportunity for immune engagement” (WOFIE).\textsuperscript{39} Clin-
ical results are discussed as they relate to the predominant intervention used, with the recognition that there is overlap in the methods and effects.

The Allograft

The earliest clinical experience with kidney transplantation is interestingly the earliest experience with tolerance. In the initial experience of Starzl et al.,40,41 patients who were treated with azathioprine and glucocorticosteroids had a high rate of rejection that required repeated bolus steroids but also had a remarkably high rate of tolerance, with nine (19.6%) of 46 patients enjoying indefinite drug-free survival. A recent reanalysis of this population considered the graft itself as the protagonist of tolerance. A weakening of the donor-specific response using immunosuppressive drugs attenuates the destructive potential of the immune interface with the graft while allowing exposure to mediate sufficient effector cell activation for apoptosis or compensatory regulation. In situations of appropriate balance, the effector arm is depleted without substantial damage to the organ, and tolerance prevails. Importantly, this balance depends on some degree of activation such that overimmunosuppression interferes with the process of deletion and prevents both rejection and tolerance.

This concept of a balance between rejection and tolerance remains theoretical but is conceptually attractive. There is ample experimental evidence that graft-derived antigens foster tolerance (reviewed by Karim et al.42). However, the use of the graft inherently risks organ injury, and the stochastic nature of success has prevented this approach from being ethically testable on any large scale. Whereas true tolerance avoids both acute and chronic allograft injury, there is justifiable concern that operational tolerance will eventually give way to chronic allograft nephropathy if there is even modest intragraft inflammation. Thus, most tolerance strategies have looked to other, more expendable sources of antigen, such as hematopoietic cells, to influence the immune response. Alternatively, tolerance has been encouraged through treatments that facilitate clonal deletion or anergy in a more efficient manner, such as co-stimulation blockade.

Hematopoietic Cells

Several approaches have used hematopoietic cells as a tolerance-facilitating antigen. The methods differ in the intended role of antigen, either to drive the apoptosis of activated effector T cells (activation-induced cell death [AICD]) or to influence thymic and central lymphoid repertoire development (chimerism). These methods are frequently confused because they both involve hematopoietic cell administration but differ dramatically in the role of adjuvant therapies and conceptual implementation.

In general, T cell activation that occurs in the absence of sufficient supporting adhesion, cytokine stimulation, or costimulatory signals leads to T cell death predicated on the antigen–T cell receptor interaction (reviewed by Green et al.43). This hypothetically has the effect of selectively eliminating alloreactive T cells without eliminating environmental specificities and is conceptually relevant to the arguments favoring the graft as the tolerizing antigen. It is a peripheral (thymus-independent) mechanism. In this case, expendable hematopoietic cells function by being eliminated and stimulating an effector response, sparing the graft the brunt of the immune attack.

The use of hematopoietic cells as a surrogate antigen is to be distinguished from bone marrow engraftment. Engraftment seeks not to have the cells rejected but rather to have them permanently incorporated into the central lymphoid organs of the host (see the Chimerism section). Given the different mechanisms involved, differing immune therapies can function as either antagonists or protagonists of success. For example, a calcineurin inhibitor prevents marrow rejection, allowing eventual thymic presentation, but inhibits T cell receptor function and AICD.44-47

The most direct use of hematopoietic elements to prevent rejection involves the use of random or donor-specific blood transfusions. It has been well established that recipients of blood transfusions have somewhat improved allograft survivals.48-50 Similarly, mixed lymphocyte unresponsiveness and improved allograft survivals have been shown using donor bone marrow infusion, albeit with ongoing maintenance immunosuppression.50-52 Although these approaches have not produced clinical tolerance, their salutary effects are critically established in the experimental literature (reviewed by Wood et al.53) and clinically well documented.48-50

It is interesting that the clinical effects of donor blood transfusion became less reproducible in the cyclosporine era. This is perhaps due to the efficacy of calcineurin inhibition’s overshadowing minor transfusion-mediated effects but may also reflect inhibitory effects on AICD.44,45 Interest in the use of nonengrafting donor antigen administration is now resurfacing with calcineurin inhibitor-free protocols.54,55 Donor antigen infusion and antigen from the organ itself have been associated with microchimerism—trace numbers of donor cells (<1% of circulating cells) residing outside replicating hematopoietic niches. Although, strictly speaking, every transplant recipient is a chimera, microchimerism has been viewed as a special circumstance in many experimental studies.56-58 Clinically, only one study has suggested an association between microchimerism and tolerance.59 In general, microchimerism seems to be a property of transplant recipients and not a mechanistic harbinger of immune tolerance.59,60

Chimerism

In complete distinction from hematopoietic cell infusion and microchimerism, hematopoietic cell engraftment and establishment of macrochimerism is a durable mechanism for central tolerance. Ideally, full chimerism provides the best condition for allograft tolerance, with successful marrow replacement from a kidney donor ensuring tolerance.61-66 However, the significant morbidity of marrow transplantation makes it realistically achievable in only a small
number of cases, with morbidity clearly exceeding that of standard immunosuppressive regimens.

