Letters

Transplant tolerance: is it really free of concerns?

Yong Zhao[1] and Xian Chang Li[2]

[1] Transplantation Biology Research Division, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
[2] Harvard Medical School, Transplant Research Center, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

Tolerance is the 'holy grail' of organ transplantation and, therefore, induction of a tolerant state has been actively pursued by clinicians and scientists for decades [1]. The promise of tolerance is that transplant patients will no longer require lifelong immunosuppression, which imposes considerable side effects on the patients, yet will still enjoy stable allograft survival [2]. The basic belief is that transplant tolerance can be achieved either by completely destroying the T cell clones that can attack the transplanted graft [3] or by reprogramming them into a benign or even a suppressive phenotype [4]. In fact, both mechanisms might be required for the induction and maintenance of a tolerant state [5]. If transplant tolerance was successfully established, few worries about any attendant problems would exist. However, there might be significant flaws in this thinking. A major concern is that T cell clones that respond to transplant antigens often exhibit extensive cross-reactivity with nominal antigens including pathogens, and such cross-reactivity is demonstrated in both the naive and the memory T cell compartments. Perhaps a compelling piece of evidence in this regard is the demonstration that T cell clones generated by alloantigens can respond to a peptide derived from an exogenous antigen ovalbumin (OVA) with a very high frequency (up to 30%) [6]. In animal models, challenging B6 mice with parasitic or viral antigens can produce effector T cells that are highly responsive to a defined set of transplant antigens, and transplantation of allografts to such pathogen-challenged mice often results in accelerated rejection [7,8]. Although pathogen-initiated 'danger signals' could contribute to the accelerated rejection response, the pathogen-activated effector T cells selected from recipient mice are clearly responsive to the donor alloantigens [7,8]. In humans, there is solid evidence demonstrating that pathogen-reactive T cells are an integral component of the alloreactive repertoire [9]. A typical example is the finding that T cell clones specific for the Epstein–Barr virus (EBV) peptide presented by HLA-B8 respond to three allogeneic HLA molecules (B14, B35 or B44) [10], suggesting that such EBV-responsive T cells are potentially alloreactive in transplant settings. Importantly, a significant proportion of memory T cells that develop in response to pathogens can also be alloreactive in transplant models [11]. In fact, the presence of a higher level of pathogen-induced memory T cells in patients is associated with a much higher rejection rate in clinical kidney transplantation [12]. Such cross-reactive memory T cells could have been the consequence of deliberate immunizations, heterologous immunity [8] or homeostatic proliferation [13]. The clinical implication is that in humans who have a usual history of infections and vaccinations, memory T cells that are potentially alloreactive to transplant antigens are likely to be numerous. Indeed, as much as 50% of the T cells in the periphery that are reactive to transplant antigens are T cells with a memory phenotype [14].

Clearly, the alloreactive repertoire consists of T cells that are inherently reactive to alloantigens, and T cells that are pathogen-specific but cross-reactive with alloantigens; such cross-reactive clones include both naive and memory T cells. As induction of transplant tolerance demands tolerization of all alloreactive T cells that can attack the transplants, eliminating these cross-reactive T cell clones, although beneficial to graft survival, could create unwanted risks, and certain pathogens that are normally controlled by such cross-reactive T cell clones might take advantage of the tolerant state and thrive in tolerant patients [9]. In this case, stable graft survival might be at the cost of compromised host protective immunity and immunosurveillance (Figure 1). Indeed, in a small cohort of 'operationally tolerant' patients who enjoyed stable kidney allograft function following a waning of the immunosuppression treatment, some exhibited obvious immunodeficiency in response to influenza vaccination [15], suggesting that the protective immune repertoire in some tolerant patients might have been altered.

