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REVIEW

Cross-immune tolerance: conception and its potential significance on transplantation tolerance

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The diversity of alloreactive T cells in graft rejection and the presence of extensive crossreactivity among alloreactive T cells indicate that the induction of transplantation tolerance may fundamentally alter the size of host T-cell repertoire involved in protective immunity and immune surveillance, especially those that are crossreactive to conventional antigens. We herein highlight the crossreactive nature of alloreactive T cells and the potential risks of altered T-cell repertoire associated with the induction of transplantation tolerance. The possibility that T-cell tolerance to one set of antigens results in their tolerance to other unrelated antigens due to T-cell crossreactivity and/or heterogeneity is defined as 'cross-immune tolerance'. The definition and significance of this concept were discussed in details.
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INTRODUCTION

Induction of transplantation tolerance in the clinic without the needs for chronic immunosuppression remains the ultimate goal in transplantation research. Tolerance in transplant models is traditionally defined as permanent acceptance of donor organs following a period of inductive therapy, but the host immune response to other antigens is preserved. Multiple mechanisms including T-cell deletion, anergy and active immune regulation collectively contribute to the induction and maintenance of transplantation tolerance. It is well known that the frequency of T cells that respond to transplant antigens is remarkably high,[1,2] and such high responder frequency constitutes one of the potent barriers to the induction of transplantation tolerance.[3] We now know that the alloreactive T-cell repertoire involved in transplant rejection is far more complex and diverse than initially anticipated. Besides T cells that are intrinsically reactive to alloantigens, a considerable fraction of T cells that are programmed to respond to nominal antigens can also be crossreactive to alloantigens.[4–6] Importantly, recent studies have convincingly demonstrated that certain memory T cells that are specific to conventional pathogens are highly alloreactive in transplant models.[3] The crossreactive nature of alloreactive T cells clearly highlights the flexibility of T-cell receptors (TCR) in their recognition of transplant antigens. In fact, such TCR flexibility is not unique to transplant models and may represent a general feature of T-cell recognition of a wide variety of antigenic epitopes.[7,8]

The high responder frequency of alloreactive T cells in the T-cell repertoire, the presence of extensive crossreactivity to other nominal antigens and the involvement of memory T cells make the induction of transplantation tolerance a challenging task.[9,10] To accomplish this task, all the T cells that are potentially reactive to transplant antigens need to be tolerized by means of deletion, anergy or regulatory T (Treg)-cell-mediated active suppression. As more and more powerful approaches are developed to overcome such barriers, it is believed that transplantation tolerance can be achieved in the clinic in the future. However, the impact of transplantation tolerance on the host's T-cell repertoire involved in protective immunity needs to be carefully considered, especially those T cells that are specific to nominal antigens but are crossreactive with alloantigens. It is conceivable that tolerization of those T cells in transplant recipients, though beneficial to graft survival, may have significant impact on the host's protective immunity or immune surveillance. In most animal models, when transplantation tolerance is successfully established, transplant recipients often only exhibit normal cellular immunity against the third-party alloantigens. However, the potential impact of tolerance on the host's entire protective T-cell repertoire has not been carefully examined. Thus we recently emphasized the potential risks of altered host T-cell repertoire associated with the creation of transplantation tolerance.[11] We herein, for the first time, define the possibility that T-cell tolerance to one set of antigens results in their tolerance to other unrelated antigens due to T-cell crossreactivity and/or heterogeneity as 'cross-immune tolerance'. Furthermore, we will discuss in details about the specificity of T-cell tolerance and recall the urgent needs to determine the immunity against an array of pathogenic antigens besides against the third-party major histocompatibility complex (MHC) antigens in transplantation-tolerance models.

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Specificity and plasticity of TCRs

To defend against an ever-changing panoply of pathogens, the immune system has the capacity to generate extraordinarily diverse TCR repertoire that is capable of recognizing a plethora of foreign antigenic peptides presented in the context of self-MHC molecules. It is well known that antigen specificity is a key feature of TCR recognition during T-cell activation, and therefore, a given antigen only stimulates and activates T cells that express the corresponding clonotypic TCR. However, this dogma has now been challenged by studies using $CD4^+$ or $CD8^+$ T-cell clone assays[12–15] or TCR-transgenic animal models.[16] Mathematical modeling predicts that a mouse may have $1–2\times10^8$ mature T cells in the periphery displaying about 2×10^6 different clonotypic TCRs, and each naive T-cell clone is likely to express about 50 copies of a particular TCR.[17] The human T-cell repertoire is estimated to contain about 3×10^{11} T cells, and the total repertoire comprises about 10^8 distinct clonotypic TCRs, with each naive T-cell clone having a clonal size of approximately 1000 individual T cells.[18,19] Although the upper limit of the speculated TCR diversity may well exceed 1×10^{13}, it is estimated that the actual number of different clonotypic TCRs expressed by T cells is close to 2×10^6 in mice and 2.5×10^7 in humans.[20] While the specificity of TCR recognition to a given antigen is often recognized as a hallmark of adaptive immunity, the question remains that if each clonotypic TCR recognizes only a single antigenic peptide, the actual T-cell repertoire would be incapable of having all the clonotypic TCRs to cover the entire 'antigenic universe'.[20]