To achieve the same outcome as marrow transplantation with less morbidity, investigators have developed mixed chimerism\(^\text{67,68}\) (reviewed by Sykes\(^\text{38}\) and Wekerle and Sykes\(^\text{59}\)). In mixed chimerism, the recipient marrow is largely preserved but modified such that both donor and recipient hematopoietic components coexist. By avoiding donor marrow rejection, transferred hematopoietic cells populate the recipient thymus and marrow and facilitate central deletion of donor alloreactive T and B cells.\(^\text{67,69}\) This nonmyeloablative approach has the advantages of being less toxic, preserving immunocompetence, and lessening the risk for graft-versus-host disease. It does, however, depend on a rigorous early regimen that variably includes T cell depletion, transient maintenance immunosuppression, and thymic or total lymphoid irradiation to prevent marrow rejection.\(^\text{70,71}\) Once established, mixed chimerism leads to a continuous influence on the developing T cell repertoire and thus seems more durable than the comparatively acute effects of peripheral AICD. Persistent cells may also promote the peripheral effects described with marrow infusion.

Mixed chimerism has been richly investigated and proved to be a means of achieving durable tolerance in animals (reviewed by Sykes,\(^\text{38}\) and Wekerle and Sykes,\(^\text{59}\) and Sykes et al.\(^\text{72}\)). Initial clinical success was reported by Strober using a conditioning regimen based on total lymphoid irradiation (TLI; see the TLI section) and has more recently been achieved in patients who require marrow replacement for multiple myeloma.\(^\text{70,71,73,74}\) In the latter cases, the marrow donor was HLA identical with the recipient, and the conditioning regimen and marrow transplant were medically indicated regardless of the renal transplant. Pilot trials using haplodisparate donor–recipient pairs without underlying malignancy are now ongoing with cautiously optimistic preliminary results.\(^\text{75}\) Mixed chimerism seems to be a promising but practically complex approach to tolerance. Unlike in small animal models, in which tolerance depends on the persistence of macrochimerism, in HLA-disparate primates (human and nonhuman), transient chimerism has been sufficient to induce tolerance in some patients.\(^\text{32–70}\) Thus, the mechanisms involved in the clinical experience may be somewhat different from those in the experimental setting and take on some of the peripheral properties of AICD already discussed and vigorous depletion discussed next.

**TLI**

Lymph nodes and the spleen are critical to the normal development of an alloimmune response.\(^\text{76}\) As such, targeted irradiation to lymphoid tissue has been used to control the immune response after transplantation in animals\(^\text{8,31,77}\) and in patients.\(^\text{29,78,79}\) When used alone, this approach has had the effect of vigorous T cell depletion (see next section). However, more recently, TLI has been used as a means of immunosuppression to facilitate marrow engraftment and subsequent mixed chimerism.\(^\text{79}\) As a pretransplantation conditioning regimen, TLI has induced tolerance in chimeric and nonchimeric humans, whereas post-transplantation irradiation has failed to produce stable chimeras or tolerance.\(^\text{80}\)

**Lymphocyte Depletion**

Tolerance strategies all have a common aim of controlling allospecific T cell precursor frequency. Between 1 and 10% of peripheral T cells are able to recognize alloantigen,\(^\text{81,82}\) which far exceeds the frequency of T cells for any given nominal antigen. Lymphocyte depletion with polyclonal or monoclonal antibodies has been envisioned as a strategy to reduce nonspecifically the precursor frequency, allowing peripheral mechanisms to proceed with less risk for allograft injury. These agents have been used both as induction and as rescue therapy of acute rejection (reviewed by Kirk\(^\text{37}\)). Although nonspecific, polyclonal depletion is not homogeneous. For example, effector memory T cells seem to be relatively resistant to depletion.\(^\text{83}\) In addition, remaining T cells after depletion undergo homeostatic repopulation, and this has been recognized as a barrier to the development of tolerance.\(^\text{84,85}\) Thus, even vigorous depletion alone is not a reliable means toward tolerance.\(^\text{86}\) Depletion is best viewed as a component of other approaches. However, depletion does facilitate reduced maintenance immunosuppressive requirements in many patients, a condition now described as “prope” or “almost” tolerance.\(^\text{87}\)

