

more apoptosis. The deciphering of downstream signals such as the activity of PI3K in the endothelium in response to relative hypoxia might also shed some light on this provocative result.

### Looking forward therapeutically

The observations that loss of the IR and IGF-1R signaling pathways appear to counter the response to relative hypoxia and do not alter vasculogenesis in the absence of relative hypoxia suggest that it is not insulin and IGF-1 signaling in endothelial cells alone that promotes neovascularization in diabetes. It also prompts us to look to other cell types in the retina for a response to diminished insulin action. The experiments of Kondo et al. (6) do not address the role of retinal glia or neurons in vascular regulation. Answers to these questions will directly impact therapeutic choices for diabetic patients. Kondo et al. suggest that, in diabetes, inhibition of retinal insulin or IGF-1 signaling in the eye might be beneficial, however, in the context of

what is understood to be systemically reduced insulin signaling in these disease states, therapeutic blocking of insulin signaling appears counterintuitive. Identification of specific molecules at the intersection of the HIF-1 and insulin and IGF-1 signals, as well as a thorough understanding of how the varied cell types in the retina respond to the diabetic state, will necessarily precede therapeutic trials to prevent loss of vision.

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## Tolerance: Of mice and men

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Little is known about the effect of an individual's immune history on his or her response to an allogeneic tissue transplant. An important study (see the related article beginning on page 1887) now reveals that individuals harboring virally-induced memory T cells that are cross reactive with donor alloantigen are resistant to conventional strategies designed to induce transplant tolerance.

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Enormous progress has been made in the field of transplantation during the past three decades, due in large part to the availability of effective

immunosuppressive drugs. Although all of these agents suppress the immune response nonspecifically with respect to antigen, the most effective ones exhibit sufficient selectivity so that rejection can be avoided without undue compromise of the host's ability to respond to microbial pathogens. Nevertheless, patients on immunosuppressive medications are constantly walking a tightrope between the consequences of too little suppression (i.e., rejection) and of

too much suppression (infections or cancer) of their immune system. In addition, even in patients without complications due to their immunosuppression, there is an inexorable loss of transplanted organs due to chronic rejection at a rate of approximately 5% per year (1).

For these reasons, ever since the description of acquired tolerance to allografts in mice by Medawar and colleagues appeared in 1953 (2), a major goal of both clinicians and immunologists in the field of transplantation has been the induction of tolerance in transplant recipients. What has been most frustrating about this quest has been the fact that a very large number of successful approaches to the induction of tolerance have been reported in rodent models, but have failed when attempted in large animals, especially in nonhuman primates and in humans (Table 1). Indeed, as clinical results of organ transplants using standard immunosuppression are so good, at least in the short term, many clinicians are no longer interested in

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**Table 1**  
Methods that have induced transplantation tolerance

Method	Mice	Primates and humans
Enhancement	+	-
DST	+	-
Peptides	+	-
Anti-MHC mAB's	+	-
Calcineurin inhibitors	+	-
ALS	+	-
Anti-CD24	+	-
Anti-CD25	+	-
Total lymphoid irradiation	+	+/-
Anti-CD3 toxin	+	+/-
Costimulatory blockade	+	+/-
Chimerism	+	+

ALS, antilymphocyte serum; DST, donor-specific transfusion.

testing new approaches to tolerance induction unless their effectiveness has already been demonstrated in large animal models.

But why should there be such a difference in the ability to induce tolerance in mice versus large animal species? Since it is much easier and far less expensive to carry out experiments in mice than in large animals, an answer to this question could have important practical as well as theoretical implications. In this issue of the *JCI*, Adams et al. (3) propose a potential reason for the discrepancy. In an elegant series of experiments, they show that sequential exposure to sublethal infections by certain pathogenic viruses makes mice more resistant to tolerance induction by a protocol previously shown by this group to result reproducibly in mixed chimerism and tolerance in mice (4). This protocol involves treatment of recipients with a short course of costimulatory blockade, busulfan, and a donor bone marrow infusion. Assays for alloantigen-primed T cells in vitro following viral exposure confirmed that the priming led to cells with specificity cross-reactive between the pathogens and the allogeneic cells, a hypothesis, which, as they point out, has been proposed before as a possible reason for the frequent association of clinical rejection episodes with intercurrent viral infections (5). Adams and colleagues argue that the reason that resistance to tolerance induction is not complete

following viral exposures is that the ability to overcome tolerance induction in this protocol is dependent on the dose of sensitized cells. To substantiate this hypothesis, they demonstrate a dose-dependence of inhibition of tolerance induction by adoptive transfer of sensitized recipient cells to animals that are then exposed to the tolerance-inducing regimen. The only caveat to their conclusion is that the sensitized cells used for the adoptive transfer were from animals sensitized by previous skin grafts, not by viral exposures, and whether the effectiveness of these two cell populations is equivalent in vivo remains to be demonstrated.

Nevertheless, this study makes a strong argument for the importance of previous antigen exposure in determining the outcome of protocols designed to induce tolerance through mixed chimerism. The data clearly support the practice of testing for potential cellular as well as humoral sensitization against the donor prior to carrying out such protocols clinically, even in cases for which there has been no known exposure to the donor antigens.

On the other hand, the implications of these studies for the more general question of why it is more difficult to induce tolerance in large versus small animals, are not entirely clear. Indeed, the induction of tolerance through mixed chimerism is one of the few methodologies (Table 1) that has been shown to work not

only in mice, but also in large animals (6, 7) and most recently in humans (8, 9). Furthermore, the most obvious difference between small and large animal species with regard to tolerance induction is in the response to vascularized organ allografts (10). Skin graft survival is the hardest to prolong (11) unless the grafts are placed after a vascularized graft from the same donor strain, which suggests that vascularized grafts are themselves tolerogenic (12). Thus, an alternative study design, utilizing a protocol for induction of tolerance to a vascularized organ allograft, might have been more suitable for answering this general question.

Among the differences between rodents and large animals that have been suggested to account for this discrepant behavior in response to vascularized grafts are the markedly different tissue expression patterns of class II MHC antigens (13). These antigens, which are the most potent stimulators of the helper pathway in rejection reactions, are notably absent from the vascular endothelial cells of rodents, but expressed constitutively in all large animals that have been studied, including humans. Indeed, in our own laboratory, we have shown, using intra-MHC recombinant lines of pigs, that matching for class II antigens permits uniform induction of tolerance to renal allografts by a short course of cyclosporin (14), one of the many methods that allows tolerance induction to vascularized organ allografts in mice across full MHC barriers (Table 1). We have also demonstrated the importance of an intact thymus to the induction of tolerance by this route (15), something that is markedly affected by age, stress, drugs, and infection – all of which may also be relevant to the difference between large and small animal models.

Thus, I congratulate the authors of this paper for emphasizing the importance of previous antigen exposure on the outcome of allogeneic bone marrow transplantation and for helping to elucidate the mechanism of this relationship. However, I expect that differences in prior antigen exposure will be only

one of the potential reasons for the marked differences that have been encountered between mice and primates in the ease with which tolerance can be induced.

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