An alternative hypothesis suggests that a typical clonotypic TCR may have the capacity to recognize a limited pool of different antigenic epitopes. Indeed, there is mounting evidence that a given TCR often displays a considerable degree of crossreactivity in their recognition of antigenic peptide/MHC complexes.[7,8,21–23] In some cases, the different antigenic peptides recognized by a given clonotypic TCR do not share obvious sequence homology.[24,25] Furthermore, such TCR crossreactivity involves not only the recognition of different antigenic peptides bound to the same MHC molecule, but also the recognition of antigenic peptides bound to other MHC molecules.[26] Thus, besides being antigen-specific, the TCRs also exhibit considerable plasticity in their response to antigenic stimulations.

An obvious advantage of this TCR crossreactivity is that a relatively high frequency of responding T cells can be readily stimulated by any foreign antigens, which allows the protective immunity to proceed in a much more efficient way.[8] As viewed from a high vintage point, such TCR crossreactivity may be developmentally programmed in the thymus. Positive selection in the thymus is well known to be self-antigen-dependent and requires weaker TCR signals. In a transgenic mouse model that is genetically engineered to express a single peptide/MHC class II ligand in the thymus, the total number of $CD4^+$ T cells is about 20% of those in the wild-type mice. Surprisingly, these $CD4^+$ T cells expresses a wide variety of different Vβ segments in their TCRs, indicating that relatively diverse T-cell repertoire can develop in the presence of a single peptide/MHC class II ligand.[27] Furthermore, these $CD4^+$ T cells reacted with the same MHC class II molecule bound to other antigenic peptides, including one that was quite different from the selecting peptide in TCR-binding residues.[28] Thus, the TCR crossreactivity is not an unusual phenomenon and may represent an important aspect of TCR recognition in the thymus as well as in the periphery.[23,29,30] However, the presence of the TCR crossreactivity also constitutes the structural basis for heterologous immunity in which a particular T-cell response to a given antigen may influence their responses to other unrelated antigens.[31] Thus, the TCR specificity and crossreactivity likely represent a dynamic balance, and such balance can permit a sufficient number of T cells to respond to a particular pathogen challenge while at the same time maintain self-tolerance.

Several possible mechanisms may help explain the crossreactivity of TCRs, and such mechanisms include molecular mimicry,[32] epitope spreading,[33] reconfiguration of cryptic epitopes, and so on.[34] The molecular mimicry theory is evolving from a primary sequence homology to a tertiary structural homology or to a functional homology between different antigenic peptides. Recent studies showed that a given TCR could recognize a number of different peptides that may be rather distinct in their primary sequences. Thus, structural criteria rather than primary sequences may dictate the TCR recognition of different antigenic epitopes, which makes it exceedingly difficult to predict the crossreactive nature of TCRs.[7,23,35,36]

Crossreactivity of alloreactive T cells

It is well known that the frequency of T cells in the periphery that can respond to transplant antigens is astonishingly high, even without prior exposure to donor antigens.[1,2] For example, it is estimated that about 0.1–1% of the peripheral T cells are responsive to alloantigens, and this responder frequency is several logs higher than the precursor frequency to nominal antigens in naive individuals.[2,37,38] At a population level, all the T cells that can respond to transplant antigens, and therefore, participate in the rejection response are collectively called alloreactive T cells. On a per-cell basis, however, the alloreactive repertoire is likely to be extremely diverse, and may include not only T cells that are intrinsically alloreactive but also T cells that are programmed to respond to other conventional antigens but are crossreactive to transplant antigens. For example, mouse T-cell clones that are specific to poly(Glu60,Ala30,Tyr10), pigeon cytochrome c or sheep insulin can respond to a variety of allogeneic stimulator cells with a very high frequency (61% responded to at least one allogeneic haplotype, and 39% responded to more than one allogeneic stimulator).[1] In animal models, infection of B6 mice with *Leishmania major* can induce vigorous rejection of P/J skin allografts,[5] suggesting that T cells activated by the parasitic antigens can readily attack the skin allografts. Moreover, mice challenged with lymphocytic choriomeningitis virus prior to or concurrently with skin transplantation can significantly accelerate allograft rejection and prevent transplantation-tolerance induction.[3,39,40] This type of crossreactivity is not confined to animal models. There is compelling evidence to suggest that the crossreactive T cells also contribute significantly to the alloreactive repertoire in humans. One of the typical examples in this regard is the finding that human T-cell clones specific for the Epstein–Barr virus peptide presented by human leukocyte antigen-B8 also respond to three common allogeneic human leukocyte antigen molecules (B14, B35 or B44),[41,42] suggesting that such Epstein–Barr virus-responsive T cells are potentially alloreactive. Indeed, a strong correlation between virus infections (e.g., Sendai virus, cytomegalovirus or Epstein–Barr virus) and acute allograft rejection has been reported in humans.[4,41,43–45]