**Co-Stimulation Blockade**

There is considerable experimental evidence that costimulation blockade facilitates tolerance induction (reviewed by Gudmundsdottir and Turka,\(^\text{33}\) Larsen et al.,\(^\text{88}\) Alegre and Najafian,\(^\text{89}\) Yamada et al.,\(^\text{90}\) and Harlan and Kirk\(^\text{91}\)). Co-stimulation blockade is based on the paradigm that specific immune responses require two signals for optimal activation.\(^\text{92}\) In the absence of a facilitating co-stimulatory signal, antigen stimulation induces anergy or apoptosis. Thus, antigen exposure, combined with co-stimulation molecule inhibition, has the effect of eliminating cells in an antigen-specific manner. Co-stimulation blockade has been used in robust preclinical models to facilitate the protolerant mechanisms at play during peripheral antigen encounter, either with the graft itself\(^\text{93–96}\) or with infused hematopoietic cells.\(^\text{97}\) It has also been used as a means to facilitate chimerism.\(^\text{98,99}\)

Although many co-stimulatory molecules have been identified and tested experimentally, only one, CD28, is targeted by a clinically available agent. CD28 is the most studied T cell co-stimulatory receptor, and its inhibition has been shown to mediate the classic effects of costimulation blockade (reviewed by Salomon and Bluestone\(^\text{100}\)). Two fusion proteins, abatacept and belatacept, have been developed to bind the ligands for CD28, the B7 molecules CD80 and CD86.\(^\text{101}\) Neither agent has been tested in clinical tolerance trials, although their promise for this eventual application is immense. A recent randomized clinical trial on the use of belatacept in renal transplantation was published with promising results for its use as an immu-
nosuppressive agent. Although designed to study its efficacy in preventing rejection compared with cyclosporine and not to address tolerance, this early study suggests that co-stimulation blockade will have a major role in future tolerance strategies.

**Thymic Manipulation**

The thymus has a central role in shaping the T cell repertoire via positive and negative selection. Thymic transplantation has been performed clinically in children with thymic agenesis (DiGeorge syndrome). In this application, it successfully restores immunocompetence and shapes the T cell repertoire. The maturation of T cells in a thymic microenvironment harboring alloantigen (donor murine islet cells or bone marrow cells) induces selective unresponsiveness in rats. Other studies have demonstrated this phenomenon after intrathymic injection of donor antigen.

In animals, the thymus itself has been transplanted in different ways to induce tolerance: As nonvascularized allogeneic thymic tissue, in composite organs (“thymokidney”), and as vascularized thymic lobe transplants. Even adult thymic grafts, after the age of thymic involution, can induce transplantation tolerance in animals. These thymus-dependent strategies of tolerance induction are still in the preclinical phase, but the experience in DiGeorge syndrome has provided some clinical proof of concept.

**Table 2. Immunologic assays used in tolerance studies**

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**MONITORING FOR TOLERANCE**

A major barrier to clinical tolerance is the absence of a method to detect it prospectively. In animal models, donor and third-party skin grafting has been used as a robust test, but this is not a practical clinical approach for many reasons. In humans, many immunologic assays have been used as surrogate tests (Table 2) to monitor the immune response after transplantation (reviewed by Newell and Larson and Najafian). Tests of antigen-specific T cell responses (mixed lymphocyte reaction, limiting dilution) have not been shown to predict the development of tolerance or been helpful guides for immunosuppression withdrawal. More recent tests of precursor frequency (enzyme-linked immunosorbent spot, tetramer analysis, and others), although promising during immunosuppression, have not yet been applied in tolerant patients. Very sensitive molecular techniques have become available to quantify relevant gene expression patterns and protein signatures in biologic samples. Using these tests, the search for a signature of tolerance is very active but remains elusive. It remains to be seen whether definition of tolerance at a given point in time will predict one’s future risk of alloimmune activation.

**FUTURE DIRECTIONS**

Since the earliest days of clinical transplantation, clinicians have sought a means of tolerance induction and been tantalized by sporadic successes. As the factors that shape an aggregate immune response have been defined, the successes of the past have become more understandable and prospective attempts have become reasonable goals. We are now in a position to make significant strides toward this goal, recognizing that we have much to do. Success will depend on multiple approaches that are rationally applied to individuals, as opposed to single approaches that are generalized for all. It is critical to recognize, however, that transplantation has become a relatively safe and effective therapy. Thus, the bar for comparative success has been raised, and tolerance must compete with valid attempts to improve long-term outcomes through dosage reduction and novel maintenance strategies.

Regimens that will likely continue to be investigated include those that are based on mechanisms of chimerism, depletion, and co-stimulation blockade. In addition, continuous monitoring of patients who achieved spontaneous tolerance and of those who did not will improve our understanding of tolerance. Finally, given the almost universal elimination of acute rejection as a cause of graft loss with current immunosuppression regimens, more attention will need to be devoted to the development of tolerance strategies that are aimed at the prevention of chronic allograft damage. Indeed, the relationship between existing immunosuppression strategies and the mechanisms of tolerance cited herein will likely have relevance in developing enduring means of avoiding chronic graft fibrosis and tubular atrophy. Funding mechanisms such as the Immune Tolerance Network and other concerted efforts to facilitate the attack on this worthy but challenging goal in transplantation will be required well into the future for success to be realized and validated.

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**DISCLOSURES**

None.

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