What about tolerizing therapies that are not based on depletion of the alloreactive cells? In fact, such therapies are highly sought after for clinical transplantation, and most of these tolerizing protocols are designed to induce regulatory T (Treg) cells and/or boost their activity. Treg cells are an important cell type dedicated to maintaining self-tolerance and acquired tolerance to foreign antigens [4]. The hope is to lock Treg cells to a transplant antigen-specific state, so that they are specific to transplant antigens but do not interfere with immune responses stimulated by other nominal antigens. However, this might not always be the case, and under certain circumstances, Treg cells can suppress across the boundary of donor antigen specificities to other antigens. For example, Treg cells that are specific for the OVA peptide antigen can suppress heart allograft rejection *in vivo* after they are stimulated by the OVA peptide [16]. Thus, it is conceivable that Treg cells that are specific to a given antigen could also suppress T cells that are cross-reactive to other antigens. In cases where the tolerant status is imposed by Treg cells that are cross-reactive to other antigens, persistent immunosuppression mediated by Treg cells across the border of antigen

Corresponding author: Zhao, Y. (zhaoy@ioz.ac.cn); Li, X.C. (xli@bidmc.harvard.edu).
Available online 26 July 2007.

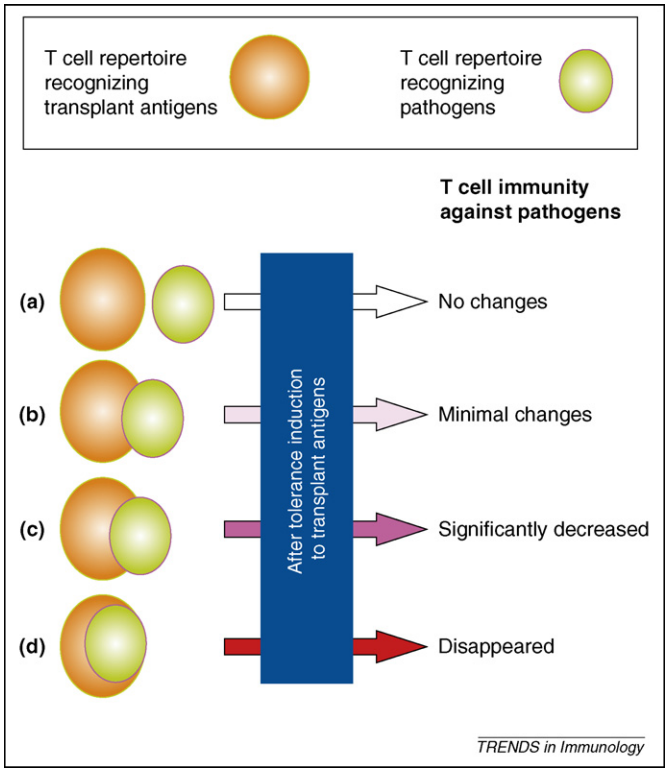

Figure 1. T cells responsive to transplant antigens can also cross-react with other nominal antigens, including pathogens. The degree of cross-reactivity could significantly affect the protective T cell repertoire after tolerance induction to transplant antigens.

specificities might also be a concern in tolerant patients. It is still entirely unclear whether Treg cells would be faithful to the specific set of transplant antigens to which they are induced. This is an important and clinically relevant issue that needs to be explored carefully.

These concerns might not be confined uniquely to transplantation; they could also apply to therapies that target T cells in other situations, including autoimmune diseases, allergy, and gene therapy strategies. However, it is important to emphasize that the precursor frequency, that is, the number of T cell clones that respond to transplant antigens, is astonishingly large [17]. It is estimated that as much as 10% of the mature T cells in the periphery are alloreactive in transplant settings, and such a responder frequency is 2–3 logs higher than T cells reactive to nominal antigens in other models [18,19]. Thus, it is conceivable that the likely detrimental impact of tolerance induction on the T cell repertoire is much greater in transplant models than in other models.

We are then left with a worrying question: is lasting transplant tolerance accompanied by an altered T cell repertoire? We therefore urge the transplant community to assess carefully the magnitude of this concern in tolerant models. Tests in this regard should go beyond the traditional analyses of responses to a third set of transplant antigens and include responses to nominal antigens in tolerant recipients [20]. Such studies might reveal likely defects associated with transplant tolerance and might also expand the concept of transplant tolerance beyond the traditional boundaries. Hopefully, studies in this area could lead to the design of greatly improved tolerizing therapies in the clinic.