It is important to emphasize that certain memory T cells that are programmed to respond to conventional pathogens can also be alloreactive in transplant models, thus contributing significantly to the alloreactive T-cell pool, especially in primate models and humans.[9] Such alloreactive memory T cells may have developed by crossreaction after viral or other pathogen infections, or by other mechanisms like homeostatic proliferation and heterologous immunity.[9] In animal models, memory T cells developed after lymphocytic choriomeningitis virus infection or after homeostatic proliferation can initiate a

robust transplant rejection response.[46] Importantly, rejection mediated by such memory T cells is more resistant to tolerance induction by protocols that can readily tolerize naive alloreactive T cells.[47] The clinical importance of crossreactive memory T cells has been provided by Heeger *et al.*,[48] who have shown that a higher level of environmentally induced antidonor memory is associated with a higher rejection rate in clinical renal transplantation.

The contribution of memory T cells that are crossreactive to alloantigens to the alloreactive repertoire in both human and animal models is a critical issue in transplantation. This finding predicts that, in normal humans who have a rich history of infections and vaccinations, memory T cells that are potentially alloreactive are likely to be numerous, and therefore, the impact of the presence of alloreactive memory T cells on tolerance induction in the clinic is likely to be far-reaching. On the one hand, the induction of long-lasting tolerance demands tolerization of not only naive alloreactive T cells but also memory T cells that are alloreactive. It has been suggested that memory T cells are not as amenable as naive T cells in tolerance induction,[47] and memory T cells are often resistant to antibody-mediated depletion or Treg cell-mediated immunosuppression;[49,50] therefore, new alternative approaches may need to be considered and developed for the induction of transplantation tolerance. This is certainly a highly contested area of research and new exciting findings are being made.[51] On the other hand, tolerization of alloreactive T cells that are crossreactive with other antigens, especially the memory T cells that are crossreactive with transplant antigens, may also generate unwanted consequences. If the entire alloreactive repertoire is rendered tolerant by clonal deletion, anergy or induction of antigen-specific Treg cells, what happens to the protective immunity or immune surveillance conferred by such crossreactive T cells then? This issue has not been directly and fully addressed yet.

The definition for 'cross-immune tolerance' in transplant models

Similar to self-tolerance, acquired tolerance to foreign antigens can be induced by several different mechanisms.[52] In the case of transplantation tolerance, the alloreactive T-cell clones are destroyed and/or functionally altered by tolerizing therapies in order to establish the tolerant state. In some cases, the alloreactive T cells need to be reprogrammed to become Treg cells to maintain the tolerant state over time.[53] Clearly, the induction of long-lasting tolerance demands tolerization of the entire alloreactive T-cell repertoire.

However, the presence of crossreactive T cells (both naive and memory) in the alloreactive repertoire may present a significant problem in transplant-tolerance induction. We speculate that if all the alloreactive T cells, including the crossreactive ones, are tolerized against transplant antigens, the tolerant state may spread to other antigens that are crossreactive with such transplant antigens. For the simplicity of this discussion, the possibility that T-cell tolerance to one set of antigens results in their tolerance to other unrelated antigens due to T-cell crossreactivity and/or heterogeneity is called 'cross-immune tolerance'. It is conceptually different from the term 'cross-presentation' which refers to recipient T cells to donor antigens that are cross-presented by recipient antigen-presenting cells.[54,55] It is also distinguished from 'linked suppression' and 'infectious tolerance'.[56]

The risks of altered T-cell repertoire in transplantation tolerance

There is no doubt that the induction of transplantation tolerance offers tremendous advantages over chronic immunosuppression in the clinic.[52] However, the sheer size of the alloreactive T-cell repertoire, its cellular diversity and the presence of extensive crossreactivity may present new challenges, even if tolerance is successfully established. It is highly desirable that tolerization of alloreactive T cells in transplant recipients should avoid marked alterations of the host's T-cell repertoire and the loss of protective immunity against certain detrimental pathogens. Otherwise, the risk of compromised T-cell repertoire will be high and the obvious advantage of transplantation tolerance may be questioned.

The potential risk of transplantation tolerance may depend on the size and the identity of the host's crossreactive T-cell clones within the alloreactive repertoire (Figure 1). The following factors may affect the size of the crossreactive T-cell clones. First, the degree of MHC mis-

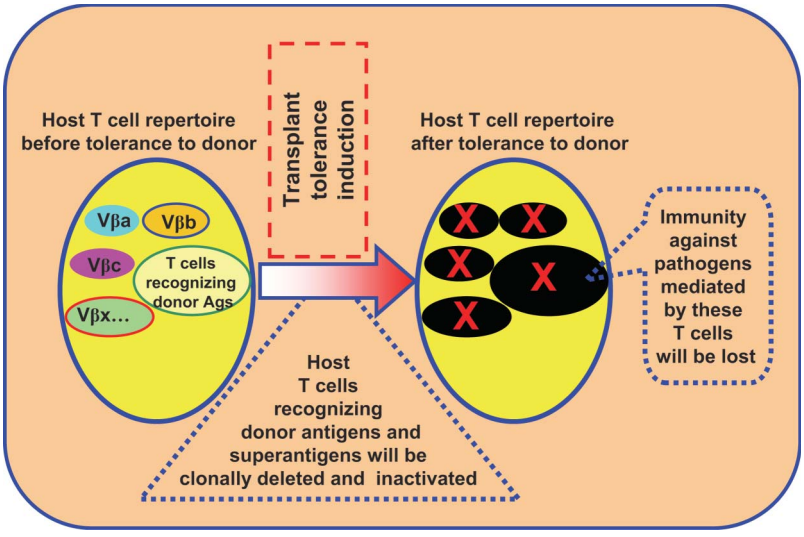

Figure 1 The host will exhibit immunodeficiency to certain pathogens if the T-cell clones recognizing these pathogens are deleted during transplantation-tolerance induction. Certain Vβ families and T-cell receptor (TCR) clones will be deleted during tolerance induction to donor antigens. If one pathogen is recognized by some of those T cells, the host will display poor cellular immune response to it. The T-cell clones recognizing both donor and pathogen antigens via crossreaction are deleted during transplantation-tolerance induction. The decreased immunity against pathogens caused by transplantation tolerance via crossreaction is called 'cross-tolerance'.

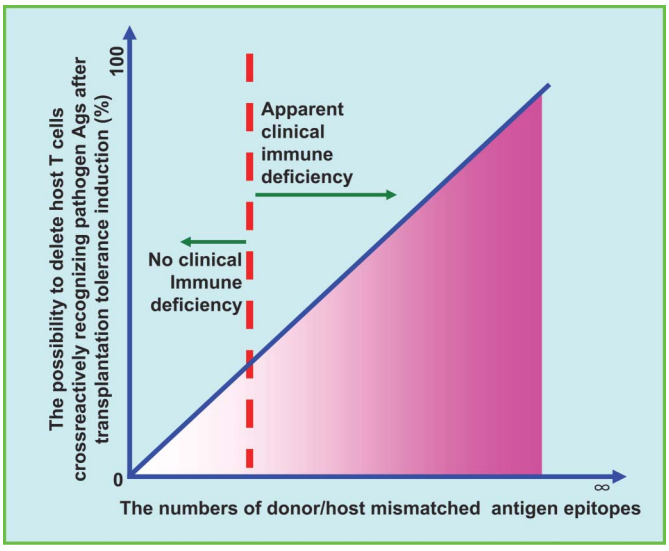

Figure 2 A relationship between the number of the donor/the host mismatched antigen epitopes and the possibility to delete the host's crossreactive T cells after transplantation-tolerance induction. The maximum number for the mismatched epitopes between the donor and the host is proposed as ∞, and the deletion of all the T cells is recognized as 100%.

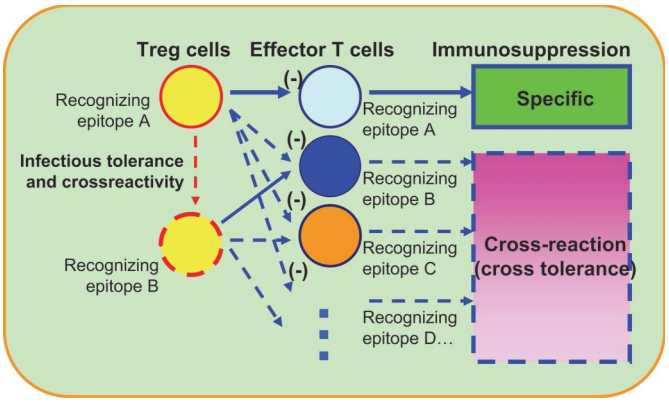

Figure 3 Regulatory T (Treg) cells may play a role in immune responses to other antigens via crossreactivity and/or infectious tolerance. Donor-antigen-specific Treg cells induced during transplantation-tolerance induction may also have inhibiting ability to other antigen epitopes via crossreactivity. In addition, the Treg cells may induce new Treg cells via crossreactivity and infectious tolerance, so the cross-immune regulating effect will be enlarged and may contribute to the establishment of cross-immune tolerance in certain level.