Acknowledgements

We thank Fritz Bach for critical reading of the manuscript.

References

1 Sayegh, M.H. and Carpenter, C.B. (2004) Transplantation 50 years later – progress, challenges, and promises. *New Engl. J. Med.* 351, 2761–2766
2 Lechler, R.I. *et al.* (2005) Organ transplantation – how much of the promise has been realized? *Nat. Med.* 11, 605–613
3 Li, X.C. *et al.* (2001) T cell death and transplantation tolerance. *Immunity* 14, 407–416
4 Wood, K.J. and Sagaguchi, S. (2003) Regulatory T cells in transplantation tolerance. *Nat. Rev. Immunol.* 3, 199–210
5 Lechler, R.I. *et al.* (2003) The complementary roles of deletion and regulation in transplantation tolerance. *Nat. Rev. Immunol.* 3, 147–158
6 Ashwell, J.D. *et al.* (1986) High frequency and nonrandom distribution of alloreactivity in T cell clones selected for recognition of foreign antigen in association with self class II molecules. *J. Immunol.* 136, 389–395
7 Pantenburg, B. *et al.* (2002) T cells primed by *Leishmania major* infection cross-react with alloantigens and alter the course of allograft rejection. *J. Immunol.* 169, 3686–3693
8 Adams, A.B. *et al.* (2003) Heterologous immunity provides a potent barrier to transplantation tolerance. *J. Clin. Invest.* 111, 1887–1895
9 Koehn, B. *et al.* (2006) Patients, pathogens, and protective immunity: The relevance of virus-induced alloreactivity in transplantation. *J. Immunol.* 176, 2691–2696
10 Landais, E. *et al.* (2006) EBV-specific $CD4^+$ T cell clones exhibit vigorous allogeneic responses. *J. Immunol.* 177, 1427–1433
11 Selin, L.K. and Welsh, R.M. (2004) Plasticity of T cell memory responses to viruses. *Immunity* 20, 5–16
12 Heeger, P.S. *et al.* (1999) Pretransplant frequency of donor-specific, IFN-γ-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J. Immunol.* 163, 2267–2275
13 Wu, Z. *et al.* (2004) Homeostatic proliferation is a barrier to transplantation tolerance. *Nat. Med.* 10, 21–23
14 Lombardi, G. *et al.* (1989) Are primary alloresponses truly primary? *Int. Immunol.* 2, 9–13
15 Ballet, C. *et al.* (2006) Humoral and cellular responses to influenza vaccination in human recipients naturally tolerant to a kidney allograft. *Am. J. Transplant.* 6, 2796–2801
16 Karim, M. *et al.* (2005) $CD25^+CD4^+$ regulatory T cells generated by exposure to a model protein antigen prevent allograft rejection: antigen-specific reactivation *in vivo* is critical for bystander regulation. *Blood* 105, 4871–4877
17 Warrens, A.N. *et al.* (1994) Presentation and recognition of major and minor histocompatibility antigens. *Transpl. Immunol.* 2, 103–107
18 Suchin, E.J. *et al.* (2001) Quantifying the frequency of alloreactive T cells *in vivo*: new answers to an old question. *J. Immunol.* 166, 973–981
19 Song, H.K. *et al.* (1999) Alloimmune responses against major and minor histocompatibility antigens: distinct division kinetics and requirement for CD28 costimulation. *J. Immunol.* 162, 2467–2471
20 Guillot, C. *et al.* (2002) Prolonged blockade of CD40–CD40 ligand interactions by gene transfer of CD40Ig results in long-term heart allograft survival and donor-specific hyporesponsiveness, but does not prevent chronic rejection. *J. Immunol.* 168, 1600–1609

1471-4906/$ – see front matter
doi:10.1016/j.it.2007.07.001