matched between donors and recipients may be significant in this regard. Apparently, the mass of alloreactive T cells in transplant recipients is strikingly different between complete MHC-mismatched, partial MHC-mismatched or MHC-matched transplants, and therefore, the crossreactive T-cell clones in these different circumstances are likely to be different. If the transplant recipients have minimal crossreactive clones that potentially respond to other antigens in the alloreactive repertoire, the induction of tolerance may have little impact on the host's T-cell repertoire, and the hosts are likely to have normal cellular immunity against all the other antigens including pathogens.[11] In cases where the crossreactive clones in the alloreactive repertoire are large, and some of the crossreactive clones are reactive to pathogen-derived antigenic epitopes, the host's cellular immunity against these pathogens can be seriously compromised or diminished when transplantation tolerance is established.[11] Under such conditions, transplantation tolerance may actually create an unwanted opportunity for viruses and other infectious agents to gain access to the host's immune system. Second, the immune history of an individual may dictate the size and the composition of memory T cells in the immune system, thus affecting the number of memory T cells that are crossreactive to transplant antigens. This issue is directly related to the protective immunity of transplant recipients, and may have broad impact on clinical transplantation. Finally, the mismatched superantigens between the donor and the host may cause dramatic alteration of the T-cell repertoire after transplantation tolerance is achieved, as some superantigens can cause as high as 20% of T cells to proliferate. Thus, the impacts of tolerance on the T-cell repertoire in individuals with different types of transplant are likely to be different. The T-cell-repertoire changes may be even more complex in xenotransplantation-tolerance settings.

Clearly, with the increasing number of mismatched antigenic epitopes (MHC, superantigens, tissue antigens, and so on) between donors and recipients, the possibility to delete host T cells that are recognizing pathogenic antigens by crossreactivity will certainly increase by transplantation-tolerance induction (Figure 2). If the number of mismatched epitopes between the donor and the host is below a certain level, obvious immunodeficiency against environmental pathogens will not be observed in tolerant recipients. However, when the number of mismatched epitopes is high between donors and recipients, the number of T-cell clones that are deleted or inactivated by tolerizing therapies may be high enough to render a certain degree of immunodeficiency in transplant recipients.

The contribution of Treg cells to the acquisition of transplantation tolerance is well established.[53] However, the precise role of Treg cells in the induction and the possible consequence of cross-immune tolerance are largely unknown, which need further investigation. It is well known that Treg cells are dedicated to the creation of self-tolerance and acquired tolerance to foreign antigens, and are supposed to be exquisitely antigen-specific.[57] There are cases where Treg-cell-mediated immunosuppression can be antigen-nonspecific, albeit activation of such Treg cells depends on stimulation with specific antigens. For example, Treg cells that are specific for ovalbumin antigens can suppress heart-allograft rejection *in vivo* once they are activated by ovalbumin peptide.[58] In addition, it is reasonable to speculate that Treg cells that are specific to a given antigen may also suppress the activation of effector T cells that are crossreactive to that particular antigen (Figure 3). In the case of transplantation tolerance induced and maintained by Treg cells that are also crossreactive to other antigens or actively suppress crossreactive T-cell clones, immunosuppression mediated by Treg cells on the cellular immunity against potential pathogens, may be amplified when transplantation tolerance is established (Figure 3). Furthermore, 'linked suppression' and 'infectious tolerance' may further increase the possibility to suppress more clones of crossreactive T cells. Certainly, this notion needs to be vigorously tested.

Indeed, in a small cohort of 'operational tolerance' patients with stable functional kidney allografts after the immunosuppression treatment is stopped, some, albeit not all, tolerant patients exhibit obvious immunodeficiency in response to influenza vaccination,[59] indicating that the protective immune repertoire in some tolerant patients may have been altered. It will be interesting to determine whether the immune responses of these transplant-tolerant patients to other vaccinations display some deficiency or not.

The significance of the conception, cross-immune tolerance, on experimental and clinical transplantation-tolerance induction

The recognition of the potential risks of cross-immune tolerance in transplant models is critically important in the clinic. To determine the specificity of transplantation tolerance by detecting the immune response of recipients to the third-part allogeneic antigens, which was often used in our transplant models, may be far from being sufficient. Intensive assays for the immune responses to an array of pathogen antigens or eiptopes with high-speed screening techniques for transplant-tolerant recipients are needed. Furthermore, even with successful transplantation-tolerance induction in all MHC-mismatched settings or in xenotransplantation, the efforts to increase MHC matching between the donor and the host may still be needed so that the recipient's T-cell repertoire is not tremendously changed to open an immunodeficient hole for pathogen infection. Importantly, determining the crossreactivity of the recipient immunity against the unmatched tissue antigens including MHC, tissue antigens, superantiges, and so on in donors to potential pathogen antigens before transplantation-tolerance induction may be critical and essential in the future. It will significantly increase the safety and advantages of transplant-tolerance induction in clinics.

In addition, it is possible that the risks caused by cross-immune tolerance in xenotransplantation cases, in which more mismatched antigens exist, may be more serious. However, it is important to point out that even with the presence of cross-immune tolerance and the potential risks for the decreased immunity against pathogens, the induction of transplantation tolerance remains an attractive and highly sought-after approach to avoid chronic nonspecific immunosuppression and graft loss. Furthermore, we believe that the concept of cross-immune tolerance may also have clinical implications in therapies targeting T cells in patients with autoimmune diseases. However, the markedly lower percentages and less T cell clonals responding to certain autoantigens, compared with those to MHC-mismatched allo- or xeno-donor antigens, possibly make the impact of cross-immune tolerance on immunity against pathogens less significant.

Concluding remarks

With our continuing efforts to overcome the barriers that impede transplantation-tolerance induction, we have made remarkable progresses in developing more potent transplantation-tolerance-induction strategies, some of which are already in the process of clinical trails. However, we are ill-informed about the potential impact of transplantation tolerance on the overall T-cell repertoire in immune-competent individuals. With the presence of extensive cross-reactivity of alloreactive T cells and the complexity of memory T cells in the graft rejection response, the possibility that the induction of transplantation tolerance may fundamentally reshape the immune repertoire is likely to be biologically significant. While transplantation-tolerance induction demands tolerization of all T cells that are potentially alloreactive, the establishment of such tolerant state may also compromise the hosts' protective immunity and normal immune surveillance by deleting or tolerizing the crossreactive T cells. This notion needs to be carefully considered and vigorously tested. With the establishment of large databases of protein sequences and their three-dimensional structures as well as the high-speed screening biotechnology, the hosts' cellular immunity against an array of pathogens or epitopes that are potentially crossreactive to transplant antigens may be tested for prognostic purpose. This will help us better understand the T-cell crossreactivity and the cross-immune tolerance, predicting the potential risk of viral infection, vaccination and tolerance induction, as well as evaluating the sensitivity for transplantation-tolerance induction.

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1 Ashwell JD, Chen C, Schwartz RH. 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* 1986; **136**: 389–395.
2 Suchin EJ, Langmuir PB, Palmer E, Sayegh MH, Wells AD, Turka LA. Quantifying the frequency of alloreactive T cells *in vivo*: new answers to an old question. *J Immunol* 2001; **166**: 973–981.
3 Adams AB, Williams MA, Jones TR, Shirasugi N, Durham MM, Kaech SM *et al*. Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest* 2003; **111**: 1887–1895.
4 Finberg R, Burakoff SJ, Cantor H, Benacerraf B. Biological significance of alloreactivity: T cells stimulated by Sendai virus-coated syngeneic cells specifically lyse allogeneic target cells. *Proc Natl Acad Sci USA* 1978; **75**: 5145–5149.
5 Pantenburg B, Heinzel F, Das L, Heeger PS, Valujskikh A. T cells primed by *Leishmania* major infection cross-react with alloantigens and alter the course of allograft rejection. *J Immunol* 2002; **169**: 3686–3693.
6 Taylor DK, Neujahr D, Turka LA. Heterologous immunity and homeostatic proliferation as barriers to tolerance. *Curr Opin Immunol* 2004; **16**: 558–564.
7 Brehm MA, Pinto AK, Daniels KA, Schneck JP, Welsh RM, Selin LK. T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. *Nat Immunol* 2002; **3**: 627–634.
8 Mason D. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol Today* 1998; **19**: 395–404.
9 Adams AB, Pearson TC, Larsen CP. Heterologous immunity: an overlooked barrier to tolerance. *Immunol Rev* 2003; **196**: 147–160.
10 Sachs DH. Tolerance: of mice and men. *J Clin Invest* 2003; **111**: 1819–1821.
11 Zhao Y, Li XC. Transplant tolerance: is it really free of concerns? *Trends Immunol* 2007; **28**: 376–377.
12 Markovic-Plese S, Hemmer B, Zhao Y, Simon R, Pinilla C, Martin R. High level of cross-reactivity in influenza virus hemagglutinin-specific $CD4^+$ T-cell response: implications for the initiation of autoimmune response in multiple sclerosis. *J Neuroimmunol* 2005; **169**: 31–38.
13 Hausmann S, Martin M, Gauthier L, Wucherpfennig KW. Structural features of autoreactive TCR that determine the degree of degeneracy in peptide recognition. *J Immunol* 1999; **162**: 338–344.
14 Zhao Y, Gran B, Pinilla C, Markovic-Plese S, Hemmer B, Tzou A *et al*. Combinatorial peptide libraries and biometric score matrices permit the quantitative analysis of specific and degenerate interactions between clonotypic TCR and MHC peptide ligands. *J Immunol* 2001; **167**: 2130–2141.
15 Bhardwaj V, Kumar V, Geysen HM, Sercarz EE. Degenerate recognition of a dissimilar antigenic peptide by myelin basic protein-reactive T cells. Implications for thymic education and autoimmunity. *J Immunol* 1993; **151**: 5000–5010.
16 Oldstone MB, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* 1991; **65**: 319–331.
17 Casrouge A, Beaudoing E, Dalle S, Pannetier C, Kanellopoulos J, Kourilsky P. Size estimate of the alpha beta TCR repertoire of naive mouse splenocytes. *J Immunol* 2000; **164**: 5782–5787.
18 Wagner UG, Koetz K, Weyand CM, Goronzy JJ. Perturbation of the T cell repertoire in rheumatoid arthritis. *Proc Natl Acad Sci USA* 1998; **95**: 14447–14452.
19 Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human alphabeta T cell receptor diversity. *Science* 1999; **286**: 958–961.
20 Nikolich-Zugich J, Slifka MK, Messaoudi I. The many important facets of T-cell repertoire diversity. *Nat Rev Immunol* 2004; **4**: 123–132.
21 Reiser JB, Darnault C, Gregoire C, Mosser T, Mazza G, Kearney A *et al*. CDR3 loop flexibility contributes to the degeneracy of TCR recognition. *Nat Immunol* 2003; **4**: 241–247.
22 Goldrath AW, Bevan MJ. Selecting and maintaining a diverse T-cell repertoire. *Nature* 1999; **402**: 255–262.
23 Kersh GJ, Allen PM. Essential flexibility in the T-cell recognition of antigen. *Nature* 1996; **380**: 495–498.
24 Reay PA, Kantor RM, Davis MM. Use of global amino acid replacements to define the requirements for MHC binding and T cell recognition of moth cytochrome c (93–103). *J Immunol* 1994; **152**: 3946–3957.
25 Gautam AM, Lock CB, Smilek DE, Pearson CI, Steinman L, McDevitt HO. Minimum structural requirements for peptide presentation by major histocompatibility complex

class II molecules: implications in induction of autoimmunity. *Proc Natl Acad Sci USA* 1994; **91**: 767–771.
26 Lang HL, Jacobsen H, Ikemizu S, Andersson C, Harlos K, Madsen L *et al*. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. *Nat Immunol* 2002; **3**: 940–943.
27 Ignatowicz L, Kappler J, Marrack P. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell* 1996; **84**: 521–529.
28 Ignatowicz L, Rees W, Pacholczyk R, Ignatowicz H, Kushnir E, Kappler J *et al*. T cells can be activated by peptides that are unrelated in sequence to their selecting peptide. *Immunity* 1997; **7**: 179–186.
29 Wucherpfennig KW. T cell receptor crossreactivity as a general property of T cell recognition. *Mol Immunol* 2004; **40**: 1009–1017.
30 Cohn M. Degeneracy, mimicry and crossreactivity in immune recognition. *Mol Immunol* 2005; **42**: 651–655.
31 Page KR, Scott AL, Manabe YC. The expanding realm of heterologous immunity: friend or foe? *Cell Microbiol* 2006; **8**: 185–196.
32 Fujinami RS, Oldstone MB. Molecular mimicry as a mechanism for virus-induced autoimmunity. *Immunol Res* 1989; **8**: 3–15.
33 Vanderlugt CL, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat Rev Immunol* 2002; **2**: 85–95.
34 Warnock MG, Goodacre JA. Cryptic T-cell epitopes and their role in the pathogenesis of autoimmune diseases. *Br J Rheumatol* 1997; **36**: 1144–1150.
35 Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, Chairunsri A *et al*. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat Med* 2003; **9**: 921–927.
36 Daniel C, Horvath S, Allen PM. A basis for alloreactivity: MHC helical residues broaden peptide recognition by the TCR. *Immunity* 1998; **8**: 543–552.
37 Blattman JN, Antia R, Sourdive DJ, Wang X, Kaech SM, Murali-Krishna K *et al*. Estimating the precursor frequency of naive antigen-specific CD8 T cells. *J Exp Med* 2002; **195**: 657–664.
38 Hornick PI, Brookes PA, Mason PD, Taylor KM, Yacoub MH, Rose ML *et al*. Optimizing a limiting dilution culture system for quantifying the frequency of interleukin-2-producing alloreactive T helper lymphocytes. *Transplantation* 1997; **64**: 472–479.
39 Welsh RM, Markees TG, Woda BA, Daniels KA, Brehm MA, Mordes JP *et al*. Virus-induced abrogation of transplantation tolerance induced by donor-specific transfusion and anti-CD154 antibody. *J Virol* 2000; **74**: 2210–2218.
40 Williams MA, Onami TM, Adams AB, Durham MM, Pearson TC, Ahmed R *et al*. Cutting edge: persistent viral infection prevents tolerance induction and escapes immune control following CD28/CD40 blockade-based regimen. *J Immunol* 2002; **169**: 5387–5391.
41 Burrows SR, Silins SL, Khanna R, Burrows JM, Rischmueller M, McCluskey J *et al*. Cross-reactive memory T cells for Epstein–Barr virus augment the alloresponse to common human leukocyte antigens: degenerate recognition of major histocompatibility complex-bound peptide by T cells and its role in alloreactivity. *Eur J Immunol* 1997; **27**: 1726–1736.
42 Burrows SR, Khanna R, Burrows JM, Moss DJ. An alloresponse in humans is dominated by cytotoxic T lymphocytes (CTL) cross-reactive with a single Epstein–Barr virus CTL epitope: implications for graft-versus-host disease. *J Exp Med* 1994; **179**: 1155–1161.
43 Yang H, Welsh RM. Induction of alloreactive cytotoxic T cells by acute virus infection of mice. *J Immunol* 1986; **136**: 1186–1193.
44 Carlquist JF, Shelby J, Shao YL, Greenwood JH, Hammond ME, Anderson JL. Accelerated rejection of murine cardiac allografts by murine cytomegalovirus-infected recipients. Lack of haplotype specificity. *J Clin Invest* 1993; **91**: 2602–2608.
45 Landais E, Morice A, Long HM, Haigh TA, Charreau B, Bonneville M *et al*. EBV-specific $CD4^+$ T cell clones exhibit vigorous allogeneic responses. *J Immunol* 2006; **177**: 1427–1433.
46 Williams MA, Tan JT, Adams AB, Durham MM, Shirasugi N, Whitmire JK *et al*. Characterization of virus-mediated inhibition of mixed chimerism and allospecific tolerance. *J Immunol* 2001; **167**: 4987–4995.
47 Vu MD, Clarkson MR, Yagita H, Turka LA, Sayegh MH, Li XC. Critical, but conditional, role of OX40 in memory T cell-mediated rejection. *J Immunol* 2006; **176**: 1394–1401.
48 Heeger PS, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA *et al*. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 1999; **163**: 2267–2275.
49 Neujahr DC, Chen C, Huang X, Markmann JF, Cobbold S, Waldmann H *et al*. Accelerated memory cell homeostasis during T cell depletion and approaches to overcome it. *J Immunol* 2006; **176**: 4632–4639.
50 Zhao Y, Sykes M. Resistance to monoclonal antibody-induced $CD8^+$ T-cell depletion in thymectomized MHC class II-deficient mice. *Transplantation* 1997; **64**: 489–494.
51 Kean LS, Gangappa S, Pearson TC, Larsen CP. Transplant tolerance in non-human primates: progress, current challenges and unmet needs. *Am J Transplant* 2006; **6**: 884–893.
52 Sykes M. Mixed chimerism and transplant tolerance. *Immunity* 2001; **14**: 417–424.
53 Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003; **3**: 199–210.
54 Blankenstein T, Schuler T. Cross-priming versus cross-tolerance: are two signals enough? *Trends Immunol* 2002; **23**: 171–173.
55 Heath WR, Kurts C, Miller JF, Carbone FR. Cross-tolerance: a pathway for inducing tolerance to peripheral tissue antigens. *J Exp Med* 1998; **187**: 1549–1553.
56 Waldmann H, Adams E, Fairchild P, Cobbold S. Infectious tolerance and the long-term acceptance of transplanted tissue. *Immunol Rev* 2006; **212**: 301–313.
57 Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M *et al*. Immunologic tolerance maintained by $CD25^+$ $CD4^+$ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 2001; **182**: 18–32.
58 Karim M, Feng G, Wood KJ, Bushell AR. $CD25^+CD4^+$ 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* 2005; **105**: 4871–4877.
59 Ballet C, Roussey-Kesler G, Aubin JT, Brouard S, Giral M, Miqueu P *et al*. Humoral and cellular responses to influenza vaccination in human recipients naturally tolerant to a kidney allograft. *Am J Transplant* 2006; **6**: 2796–2801.